National Agricultural Biotechnology Council Report

Cover: NABC 17 focused on agricultural biotechnology products and processes beyond food and energy. The bottom illustrations represent examples that are under research and development. The top illustrations represent areas of expected benefit, including improved economics and decreased environmental impact, as well as expanded opportunities for agriculture and rural communities. As identified at NABC 17, governmental regulations are needed for commercialization and societal benefits to be realized, as represented by the disconnection between the segments.

Trees are being genetically engineered (represented bottom left) for faster growth to diminish demands on natural forest stands, to clean up toxic wastes, and with lignin content modified to improve efficiency of pulp production. Chinese brake fern (bottom middle) is being appraised as a means of removing arsenic from contaminated soils, and genetically engineered (GE) species of *Nicotiana* (bottom right), related to tobacco, are under study as vehicles for the production of pharmaceuticals—vaccines, antibodies, *etc*. Trees, genetically engineered for reduced lignin content, will require less chlorine for bleaching of pulp from dark brown to white (top left) for paper manufacture. Benefits to the environment (represented top middle) will accrue also as a result of using GE plants for phytoremediation of soils contaminated with metals such as mercury, lead and cadmium, and/or toxic organic compounds and explosive materials. Using plants as vehicles for production of long-shelf-life pharmaceuticals (and industrial compounds) holds promise for developed and developing countries alike, as well as possibilities for improving farm incomes and rural economies (top right).

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Top: Quak F. Lee (University of British Columbia) / Allan Eaglesham (NABC) / Allan Eaglesham (NABC)

Bottom: Dawn Parks (ArborGen) / Michael Blaylock (Edenspace) / Maelor Davies (Kentucky Tobacco Research and Development Center)

NABC Report 17

Agricultural Biotechnology: Beyond Food and Energy to Health and the Environment

Edited by
Allan Eaglesham, Ricardo Bessin, Robert Trigiano, and Ralph W.F. Hardy

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NABC Report 17
Agricultural Biotechnology:
Beyond Food and Energy to Health and the Environment

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National Agricultural Biotechnology Council

Providing an open forum
for exploring issues in
agricultural biotechnology

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Thanks are due also to the other members of the Program Committee for the excellent agenda and choice of speakers: Glenn Collins, Lisa Collins, Nancy Cox, Maelor Davies, Haven Miller, Scott Smith and Gabriel Wilmoth (all of the University of Kentucky), Jack Britt, Alan Brown, Max Cheng, Kurt Lamour and Neal Stewart (all of the University of Tennessee), and Tony Shelton (Cornell University). A special expression of gratitude goes to Lori Garkovich and Randy Weckman (both of the University of Kentucky) and to Kim Jensen and Bill Park (both of the University of Tennessee) for facilitating the discussions at the breakout sessions and for summarizing the recommendations. Thanks also to Katie Russell (University of Kentucky) and to Jason Abercrombie, Renae DeVries, Lori Osborne, Murali Rao and Lisa Vito (all of the University of Tennessee).

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And we conclude with thanks to Nancy Cox (University of Kentucky) on behalf of NABC for her exemplary leadership as Chair, 2004–2005.

Ralph W.F. Hardy
President
NABC

Allan Eaglesham
Executive Director
NABC

December 2005

PREFACE

The seventeenth annual meeting of the National Agricultural Biotechnolongy Council (NABC 17)—*Agricultural Biotechnology: Beyond Food and Energy to Health and the Environment*—focused on the following areas:

- Plants as new sources of medicinals,
- Bioremediation, phytosensing, and ecorestoration,
- Gene-to-product development, and
- Regulation, consumer acceptance, and risk management.

Prior to addressing these issues, the stage was set with plenary presentations from Roger Beachy (Donald Danforth Plant Science Center) and Michael Rodemeyer (Pew Initiative on Food and Biotechnology).

NABC 17 was our second meeting to focus on emerging product and/or process opportunities for agriculture outside of the traditional food, feed and fiber markets. The first, NABC 12—*The Biobased Economy of the Twenty-First Century: Agriculture Expanding into Health, Energy, Chemicals, and Materials*—led to the initiation of the *World Congress on Industrial Biotechnology and Bioprocessing: Linking Biotechnology, Chemistry and Agriculture to Create New Value Chains.* Summary Proceedings of the 2004 and 2005 World Congresses, edited by NABC's Executive Director Allan Eaglesham, are available from NABC.

The products and processes of NABC 17 are almost all at the research stage, whereas there is up to 15 years experience with commercial products in the enzyme and crop areas. Economic and environmental benefits of the products discussed at NABC 17 could be large, *e.g.* plants engineered to produce low-cost medicinals with ease of scale-up stated as a unique advantage compared to traditional methods of manufacturing pharmaceuticals, plants that remediate soils *in situ* instead of wholesale excavation and landfill placement, and trees modified for lower lignin content so as to decrease processing costs while increasing pulp yields with less environmental impact. However, all have major not-yet-well-defined regulatory hurdles to navigate. This report provides cutting-edge information on a cross-section of these novel products and processes and includes open dialog on regulatory and related issues. It emerged that some academic scientists believe that biotechnology products are over-regulated, because regulation is based on process not trait. All, including those from industry and the Biotechnology Industry Organization, support the necessity for regulation. The bottom line is cautious optimism for commercial use of these products; at this time there are few green lights, many yellow and some red.

NABC 17, hosted jointly by the Universities of Kentucky and Tennessee June 27–29, 2005, had 151 attendees, including representation from twenty-one countries.

At the conclusion of the formal presentations, expert panelists made brief statements followed by audience Q&A. Attendees then convened in breakout sessions for further discussion of issues raised by the speakers and the panelists, and to make recommendations to policymakers.

This volume contains an overview of the meeting, a summary of the breakout workshops and the recommendations, the plenary and module presentations, and presentations made during the banquet and luncheons. Transcripts of panelists' statements and the Q&A sessions are also provided.

NABC 18, *Agricultural Biotechnology: Economic Development through New Products, Partnerships, and Workforce Development,* will be hosted by Cornell University June 12–14, 2006, with sessions on the Ithaca and Geneva campuses. Further information may be accessed via http://nabc.cals.cornell.edu.

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PART I

Meeting Summary

Agricultural Biotechnology: Beyond Food and Energy to Health and the Environment

Allan Eaglesham
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Ithaca, NY

NABC's seventeenth annual meeting, co-hosted by the Universities of Kentucky and Tennessee, convened at the Renaissance Hotel, Nashville, TN, June 27–29, 2005, a few blocks from the Ryman Auditorium (the original venue of the Grand Ole Opry), Broadway (Music City's famous "heartbeat") and the Country Music Hall of Fame. The modular structure of NABC 17 juxtaposed sessions on plant-made pharmaceuticals, bioremediation and associated issues, product development, and regulatory and risk-management considerations. The 151 attendees included university and industry researchers and administrators, educators, members of federal agencies and non-governmental organizations, and journalists.

In the plenary session, keynote addresses were delivered by Roger Beachy (Donald Danforth Plant Science Center) and Michael Rodemeyer (Pew Initiative on Food and Biotechnology). These were followed by modules composed of formal presentations and brief contributions from panelists:

- Module I–*Plants as New Sources of Medicinals: Production of Protein Pharmaceuticals in Food and Non-Food Plants*
  - Speakers: Maelor Davies (University of Kentucky) and Schuyler Korban (University of Illinois)
  - Panelists: Henry Miller (Hoover Institution) and Mark Nelson (Grocery Manufacturers of America)
- Module II–*Bioremediation, Phytosensing, and Ecorestoration*
  - Speakers: Bruce Ferguson (Edenspace Systems Corporation), Jacqueline Shanks (Iowa State University) and Scott Merkle (University of Georgia)
  - Panelists: Lena Ma (University of Florida), Steve Rock (EPA Cincinnati) and Neal Stewart (University of Tennessee)

- Module III–*Gene-to-Product Development*
  - Speakers: Maud Hinchee (ArborGen), Vincent Chiang (North Carolina State University) and Elizabeth Hood (Arkansas State University)
  - Panelists: Roger Conway (USDA Office of Energy Policy and New Uses), Alex Day (Kentucky Life Sciences Organization) and William Goldner (USDA Small Business Business Innovation Research)
- Module IV–*Regulation, Consumer Acceptance, and Risk Management*
  - Speakers: Cindy Smith (USDA-APHIS Biotechnology Regulatory Services), Thomas Redick (Gallop, Johnson & Neuman LC) and Kim Waddell (American Vineyard Association)
  - Panelists: Thomas Hoban (North Carolina State University), Canice Nolan (European Commission to the United States) and Allan Bennett (University of California at Davis)

Banquet and luncheon presentations were delivered by Wayne Parrott (University of Georgia), Gregory Jaffe (Center for Science in the Public Interest) and Michael Phillips (Biotechnology Industry Organization).

Discussion among the participants occurred within three breakout sessions composed of four smaller groups. A summary of those discussions and emerging recommendations is provided elsewhere..

Plenary Session

Roger Beachy ("Controlling Traits in Transgenic Plants: Tools that Enhance Value and Reduce Environmental Release") discussed the benefits of controlling expression of transgenes in plants, for example to maximize the effect of a gene product in a specific tissue at a specific growth stage as a means of eliminating adventitious presence of the product or nonessential release into the environment. He described the development of systems to control gene expression at will, upon induction by environmental conditions or by chemical (small molecule) application to cause a gene to be turned on—at a high, medium or low level like a rheostat—or shut off. The basic components of a good gene-switching system are a suitable inducer and a receptor-like protein that binds to a ligand that regulates the inducer. A promoter that responds to the inducer increases or decreases expression of the gene of interest. An EcR (ecdysone receptor) approach has been developed, employing receptors found in lepidopterous insects that are activated by specific juvenile hormones. When the EcR protein in the cytoplasm complexes with the ligand, it is transported to the nucleus and binds with the gene of interest inducing expression. In this case, the ligand is the insecticide methoxyfenozide (Mimic®). Arabidopsis plants have been engineered with a gene-switching unit that causes production of transcription factors that bind to chimeric promoters that are expected to activate eight or ten different promoters and, therefore, eight or ten different reporter genes. It may soon be possible to modify metabolism in several biochemical pathways simultaneously. How science

becomes relevant to the public and to commercialization depends upon many factors. Gene switching may assist consumer acceptance.

Michael Rodemeyer ("Can You Get There From Here? Speed Bumps in the Road To Health And Environmental Biotech Applications") questioned whether plant biotechnology can be harnessed to provide benefits outside of the area of food and feed. Can the kind of global deadlock that has emerged from the introduction of genetically modified (GM) crops and food be avoided? Is the opposition to the use of plant biotechnology limited to its use in food? Can the potential health and environmental benefits of the next generation of plant biotechnology change the contours of the global debate? What obstacles await the commercialization of health and environmental applications of plant biotechnology? Experience and common sense suggest that every application is likely to have its own opportunities and challenges; Rodemeyer suggested that across-the-board predictions are likely to be misleading. Although the hurdles to commercialization of health and environmental plant-biotechnology applications are significant, he sees reason for cautious optimism. The regulatory system is slowly responding to the need to evolve for new and different types of biotechnology products. Management and stewardship requirements are becoming clearer. And experience suggests that the market welcomes safe, innovative products that provide perceived benefits to buyers and to the public. The challenge to developers is to ensure that the potential benefits of this technology are clearly explained to the public while the government continues to ensure safety. If developers can do that, then there is indeed a way to get there from here.

Plants as New Sources of Medicinals: Production of Protein Pharmaceuticals in Food and Non-Food Plants

The concept of "molecular farming" was born in the early 1980s when it became possible to envisage crops as sources of proteins that originally derived from microbial or animal sources. Maelor Davies ("Plant-Made Pharmaceuticals: An Overview and Update") described the advantages of plant-made pharmaceuticals: overall economy of production, lack of need for major capital investment (*e.g.* in fermentation bioreactors), ease and economy of scale-up, lack of risk of contamination with human pathogens, *etc*. However, significant markets for plant-made proteins failed to develop; by the mid-1990s plant molecular farming was essentially stalled. Concerns about contamination of existing crops—and food or feed products—with compounds from the corresponding transgenic crop would be moot if pharmaceuticals, for example, were synthesized with "vehicle" plants that had hitherto not been developed for food or feed. Tobacco and related *Nicotiana* species offer excellent potential for development of a new, dedicated system for crop synthesis of pharmaceuticals and other useful products.

Schuyler Korban ("Opportunities and Challenges for Plant-Based Vaccines") described the move, in recent years, towards developing subunit vaccines whereby linear immunogenic epitopes of a pathogen elicit production of antibodies. These alleviate concerns over risk of reversion of attenuated strains to aggressive forms of the pathogen. And a novel approach for developing subunit vaccines has emerged as a result of genetic engineering technology: use of plants as vehicles for developing new products. As the technology to

produce vaccines in plants goes through the regulatory pathway and demonstrates its economic feasibility, it may also overcome public-perception concerns that have dogged agricultural biotechnology in the past decade. The likelihood that plant-based vaccines can be administered via oral or intranasal routes—rather than via the hypodermic needle—will add to their desirability as well as their economic benefits. All this will have a major impact on public health, particularly in developing countries. However, much work remains to be done, including the establishment of standardized safety-assessment models. Risk assessment must be science-based for the results to be believable and trustworthy. Increased funding of research in this field will accelerate the advances made thus far, and bring this technology closer to commercialization and worldwide use.

Henry Miller, who spent several years with FDA, made the case against regulation of transgenic organisms based on the process used to produce them rather than on the host and the added trait. He criticized both highly activist and more moderate public-interest organizations, and emphasized that the regulatory system and associated costs have debilitated public-sector agricultural biotechnology.

Mark Nelson expressed concern over the possibility of adulteration of food if PMPs are produced in crops such as corn, soy and canola. The Grocery Manufacturers of America has asked for a safety evaluation of the implications if PMP-producing food crops are commingled in the food supply; reasonable standards are needed.

Bioremediation, Phytosensing, and Ecorestoration

Bruce Ferguson ("Systems Agriculture: Towards a Sustainable Agricultural and Environmental Policy") described Edenspace's projects in phytotechnology: using ferns to remove arsenic from soils; engineering plant biosensors—"phytosensors"—to detect and monitor environmental parameters such as heavy metals; engineering plants to produce higher yields of ethanol per acre; and forming a new agricultural cooperative to provide additional income to producers who work on environmental projects. These and similar projects have afforded a broad range of experiences, including site characterization and environmental remediation, plant genetic engineering and APHIS field permitting, market research, and marketing and sales. From this experience Ferguson offered the following observations and recommendations:

- Change agricultural policy from insulation to innovation.
  - When subsidies cease, farmers may be left without a means of competing with lower-cost food imports. To address this problem, rapid innovation and product development should be encouraged allowing farmers to compete by offering higher-margin value-added products.
- Promote "systems agriculture."
  - Systems agriculture is the engineering of plant traits and agricultural protocols on an integrated basis with other production technologies so as to minimize total costs of end-user products and services. The approach requires that new agricultural products and techniques be developed by considering

multiple areas of upstream and downstream production expertise together—on an integrated basis—that are now considered separately.

- Create more-receptive public opinion.
  - Traditional breeding methods are too imprecise and too slow to achieve the rapid product development needed to support a competitive US agricultural sector. A key element is to develop public demand for new transgenic plant products that directly promote human health, provide low-cost energy, *etc.*, rather than simply (though importantly) reduce producer costs with no significant benefit perceived by consumers.
- Increase government R&D funding.
  - Transgenic plant development is under-funded by the private sector, largely because of the divisiveness of the transgenic crop wars of the last 10 years. To address this market imbalance, the plant biotechnology budget should be at least quadrupled in size. Half of the increase should be apportioned to the USDA and half to other government agencies—EPA, NIH, HUD, DOT, *etc.*—to fund crop-plant research related to their missions.

Explosive chemicals that contaminate groundwater and soil—at ammunition-production and military-training sites—are toxic to many microorganisms, mammals and plants. However, some plant species have the ability to remove and transform them into less harmful compounds. Jacqueline Shanks ["Plant Transformation Pathways of Energetic Materials (RDX, TNT, DNTs)"] described genetic and biochemical studies of pathways that transform explosives and development of transgenic plants for phytoremediation purposes. An important consideration is that explosive compounds assimilated by plants can be released from the tissues by action of water, *e.g.* rain and runoff, and thus may be returned to the environment as hazardous contaminants; research is required on post-harvest fate. Less information is available on phytoremediation of dinitrotoluenes, compared to trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).

Forest trees, with their extensive root systems and ability to rapidly accumulate biomass, would be attractive tools for remediation of soil and water contaminated with heavy metals—mercury, arsenic, *etc.*—if they could be modified to handle high levels. Scott Merkle ("Engineering Forest Trees with Heavy Metal Resistance Genes for Phytoremediation") discussed the engineering of fast-growing trees with modified bacterial genes that allow them to detoxify or sequester some heavy metals, with the objective of using them for phytoremediation. Insertion of these genes required optimization of *in vitro* culture systems followed by adaptation of *Agrobacterium*- and microprojectile-mediated gene-transfer methods for each species. Yellow poplar (*Liriodendron tulipifera*) expressing a modified bacterial mercuric-ion reductase (*merA*) showed enhanced resistance to mercuric ion *in vitro*. Eastern cottonwood (*Populus deltoides*) engineered with the same gene demonstrated the ability to tolerate ionic mercury up to 400 ppm in soil; these trees are being field-tested at a mercury-contaminated site. While eastern cottonwood engineered with the organomercurial lyase (*merB*) gene

showed only slightly enhanced tolerance of organomercury, trees engineered with both *merA* and *merB* were able to convert phenylmercury acetate to elemental mercury. Preliminary work with eastern cottonwood expressing a γ-glutamyl synthetase (γECS) gene indicated that it had slightly enhanced tolerance to arsenate *in vitro*. Continuing work involving the combination of γECS with other genes for mercury or arsenic resistance may enhance the phytoremediation ability of transgenic trees.

Lena Ma noted that phytoremediation of organic contaminants, energetic materials and metals is mostly in the demonstration stage; there are no full-scale applications to date. Problems and needs are the slowness of the process—5 to 10 years—and what to do with the resultant biomass.

Neal Stewart pointed out applications of phytosensors: as indicators of phytoremediation progress, coupled with GPS to guide management of crops, to monitor agro-security, and to detect explosives (*e.g.* buried landmines). He also suggested that we need to rebuild regulations based on our current knowledge-base.

Steve Rock called attention to the Interstate Technology Regulations Council, a group of about forty state regulatory bodies that banded together to share regulation information and eliminate repetition and duplication. He observed also that many researchers in transgenics in Europe have redirected their efforts to phytoremediation.

Gene-to-Product Development

Maud Hinchee ("The Application of Biotechnology to Sustainable Forestry") stated that forest genetics, because of long generation times, is only now reaching the stage at which genetically superior trees are being planted. Improved tree genetics is occurring through mass controlled pollination: controlled crosses to create varieties that capture superior parental qualities. Biotechnology is being applied to this new germplasm base. ArborGen's mission is to develop and commercialize technologies, products and services that will ensure sustainability of the world's forests. The first-tier products are focused on *Eucalyptus*, *Populus* and *Pinus*. The largest market for *Eucalyptus* is Brazil's pulp and paper industry, and ArborGen is examining the potential to modify lignin for improved efficiency of pulp production and to accelerate growth rate. Accelerated growth in plantations, without compromising wood quality, is an objective also for *Populus* and *Pinus*; asexual propagation technologies are being employed. ArborGen is developing transformation methods applicable to elite varieties of *Eucalyptus* spp. and hybrids, loblolly pine (*Pinus taeda*), Monterey pine (*Pinus radiata*), grown in Australasia, and eastern cottonwood. Pine is transformed using a somatic embryogenesis-based protocol, with *Agrobacterium*-mediated transformation. Eucalyptus transformation is based on micro-propagated elite material; a shoot organogenic process is used after inoculation with *Agrobacterium*. ArborGen has the capacity to generate thousands of transgenic events per year for field screening and selection for each of the species of interest.

Vincent Chiang ("Understanding Gene Functions and their Control for Lignin Formation in Wood") summarized his research on genetic engineering of lignin biosynthesis for the purposes of improving wood-pulping and bleaching efficiencies. His objectives include the production of transgenic trees of low lignin content. Using aspen (*Populus*

tremuloides) as a model species, he and colleagues have characterized the biochemical functions of various genes and kinetic properties of products involved in the monolignol biosynthetic pathway. There is strong evidence that a principle phenolic flux leads to the formation of monolignols. Biochemical evidence has further demonstrated that, in this principle flux, 4-coumarate:CoA ligase (4CL) could be the enzyme limiting total lignin accumulation, whereas coniferaldehyde 5-hydroxylase (CAld5H) might control the lignin syringyl:guaiacyl (S/G) ratio. These propositions are fully supported by the *in vivo* functions of these enzymes. Transgenic trees with inhibited 4CL enzyme activity exhibit 5% to 45% reduction in lignin content. The chemical structure of the resulting lignin is essentially unchanged. More importantly, lignin reduction is compensated for by a concomitant increase in cellulose content. When antisense *4CL* and sense *CAld5H* genes were simultaneously transferred into aspen via *Agrobacterium*, transgenic trees expressing each one and both of the transgenes were produced. Lignin reductions up to 55% were achieved in antisense *4CL* plants and up to three-fold increases in S/G were observed in sense *CAld5H* plants. These effects were independent but additive, and plants expressing both transgenes had less lignin and higher S/G ratio. These transgenics are potentially valuable for pulp production. But, more importantly, these benchmark transgenics are rich sources of information for functional genomics and metabolic engineering, allowing the generation of the ultimate raw materials for wood-pulp production.

Elizabeth Hood ("Commercialization of a Protein Product from Transgenic Maize") described the steps involved in commercializing bovine trypsin as a product synthesized in transgenic maize, including proof of concept, product development (market development, patent protection, final formulation, safety assessment, *etc.*) and public acceptance and sales. Lack of public acceptance is the major barrier to producing pharmaceutical or industrial products in plants. Response to this public distrust has driven current regulations to be quite restrictive. The scientific community and the regulatory agencies are striving to gather substantive safety data to support regulations that are based on scientific principles and will protect the public as well as allow this new industry to develop. The critical asset for general acceptance is whether the consumer sees benefits and whether these perceived benefits outweigh costs and risks. When products with obvious benefits are available to the consumer, public acceptance, science-based regulations and sales will fall into place.

William Goldner described a new initiative to assist in the navigation of regulatory requirements for specialty or minor crops: the Specialty Crops Regulatory Initiative. It should assist public-sector and small private companies in meeting regulatory requirements.

Alex Day described obstacles in bringing products to market. He mentioned the challenge of bridging scientists and business people for effective communications, and noted the lack of money for seed-stage investments.

Roger Conway listed programs that assist commercialization of industrial biotechnology products. These include the Federal Biobased Products Preferred Procurement Program, which provides government markets especially for early-stage products, and the USDA CCC Bioenergy Program, which has catalyzed investment in the biodiesel industry. Other opportunities/needs exist in capital investment and education.

Regulation, Consumer Acceptance, and Risk Management

The United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) has regulated transgenic organisms since 1987, and in 2002 established Biotechnology Regulatory Services (BRS) to place a renewed emphasis and priority on biotechnology. APHIS has authorized more than 10,000 permits and notifications for the introduction of GM organisms and deregulated over sixty products for use, establishing itself as an international leader in the safe regulation of transgenic products. Cindy Smith ("Regulating Pharmaceutical Plants: Meeting the Challenge") described the significant reorganization that has occurred at BRS since its inception, making it better prepared to anticipate and respond to challenges resulting from the evolving nature of biotechnology. The newly reorganized BRS goes beyond a staff of scientists to evaluate permit applications and petitions for deregulation. It includes a Compliance and Inspection Branch, a Communications and Capacity Building Branch, a Regulatory Analysis Branch, an Office of Science, and a forecasting function that help BRS address these challenges and keep pace with the advancing science. In addition, BRS has developed five priority areas of emphasis that set program direction and provide the foundation for decision-making. The following priority areas are key to BRS's ability to meet the challenges of regulating biotechnology in general, and, specifically, plants engineered to produce pharmaceuticals:

- maintaining rigorous regulation that thoroughly and appropriately evaluates and ensures safety and is supported by strong compliance and enforcement;
- ensuring that BRS's regulatory process and decision-making are transparent to stakeholders and the public;
- maintaining a science-based system that ensures that the best science is used to support regulatory decision-making and to assure safety;
- maintaining communication, coordination, and collaboration with the full range of stakeholders; and
- establishing international leadership to ensure that international biotechnology standards are science-based, international regulatory capacity building is supported, and international implications of domestic policy and regulatory decisions are considered.

As the science progresses, BRS will continue to evaluate the implications of new technologies, enhance its processes and procedures, and develop appropriate regulations to meet the challenges posed by this new science while continuing to safeguard American agriculture, the nation's food supply, and the environment.

Thomas Redick ("Liability Prevention and Biotechnology: A Brief History of Successful Industrial Stewardship") summed up the regulatory and liability hurdles that stand in the way of launching a new transgenic product. He briefly reviewed successes and failures and existing risk-management methods to help overcome legal barriers to entry. Despite past successes, and the knowledge gained from failures and near misses, the road to future commercial success in agricultural biotechnology remains fraught with difficulties. The European Union and its like-minded trading partners will continue to hold their zero-

adventitious-presence standards over the heads of grain exporters and will increasingly drive innovation in agricultural biotechnology into contained, closed-loop production systems. Like a game of three-dimensional chess, transgenic crops will face three levels of regulatory oversight, starting with federal approvals, but with more requirements emerging at the state level (even in counties or cities) and overseas. For many transgenic crops, international approvals may be required prior to market launch within the United States (for soybean, rice, wheat and other primarily export-bound crops). As a result, US agricultural biotechnology operations will need to maintain perfect divisions between its "green," "white" and "red" sectors—food and feed, plant-pharmaceutical, and industrial applications.

In 2000, the federal government completed an interagency review of its regulatory oversight of biotechnology products that revealed that ensuring confinement could become a regulatory requirement for approval of some transgenic organisms. In 2001, the USDA asked the National Academies to review and evaluate biological methods and report on their application in confining transgenic crop plants, shellfish, trees, grasses, fish, microbes, insects and other organisms. Kim Waddell ("Biological Confinement of Genetically Engineered Organisms: Opportunities for Reducing Environmental Risks?") summarized that report (*Biological Confinement of Genetically Engineered Organisms*) with particular emphasis on: definition of bioconfinement, when and why to consider bioconfinement, bioconfinement of plants, bioconfinement of animals, bioconfinement of microorganisms, and biological and operational considerations for bioconfinement. Recommendations in the report include:

- Evaluation of the need for bioconfinement should be considered for each transgenic organism separately.
- Early evaluation of the need for bioconfinement in the development of a transgenic organism or its products is recommended.
- Bioconfinement techniques should be assessed with reference to the temporal and spatial scales of field release.
- An adequate level of bioconfinement should be defined early in the development of a transgenic organism, after considering worst-case scenarios and the probability of their occurrence.
- An "integrated confinement system" approach (defined in the report) should be used in deployment of the transgenic organism.

Current lack of quality data and science is the single most significant factor limiting ability to assess effective bioconfinement methods. Methods need to be tested in a variety of appropriate environments and in representative genotypes of the transgenic organism under consideration. In order to implement effective bioconfinement of GM organisms, the report recommended support for additional scientific research that:

- characterizes the potential ecological risks and consequences of a failure of bioconfinement,
- develops reliable, safe, and environmentally sound bioconfinement methods, especially for transgenic organisms used in pharmaceutical production,

- designs methods for accurate assessment of the efficacy of bioconfinement,
- integrates the economic, legal, ethical, and social factors that might influence the application and regulation of specific methods, and
- models the dispersal biology of organisms targeted for genetic engineering and release, where sufficient information does not exist.

Thomas Hoban was highly critical of industry, government and universities regarding agricultural biotechnology and its products, especially those from cloned animals and PMPs from food crops.

Canice Nolan identified the major problem in European acceptance of GM foods as the consumer not the regulators; food processors aren't going to source when risk is commonly associated with GM. There is consensus in Europe that the regulatory system works well and should remain in place.

Allan Bennett described the Public Intellectual Property Resource for Agriculture (PIPRA), a public-sector consortium that hopes to provide bundles of proprietary technologies—enabling and trait—to allow the benefits of biotechnology to accrue to a broader base of crops and consumers, *e.g.* specialty crops and developing-country farmers. He noted that 25% of crop-biotech patents belong to the public sector.

Banquet and Luncheon Presentations

Wayne Parrot ("The Nature of Change: Towards Sensible Regulation of Transgenic Crops Based on Lessons from Plant Breeding, Biotechnology and Genomics") reminded the audience that the literature contains many suggestions that plant genomes are highly variable. One early indication was the discovery that maize inbreds differ in the number of rDNA copies, ranging from a low of 5,000 in "W23" to 23,000 copies in "Illinois Reverse High Protein." Total DNA content varies also within crop varieties—up to 12% for soybean, 25% for red pepper and 42% for maize. Until the advent of genetic engineering technology, it is true that scientists had not crossed the species barrier in terms of gene transfer between kingdoms. On the other hand, it must be acknowledged that DNA from unrelated species is transferred and incorporated into plant genomes. Plantain bananas contain the entire genome of the banana streak virus, rice contains DNA from the rice tungro bacilliform virus, and tomato has DNA from the tobacco vein-clearing virus. The integration of viral sequences may be widespread in the plant kingdom, having occurred for a long period of time. Genes from the bacterium, *Agrobacterium rhizogenes*, have been found incorporated into the genome of some tobacco species while DNA from unrelated higher plants has been found to be transferred between their mitochondria, and, from there, to their nuclei. Although not a common phenomenon, horizontal gene transfer does take place, at least on an evolutionary time scale, and does not appear to pose any hazards to recipient plants. This and other information lead Parrot to conclude that plant genomes are variable and dynamic, constantly changing in response to breeding efforts and even to environmental conditions. Therefore, it is a mistake to treat transgenes and their associated DNA changes as inherently dangerous. Ultimately, it is the trait imparted by the transgene that matters, and, as such, it is the trait that should

be the focus of regulatory efforts, should these be warranted. In Parrot's opinion, risk to health and the environment posed by most traits is sufficiently low as to preclude the need for regulatory oversight.

Gregory Jaffe ("Creating the Proper Environment for Acceptance of Agricultural Biotechnology") stated that the past 10 years have been extremely successful for the biotechnology industry. Several blockbuster products were marketed in the 1990s, including soybeans, corn, cotton, and canola that are herbicide-tolerant and corn and cotton that produce their own insecticide to control specific pests. These varieties have been widely adopted by farmers in the United States and, to varying extents, in seventeen other countries: over eight million farmers grew 200 million acres of GM crops in 2004. These herbicide-tolerant and insect-resistant crops—biotechnology's "first generation"—have provided benefits to farmers and the environment by increasing yields and reducing the use of insecticides. Despite this success, the introduction of new GM products has slowed considerably. In February, 2005, CSPI released a study—*Withering on the Vine: Will Agricultural Biotech's Promises Bear Fruit?*—showing that from 1995 through 1999, forty-seven crops (an average of nine per year) completed the FDA regulatory process, whereas from 2000 through 2004 only fifteen crops (an average of three per year) completed the process. Although the pipeline has slowed, international controversy over GM crops has continued. Whereas most governments and many distinguished scientists have found that these crops are safe, some people continue to be concerned over perceived risks to human and/or environmental health. The controversy over genetic engineering will only increase with the next generation of products. Biotechnology-industry and university researchers are inserting a wide range of engineered traits into many different organisms. While research on drought or salt tolerance may reduce the controversy over genetic engineering if it benefits small-scale farmers in developing countries, engineering plants to make pharmaceuticals or industrial products is particularly worrisome when food crops are employed; no one would want to eat corn flakes containing a vaccine, for example. With the current state of affairs and controversial new applications on the horizon, international debate over the pros and cons of agricultural biotechnology is likely to increase.

Michael Phillips ("The Importance of Stewardship in Agricultural Biotechnology") described a training program being developed by the Biotechnology Industry Organization (BIO), laying out principles for confining plants making pharmaceuticals and those making industrial products. Workshops dealing with compliance aspects affecting GM corn, cotton and soybean will be offered in conjunction with professional society meetings and conferences such as those organized by NABC. Not only is industry participation expected, the courses will be offered also to universities and federal research agencies to help ensure that all abide by the federal requirements and understand the legal implications involved in conducting field trials with GM crops. Furthermore, BIO is planning to provide accreditation as part of the incentive to participate. It is hoped that continuing education credits (CECs) will be offered. For biotechnology to continue to evolve, commitment to good stewardship on the part of the industrial sector will be essential, together with embracement of federal regulatory policies.

PART II

Breakout Sessions

Breakout Sessions: Summary of Discussions and Recommendations

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The NABC-17 workshop discussions focused on *Bioremediation, Phytosensing, and Ecorestoration*; *Gene-to-Product Development*; and *Regulation, Consumer Acceptance and Risk Management*. For the first two topics, the following aspects were discussed: opportunities for innovative applications, obstacles to innovative applications, how public policies could overcome the obstacles, and how research and development could be advanced. For the third topic, discussants focused on the public's beliefs and attitudes about agricultural biotechnology, actions that industry could undertake to address consumers' fears and concerns. Participants were encouraged by the facilitators to focus on non-food applications for genetically modified (GM) plant technologies in defining appropriate evaluation criteria. The workshop discussants focused largely on impediments to commercialization and public acceptance of non-food products of biotechnology. The predominant observations were that the companies involved in commercialization of new, non-food technologies tend to be small, such that the regulatory process is particularly burdensome. Broad-ranging recommendations listed below focused on industry, the regulatory agencies, public acceptance and associated policy needs, and the role of public-sector entities such as universities and the government:

Role of Industry

- Improve communications between and among scientists in the private and public sectors, and with consumers and policymakers. Pro-actively seek partnerships with groups who share mutually desired outcomes who would help with funding, regulatory approval, commercial development, and market acceptance of genetically modified products.

[1]This summary draws upon a verbal report on the workshop discussions delivered at the end of the conference by Lori Garkovich (University of Kentucky) who received input from fellow-facilitators Kim Jensen (University of Tennessee), Bill Park (University of Tennessee) and Randy Weckman (University of Kentucky).

- Create more effective messages that fairly and adequately address the costs and benefits of biotechnology, emphasizing how GM products can help address critical public concerns (*e.g.*, improvement of environmental quality).
- Acknowledge public concerns and fears by explaining how the current research, development and regulatory processes already guard against potential problems.

Regulatory Agencies

- A strong theme of the conference was that regulatory policies should be focused on the product rather than the process used to produce it. Therefore, participants recommended redesigning the regulatory process by basing decisions on a broader, different, and more clearly defined set of criteria and developing new roles for participants in the process.
- Regulatory decision processes should be transparent and should utilize cost, benefit, and liability analyses that include a cost accounting throughout the life cycle of the product to determine the speed and priority of regulatory reviews.
- Regulators should clearly define what steps must be completed and what information is necessary for approval of a genetically engineered product. Small companies in particular lack the intellectual infrastructure and financial capital to effectively negotiate a regulatory approval process that is still dynamic and often lacks clarity with respect to criteria for decision-making.
- As part of redefining the regulatory process, agencies should evaluate current definitions and standards for key concepts used in the evaluation and approval process and consider their applicability to GM plants with respect to site remediation, risk assessment, liability and adventitious presence.
- Dialogues with regulatory agencies and multiple stakeholders should be opened in an effort to identify common ground, and encourage regulators to become proactive spokespersons in explaining the decisions they render.

Consumer Acceptance and Public Policies

- For the smoothest path to commercialization to occur, a shift to a "market-driven" rather than a "science-driven" focus needs to guide the research and development process. In particular, because of issues related to GM foods, other GM technologies must be developed with a clear understanding of consumer acceptance and risk-benefit analyses. These needs are compounded when one considers international cultural, social and political environments.
- A knowledge/information gap exists between scientists who develop technologies and those who will use them. With respect to the technologies addressed at NABC 17, there is a clear opportunity to educate the public about potential risks and benefits. With bioremediation in particular, the purchaser is likely to be a government agency but perhaps still subject to the same perceptions as the general consumer.

- It is also recommended that an informed public-policy discussion begin to assure that planning and funding for bioremediation activities be placed up-front into site-planning for industrial and commercial construction sites. These activities should include preference for phytoremediation practices, where possible, at federally funded cleanup sites, tax incentives to promote the use of phytoremediation, and allocation of pollution fines or Superfund monies for this activity.
- Strategies should be employed to build on existing public support for technology applications such as the following: animal vaccines and veterinary uses, agricultural crop phytosensing to improve the efficiency of crop production, and research on plant-made pharmaceuticals (PMPs) directed at "orphan" diseases and health needs in developing countries.
- A clear recommendation to encourage continued public acceptance of PMPs is to focus such research on crops that cannot cross with other food/feed species, such as *Nicotiana* plants developed by the Kentucky Tobacco Research and Development Center.

Role of Universities, Government and Public-Sector Organizations

- Workshop participants recognized the role of public research institutions as a driving force in creating knowledge for development of new technologies. It is recommended that the reward system of universities be reoriented to encourage intellectual efforts directed towards the commercialization pipeline.
- Continued coordination and communication with regard to university-owned technologies, as conceptualized by the Public Intellectual Property Resource for Agriculture (PIPRA), is strongly recommended. The PIPRA initiative recognizes the collective strength of public research institutions with respect to the number of patents controlled. Most importantly, PIPRA not only serves in clarifying issues related to freedom to operate, but also promises to inform and streamline the regulatory approval process.
- Creation of a repository of novel GM organisms was recommended to ensure their preservation for possible future use. Development of many products is in abeyance due to the current climate of regulatory requirements and consumer acceptance, which collectively result in less venture capital investment.
- The workshop participants advocated study by a newly commissioned National Research Council committee to examine scientific, regulatory and liability issues related to non-food, non-energy uses of GM plants. The charge should include evaluating the roles of phytoremediation and PMPs in enhancing environmental quality and human health and to make recommendations on regulatory aspects.
- Increase funding to regulatory agencies sufficient to address the greater challenge posed by biotechnology research and its commercial products.

- Universities also need to expand the scope of work of their Offices of Technology Transfer to include seeking potential partners for GMO research at the beginning so that commercialization issues (such as market applications) can be incorporated into the research and development process.
- Universities should promote science literacy by developing K–12 science education modules to introduce students to biotechnology and working with state curriculum committees to include biotech issues in science curricula.
- A recurring theme of the conference was that public-sector entities like NABC, universities, and government agencies should strive to improve communications between/among scientists, industry, public and policymakers, with particular emphasis on risks and benefits of new technologies. It is considered that the extension services of land-grant universities could be important forces in reinvigorated communications efforts. These efforts should acknowledge public concerns and fears by explaining how the current research, development and regulatory processes already guard against potential problems.

PART III

Plenary Session

Controlling Traits in Transgenic Plants: Tools that Enhance Value and Reduce Environmental Release

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Having just come from Montreal where I participated in the Meeting of the Parties of the Cartagena Biosafety Protocol and then later at BIO2005, it is clear that a number of issues face plant science and biotechnology. There is much to be said about the importance of public-sector scientists speaking their minds and stating the facts with regard to the issues of applications of agriculture biotechnology. Now is an absolutely appropriate time. Last year's conference—gleaned from leafing through the NABC-16 proceedings volume—reminded us how difficult it is for scientists in the public sector to develop a product via agricultural biotechnology. The costs required to take a product from the experimental stage to commercialization are overwhelming for scientists in the public sector. The regulatory oversight policies that have accompanied this industry have, in my opinion, gotten so far out of hand that we in the public sector can no longer effectively bring products to market.

How does one capture trait value and how does one prevent the escape of a trait?

Today I will discuss the rationale for controlling the expression of transgenes—including the use of gene-switching technologies—that will reduce the transfer of transgenic traits and may reduce regulatory concerns of agricultural biotechnology. A description of the technology of chemical control of gene expression will be followed by a brief discussion of some of the potential issues of concern about the use of gene-expression technology. Lastly, I'll put the technology in the context of the topic of this conference: How does one capture trait value and how does one prevent the escape of a trait where it is not wanted,

either through theft or through out-crossing, while reducing adventitious presence of a GM product in a non-GM crop.

It is important to find ways to continue to innovate while making the products of agricultural biotechnology profitable, if the technology is to reach its potential. My concern is that if we don't find ways to capitalize on the significant investments that have been made in the basic plant sciences by federal and non-federal funding sources by developing relevant knowledge and potential products, there will eventually be reductions in research funding in the public sector.

Controlling gene expression for commercially valuable traits may be necessary as a means of capturing value.

There are several benefits of controlling expression of transgenes in plants.

- For basic studies of gene function. Many gene functions are lethal if mis-expressed and it may be desirable to exert tight control over gene expression.
- To reduce the spread of a genetic trait to a weedy relative or to non-GM varieties of the crop plant. Control of trait expression can significantly reduce likelihood of adventitious presence of a controlled product and, of course, nonessential release to the environment. For example, when considering the use of plants to produce a novel food product, an industrial or pharmaceutical material, gene switching will limit expression of the materials to plants or plant tissues in which the gene is activated.
- To capture value of the new trait. Controlling gene expression for commercially valuable traits may be necessary as a means of capturing value, in particular in crops that are inbred, or where seeds can be saved.

Methods to Control Gene Expression and Applications in Biotechnology

The regulation of gene expression is a complex process that requires the coordinated activity of proteins and nucleic acids that ultimately determine whether a gene is or is not transcribed, and if transcribed, results in production of a protein that produces a phenotype. Most of the emphases of studies of gene expression have been on regulation of gene transcription, and a number of technical methods are used to affect the control of gene expression. First, one can use a promoter that has known regulatory characteristics; for example, a promoter that is expressed only in vascular tissues, in the leaf epidermis, seed endosperm or embryo, and so on. Or one can mix and match fragments of DNA and transcription factors to develop chimeric promoters that have the desired patterns and levels of gene expression.

In my laboratory we study a promoter that is expressed in plant vascular tissues [a promoter from rice tungro bacilliform badnavirus (RTBV)] and two transcription factors (Rf2a and RF2b). The factors, in conjunction with other co-factors and components of RNA polymerase II, are responsible for tissue-specific gene expression of the RTBV promoter. The RTBV promoter is expressed only in vascular tissues in transgenic rice, *Arabidopsis* and tobacco plants. However, when genes encoding RF2a or RF2b are constitutively expressed (using the constitutive 35S promoter) the RTBV promoter was likewise expressed constitutively (Petrucelli *et al*., 2001; Dai *et al*., 2004). As a consequence of this and other research, we identified the DNA-sequence element to which RF2a and other transcription regulators bind to govern expression of the promoter (Dai *et al*., 2006). We have used these and other elements to create a regulatable gene-transcription cascade that can be "put to work" to control the expression of transgenes in plants, including using a chemical gene switch.

The remainder of the discussion will be devoted to describing systems that can be used to control expression of genes at will. The challenge with all systems is that none is perfect for all applications, and it is important to define the intended application prior to making a choice. Some switches are more appropriate than others for experimental use and for field use. To date, most research on gene switching has been applied to laboratory studies. Nevertheless, the potential applications of gene switching are numerous and it is anticipated that a number of commercial applications will be developed.

Basic Components

The basic components of a good chemical gene-switching system include: (1) a suitable inducer; (2) a receptor-like protein that binds the ligand; (3) a promoter that is activated or repressed as a consequence of binding of ligand to the receptor. Over the last 15 years, a number of gene-switching technologies have been developed, including those induced by cations, phytohormones, steroid-related molecules, antibiotics, ethanol, herbicide safeners, and other organic molecules. Several recent reviews have described the state of the science in this topic area *(e.g.*, Padidam *et al*., 2003).

A suitable chemically regulated or inducible gene-expression system will have a number of characteristics, including: (1) high specificity of the ligand for the receptor to ensure that genes are tightly regulated to be "on" or "off" in the absence of the ligand; (2) the ligand should be readily taken up by the plant and move to all organs and tissues; (3) the ligand should elicit a rapid response; (4) the ligand should be non-toxic to the target and non-target organisms; (5) the ligand should be active at low concentrations and have a favorable environmental profile; (6) the ligand should be suitable for convenient application, either as a foliar spray, seed treatment, or root drench; (7) application should be low cost.

We have experimented with and adapted a system based on the ecdysone receptor (EcR) found in lepidopteran insects

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The EcR-Methoxyfenozide System

In my laboratory, we have experimented with and adapted a system based on the ecdysone receptor (EcR). These receptors are found in lepidopteran insects that are activated by ecdysone, a compound that regulates insect growth. In this system an inactive EcR receptor remains in the cytoplasm until ecdysone, or a suitable agonist, binds, after which the ligand-receptor complex is transported to the nucleus where it binds to the responsive DNA-sequence element. Under appropriate conditions, binding of the complex causes a change in gene expression. In insects and other animal cells, the system is bi-partite, and requires an additional endogenous protein. Padidam *et al.* (2003) developed a mono-partite inducible expression system that is appropriate for use in plants and plant cells. The chimeric receptor comprises the VP16-activator domain from SV40, the *gal*-4 DNA binding domain, and EcR (the chimeric receptor is referred to as "VGE"). When the gene encoding the chimeric receptor is produced from a promoter that is either constitutive or tissue-specific, it remains inactive until ecdysone or a suitable agonist binds and causes the complex to activate gene expression by binding to the gal-4 cis element that is a component of the target promoter. Methoxyfenozide is a suitable agonist of ecdysone and a suitable ligand in this system. It is proposed that methoxyfenozide causes the formation of a dimer with the receptor, which binds to the DNA-binding site on a chimeric gene. The system is easy to use and is highly active in *Arabidopsis* and other plants. Methoxyfenozide is the active ingredient in the insecticide Mimic® (Dow AgroSciences LLC).

Methoxyfenozide has a suitable safety profile for use as a gene switch in laboratory and greenhouse conditions. Furthermore, it does not cause non-specific expression of a high number of genes in *Arabidopsis* (S. Dai, I. Ordiz and R.N. Beachy, unpublished data), an indication that the ligand has very little direct effect on the host plant. Furthermore, the level of expression of a target gene can be controlled by the concentration of the ligand. In other studies, we developed several hundred transgenic *Arabidopsis* plant lines that produce luciferase upon addition of methoxyfenozide. These studies confirmed that, like other transgenes, expression of the gene-switch system is controlled by position effects and different lines respond to different concentrations of the ligand and exhibit different rates of responsiveness (S. Dai, I. Ordiz and R.N. Beachy, unpublished data).

We used the methoxyfenozide gene-switch system to demonstrate that the ligand is taken up and systemically distributed in *Arabidopsis* plants and can induce the expression of the transgene in a variety of tissue types. These studies demonstrated that the ligand is taken up rapidly when applied to roots, is transmitted throughout the plant and causes expression in all cells. We then went on to show that the system can be used to induce expression of a gene encoding the coat protein of TMV-Cg tobamovirus, and induce coat-protein-mediated resistance against the virus following addition of methoxyfenozide (Koo *et al.*, 2004). In one plant line, the level of accumulation of coat protein exceeded the highest level produced by the enhanced 35S promoter. To date we have developed more than 750 plant lines using this system and have observed a variety of levels of gene induction, from 10-fold to more than 1,000-fold following addition of the ligand.

Applications of the Gene-Switch System

In an ongoing study, we are evaluating the methoxyfenozide gene-switch system to determine whether or not gene-switching technologies can be used to induce tissue-specific expression of genes. For these studies we selected promoters that are known to be expressed only in selected plant tissues, and constructed genes with the VGE-coding sequence. The gene was co-introduced with a *uidA (*encoding GUS) reporter gene that is under control of a minimal 35S promoter ligated with the DNA-binding site for recognition by the receptor. In these studies the reporter gene was silent in the absence of VGE and methoxyfenozide. Although the study is not yet completed, we are encouraged with the results and are confident that they will show that the gene-switch system can be used to restrict gene expression to specific tissues after addition of the ligand.

How might one use a gene-switching system? One of the experiments in progress is to develop a system to control multiple genes with application of the ligand. If successful, we will use the system to activate expression of genes that cause the repression/suppression of a gene in one or more metabolic pathways while activating other genes in the same or other pathways. If successful, this will make it possible to substantially alter primary and/or secondary metabolism in plants. We do not yet know the limits of the system, but are confident that it is sufficiently robust to make significant changes in the metabolism as well as growth and development of the target plant.

The next generation of chemical gene switches will likely be substantially different and better than current systems.

The Next Generation

The next generation of chemical gene switches will likely be substantially different and better than current systems. There will be improved receptors that eliminate proteins of animal origin, and receptors that provide active repression as well as activation of gene expression. We also anticipate that a variety of ligand:receptor pairs will be developed, and that future developments will create plants that will respond to multiple gene switches.

RheoGene Co. (Philadelphia, PA) has developed several different receptor:ligand pairs that function in animal cells. It is anticipated that some of these will function in plants either in a two-protein or one-protein gene-switch system. This may make it possible to use one ligand to turn on a target gene and a second ligand to turn the gene off. Such flexibility in the system would have other uses: for example, company A might want to use a unique receptor:ligand pair while company B will want another receptor ligand pair, and so forth.

It is likely that there will be additional opportunities for ligand-receptor development with a variety of different biological characteristics. The challenge is, of course, to identify gene-switching systems that are safe for the environment, the plant, and for the final product. And, if a chemical ligand is to be released to the environment, it must pass

standard EPA toxicology tests. For this reason, scientists anticipate that early adoption of chemical gene-switching systems will involve ligands that have been approved as safe or that can be thus approved with modest investment.

Regulatory Considerations

The development of safe and reliable gene switches that are used commercially will rely on new applications of existing chemistry or on new chemistry; regulatory approvals will be required either for new use or for new chemicals that will be used. Furthermore, one must ensure that there is strict on/off control of gene expression; leakiness of gene expression will not be acceptable. This is essential if one expects the public to agree to selected types of agricultural biotechnology.

In order for chemical gene switching to be widely used, it will be necessary for regulatory agencies to adapt and undergo certain types of change. At the present time, the agencies regulate transgenic organisms on the basis of the presence or absence of transgene DNA and/or the presence of the gene product. Many scientists agree that the most important criterion for phenotype is not the presence or absence of a transgene *per se*, but whether or not the product of gene expression (*i.e.*, the RNA or a protein product) and the resulting phenotype are produced. Thus, the manner in which products are subjected to regulatory control will need to be established.

There is concern amongst some parties that limitations on trait expression will limit access of some technologies to those farmer/producers that can afford to pay high technology fees.

Public Discussion

As with any new technology, it is important to engage the public, both academic and non-academic, in discussions related to issues that may have impact on regulatory structures, on environmental safety, and with regard to possible ethical issues that may arise. It's perhaps more important now than it was in following the first breakthroughs in agricultural biotechnology in the 1980s. In January, 2005, we held a workshop at the Danforth to discuss chemical gene switching with ethicists and environmentalists to help us better understand the challenges that might be faced in bringing forward a viable gene-control system. I think that engaging the public and non-scientists in such discussions is important for all of us.

There is concern amongst some parties that limitations on trait expression will limit access of some technologies to those farmer/producers that can afford to pay high technology fees. It is considered likely that gene-switching systems will be first used on crops that will produce high-value materials; uses on field crops or other food crops are much less likely, except to restrict trait flow to non-GM crops. It is highly unlikely that gene switching will be used in the foreseeable future in commodity crops or crops that

are produced by small-scale, economically disadvantaged farmers. Unlike the so-called sterile seed technologies, gene switching as outlined here will not be applied to restrict seed germination *per se*.

I am convinced that we have an opportunity to make outstanding strides forward in biotechnology, but am increasingly concerned that much of the potential will not be realized unless we learn to deal with some of the issues that can be addressed by gene switching. Whether or not gene-switching technologies emerge as a tool to bring new agricultural biotechnologies to the public marketplace depends on many factors. Challenges notwithstanding, the potential of the technology is high and I am confident that it will be an important component of agricultural biotechnology.

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Dr. Beachy received a PhD in botany and plant pathology from Michigan State University in 1972. After postdoctoral work at the University of Arizona and Cornell University, in 1978 he was appointed to the faculty at Washington University, St. Louis. In 1991 he joined The Scripps Research Institute in La Jolla, CA, as Head of the Division of Plant Biology holding the Scripps Family Chair in the Department of Cell Biology. In 1999 he accepted the position as founding president of the Danforth Center.

Beachy was elected to the National Academy of Sciences in 1997 and received the Wolf Prize in Agriculture in 2001. He is a Fellow of the American Society for Microbiology and of the American Association for the Advancement of Science. He is a strong proponent for training of, and cooperative research with, scientists in developing countries and is an advocate for implementation of policies of technology management that encourage sharing of intellectual property, and research for the public good.

Can You Get There From Here? Speed Bumps in the Road To Health And Environmental Biotech Applications

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The global division over genetically modified (GM) foods has, by now, assumed a familiar dimension. In the United States and Canada, farmers routinely grow GM varieties of crops and consumers readily (if unknowingly) eat foods containing ingredients derived from GM crops. The US media have paid relatively little attention to GM foods, and while one cannot say that the public has accepted GM foods, it is clear that the majority of US consumers do not view GM foods with active concern. Indeed, the most salient finding of numerous polls is that US consumers remain largely uninformed about GM foods and their presence in the food supply (Hallman, 2005; PIFB, 2005).

The situation is far different in other parts of the world—parts of the world that also happen to be major markets for US farm exports. European consumers in particular are hostile to GM crops and food. Even when approved as safe by European Commission regulators, few GM foods are available for sale in the EU because retailers and manufacturers fear hostile consumer reaction to foods labeled as containing genetically modified organisms (GMOs) (USDA, 2005). As a consequence, the global market for commodities like corn has been divided into GM and non-GM zones, complicating trade. In part because of these trade disputes and market uncertainties, the future for new GM-food crops is clouded.

The question is whether plant biotechnology can be harnessed to provide benefits outside of the area of food and feed.

The question is whether plant biotechnology[1] can be harnessed to provide benefits outside of the area of food and feed. Can the kind of global deadlock that has emerged from the introduction of GM crops and food be avoided? Is the opposition to the use of plant biotechnology limited to its use in food, or can the potential health and environmental benefits of the next-generation of plant biotechnology change the contours of the global debate? What are some of the obstacles that await the commercialization of health and environmental applications of plant biotechnology?

The temptation to generalize too much should be resisted. Experience and common sense suggest that every application is likely to have its own opportunities and challenges, and across-the-board predictions are likely to be misleading. Some of the issues specific to different types of applications are explored later in this paper. Nevertheless, any new GM plant is likely to have to face four critical hurdles that will require both time and money to overcome on the road to commercialization. Some of these hurdles are no different from those faced by any novel product, while others are unique to products developed through biotechnology. First, of course, is the development of the product itself—proving technical and economic feasibility. Second, products of plant biotechnology need stewardship and management beyond that required for plants developed through conventional breeding, both as a requirement of regulators as well as the necessity of sound business practice. Third, plant biotechnology products need to pass through a regulatory review and approval process that involve both direct and indirect costs. Finally, as with any product, a plant biotechnology product must meet the ultimate marketplace test: are there buyers willing to buy it at a price that delivers a profit to the developer?

The Market Potential of Health and Environmental Biotech Applications

A number of products are being developed through plant biotechnology that could have significant health or environmental benefits beyond food or feed. Understanding what the potential market may be for these applications is an important starting point in understanding the hurdles that they face on the road to commercialization. Other contributors to this volume will develop these points in much greater detail, so only a summary is offered here.

Plant-Made Pharmaceuticals

The potential economic and safety benefits of producing therapeutic proteins from plants have been explored in a number of venues (PIFB, 2002; BIO, 2005a). The market for antibodies is projected to be $26 billion by the year 2010 (Novis, 2005), but current production practices for antibodies cannot keep pace with demand and there appears to be a significant supply shortfall. In particular, the costs associated with scaling up traditional bioreactors using animal or microbial cells create a significant bottleneck in

[1]For the purpose of this paper, the term "biotechnology" is used in the popular (rather than scientific) sense to refer to recombinant DNA techniques. Similarly, the terms "genetically modified" and "transgenic" are used interchangeably and refer to plants modified through recombinant DNA technology to introduce novel or enhanced traits.

The market for antibodies is projected to be $26 billion by the year 2010, but current production practices for antibodies cannot keep pace with demand.

the development of therapeutic proteins. One of the potential advantages of plant-made pharmaceuticals (PMPs) would be the ability to scale up relatively quickly and at relatively low cost. In addition, there may be fewer safety concerns about proteins derived from plants rather than from animal cells.

Unlike food-biotech applications, PMPs potentially distribute benefits along the value chain. For farmers, growing a high-value crop from low-cost commodity species could offer a way to enhance farm income. For consumers, the potential lower cost of therapeutic proteins would be of considerable benefit. For that reason, it is not surprising that the use of biotechnology to create lower-cost pharmaceuticals remains one of the reasons most strongly supported by the US public (PIFB, 2005). The potential application of the technology to develop vaccines that may be of particular benefit to developing countries is also the subject of considerable research and development effort in the non-profit arena (Mason *et al.*, 2002).

Forestry Applications

The application of biotechnology to forestry would also appear to have significant market advantages. Increasing demand for wood and wood products from a growing world population poses a challenge for forestry management and forest-product companies, which are increasingly under pressure to reduce logging in natural forests and to adopt environmentally sustainable practices (Hardaker, 1997; Brooks, 2001; PIFB, 2001). While these pressures have led to the development of forest plantations carefully managed to enhance growth, commercial forestry has not yet captured the benefits of improved genetics that have accounted for significant productivity gains in crop agriculture. The use of biotechnology may provide an opportunity for forestry to make genetic improvements more quickly that could help increase yields by reducing disease, improving pest resistance, and promoting faster growth. In addition, the use of biotechnology to control certain traits more directly could lead to the introduction of trees better suited for processing in specific applications, such as pulp and paper (PIFB, 2001; ArborGen, 2004; El-Lakany, 2004).

The spread of disease among major species of trees in the United States, including elms, chestnuts, oaks and the eastern dogwood, has also created an urgent need to develop disease-resistant varieties. While research using conventional breeding techniques continues, biotechnology may offer a way to introduce desirable disease-resistance traits more quickly (Osusky, 2000; PIFB, 2001; ArborGen, 2004;).

Phytoremediation

The clean up of environmentally contaminated sites remains a huge challenge in this

country. The Environmental Protection Agency (EPA) estimates that tens of thousands of contaminated sites still need clean up in the United States (EPA, 2004). In most cases, the technology of choice is simply to dig up contaminated soil and cart it to some other place. Technologies for treatment *in situ* remain costly and controversial. Phytoremediation offers a number of potential benefits, including lower costs, better performance and greater public acceptability (EPA, 2005). According to EPA (2005), field trials of phytoremediation techniques have reached a promising stage, and estimated costs of various phytoremediation techniques vary from 10% to 50% of physical, chemical, or thermal clean-up techniques. At the same time, phytoremediation is likely to be useful for only a small subset of affected sites where the contaminants lie within the root zone (EPA, 2005). Estimates made in the late 1990s suggested that the domestic market for phytoremediation ranged from $3 million to $30 million, with projections ranging as high as $370 million by 2005 (Kidney, 1997; Glass, 1998). Given this extraordinarily wide range of estimates, it is clear that there is still significant uncertainty about the potential market for the application of phytoremediation, and that much will depend on how well phytoremediation actually performs in-site clean ups. The goal of research is to use biotechnology to develop plants that are more efficient, further reducing costs and potentially decreasing the time it takes to decontaminate a site.

There is still significant uncertainty about the potential market for the application of phytoremediation.

Threshold Question: Who Bears the Cost of Product Development?

Clearly, there appear to be significant market opportunities for applications in these three areas. However, the threshold question faced by any developer is easy enough to state: who is going to pay the cost of taking the product through all of the critical stages of proof of concept, development, testing, regulatory approvals and marketing? For the private sector, products can be self-financed if the developer is a large, well capitalized company with R&D budgets, but small businesses and start-ups will need to look to venture capital and partnerships to sustain them through the development and approval processes.

The willingness of the private sector to invest in product development will depend largely on the anticipated return on investment, which includes not only consideration of potential revenues downstream, but also the costs associated with the process of bringing a product to market. Products that are likely to be commercialized through traditional private-sector incentives are those for which there is a well defined and profitable market. In addition, the private sector will tend to invest in products only where there is strong intellectual-property protection to prevent potential "free rider" and competition problems. On the cost side of the equation, some of the uncertainties unique to plant-biotechnology products make predicting development costs more difficult and raise the risk for investors.

As the history of the development of plant biotechnology demonstrates, many potential applications are unlikely to meet the conditions for private-sector development and investment. In some instances, products may lack a viable market capable of returning sufficient revenues—such as in the case of niche food crops or the development of plants modified to provide vaccines for endemic diseases in the developing world. In other instances, steep development or regulatory costs or uncertainty about market acceptance could deter private-sector development. If products with potential "public" value are going to be developed, they will have to come from the non-profit sectors: government, university, and other non-profit research institutions.

But the non-profit developer faces the same question: where does the money come from to pay the cost of taking a plant-biotechnology product through all the required steps? Since non-profit developers tend to focus on the "public goods" that are unlikely to return a profit to a private investor, they must rely on sources of funding from governments, foundations and other donors. The funding plight of non-profit plant-breeding research in the United States and throughout the world has been well documented (Frey, 1994; Heisey *et al.*, 2001). While most plant breeding used to be in the public sector, private-sector research now dominates as a result of declining public funding and new forms of intellectual-property rights and modern biotechnology that spurred increased private investment (Alston, 2004).

The funding support for plant-biotechnology products that are truly "public goods" remains a serious problem.

Non-profit institutions face additional challenges when it comes to the use of agricultural biotechnology and plant-breeding programs. Such institutions traditionally have little experience with the stewardship and regulatory issues associated with the management and development of bioengineered crops. In an environment characterized by scarce resources, the increased costs and uncertainties faced by products of plant biotechnology also operate as a significant constraint. In some instances, particularly where a product has some potential for commercialization, non-profit organizations may enter into partnerships or licensing agreements with private-sector entities that have more experience in commercialization as well as the management capabilities to deal with stewardship and regulatory issues. However, the interest of the private sector in such partnerships will still be limited by the potential profitability of the product. As a result, the funding support for plant-biotechnology products that are truly "public goods" remains a serious problem.

Speed Bumps in the Road to Market

The threshold question, stated above, is simple: who pays? The next question, of course, is: how much? Surmounting the hurdles of development, management, regulatory approval and commercialization all require investments of time, resources, and money. Having a

clear understanding of those costs is critical to all developers, but is of particular interest to private-sector developers and investors who are making business decisions about whether or not to invest in the technology. Below, the potential costs—and uncertainties—associated with each stage, with reference to health and environmental plant biotechnology applications, are considered in more detail.

Technical and Economic Feasibility

The initial hurdle, of course, is technical feasibility—that is, simply getting the technology to work. It is one thing to get a protein expressed in a plant in a laboratory; it's another thing altogether to get the trait expressed in a plant in the real world. Proponents of biotechnology have been talking about the remarkable promise of this technology for more than 20 years, but the only two commercially significant traits on the market today are herbicide tolerance and insect resistance. Part of the reason for the relatively few traits is that getting plants to do some of the things that developers long ago envisioned has proven to be more difficult than originally expected.

For example, Ingo Potrykus's development saga of "golden rice" continues today, years after the original concepts and early products were tested. Researchers are now following up on the recent development of SGR2, a golden rice variety developed by Syngenta that may produce ten times as much beta-carotene as the original SGR1 variety (Derham, 2005). Drought tolerance, a trait long pursued by plant-biotech developers in the private and non-profit sectors, appears at last to be close to moving toward the regulatory approval phase (Melcer, 2004). Even when gene sequences are successfully identified, it takes time to integrate that trait successfully into a variety with desirable agronomic or output traits. The science of plant genomics is moving ahead quite rapidly, but the complexity of gene modification to achieve commercially acceptable output or input traits is still a time-consuming and somewhat uncertain process.

One issue related to technical feasibility is access to intellectual property (IP). The impact of the introduction of strong IP-protection schemes in the plant-breeding and plant-biotech world remains a hotly debated issue. However, it seems fairly clear that, at a minimum, the development of strong IP-protection schemes raises the transaction costs (in time and money) for non-profit developers by requiring due-diligence searches to avoid infringement and to negotiate licensing arrangements when needed. On the other hand, strong IP protection is a precondition to investment by the private sector (Alston, 2004; BIO, 2005b).

Proving technical feasibility clearly remains a challenge for a number of specific non-food health and environmental plant-biotech applications. For pharmaceutical crops, for example, it remains to be seen whether plants can be modified and grown in a manner that allows consistent expression of the protein, and whether the protein will prove to be clinically equivalent and equally safe and effective as those grown in animal-cell cultures. Merispase®, a PMP designed to treat a condition that affects patients with cystic fibrosis, has been through some phase-II clinical trials intended to answer these types of questions (Meristem, 2005). Whether the predicted cost efficiencies will be realized is another key issue associated with proof of concept for PMPs.

Biotechnology applications to forestry are complicated by limited scientific knowledge of tree genomics as well as the inherent complexity of engineering an organism intended to grow for years before harvesting (PIFB, 2001). For example, it is possible that genetic changes could result in undesirable effects that would not be observed until after several years growth. Ensuring consistent expression of traits over the lifetime of a tree is also important for traits like insect and disease resistance (PIFB, 2001).

In the area of phytoremediation, there are promising laboratory and field-trial developments using genetic modifications to enhance plants' abilities to take up environmental contaminants such as metals (Bañuelos *et al.*, 2005). The question, of course, is how well such plants will work in the real world of contaminated sites; to date, field-trial data have been limited to simulated contaminated sites. For reasons discussed later in this paper, more compelling data of the efficacy and efficiency of this technology are likely to be needed before it will be applied in real-world environmental clean ups.

Product Management and Stewardship

The second hurdle is the cost of management and stewardship associated particularly with the development of bioengineered plants. Because of the environmental, food-safety, and marketing issues associated with bioengineered plants, they require special handling and management to ensure containment and, in some cases, tracking and identity preservation. Management and stewardship requirements start early in the development process, long before a plant may be ready to be commercialized; indeed, key product-development phases, including field trials, will be required to be under US Department of Agriculture (USDA) permit.

Arguably, these stewardship and management costs could be considered a part of regulatory compliance costs, because in many cases these requirements are actually mandated by regulatory agencies—as with USDA transportation and field-trial permits or notifications. On the other hand, it could also be argued that bioengineered crops would be subject to special handling and management even in the absence of regulatory requirements given potential concerns about liability under the common law of torts (Kershen, 2002) or to fulfill private contractual requirements. Particularly in the wake of the StarLink™ episode, seed companies, farmers, grain distributors and processors, and others in the food-production chain have become more conscious of the need to adopt best management practices to ensure that customers are getting what has been represented, and to avoid potential liability for GM-plant products mixing with plants where GM components are undesirable for any number of reasons.

However, as a representative of ProdiGene (2004) noted in a recent comment to USDA:

> *…no matter what system of production is employed, accidents, natural disasters, or other unforeseen events may allow the loss of containment despite best efforts.... [D]espite adherence to rigorous containment protocols, low level products not intended for food or feed have the potential to be present in commercial crops at some time.*

Given this potential, developers and investors seeking to reduce potential exposure to risk are likely to consider the availability of liability insurance in this area.

Part of the difficulty faced by developers and investors in this area is the lack of clarity and certainty about the standards to which they are being held accountable. At the present time, there are no legally binding standards or even guidelines to provide developers a "clear harbor" for adventitious presence. In the absence of legislation or regulation, liability will be determined through the rather ineffective process of litigation. To date, few cases have been litigated that shed any light on appropriate duties and responsibilities, leaving the field ripe for speculation by lawyers in law-review articles (Kershen, 2002).

Not even the regulatory agencies have taken on the task of defining tolerances or thresholds for materials from GM plants that have not completed the regulatory review process. Instead, USDA and EPA have imposed conditions on field trials that are, as a practical matter, intended to prevent any gene flow and thereby achieve a zero-tolerance level (PIFB, 2004b). Not only are these conditions expensive to follow, their existence implies there may be a legal liability for even a *de-minimus* level of contamination. It is also unclear if these conditions will be successful 100% of the time.

This issue has already been a particular challenge for developers of PMPs. Farmers, food manufacturers and others have expressed concern about any mixing of PMPs with food or feed crops, even if such mixing is unlikely to raise any environmental or health concern (Nutraceuticals International, 2003). Clearly, food manufacturers are concerned about the potential economic damage to their brands in the event of a publicized event where PMPs are found in their products. Growers are concerned that even the remote possibility of adventitious presence of PMPs in their food or feed crops could dry up lucrative markets, particularly in nations with markets hostile toward GM crops. This concern recently became a reality when Ventria's proposal to grow rice that has been genetically modified to produce a pharmaceutical compound in Missouri prompted Anheuser-Busch and Riceland Foods to threaten to boycott all rice produced in Missouri (Kasler, 2005).

Stewardship and management issues may also pose a challenge to the developers of GM-forestry applications, particularly given the long lifetimes of plantation trees. While somewhat different, given the food application, there have already been two incidents over gene flow from GM papaya trees to conventional papaya trees in Hawaii and Thailand (Creamer, 2004; Elias, 2004; Mathes, 2005). As the technology moves forward, there almost certainly will be issues associated with managing gene flow from GM trees in plantations to trees in unmanaged forests.

Managing plants to prevent unintended gene flow is less likely to be a concern with GM plants intended for phytoremediation since such plants are intended to be used on contaminated sites, far from any food or feed crops. How these plants are disposed of, however, will need to be the subject of careful consideration so that further soil contamination does not take place and to ensure these plants do not inadvertently move into the food or feed chain.

Management and stewardship issues are particularly troubling for university and other non-profit researchers who generally lack the experience of navigating regulatory requirements and managing long-term field trials under conditions of strict confinement.

Moreover, university researchers frequently lack the infrastructure and funding for such activities, an issue discussed in more detail below.

Management and stewardship requirements have clearly emerged as a significant hurdle—in money, resources, and time—on the road to product commercialization or deployment. What has made it even more difficult, of course, for developers and investors trying to make judgments about development costs, is that there remain significant uncertainties about the standards to which developers are expected to adhere. Clearly, the development of gene-expression restriction technologies, like those described by Roger Beachy elsewhere in this volume, would go a long way to reducing the costs of managing unwanted gene flow.

Regulatory Requirements—Direct Costs

The third major "speed bump" in the road to product commercialization is the regulatory review and approval process. Regulations impose additional costs on the development of bioengineered plants compared to improved varieties created through conventional breeding, but it is difficult to estimate the cost with any great precision (Alston and Kalaitzandonakes, 2005). Certainly much of the product-testing and development work required by regulators would be conducted by developers in any case, simply as a part of ensuring the quality, safety and performance of a new GM plant. As noted above, management and stewardship costs would, in many cases, be required by prudent business practices even in the absence of regulation. But, plainly, the costs associated with additional testing, data production, data-package submission, and the time associated with regulatory review, are significant. Costs for some of the initial GM-crop approvals have been estimated at $5 million to $15 million (Alston, 2004). Some analysts have estimated that half of all total development costs are associated with regulatory requirements (PIFB, 2004a). However, these costs have not been well characterized and studies are ongoing to obtain some independent analysis of those estimates.

Costs for some of the initial GM-crop approvals have been estimated at $5 million to $15 million.

One of the factors affecting the costs of regulatory approval is the novelty of the trait or the novelty of the product in which the trait is being inserted. Not surprisingly, regulators tend to approach novel issues with greater caution, often demanding more studies and additional information to help answer their questions. As a result, first products through the regulatory system unquestionably bear a disproportionate amount of the regulatory burden. Today, it is unlikely that approval of a commodity food crop with a genetic construct already approved by the regulatory agencies would cost as much or take as long as the initial approval. On the other hand, a recent report by the Center for Science in the Public Interest noted that the time for regulatory approval of a new GM plant appears to be growing longer, not shorter, even though the plants being reviewed did not seem to present novel regulatory issues (Jaffe, 2005, this volume).

Once again, the regulatory approval hurdle places a disproportionate burden on university and other non-profit researchers who lack the experience with navigating the regulatory agencies and, perhaps more importantly, often lack the funding to carry out the required testing and to prepare the regulatory approval documents. Most non-profit researchers rely on government or foundation grants that typically support basic research, but not the kind of "regulatory science" needed to develop the data package to obtain product approvals (PIFB, 2004a).

Regulatory Requirements—Indirect Costs

The most visible cost of regulation is the direct cost of testing, data submission, and delay. Delaying the time in which the product can come to market imposes real costs, particularly for products that have time-limited intellectual property protection.

Beyond these direct costs is another, perhaps less obvious set of costs associated with regulatory uncertainty. In this case, the issue is not so much about what the regulations currently require, but uncertainty about what the regulations might require in the future. Without clarity from an agency about what a product approval requires, it is impossible for developers and potential investors to estimate the total costs of bringing a product to market. Today, for example, a developer may have a fairly good sense of what it would cost to bring another *Bt* or herbicide-tolerance gene through the regulatory system. But the question of what it will cost to approve a different type of trait—such as a drought-resistance gene—is much less certain. That kind of uncertainty discourages private-sector investment.

While some parts of the regulatory framework are relatively clear, others are not. As noted previously, agencies have not addressed the issue of adventitious presence except through permit requirements intended to prevent it from occurring. Even here, the rules continue to shift, as occurred in 2003 when USDA increased setback requirements and other conditions on PMP permits, sharply limiting where PMP field trials could be conducted. The White House Office of Science and Technology Policy has called on EPA, FDA and USDA to adopt rules to address adventitious presence resulting from field trials of GM crops intended for use as food or feed (OSTP, 2002), but there has been no similar call for guidance on plants not intended for use as food or feed—such as PMPs. While USDA has indicated that PMPs will always remain under APHIS permit, the Food and Drug Administration (FDA) could also exercise its authority over the drug-manufacturing process to oversee the planting, growing, harvesting, and transporting of PMPs (FDA, 2002).

New products inevitably raise novel issues for regulators. For example, it is not clear how plants modified through biotechnology for phytoremediation purposes will be regulated. While USDA's rules with respect to transport and field testing would certainly appear to apply, EPA has asserted that it has the authority—not exercised to date—to regulate plants intended for commercial bioremediation under the Toxic Substances Control Act as "new chemical substances" (EPA, 2005).

Likewise, while USDA's authority over GM trees is fairly clear, whether USDA will "deregulate" long-lived trees intended for plantations—or what information it would

require to make that decision—is much less clear. (EPA would presumably be in charge of approving pest-resistant trees under the pesticide laws.) Even more opaque is how USDA would make a decision to approve the release of a GM disease-resistant chestnut intended to grow and spread in unmanaged forests.

It's worth noting here that the regulatory system for GM plants is a paragon of clarity compared to the regulatory system for transgenic animals, where we still lack any formal statement from the administration as to what agency is responsible for what decisions about transgenic animals.

Finally, the ever-changing international regulatory environment poses an additional set of challenges for plant products that move out of the United States. In addition to specific laws adopted by countries with respect to GM foods and GM crops, the Cartagena Protocol on Biosafety continues to evolve and will certainly affect the inter-boundary transportation of any type of genetically modified organism, including plants and trees. How the Cartagena Protocol will continue to evolve and whether it will impose new legal or regulatory requirements remain a major source of uncertainty.

Marketplace Acceptance

The final hurdle is, of course, the test of the marketplace. As with any new product, the question will be whether buyers are willing to pay a price for it that returns a profit to its developers.

Are there any unique marketplace challenges that face health and environmental applications of plant biotechnology? The history of the introduction of GM foods offers a cautionary tale. Regardless of regulatory approvals, consumers in a number of countries remain suspicious about, and hostile to, GM crops and foods. In a market where consumers have alternative choices, their rejection of GM foods has had an enormous impact on trade and has dramatically slowed the introduction of new varieties of GM foods. Farmers, food manufacturers, grain processors and distributors and others have balked at the introduction of new GM varieties out of concern over negative consumer and marketplace reaction. GM potatoes, GM wheat, and GM sugar beets are all examples of products that made it through the regulatory process, but were rejected in the marketplace.

There are a number of reasons to believe that consumer and market attitudes toward non-food products of plant biotechnology may be different.

First, there is some reason to believe that the opposition to biotechnology is tied to its use as food. While there are environmental and other concerns about GM plants in Europe, the strongest opposition is associated with GM food products, and the opposition is based in large part on fears about safety (Allum *et al.*, 2003). In comparison, there has been little opposition to the non-food products of GM plants, such as cotton. For example, there has been little consumer opposition to blue jeans and few demands that they be labeled. So there is some reason to believe that the stigma attached to food biotechnology in some parts of the world may not automatically translate to other non-food applications of plant biotechnology.

Second, since the public is the ultimate buyer and consumer of GM foods, its choices have enormous influence on the food-marketing chain. Farmers may be enthusiastic

buyers of GM seeds, but unless they can find markets for their crops, they will buy something else. Interestingly, consumers appear to be most concerned about foods that contain GM ingredients but there is far less concern about meat or milk from animals fed with GM grains.

In most of the non-food health and environmental applications of biotechnology, however, the public is not the buyer, and the products and services they ultimately receive are not "genetically modified." For example, the buyers of lumber and pulp are simply businesses that, like farmers, are concerned primarily with cost and performance. The forestry products ultimately bought by consumers—paper, cardboard, houses—do not contain "GMOs."

Similarly, the "product" bought and used by consumers from GM plants that produce PMPs is the drug or therapeutic protein itself—typically prescribed by a doctor and approved by the FDA. Again, the product will be long divorced from the process by which it was made.

Third, at least some of the consumer opposition to GM food has been the result of a risk-benefit consideration where consumers see no benefit in the current generation of GM foods and elevated risk. Health and environmental plant-biotechnology applications, almost by definition, offer the prospects either for public benefits or direct consumer benefits. Using plants to produce lower-cost, potentially safer drugs has the strong support of a number of disease-research advocacy groups.

The fact is, consumers do make distinctions among applications of plant biotechnology (PIFB, 2005). It should not be surprising, for instance, that in light of the above discussion, the applications of plant biotechnology most strongly supported by Americans are those that would provide lower-cost pharmaceuticals or that would reduce world hunger (Figure 1).

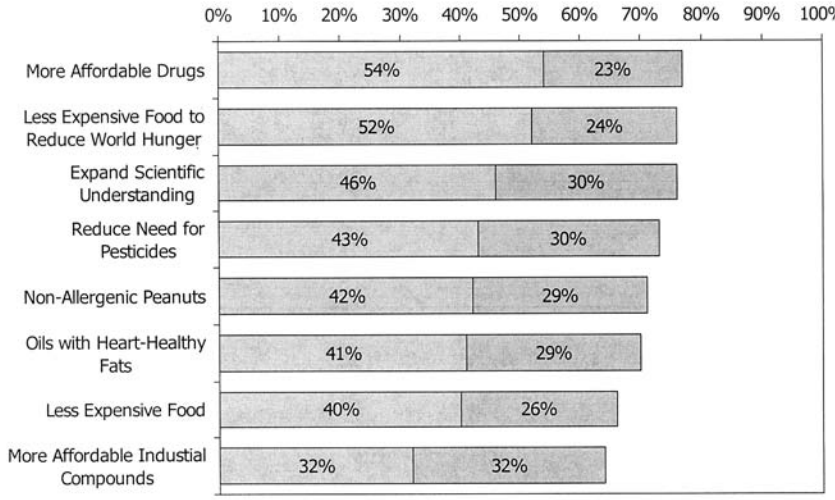

Figure 1. Ratings of "very good" or "somewhat good" reasons, respectively, to produce GM plants (PIFB, 2005).

Nevertheless, given the history of predictions about biotechnology, one must be humble when predicting the future. It frankly is too early to know whether the stigma against GM technology in some parts of the world will cling to these health and environmental applications. Mixed in with concerns about food safety are environmental concerns and embedded cultural, social, and economic issues that are often not clearly expressed. Even in the absence of a food-safety issue and direct consumer concerns, some of these other issues could still surface as opposition that could impact the market acceptability of these products.

In particular, the use of biotechnology in forestry will almost certainly be controversial, if for no other reason than, as with aquaculture and agriculture, there already are strong disagreements about the role of intensive forestry practices. To the extent that biotechnology makes plantation forests more economically viable, it is likely that it will be opposed by those who are already critical of existing forestry practices. In addition, forests have a cultural significance that row crops do not. In a PIFB-sponsored conference in 2001, a number of speakers referred to the emotional and moral value that people place on forests as natural places worthy of protection and respect. As a consequence, people are more likely to view the use of genetic modification technologies in forestry as unnatural, which could conceivably translate into opposition to forest products derived from GM trees along the lines of similar campaigns relating to "sustainable" forestry (PIFB, 2001).

On the other hand, potential environmental benefits from this technology may be appealing to some of the same segment of the public. For example, the ability to grow trees that require less energy to produce paper and pulp could be seen as an environmental benefit, not to mention the development of disease-resistant varieties of elm, chestnut, and dogwood. And the ability to create disease-resistant strains of key tree species could introduce the unique ability to preserve species that otherwise might become extinct. As noted, concerns about PMPs have little to do with the products, but rather with the potential that gene flow could move unwanted biological materials into food or feed crops.

GM plants intended for use in environmental clean ups present a different set of marketing issues, since the primary buyer is the government or a clean up contractor working under government standards. As with any treatment technology, regulators choosing a particular remediation technology must find the product to be "protective of human health and environment, maintain protection over time, and minimize untreated waste" (40 CFR 300. 430). For example, if a treatment technology is being selected for use in the clean up of a Superfund site, the EPA remedial project manager is required to consider nine factors to evaluate alternatives and determine the remedy preference, with cost being merely one of the considerations (EPA, 1990).

The environmental-technology market is highly risk-adverse (OTA, 1985). Government and their contractors do not want to take a chance in adopting a technology that does not work and risks making a problem worse. EPA has noted that clean up-project managers will need strong assurances—and a viable backup plan in the event of failure—before they are likely to select phytoremediation as an option (EPA, 2005). Community support is an additional factor in remediation-technology choices. If there is concern about the use of GM plants, public opposition could constrain the use of this particular technology.

On the other hand, communities could embrace GM phytoremediation as a cost-effective, quick, and more "natural" process than employing chemical or thermal destruction treatment processes, or more desirable than typical dig-and-dump techniques. It simply is too early, particularly without experience using GM phytoremediation in real-world clean up tests, to know what the public will accept.

Conclusion

The hurdles to commercialization of health and environmental plant-biotechnology applications are significant. Much about them is uncertain. Few developers have the kind of financial security or "bet the company" attitude to risk being the first product to "test" the system. Unquestionably, some potentially valuable applications remain sitting on bench shelves in universities and companies around the country waiting for someone else to go first.

Few developers have the kind of financial security or "bet the company" attitude to risk being the first product to "test" the system.

Nevertheless, there are reasons for cautious optimism. The regulatory system is slowly responding to the need to evolve for new and different types of biotechnology products. Management and stewardship requirements are becoming more clear. And experience suggests that the market welcomes safe, innovative products that provide perceived benefits to buyers and to the public. The marketplace makes distinctions between products—even between products made with biotechnology.

Not surprisingly, Pew Initiative polls tend to show that when consumers see a strong benefit for themselves, their families, or their community, they respond positively. As this technology moves forward, the bulk of the concern and opposition may prove to be rooted in food and the unwillingness of affluent consumers to take a small perceived risk in the absence of a clear benefit. The challenge then to the developers is to ensure that the potential benefits of this technology are clearly explained to the public while the government continues to ensure safety. If developers can do that, then there is indeed a way to "get there from here."

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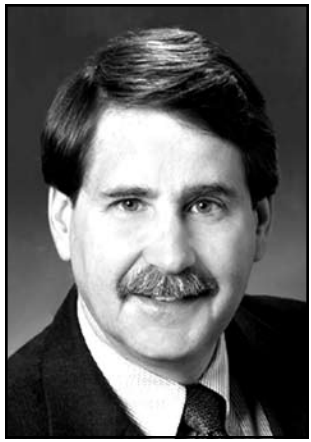

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Mr. Rodemeyer also served for more than fifteen years on the staff of the US House of Representatives' Committee on Science, including positions as chief counsel, and staff director and counsel of the Subcommittee on Natural Resources, Agriculture Research and Environment. From 1976 to 1984, he also worked on antitrust and consumer protection issues at the Federal Trade Commission.

Rodemeyer received his law degree from Harvard Law School in 1975 and his undergraduate degree from Princeton University in 1972.

Plenary Session Q&A

Moderator:
Anthony M. Shelton
Cornell University
Ithaca, NY

Dann Okoth (Nairobi, Kenya): I'm a journalist from the same country as the current Nobel Peace Prize holder, environmentalist Wangari Maathai. This question is directed to Dr. Rodemeyer. Perhaps I should get some information to take back to Wangari Maathai on environmental conservation. How can GM help us conserve our environment? Thank you very much.

Michael Rodemeyer: You'll hear more about that in the next few days from people who are working on some of those applications. But certainly, one of the arguments, for example is in forestry. If we can develop ways to meet the needs of populations for timber and wood products from more intensively managed plantations, harnessing genetics to make those trees more appropriate for those uses, then we can begin to take some pressure from the destruction of natural forests, which is clearly an issue in many countries in the developing world. So that's certainly one part of the issue. *Bt* crops potentially reduce the use of insecticides and drought resistance has the potential to conserve scarce water resources. There is a number of possible environmental applications that could be of benefit in the developing world.

Roger Beachy: I think you can't avoid the obvious. By increasing food yields per acre you also reduce the pressure to require more land. I listened recently to the governor of one of the states in Madagascar where very fine centers of biodiversity are in operation, and he was lauding the efforts of agencies, including the US government and the United Nations, for working with his state for conservation, although it was a very small piece, about 380 hectares. He said, "You know the problem is that our farmers grow cassava that are severely diseased, causing continuous need for more and more land. Can you help us get disease-resistant cassava so we don't have to do that?" It's a continuing challenge in these economies—struggling to maintain this vast wealth of important biodiversity along with food production. While we are considering value-added traits, such as plant-made pharmaceuticals, there is the expectation that our food needs will be met. Not instead of, but on top of.

Okoth: In Kenya, we don't have regulations in place to govern the agricultural biotechnology sector. It's in parliament, but the legislators keep turning it around every other year. Hopefully soon they will be able to debate and decide what to do. In terms of biotechnology research we are very well established, but the regulation part of it is dragging us down. I hope very soon they will formulate laws to regulate this and this economy will be on the way.

Oyeley Olukayode (Lagos, Nigeria): I don't know whether to classify this as a question or an observation. In Nigeria we have the problem of bush burning and I think we are losing species of potential economic importance. Are there ways through biotechnology application and and regulation to conserve species that are at risk of extinction through bush-burning?

Rodemeyer: Part of the response to that is similar to what we've said before. Most of the diversity exists in unmanaged and natural areas, and to the extent that you save that habitat by doing agriculture and forestry in other places, you help take the pressure off development of those areas. That's one answer. But there is a broader question—and this is certainly not something that I'm an expert on—of trying to identify and save, for example, seeds and other resources, creating a biological diversity type of inventory. Certainly others have tried to ensure that there is a "savings bank," so that those genetic resources are at least available. But there is larger debate. Are we trying to preserve these things in place or are we trying to simply preserve them as germplasm for future development?

Beachy: In African countries where we've had discussions *vis-à-vis* biosafety, there was confusion between the importance of having biodiversity solved before implementing biosafety regulations. The issues are very separable. You are talking about agriculture in one case and biodiversity in the other, and the issue is not biotechnology's impact on biodiversity. The question is, "Is agriculture affecting biodiversity?" And sometimes in countries where a scientist is not placed at the head of the regulatory agency, then other influences, other externalities and philosophies, enter the regulatory process from the standpoint of the environment that are not based in science. Our concern is, as science is respected at high levels in all countries, so science should make an informed impact on policies. That's the challenge that we see in Africa. There's concern that biodiversity is so important—and it really is—but the issue is biodiversity and agriculture, not biodiversity and biotechnology. Although those are very separable they are too often confused, and that stops countries from making decisions with regard to biotechnology. We heard that over and over and over at the Montreal Cartagena Biosafety Protocol meetings. These are important issues, but separable, and need to be dealt with differently.

Milt Zaitlin (Cornell University, Ithaca, NY): Dr. Rodemeyer, I'm not quite as comfortable as you are with your assertion that food system fears won't carry over to these PMPs. I mean, let's face it, the objection to bioengineered food is not based on science and is not always based on risk. There are activist organizations that are very effective in combating…[audio

lost]…rice and Anheuser Busch is a good example of that.

Shelton: Michael, it would be good if you state what the example is. I'm not sure everyone in the audience knows about rice and Anheuser Busch.

Rodemeyer: A company called Ventria is developing a transgenic rice to produce a number of proteins, including lactoferrin. There was opposition in California from conventional rice growers and they made an agreement with the University of Missouri, to carry out some field trials there. There was opposition from environmental consumer groups and, interestingly, from food companies and other producers. It's not that people are concerned about the safety of the protein that is developed from the pharmaceutical plant. The concern is over how to protect the food supply. Until there is some additional guidance on what those standards are going to be, there will be a clash. Some in the food industry and others have called for a ban on the use of pharmaceuticals produced in any food crop, to preclude adventitious presence in the food chain. On the other hand, some say that these proteins are ubiquitous and it makes no difference whether they get into our cornflakes or not. But that decision hasn't been made yet, and that is part of the problem.

Beachy: This is an issue of marketing not an issue of safety. Riceland is a major buyer of rice in the delta—in the Mississippi region and throughout Arkansas and Missouri and other growing areas—and the question is who buys their rice? Well, if it's Kellogg and Kellogg said that there might be accidental mixing, whether it's safe or not is not the issue. It's a marketing issue because somebody in another company can say, "We grow our rice elsewhere, therefore, ours is not mixed." Whether the contamination is 0.001% or 0.1%, it becomes marketing, not safety. We certainly don't want to be glib and think that the future for value-added traits will be smooth. It won't. We've heard for the last 20 years, "Just bring us a consumer product and the consumers will approve GM crops." I think that's a red herring and meant to put us off, meant to make us all feel like we are on the right track. I'm cynical at this point, after 20 years of agbiotech and still not getting new products out very often. You have to be ready for every eventuality. There are so many products in development that are in great states of readiness that aren't coming to market. There is such great fear of bringing things into foods. I must say I was very pleased to hear Michael's positive leaning because that is not what I expected. I'm hopeful, but I wouldn't say I'm optimistic.

Neal Stewart (University of Tennessee, Knoxville, TN): I've often wanted to ask this question but I've always hesitated and especially now that we've had such heavy stuff —bush burning and people starving. Well anyway, this is it. We are always thinking about benefits of, potential benefits of biotechnology in products. What about just fun stuff? For example, flowers with novel fluorescent traits or bioluminescence or just new colors? There was a company that spun out of Carnegie Mellon a few years ago that had bioluminescent squirt guns. So I guess my question is, "Would consumers actually need benefits if something was fun?" A genetically modified golf ball that would fly a thousand yards?

Rodemeyer: Now you're talking benefits!

Stewart: But that would not necessarily be very safe, especially if I was hitting it. Are there any scale effects of risks and benefits that we apply to food vs. nonfood and then just frivolous stuff that is fun?

Beachy: Remember that regulatory agencies no longer capture on just outcome, they now capture based upon method of production. That's the major challenge. We're not regulating product, we're regulating process. As long as that's the overriding consideration, then a golf ball that is locatable with a radio beam because of genes or luminescence will be regulated by those agencies and the cost goes up as a consequence. So, I think it gets back to this issue of rationalization of regulation. There is a way to rationalize and so far we've been in a reactive mode listening so much to every potential doomsday scenario in response to every product that comes out that we keep imposing more regulations. And so a few years ago the cost for a new product was a million dollars, then it went to five, now its twenty so what will it be in 5 years unless you bring regulation under rationalization?

Stewart: Of course, unless it's a zebra fish—then there's no regulation, right?

Rodemeyer: Right. But the glowfish is, in fact, partly the answer to your question. I am unaware of any protests or consumer or environmental or ethical issues with response to having a genetically modified aquarium fish.

Beachy: Yet they were banned from certain counties in California.

Rodemeyer: Well, except for California; maybe that's an outlier. But it's an interesting story. From consumers, from the public, I've heard absolutely nothing and I have talked to the folks who make this thing and they say they've heard very little.

Beachy: In the case of green roses—that's also been a nonoffender. The blue rose, I suspect, would be the same.

Rodemeyer: One of the reasons I distinguish between food products and nonfood products—I'm not aware of any efforts to boycott blue jeans that have been made from—

Beachy: Patagonia did it.

Rodemeyer: Well you're right, Patagonia maybe. There's no reason that you couldn't launch exactly the same campaigns against fabric that you launch against food and this is one of the reasons that I argue that this may be different because it's not food. So that makes me a little bit more cautiously optimistic about these applications.

Henry Miller (Hoover Institution, Stanford, CA): There's an interesting historical example that melds agriculture and the last question—fun aspects of biotech—and that's the old example of *Pseudomonas syringae*, the old ice-minus organism, that was field tested to prevent frost damage. Now, for those of you who don't recall, frost is promoted on plants by an ice-nucleation protein that is in *Pseudomonas syringae*. And so scientists at the University of California devised a mutant that lacked the gene for the ice nucleation protein and found that, indeed, it did have some protective effect. Now, the regulatory aspect of this is interesting. EPA in its wisdom decided that frost is a pest and so this deletion mutant of *Pseudomonas syringae* is a pesticide, and they regulated it as a pesticide. As a result, although it was shown to be effective in field trials, ultimately it was never commercialized because it would have to be registered as a pesticide. Now, what gets us to the last questioner is that the wild-type *Pseudomonas syringae*, which is ice-plus, is used in snowmaking equipment at ski resorts. It's sprayed into the air and there are skiers all around. And what's interesting about it is that if somebody using recombinant DNA techniques made an overproducing mutant, that would be regulated by USDA and/or the EPA at the cost of tens of millions of dollars for registration. This is the kind of thing that Roger is talking about—the need for rationalizing regulation. Currently, it makes no sense. It's extremely destructive. It limits the number of experiments that can be done, it limits what gets into the pipeline, it limits what gets through the pipeline and it raises the cost ultimately for anything that does manage to get through.

Ralph Hardy (NABC, Ithaca, NY): You were talking about the regulatory process. We have a number of people from foreign countries here that do not yet have regulatory processes. What sort of guidance might you give them, and just as a lead off to that, I might suggest the Canadian system vs. the US system. The Canadian system regulates on the basis of novel traits. The US system regulates on the basis of process. In the US system we can use ionizing radiation—all sorts of things that can be produce massive changes in genomes—that require no regulation, but if its by a molecular process it does. In the Canadian system if it's a novel trait, it doesn't matter how you got there, it requires regulation. So, I'd be interested in your comments on that and as an add-on question, you've described one way of getting containment of genes, could you assess where the other processes, like the Daphne Preuss synthetic chromosome and chloroplast modification, fit into accomplishing the same end point?

Rodemeyer: Well Ralph, I think you've just thrown the entire agenda off if you want to open up what the regulatory system should look like. The initial point is that the administration, back in 1986, made a decision that we were going to regulate biotechnology using existing laws, and I would argue that part of the problem is that by having to shoehorn the review under our existing laws, we don't have an optimal system in place. Everyone was concerned that if Congress enacted a new system it would be worse than what the regulators could invent on their own. So, I'm not sure I have any great wisdom to share with you about our own system. There is no question that the regulatory system has done

pretty well with respect to the crops that are out there today. I don't think there are any major environmental issues. There are certainly no health issues. The issue that we have tried to raise is: is the system ready for the new kinds of products that are coming along? And as I said, given the nature of novelty, every time you get a new issue, regulators are going to want to ask new questions. I'm not sure there's a magic bullet. There have been arguments that we should have a system with minimal or no regulations. But—and I'm probably going to get a lot of disagreement on this—the reason that we have acceptance of biotechnology in this country is largely because people have confidence in the institutions that regulate it. That's one of the differences between us and Europe. I think no regulation would be a mistake because the people wouldn't have confidence in the technology. You have to have a rational system that's based on science and on actual risk. There's no magic bullet.

Beachy: I agree that we needed regulations early on. That was an important thing to implement. We have built a bigger system than we need and more onerous than necessary because we haven't learned from the last 10 years. I mean a *Bt* gene, the same one put in crop "X" and approved, going into a second crop, or even into another variety by a different transformation event, gets re-regulated as if it's not known before. What's the rationale for that after 10 years of safe use of the *Bt* gene? What's the rationale for limiting some of the use of the virus-resistance technology or some of the anti-fungal technology? The Canadian system regulates the protein product that gives a trait. China seems to have done it in a way that is rational and one that meets their needs. They are looking for ways to reduce the use of agriculture chemicals. So I think China has moved ahead in the regulatory process in a different way. I think Kenya and Nigeria have an opportunity to do it in a different way, learning from what we've done in the last 10 years and what China is moving to. And rather than reinventing the wheel, you start with a knowledge-base after 10 years. And that's what we don't find in countries where the regulation is not in place. Most want to start over from scratch, as if we have never had regulation. That doesn't make any sense. We don't do that when we regulate drugs. Having approved aspirin or Tylenol® in this country, they're acceptable in Kenya because there is a long history of safety.

With regard to how else to control trait flow, this issue of gene-switching versus the artificial chromosomes are apples and oranges and the reason why Daphne Preuss promotes the use of the artificial chromosome is to reduce the likelihood of insertion of the gene at a place on the chromosome where there might be a negative impact. That might be useful in some cases or to move full genes in. So there may be some advantages, but I think they are apples and oranges. They are not the same. There are other ways to get to trait-control. There might be suicide genes where with x numbers of generations you trigger the gene to come back out again. Or you may make expression dependent upon hybridization and if that hybrid outcrosses to something else it won't function. There are lots of ways to do it. I think the one that's the most effective will be one that you can control ultimately with a small molecule.

Robert Wager (Malaspina University, Naimano, BC): It seems to me that marketing of fear has been driving policies, to a greater or lesser extent in North America, but certainly to a great extent in international policies. The MOP 1 talks seemed almost totally driven by fear as opposed to science and I'm curious what your feeling is about the MOP 2 talks on the Cartagena Protocol.

Beachy: I was there for only 24 hours, which was a long enough time for me to be there. I was there with the Public Research and Regulation Foundation. This is a move that started in Holland and England to try to engage public-sector scientists from all around the world including in Africa, Latin America, Asia, South Asia, Southeast Asia, in the discussion of the impact that the Cartagena Biosafety Protocol has on public researchers like us. What are the impacts and how do they stop innovation? How are they preventing us from doing our jobs? As Ralph indicated earlier, more than 70% of the biotechnology that is known about in the United States is done by his member organizations. If you look in developing countries, far more biotech is done in the public sector at KARI for example, or at universities in Nairobi and Abuja and other places around West, East and South Africa. That's where it's done. Yet lack of understanding of what biosafety is has prevented public-sector scientists from doing their jobs and delivering improved vegetables and grains for their populations. So, for the first time, public-sector scientists were not afraid to speak out, at MOP 2. We had a preliminary meeting at the Danforth Center in March and that group and more were in Montreal to understand the process and make inputs when given the opportunity to do so, to correct some of the misunderstandings. The feeling that I had there—the accusation is—this is a big multi-national way to take charge of food production worldwide, and by George we're not going to have that happen and we're going to put in place regulations to prevent that, while doing so under the guise of preventing transfer of DNA between my country and your country. In the past there was an intention to use the process to listen to the antitechnologist and to block applications of relevant biotechnology around the world. I do agree that that was the goal at MOP 1. I think we've seen a slowdown towards that, and maybe a realization that the public sector has a lot to contribute here. Some of the delegates at the conference expressed surprise that university people are actually doing biotechnology! They didn't know—so that was a learning. We should have been there the last time. The public sector should have been a lot more vocal over the last 10 years than it has. We bear some of the responsibility for how far the Cartagena Protocol has already gone because we've not been there often enough. We haven't spoken out. We haven't made our voices heard. We need to find better ways to get our message across or we'll look a lot like Austria or Switzerland in the next few years. There is a possibility we'll see ourselves slip back.

Shelton: Michael, in the title of your talk you used the words "speed bumps." You didn't use "wrong streets" or "crashes," so I'm taking that as an indication of an optimistic future for some of these products. I'd like to thank both speakers. It was a great session.

PART IV

Module I–Plants as New Sources of Medicinals:

Production of Protein Pharmaceuticals in Food and Non-Food Plants

Plant-Made Pharmaceuticals: An Overview and Update

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University of Kentucky
Lexington, KY

The development of plant genetic transformation in the early 1980s introduced the possibility of having plants express non-native ("foreign") genes, and thereby accumulate non-native proteins in their cells and tissues. Thus the concept of "plant molecular farming" was born, envisaging crop plants as production "vehicles" for useful and/or valuable proteins that originally derived from microbial or animal sources. Several advantages were claimed for such plant-based production, relative to bulk production of the natural source for the corresponding protein, including overall economy of production, lack of need for major capital investment (*e.g.* in fermentation bioreactors), ease and economy of scale-up, lack of risk of contamination with human pathogens, *etc.* (For a background overview of plant molecular farming, see Collins and Shepherd, 1996). Because the case for using plants as the production system became more compelling as the yield of protein per plant increased, proprietary gene-expression technologies were developed specifically for achieving very high concentrations of "foreign" proteins in plant tissues. These technologies, in turn, resulted in the emergence of several agricultural biotechnology companies specializing in plant molecular farming. A wide variety of proteins were expressed in a number of plant species, illustrating the potential of the approach to supply products for pharmaceutical, industrial-enzyme, structural-polymer, *etc.*, markets. Clinical trials in humans were conducted, successfully, with protein pharmaceuticals generated in plants (*e.g.* Ma *et al.*, 1998). A particularly attractive feature of the plant molecular farming concept, which was apparent from its outset, is its potential to provide opportunities for both the agricultural sector and the biotechnology business sector simultaneously.

A variant on the molecular farming theme, which developed contemporarily with it, is the concept of "edible vaccines." In this strategy, the entire plant tissue or organ (such as a fruit) in which the protein pharmaceutical accumulates is also the final delivery device for the protein product, and no extraction of the protein from the plant material

is necessary. Edible vaccines are considered elsewhere in this conference, and will not be discussed further here.

In contrast with the spectacular scientific progress in "proving" the principle, however, the development of significant markets for plant-manufactured proteins failed to happen, so that by the mid-1990s the plant molecular farming field was essentially stalled. The companies that were based on appropriate gene-expression technologies were in a survival-oriented mode rather than in a state of vibrant growth, and little was happening in terms of further technology enhancements. Most troubling was the sense that the end-users of the technology, *i.e.* those companies who owned protein pharmaceuticals, enzymes and other possible product "targets" for plant-based production, appeared to be unconvinced regarding the plant manufacturing platform. Field-level production had been demonstrated on a pilot scale, and some post-harvest bioprocessing capability was constructed. But, in spite of these advances towards scaled-up commercial applications, the plant molecular farming biotechnology sector continue to lack much-needed endorsement by significant clients whose protein medicinals and protein reagents addressed substantial and sustainable markets. The plant-based production "platform" was, in effect, a technology searching for an application.

Fortunately, much has changed in the last 5 years or so, largely as a result of biotechnology's increasing impact on drug development. The fledgling molecular farming industry is ideally placed to address the resulting demand for protein pharmaceuticals, and consequently has re-characterized its technology platform as "plant-made pharmaceuticals" (PMPs). Applications to other proteins such as industrial enzymes, now referred to as "plant-made industrial products," are seen as a future priority.

It now seems much more likely that a new, specialized crop agriculture will be constructed specifically to service the PMP opportunity.

This brief overview will consider the current status of the PMP industry and the challenges facing large-scale implementation of the PMP opportunity today. Using some of our own research at Kentucky Tobacco Research and Development Center (KTRDC) as an example, I will also discuss the interface between the PMP industry and conventional crop agriculture. This is an aspect that has often been neglected in the past, but one that comes sharply into focus when one recalls that conventional crops are not optimized for these new applications and that today's regulatory, containment considerations render them even less suitable. Rather than providing new plant varieties and corresponding new markets for production through existing crop agriculture—as was at one time envisaged—it now seems much more likely that a new, specialized crop agriculture will be constructed specifically to service the PMP opportunity.

The PMP Opportunity Today

To appreciate the current status of the PMP industry, we need to review both the capabilities of the industry itself and the condition of the protein-pharmaceutical markets that it is designed to address.

The basic philosophy of the PMP opportunity today can be stated in the following way:

- there is a substantial, and increasing, worldwide demand for protein pharmaceuticals and therapeutics;
- existing and traditional methods of manufacturing proteins in bulk to the required level of purity and quality are stretched to capacity and will soon become limiting in protein-drug manufacturing;
- specialized plant-based gene-expression technologies are ready to provide an alternative manufacturing platform that can help meet the demand and thus overcome the perceived "bottleneck" in protein production; and
- taken together, these situations make a strong case for commercial development of PMPs.

The following summary reviews each of the above aspects in relation to the current status of PMP commercialization.

Protein Demand

The development of novel protein pharmaceuticals, vaccines, therapeutics and other medically useful molecules is an expected consequence of biotechnology-driven drug discovery. Accordingly, it is not surprising that proteins feature prominently in new drug development today, and that they are predicted to comprise a larger and larger proportion of new medicines over the next decade or so. For example, an estimate of 14% of the pharmaceutical market as proteins in the year 2000 is projected to expand to 40% by 2010 (Price, 2003). A 2004 survey by the Pharmaceuticals Research and Manufacturers' Association (http://www.phrma.org) indicated that 324 "biotech medicines" were in clinical trials or in advanced development (such as preparation for clinical trials) in the United States alone, in that year. Most of these prospective new drugs were proteins such as antibodies, enzymes, peptides, *etc*. Worldwide, many hundreds of monoclonal antibodies and other medicinally active proteins are in all stages of development from discovery through human trials. Moreover, some of these proteins will be administered in large amounts (*e.g.* mg/dose) that will necessitate large production volumes (Garber, 2001), and the prospect of personalized or individualized medicines for some products will pose particularly tough challenges for economical, custom, batch-wise production (*e.g.* Alison *et al.*, 2003). Thus, the demand for pharmaceutical proteins is considerable, and growing, creating a market opportunity that was almost nonexistent when plant molecular farming was conceived in the 1980s.

Protein-Manufacturing Capacity

Next, let's consider the extent to which this demand has created a supply-level crisis that

might stimulate the expansion of PMPs. An increasing demand for proteins creates opportunity for all possible production platforms, including the established way to manufacture proteins to the stringent standards laid down for veterinary and human applications, *i.e.* via fermentation of specialized microbial (bacteria and yeast) and mammalian (*e.g.* Chinese hamster ovary) cell lines, engineered to express the appropriate genes. We can, therefore, expect some expansion in this fermentation industry. While this will represent competition, the increasing need for proteins may also provide a chance for PMP technology not only to prove its fundamental capability but also to illustrate its efficiency in responding quickly, flexibly and perhaps economically to a rapidly evolving demand for product quantity and diversity.

Fermentation facilities require considerable capital investment and have finite capacity so that expansion also requires substantial capital. Such facilities are typically said to cost $300–$500 million to build, with a time frame of 4 to 5 years to cover construction, validation, and licensing (Thiel, 2004). Not surprisingly, therefore, the initial surge of progress in protein drug development stressed existing fermentation capacity, raising serious concerns about a coming "capacity bottleneck." As recently as 2001, a representative from a contract manufacturing organization (CMO) in this field was quoted as saying (cited in Garber, 2001):

> *…the only long-term solution is to shift some production (from fermentation systems) to transgenic animals and plants, which can in theory be scaled up much more efficiently to virtually any level.*

In our opinion at KTRDC, this period of "protein crisis" (Garber, 2001) in the late 1990s and over the last few years generated considerable new interest in the PMP concept. Two more gene-expression technologies emerged as PMP companies during that time, and pharmaceutical companies began to examine the PMP-manufacturing option more seriously. The PMP industry and associated organizations (*e.g.* KTRDC and other PMP-relevant research programs) also became more recognizable as a biotechnology sector in its own right, featuring prominently in the Biotechnology Industry Organization (BIO; http://www.bio.org) and holding a biannual conference (*Conference on Plant-Made Pharmaceuticals*; http://archives.cpmp2005.org).

The protein crisis might have become a forceful creator of new end-users and significant markets for the PMP industry. However, sufficient expansion has taken place in the fermentation industry that the crisis appears to be over, at least for now. Indeed, the biomanufacturing news spotlight is now focused on the end of the capacity-expansion boom, and industry analysts opine that "…there is little immediate pressure for companies to move to alternative platforms (*e.g.* PMP) that are as yet commercially unproven" (Thiel, 2004). Whereas just a few years earlier the transgenic animal- and plant-production systems were viewed with new interest as potential ways around the bottleneck, they are again left to make their own case for advantages, economy, *etc.*, relative to the traditional methods of making proteins.

While it seems unlikely that there will be another sudden surge in demand for proteins, the steady growth in demand will continue to apply pressure to the manufacturing

industry. It will be interesting to see if the CMOs will repeatedly be able to raise financing for expansions, and whether the larger pharmaceutical companies that have their own protein products will invest further in fermentation capacity. Another possibility is that protein production will grow sufficiently as a business sector that it will become much more internally competitive, triggering new interest in more economical methods of production and hence, potentially, in PMPs.

For a commercial PMP, an accumulation of product to a level of at least 1% of total soluble protein by weight is considered necessary, with enrichments of 5–10% of TSP being preferable.

PMP Technologies

The need, once again, for the PMP industry to convince the pharmaceutical community that PMPs can become an established route of large-scale production, brings us conveniently to a short overview of PMP technologies, their capabilities, characteristics, strengths and weaknesses. Table 1 lists the more prominently visible PMP companies, together with the kinds of gene-expression technologies on which they are based and the plant species with which they are compatible. The majority, if not all, of these proprietary technologies were derived originally from research conducted in the academic sector. The majority of them share an important attribute, namely the ability to drive protein accumulation in the appropriate plant tissue to very high enrichment levels relative to the native plant proteins in that tissue. Indeed, for a commercial PMP, an accumulation of product to a level of at least 1% of total soluble protein (TSP) by weight is considered necessary, with enrichments of 5–10% of TSP being preferable. With certain gene products that are particularly stable in the plant cell, and/or with certain technologies, enrichments approaching 80% of TSP may be attainable (*e.g.* Marillonnet *et al.,* 2004).

Table 1. Some* examples of PMP companies and technologies

Company name	"Vehicle" plant	Proprietary technology
Large Scale Biology	Tobacco	Viral transfection vector
Icon Genetics	Tobacco	Viral vector (and others)
Chlorogen	Tobacco	Chloroplast-based expression
Planet Biotechnology	Tobacco	Antibody production
SemBioSys	Safflower	Oilseed-based expression
Ventria Bioscience	Rice	Seed-based expression
Medicago	Alfalfa	Expression in forage crop
Biolex	Duckweed	Expression in aquatic plant

*This is necessarily an incomplete list, on account of limited space and scope of this review article; omission of any company or technology does not imply any negative assessment or view.

While sharing a common feature of highly productive gene expression, the PMP technologies otherwise exhibit considerable diversity in regard to other characteristics and perceived advantages. Expression in stably transformed plants contrasts with expression through transfection with modified virus particles. Many of the expression technologies are restricted to certain plant species, but the overall list includes food/feed plants such as corn and canola, feed crops such as alfalfa, and non-food species such as tobacco and duckweed. Depending on the particular technology, expression may occur in the leaf and hence in the bulk of the plant's above-ground biomass, or it may occur exclusively in seed or fruit tissues. Also, expression in the transgenic systems may be from vectors inserted in the nuclear genome or in the chloroplast genome.

An in-depth comparative analysis of all these different PMP technologies is beyond the scope of this paper, but suffice it to say that each has particular advantages or unique features. Expression in the chloroplast, for example, may offer advantages in regard to containment and regulatory compliance when deployed in plants that exhibit little or no transfer of chloroplasts through pollen [for a review of chloroplast transformation, see Bogorad (2000)]. Viral transfection systems (Lacomme *et al.*, 2001; Marillonnet *et al.*, 2004) will pose different regulatory approval considerations from those required for transgenic plants, and this may be advantageous in some circumstances. Ease of post-harvest isolation of the protein product, and subsequent bioprocessing is a unique claim made by an oilseed-based system (Moloney, 2000). And at least one system is proven in the production of complex antibody molecules requiring simultaneous and balanced expression of several genes in the same plant cell (Wycoff, 2005). Overall, this diversity of characteristics and features should work to the advantage of the PMP strategy, enabling it to address a wider range of product "targets" and production constraints (*e.g.* growing location, farming know-how, and special regulatory/containment considerations) than might be possible with only one or two gene-expression technologies.

The PMP industry is still working towards its first truly large-scale (100 acres or more), preferably ongoing, provision of a protein drug to a client company or to consumers.

PMP Engagement of Protein Markets

The status of the PMP opportunity today is largely consistent with the ideal philosophy presented at the beginning of this section. Proteins represent an increasing proportion of pharmaceutical products, worldwide. While there is currently less concern about production capacity for those proteins relative to the situation a few years ago, it seems possible that growth in markets for protein drugs, along with dosage and personalization issues, will continue to pose challenges for the capital-intensive, cell-culture-based manufacturing platforms. The PMP platform comprises a range of impressive technologies with a

broad range of capabilities, ready to address these opportunities. But while there have been a few instances of commercial products' being made via the PMP route, most have been small-scale (1–100 acres), one-time, or experimental endeavors. To date, the PMP industry is still working towards its first truly large-scale (100 acres or more), preferably ongoing, provision of a protein drug to a client company or to consumers.

We will next examine the challenges that must be met for the industry to break through into such mainstream protein-drug manufacturing.

Challenges to PMP Development

The basic scientific and technical challenges of making "foreign" proteins in plants were met many years ago. And while it is true that plant-based production may not be feasible for some proteins [*e.g.* on account of different post-translational modifications such as glycosylation (Gomord *et al.*, 2004)], and that technical advances continue to be made in expression levels and other aspects that may improve the range of products that can be made in plants, there is no shortage of viable PMP targets today.

Rather, the factors that most influence the growth rate of the industry at the present time are financial and business related. PMP companies must compete for the attention of investors who also review business models formed around other manufacturing platforms, and who may be more inclined to invest in companies that own the innovative new protein drugs. (PMP companies sometimes own the product target as well as the production technology, but all seek to partner with companies who own additional prospective targets.)

This competition may become easier to beat once the PMP approach is embraced by the pharmaceutical industry as an established production platform. Factors that influence the pharmaceutical community's acceptance of PMPs include the "cultural" differences between the pharmaceutical sector and the agricultural biotechnology sector, an overall lack of understanding of the economics of plant-based production (discussed further below), the lack of familiarity with large-scale purification of proteins from plants to the stringent specifications required for clinical application, and quite possibly a desire not to be the first drug company to adopt the PMP concept. To appreciate the cultural differences, one has only to contrast the highly controlled, highly contained, nature of bulk cell-culture systems in which every cell is almost identical to every other, with the PMP image of a greenhouse or a field of plants; it must be tempting for those engaged in protein-pharmaceutical manufacturing to persist with their established, long-proven, methods for reasons of familiarity and "comfort-level" alone.

If a protein-supply crisis does not drive a wider adoption of plant-based manufacturing, attractive and superior economics would certainly be expected to do so. Indeed, the PMP industry has recently been challenged to demonstrate the economic advantages of its platform, particularly in regard to production scale-up where the relationship between capital investment and capacity increase may be distinctly advantageous (Thiel, 2004). Unfortunately, many published papers and review articles over the years have misleadingly characterized the PMP technology as a "cheap" way to make a protein. There is, of course, always a finite cost associated with producing a crop, and with PMPs there can

be specific additional costs associated with containment (at least until the plant becomes deregulated), particular harvesting practices, *etc*. Moreover, a complete estimate of cost-of-production for a plant-derived pharmaceutical cannot be made without data on extraction and bioprocessing expenses. For any particular PMP product under consideration, these post-harvest details are usually either unknown or based only on experience at small scale. Thus, the majority of statements in the literature that proclaim an inherent cheapness for PMPs, without justifying that claim, cannot be taken seriously. On the other hand, incremental costs associated with PMP scale-up should be appealing, for the simple reason that planting more acres in the field, or constructing additional contained facilities such as greenhouses, has to be significantly cheaper than building bioreactors for fermentation. (Bioprocessing expansion costs would likely be very similar for all platforms.) It is in this aspect of flexibilty and expansion that the economics of PMPs deserve careful consideration.

Most recently the PMP industry has found that its potential pharmaceutical-industry clients are not the only stakeholders needing to be convinced. The potential use of food/feed plants as the crop species of choice for some PMP systems has drawn criticism from the respective food industry organizations, as well as from environmentalists and biotechnology-opposing groups (*e.g.* see the editorial on page 22 of Nature Biotechnology 133, 2004, for an overview, and http://www.gmabrands.com/news/docs/NewsRelease.cfm?docid=1029 for an example of food-industry perspective). Concern has been expressed over the degree of protection and assurance that could be provided to obviate an envisaged possibility of contamination of the food/feed supply with pharmaceutical proteins from the PMP variant of the crop, either via genetic means (cross-pollination) or by direct mixing of plants or harvested material. Anxiety on this point has generated local/regional opposition to one PMP platform that uses a food plant, resulting in that company's relocation of its pilot production trials and the resulting loss of a new opportunity for farmers at the original site. The fear of food contamination is also reflected in the presently very high level of governmental regulation and risk assessment applied to field releases (field trials, pilot production) of PMP plants (Peterson and Arntzen, 2004).

Despite these concerns, it has been speculated that PMP crops might fare better than most genetically-modified (GM) crops in terms of public perception and acceptance in those countries that are uneasy about GM plants in general [for further discussion and relevant citations see Einsiedel and Medlock (2005)]. The often-heard criticism that input traits such as herbicide tolerance do not provide sufficiently obvious benefit for the consumer no longer applies, and the product is readily understood as a beneficial medicine.

Developing a Dedicated Crop System for PMPs

Concerns about contamination of existing crops with pharmaceutical products coming from the corresponding PMP crops would be moot if PMPs were produced using "vehicle" species that had hitherto not been developed into crops, *i.e.* plants that had not previously been domesticated. However, suitable gene-expression technologies would have to be adapted for use with these previously uncultivated species, or developed from scratch,

and the domestication aspect alone might take many years. Even with the non-food plant, tobacco, there is concern to prevent any possible contamination of the traditional crop; the tobacco-production industry in the United States currently has zero tolerance for GM contamination.

There is an additional reason to consider the development of a dedicated vehicle plant exclusively for PMP applications, namely the lack of desirable characteristics in existing crop varieties. Modern crop cultivars are highly customized to the particular applications for which they are used. Adaptive enhancements continue to be made through advanced breeding and genomics research, improving yield, resistance to pests, and other useful traits. Some of these qualities, particularly the more agronomically oriented ones such as disease resistance, will still be relevant when the plant is used as a protein-production vehicle. However, other traits specific to the PMP application may also be lacking in the commonly grown varieties. Examples include productive response and systemic spread when infected with virus-based PMP gene vectors, sterility or limited (or late) flowering to minimize gene flow via pollen, special morphology and growth habit suited to a specific harvesting method that will be used with the PMP application, absence of certain metabolites that may compromise product integrity or quality during bioprocessing, *etc*. Indeed, the design and development of new plant varieties customized for PMP applications is to be expected, given the general practice of variety development in crop agriculture.

Tobacco is convenient for illustrating vehicle-plant customization. The *Nicotiana* genus contains a large number of species that are found in diverse regions of the world and which exhibit a wide range of morphological and other relevant characteristics. Most importantly, many of these undomesticated species are already large, bushy and productive plants, in contrast to the "wild" relatives of many crop plants, which so often exhibit small, low-yielding forms. Moreover, many *Nicotiana* species have disease-resistance traits not found in the traditional, commercial *N. tabacum* cultivars. Thus, there is an extensive and useful germplasm resource, and the domestication breeding path is conveniently short. Most of this germplasm has been ignored in breeding traditional tobacco types, presumably because the exotic species and associated variants are unsuited to the manufacture of traditional products such as cigarettes. Naturally, these issues have no relevance in the new PMP applications of the plant.

Table 2 provides a listing of the most desirable characteristics to be incorporated into the new vehicle plant that we are constructing at KTRDC for use with all tobacco-specific PMP gene-expression technologies. In order to achieve optimal compliance with federal regulations concerning limitation of gene flow (genetic "containment"), we have adopted a hybridization strategy. Thus the plants set out in the field for production will be interspecific hybrids that exhibit a high degree of sterility. One parent of the hybrid is an appropriate cultivar of *N. tabacum*, whereas the other is a different species of *Nicotiana*. Each parent also contributes different characteristics that are important features of the final custom hybrid. For example, for transgenic PMP strategies one parent will be expressing the PMP transgene via the appropriate high-level expression system. For chloroplast-based expression, this would be the maternal parent. The other parent may also be transgenic for other characteristics such as herbicide tolerance, *etc*.

Table 2. Desirable traits and characteristics of a new crop plant customized for use with tobacco-based PMP gene-expression technologies.

Performance traits	Production-related traits
Transformable	Eonomical production*
Regenerable from cell culture	Vigorous regrowth*
PMP-vector-system compatible	Disease resistance
Sterile	Herbicide tolerance
Identity-preserved	Insect resistance
Desirable metabolite profile	High biomass-yield*
Bioprocessing-optimal	Good protein production
	Suited to mechanized harvesting

*Vigorous regrowth (for multiple harvests) and high biomass-yield contribute directly and importantly to economical production, but the latter is listed separately as well so as to include improved economy of seedling production, transplanting, and many aspects of crop maintenance, as well as economical disposal of waste material post-bioprocessing, *etc.*

It will be noted that the table of characteristics includes economy of production. One disadvantage of conventional tobacco for PMP applications is the very high cost of production of the traditional crop, resulting from the use of transplants, wide plant spacing, and considerable manual labor even pre-harvest. In developing the customized *Nicotiana* hybrids for PMPs, we can also take the opportunity to address the cost-of-production issue. For example, mechanized harvesting enables the crop to be produced by sequential harvesting and regrowth, unlike traditional tobacco, which is (manually) harvested once. This contributes significantly to improved production economics, so productive regrowth becomes an important performance trait for the new PMP hybrid lines.

Along with the development of new hybrid *Nicotiana* plants, much can be done to usefully customize the production practices, further reducing the cost of production for PMPs. The mechanized harvesting mentioned above, using plants grown much closer together and employing three or four rounds of growth from the same plants over an extended growing season, are good examples. Eliminating transplants, and producing the crop by direct seeding into the field, would also lower production costs. However, at the present time transplanting is actually viewed advantageously relative to direct seeding for PMPs, as it further reduces the possibility of volunteer plants' emerging in the following year. Accordingly, we are examining the possibility of achieving more economical raising of transplants in the greenhouse, and good progress is being made with higher densities of transplant production.

We are hopeful that the increasing market opportunity (demand) and the demonstrated production capability (PMP technology) will soon converge.

In Conclusion

The continuing development of protein-based medicines worldwide bodes well for the future of the PMP concept. Gene-expression technologies for plant-based production are proven and productive. We are hopeful that the increasing market opportunity (demand) and the demonstrated production capability (PMP technology) will soon converge to achieve large-scale, and ongoing, manufacturing of valuable proteins from one or more plant-based platforms. Meanwhile, much can be done to enhance the agricultural interface with PMPs, as I have illustrated above with reference to our work with *Nicotiana*. Existing crops and associated production methods are frequently sub-optimal for application to PMPs, but the use of related germplasm that remains compatible with PMP gene-expression technologies can not only enhance production economics and facilitate regulatory compliance, but also mitigate potential conflict with traditional food and other applications of that plant.

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Since 1996, Davies has been responsible for designing and overseeing the transition of KTRDC from health-related research to its present mission of facilitating the development of new crops for Kentucky agriculture. The research in plant-made pharmaceuticals (PMPs) emphasizes the development of a new, PMP-dedicated crop plant and an associated agricultural production system that will be useful to all companies using tobacco-based gene-expression technologies for protein production. The Center also supports unique research into natural products, anticipating that the development of new applications for intrinsic, small-molecule plant metabolites will, in turn, create new crop opportunities for growers.

Opportunities and Challenges for Plant-Based Vaccines

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Vaccination has become an important and effective public-health measure for safeguarding against devastating outcomes of infectious diseases. Current vaccines rely on the use of either attenuated (weakened) or killed strains of pathogens, *e.g.* against diphtheria, tetanus, measles and mumps. For some vaccines, such as the one against human smallpox, a strain from a different species (cowpox) is used instead. Some of these vaccines (especially parenteral vaccines) contain toxic preservatives such as formaldehyde, thiomersal (a mercury-based compound), and aluminum phosphate (Buetow and Korban, 2000; Streatfield and Howard, 2003). In recent years there has been a move towards developing subunit vaccines, linear immunogenic epitopes of the pathogen that elicit production of antibodies. This alleviates concerns over risk of reversion of attenuated strains to aggressive forms in pathogen-based vaccines (Buetow and Korban, 2000). Scale-up production of current vaccines takes place either in specific pathogen-free (SPF) eggs or in mammalian cells grown in large fermentors or bioreactors. Therefore, these vaccines require purification before they are available for use. Moreover, most are delivered via intramuscular injection, and, therefore, require the use of sterile hypodermic needles.

A novel approach for developing subunit vaccines has emerged as a result of the genetic engineering technology: the use of plants as hosts—biological bioreactors.

In the last several years, a novel approach for developing subunit vaccines has emerged as a result of the genetic engineering technology: the use of plants as hosts—biological bioreactors. At this time, the most economical and technically feasible class of products using this approach involves the engineering of genes to express novel proteins. This has resulted in a $40-billion industry of new therapeutics and industrial enzymes (Howard, 2005).

Opportunities

The important features of any effective vaccine include safety, protective immunity that is sustained for long periods of time (preferably a lifetime), stability, ease of administration, low cost and few side-effects. In recent years, plants have emerged as alternative production systems for subunit vaccines as they are likely to contribute to all of these critical features of effective vaccines. Plants that have been engineered with genes encoding antigenic proteins of various pathogenic viral and bacterial organisms have been shown to correctly express the proteins that elicit production of antibodies in mammalian hosts. Plants can readily and properly handle the downstream processing of foreign proteins, including expression, folding, assembly, and glycosylation, all contributing to the fidelity of antigenic proteins (Wycoff, 2005). As a result, these proteins maintain their activity and efficacy, thus contributing to their viability as subunit-vaccine candidates (Figure 1). Plants can produce not only single, simple foreign proteins, but also complex multimeres, such as secretory proteins and antibodies (Wycoff, 2005). All these capabilities render

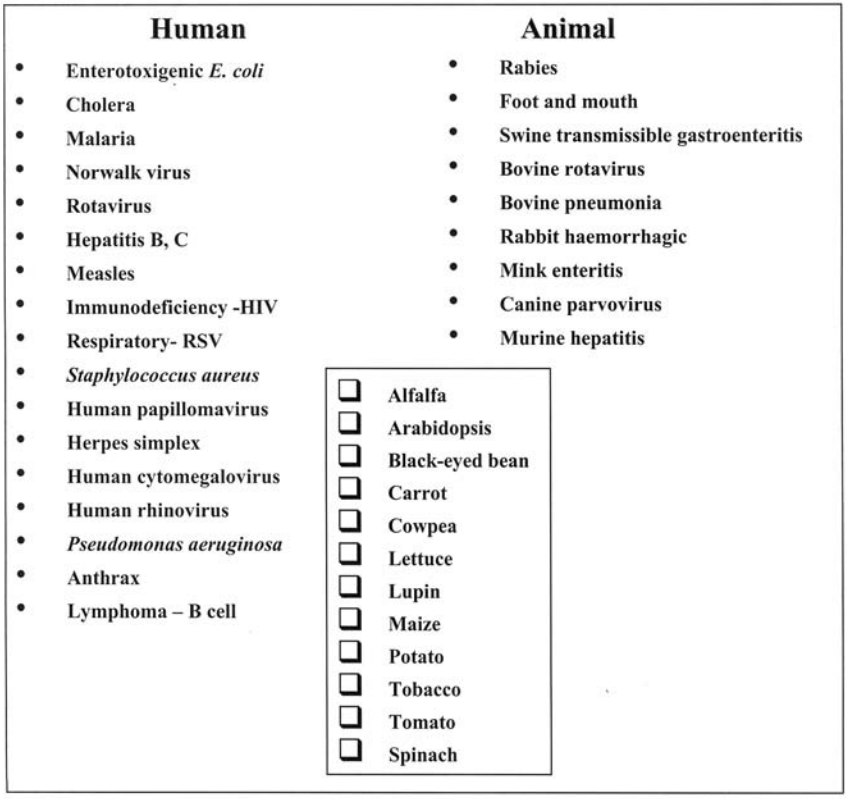

Figure 1. Subunit-vaccine candidates against human and animal diseases for possible production in various crops.

plants as targets of opportunity for marketing of high-value protein products. However, that's not all that plants have to offer.

Plant systems do not harbor human or animal pathogens (such as virions or prions) and, therefore, they do not transmit such pathogens along with the target subunit vaccine. Moreover, they cost less to produce than via fermentation or bioreactors; plants can be grown in the field or in a greenhouse relatively inexpensively (Howard, 2005). When produced in edible parts of the plant, such as grain, fruit or even leaves, subunit vaccines may not require purification. Maintaining the antigenic protein within plant cells that are edible may also contribute to stability and reduce degradation. Another advantage of producing subunit vaccines in edible parts of a plant is the potential to deliver them orally rather than intramuscularly (Streatfield and Howard, 2003), providing a simple and easy means of administration to humans and animals. Moreover, oral delivery stimulates mucosal immunity (the first line of defense) in the tissues lining the mouth, nose and esophagus (among others) that provide the first target of opportunity for pathogens to enter and infect the human body. In addition, production in plants reduces the overall cost of vaccinations, which is often prohibitive in developing countries; for example, sterile hypodermic syringes are not required.

The advantages and opportunities from producing subunit vaccines in plants may be summarized as follows:

- Elimination of risk of contamination with infectious agents
- With oral delivery, they activate the mucosal immune system—the first line of defense
- Avoidance of injections
  - Improved patient compliance
  - Reduced risk of transmission of other infectious agents through contaminated needles
- Longer shelf-life
- Cost-effective in large quantities

However, myriad challenges are yet to be overcome before the promise of this technology will be fully realized.

Challenges

The challenges facing plant-based-vaccine development include technical, regulatory and economic aspects and public perception. Among the technical challenges it is critical to select a plant system that can be grown under conditions that minimize environmental risks, such as transfer of pollen from transgenic to conventional varieties or to related

The challenges facing plant-based-vaccine development include technical, regulatory and economic aspects and public perception.

species. Expression of antigens in plants is a major regulatory concern. Whether or not the protein is confined to specific tissues will enable or nullify exposure to the environment. Targeting expression via a tissue-specific promoter driving the transgene may reduce regulatory concerns (Korban, 2002). For example, elimination of expression of the transgene in pollen will reduce dissemination of the antigenic protein to other plants and alleviate environmental contamination, although not completely.

Among other technical challenges to be considered, the crop should provide ample biomass for accumulation of a sufficient quantity of the antigenic protein. Whether it is a grain, vegetable or fruit crop, protocols will be needed to ensure transcription, translation, intracellular localization, tissue specificity, adequate gene-copy number, and metabolism and accumulation of the protein of interest (Buetow and Korban, 2000; Streatfield and Howard, 2003). Determining the level of expression of the transgene and stability of expression over generations of the transgenic line will be essential for determining the economic feasibility of a proposed plant-based vaccine. Depending on the target protein product, levels of 10 mg/kg of plant dry weight of a crop may be sufficient, although levels of 100 mg/kg or higher are more likely to be necessary (Howard, 2005). Approximately 50 kg per year of a particular antigenic protein would certainly meet economic feasibility. Other issues related to technical challenges include formulation: will the vaccine be marketed as an encapsulated powder, a concentrated liquid or a nasal aerosol? By what route will it be delivered? What will be the proper dosage? Would a single dosage suffice, or will (a) booster(s) be necessary? All of these technical questions are yet to be answered for plant-based vaccines.

Among regulatory challenges, issues relevant to any genetically modified (GM) crop that have to gain regulatory approval from the USDA, FDA and/or EPA apply to plant-based vaccines. In addition, issues related to separation of a pharmaceutical product from the original crop targeted for the food chain have become increasingly important as concerns over adventitious presence of medicinal products in the food supply have surfaced in recent years. Physical separation of dual-purpose crops is needed—whether achieved by geographical isolation or by greenhouse containment—as is dedicated equipment for harvesting and handling, as well as standardized monitoring procedures. Concerns over the use of food crops for production of plant-based vaccines have been accompanied with calls for targeting non-food crops for pharmaceutical purposes, whether for the production of therapeutic proteins or plant-based vaccines. However, as indicated above, food crops remain highly desirable as targets for production of plant-based vaccines because of their amenability for oral delivery, avoiding the necessity for isolation and purification of the subunit vaccine prior to delivery. In addition, regulatory issues related to clinical trials, going through phase I–IV trials—similar to any other pharmaceutical product—must be pursued to assess efficacy, safety and reliability, followed by FDA approvals. For more than 30 years, live attenuated vaccines have been produced in SPF eggs, and successfully used to immunize infants and adults against common diseases such as measles and mumps. So, how can we take advantage of the regulatory history already established by the vaccine industry to push for plant-based vaccines?

Expanding Markets

Until recently, the vaccine market was considered low-margin, but that is changing as technology advances and new diseases are being addressed with vaccines. Worldwide, the market is $6 billion according to Peter Young, CEO of AlphaVax (Research Triangle Park, NC), which is developing viral-vector vaccines for HIV, malaria, Marburg virus and cancer, among others. At least one plant-based vaccine must prove to be an economic success story in order to pave the way for others to make it through to commercialization. This new technology may also serve as a platform for delivery of multiple antigens against several economically important diseases. This would certainly alleviate economic concerns over the plant-based vaccine approach, and boost its impact on the market.

Three years ago, the *Partnering for Global Health Forum* [co-sponsored by the Biotechnology Industry Organization (Washington, DC) and the Bill and Melinda Gates Foundation (Seattle)] brought together individuals from biotechnology companies, government agencies, foundations, and NGOs interested in pursuing biotechnology-based solutions for overcoming malaria, tuberculosis, typhoid, cholera, dengue fever, river blindness, AIDS and other diseases that plague developing nations. This was the beginning of an ongoing process to match funding sources and biotech companies, and to influence legislation and the regulatory process to encourage drug development for impoverished markets. Many biotechnology laboratories currently have proven technology and compounds ready for late-stage development, but lack funding to bring them to fruition to assist the individuals who need them. The message of the meeting was that funding from foundations, the government, and not-for-profit groups is available to further these efforts. Unfortunately, plant-based vaccines were not specifically spelled out in the announcement for request for proposals, although it was clear that this technology has great potential to help meet the goals of this major worldwide initiative.

As for the issue of the public's acceptance of plant-based vaccine technology, it is important to point out that the pharmaceutical industry has become a target for critics, and negative opinion is reflected in public polls. In a Kasier Family Foundation poll (spring 2005), pharmaceutical companies were ranked seventh in a list of nine industries, deemed less trustworthy than HMOs, but more trustworthy than oil and tobacco companies.

The pharmaceutical industry estimates that the cost of bringing a new chemical entity to market is around $800 million (including time-value of money; *i.e.*, factoring in the interest a company has to pay to borrow capital). Therefore, it is deemed justifiable that the public pays more in order for these drug companies to see returns on their investments. This, in turn, has contributed to the public's anger over drug prices. The shortage of influenza vaccine supply in the winter of 2004–2005 revived an issue that predates the biotech industry: what is the best way to make vaccines? For the influenza vaccine, a confluence of cost, pricing legal liability, and inertia provides an odd, but now familiar answer. Influenza vaccine is produced in chicken eggs, a manufacturing process blessed by regulatory bodies worldwide despite the fact that it has not been substantially upgraded in 60 years. The plant-based technology may circumvent long-standing production problems inherent in the egg-based system.

Summary

Producing vaccines in plants offers numerous advantages over current vaccine methodologies. Among them, safety, ease of production and low cost of production provide strong justification for developing the technology. However, many challenges remain within the pharmaceutical industry; requirements for generating non-food products in transgenic plants are different from those for food products. These challenges include technical, regulatory, economic, and public-perception issues. Physical isolation, delayed planting, agronomic support, dedicated equipment and frequent monitoring all contribute to the technical challenges involved.

As the technology to produce vaccines in plants goes through the regulatory pathway and demonstrates its economic feasibility, it may also overcome public-perception concerns that seem to have been dodged by the pharmaceutical industry. The likelihood that plant-based vaccines can be administered via oral or intranasal delivery systems will also add to their desirability as well as their economic benefits. There is potential for major impacts on global health, particularly in developing countries. However, standardized safety-assessment models must meet with approval from the general public along with the regulatory agencies and other interested parties. Risk assessment must be science-based in order for the results to be believable and trustworthy. Funding of research will accelerate the advances made thus far, and bring this technology closer to commercialization and worldwide use.

Producing vaccines in plants offers numerous advantages over current vaccine methodologies. However, many challenges remain.

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His research has been featured in the mass media, including NBC's *Today* show, TVE (TV-Spain), BBC, *St. Louis Post-Dispatch*, and *US News and World Report*. He has been an invited speaker at numerous institutions and conferences in the US and abroad, and has organized several symposia and workshops for various national and international conferences. He is (co)author of a dozen patent applications and has published over a hundred refereed scientific articles and thirteen chapters/reviews.

Korban received the American Society for Horticultural Science (ASHS) Outstanding Researcher Award in 2002 and the Paul A. Funk Recognition Award from the College of Agricultural, Consumer, and Environmental Sciences at the University of Illinois in 2004. He was elected a Fellow of ASHS in 2002 and a Fellow of the American College of Nutrition in 2003.

Panel Discussion and Q&A

Moderator:
Anthony M. Shelton
Cornell University
Ithaca, NY

Panelists:
Henry Miller
Hoover Institution
Stanford, CA

Mark Nelson
Grocery Manufacturers of America
Washington, DC

Henry Miller: I'm sure you are all familiar with the adage that the three most important factors in the value of real estate are location, location and location. Well the equivalent in public policy is context, context and context. Unfortunately, public-policy discussion groups, like Pew, the misnamed Center for Science in the Public Interest, Greenpeace, the Union of Concerned Scientists and other anti-technology groups consistently omit the context that is essential for judging and crafting public policy toward recombinant DNA technology and products from it. Contrary to these organizations' claims that they are nonpartisan and agnostic and honest brokers about biotech, in fact their workshops, conferences and publications invariably show a pervasive risk-averse and pro-regulatory bias and they attempt to create a presumption of genuine controversy where none exists. Mr. Rodemeyer's comments earlier seemed very moderate, so you have to scratch beneath the surface a little bit to find the disingenuousness. He spoke about unique requirements for recombinant-DNA modified organisms. And he mentioned animals and forestry. In fact, as Roger Beachy pointed out several times, it's not the technology that should serve as the trigger for regulation, rather it's the intrinsic risks imposed by the host organism and any new traits that have been introduced, whatever the technology that's used. Genetically improved trees and forestry are not, in effect, new. Who here would say that the forests that were planted and maintained by companies like Weyerhaeuser and Georgia Pacific are not in fact genetically engineered. Animals are a good example. There are animals that are natural mutants about which we might be concerned. There is a natural mutant of the rat, that's about five times larger than normal. Regulators

aren't interested in that, but they are interested in innocuous organisms that have, say, a marker gene introduced with recombinant DNA techniques. Often on bulletin boards I see advertisements for animals that have resulted from a cross between a timber wolf and a German shepherd. Some of these are F2 generation and three-quarters timber wolf and one-quarter shepherd, and these aren't regulated. Perhaps they should be—they have potentially worrisome characteristics. What's the motive for these misrepresentations, this lack of context about biotech and about process versus product as a trigger for regulation? Activists understand that over-regulation is an effective tool to inhibit innovation and to slow the diffusion of even a superior technology or product of which they disapprove, and they've had modest success. You've heard how expensive and debilitating our current regulatory schemes are.

What is the essential context that I'm referring to? It has to do with food safety so I won't belabor this, but, as you are aware, with the exception of wild berries, wild mushrooms, wild game and fish and shellfish, virtually all of the organisms—plants, animals, microorganisms—in our food supply have been modified by one genetic technique or another, and in crude and sometimes unpredictable ways. Second, because recombinant DNA techniques are more precise than their predecessors, biotech-derived plants and foods, including those making PMPs and other industrial materials, are likely to be even more safe, more predictable than other products. Yet, they alone are much more highly regulated. And so we've ended up with a regulatory process in which there is an inverse relationship between risk and the degree of regulation, which makes absolutely no sense. Third, the FDA does not normally perform safety determinations on new food varieties, but primarily conducts surveillance of marketed foods and takes action if any are found to be adulterated or misbranded. And lastly, unwarranted excessive regulation discourages innovation, imposes costs that are passed along to the consumer and are a disproportionate burden on smaller companies and academics. Again, Roger Beachy talked about how debilitating regulation and its expense and its distraction have been to research in the public sector. Nina Federoff made that point repeatedly in her excellent book, *Mendel in the Kitchen*. In both flagrant and subtle ways, anti-biotech lobbyists and propagandists—that's really what they are—continue to perpetuate various manifestations of the big lie. That is, that agbiotech is untested, unproven and unregulated. Their agenda is not to advance environmental protection or public health, but really to arrogate control over what R&D can be done and which products and technologies can have access to the market place. You heard a very moderate sounding presentation from Mike Rodemeyer. Don't take my word for it. Go to Google and put in my name and Pew or my name and Pew and biotech and CSPI and read the articles. You will get hundreds of links. Some of these articles are peer-reviewed and you can examine them for yourself. Also look at the specious surveys that these organizations have done and the extremely poor, unscholarly quality of the reports they produce and make up your own minds.

Much of the anxiety about the kinds of products that we are concerned with here does not take into account the realities of contemporary agriculture. As you've heard and as you know, gene flow is ubiquitous. Gene transfer is an age-old consideration for farmers and plant biologists. Farmers in North America who grow hundreds of crops, virtually all of

which have been genetically improved in some way, have meticulously developed strategies for preventing cross-contamination in the field, when and if it is necessary. The demands by some, including parts of the food industry, for food plants to be largely off limits for biopharming are really quite absurd. At the same time that such a prohibition would offer minimal incremental safety, it would severely stigmatize the technology and push the development of costs of biopharm products into the stratosphere, limiting development to very high value-added substances and inflating the ultimate costs to the consumer of those few products that do reach the marketplace. USDA's approach to regulation of biopharm crops, which, by the way, largely follows the demands of the food industry and the radical environmental lobby, is unscientific and debilitating: purely technique-based, process-based triggers to regulation. Typical of federal agencies whose approach to the regulation of recombinant-DNA-modified plant varieties in general, USDA's rules impose highly prescriptive design standards that don't take into account the actual risks of a given situation, but mindlessly dictate one-size-fits-all requirements. Moreover, the regulators exclude the establishment of tolerance levels although, ironically, regulators have established tolerances for unwanted contaminants in food, including fungal toxins, insect parts and rodent droppings. Although we do have long experience in segregating crops when necessary, human error is inevitable so it's reasonable to ask what the likelihood is of consumers sustaining injury in a worse-case scenario in which biopharm crops enter the food supply. Well, consider that, first, the active substance would have to be present in the final food product, say tofu or a salad dressing made with soybean oil, or in corn chips, at sufficient levels to exert an adverse effect, the result either of direct toxicity or allergy. But as with the ProdiGene incident several years ago in which biopharm corn stalks that contaminated a subsequent crop of soy, in most instances there would likely be a huge dilution effect. For example in the ProdiGene case, miniscule amounts of biopharm corn stalks and leaves were pulled into a massive soybean harvest. Second, the active agent would need to survive milling and other processing and cooking. Third, it would need to be orally active, as, by the way, the ProdiGene product was unlikely to be. And in any case it was simply a vaccine protein that was intended for veterinary use. So the probability that all of these events would occur is extremely low.

The regulatory obstacles to many of these products are going to be prodigious. Having spent 15 years as a federal regulator, I can tell you that regulators are suspicious of innovation, they are uncomfortable with new technology and the easiest way to cope with it is simply to stall, not to approve much, to ask for more and more studies and to be extremely, conservative.

Tony Shelton: Okay. That will certainly open up some questions. I was just looking over the NABC theme, which is providing an open forum for exploring issues in agricultural biotechnology, so we will certainly have an open forum on that. Mark?

Mark Nelson: There are some people in the food industry who, if PMP plants were planted, grown, harvested and processed on the moon, that would be fine with them, but the position of the Grocery Manufacturers of America (GMA) is a little more nuanced than

that, and I will return to that. GMA is the largest food-, beverage- and consumer-products association in the world. Our 120 members have a combined sales of about $500 billion a year, with two and a half million employees in all fifty states. Somewhat above 95% of supermarket products are from GMA's members. The earlier talks in the plenary session and then what we heard from the two previous speakers, I found fascinating—intellectually very stimulating. You folks doing this every day, you reach these goals, you make these accomplishments, it has to be incredibly satisfying and no doubt I will be a beneficiary from some of these products in the future. But the concern the food industry has with this is the simple fact of adulteration. It may not be a safety issue, but while we do like to talk with our consumers, we don't like to have conversations like: "About that cancer drug in your corn flakes? Don't worry, the FDA says its *de minimis*." Our concern is not based on marketplace issues as was mentioned earlier, *e.g.* that company A would say company B's product is contaminated. There are people like that in the food industry—as there are in any industry—but most don't engage in *schadenfreude* because they know it could happen to them. They have a common approach to make sure that the food supply continues to be safe. It comes down to our pocketbooks. If you have an adulteration, it will cost a lot of money to deal with it, particularly where a safety evaluation in the food has not actually been done yet. That's why, as an organization, we have asked FDA on more than one occasion for more regulation. Now I know that's different from what Henry was just talking about and what we heard earlier, but again it depends on context. As I understood it, your concern and your frustration was with regulation of transgenic events and it makes a lot of sense to me that if it's the same event or the same gene, why do we have to reinvent the wheel every single time. We are *Homo sapiens*, we should be able to learn from experience. But, in our situation—in the food industry—we want to make sure that the food supply is safe, and, currently, as these PMPs are being reviewed for field tests there is no full safety evaluation of the implications if these are commingled in the food supply. The FDA in its own documents and proposals for various guidelines and regulations has indicated that as these field tests increase in number, the probability of commingling also is likely to increase. Dr. Davies mentioned some very compelling points about why there is increasing pressure to make pharmaceutical proteins in plants. It makes a lot of sense. And while the economics would suggest that we are not going to rush to tobacco any time soon, the fact of the matter is we do know a lot more about some of the food crops and have experience in handling and managing them, and so they make perfect vehicles. Dr. Korban mentioned oral vaccines with fresh tomatoes and carrots, providing a new take on the concept of health-food stores. But regulations already exist for those, which would be considered drug-delivery vehicles and they would have to be separated; they wouldn't be in our food supply. So what have we asked FDA to do to help maintain the safety of the food supply, to allow PMPs to deliver benefits but at the same time make sure that the food supply is maintained in a safe way? We have asked for a mandatory food-safety review for these. Currently, a PMP would be evaluated by USDA by the APHIS Biotechnology Regulatory Services and it would deal with the center in the Food and Drug Administration responsible for drugs. [audio lost] Specifically, we have asked FDA to evaluate the compound, look at the quantity in the food, the impact of the

food in the diet, look at the possibility that it would be changed or degraded or denatured by processing and then assess it against existing regulations. Some of these proteins may in fact already be approved food additives or GRAS[1] substances, in which case if they got into the food supply they wouldn't be a problem, unless they were in foods that they are not supposed to be in or if they were at levels that were beyond the regulatory limits. But those probably are few and far between. If it is not a GRAS substance or a food additive then by definition it's an adulterant and what we have asked then is for FDA to set up reasonable approximate standards. If a crop contains this compound at X level, at 10 or 15% and there is no health problem, then perhaps you just need a simple Class-1 recall. On the other hand, if the amount in the food supply is at a dangerous level then there should be a Class-3 recall if it's in the food supply through commingling. Then that food-safety assessment should be used to inform the permit requirements. So perhaps there are different levels of physical separation or biological separation. The gene-control cascade concepts we heard of earlier would probably be something that would be very helpful to limit that. Dedicated equipment, trained employees with standard protocols are already being asked for by USDA and those would make perfect sense in our estimation as well. So, as I said earlier, some people in the food industry would like to see these things grown on the moon, but we recognize that that's not feasible but we do think that we can use our experience and our knowledge and existing regulations to take a much more reasoned and nuanced approach to help the biotechnology industry—and particularly the PMP part—develop and also maintain a safe food supply.

Shelton: Are there questions from the audience or questions that panelists would like to ask each other?

Steve Rock (Environmental Protection Agency, Cincinatti, OH): As a research scientist, the definition of "side-effect" was something I didn't think about or didn't want to think about. And I want to ask the panelists to think about what happens if you change your mind. And in terms of what Mark was saying about recalls—we have had recalls. We have had products that we put into the food supply that we decided we didn't want in the food supply and were able to recall at some point—cyclamates, red dye #4. Those things were fairly easy to extract from the process. And I wondered if you all have thought about how easy it would be to extract these from the process? As a scientist in research and development, I don't know anybody who is against innovation. Most of my colleagues in the research world are all for innovation as long as there is a significant probability of getting it right, and when you miss there is some way to correct the error. I spend a lot of my time cleaning up historical contamination on hazardous waste sites, specifically oil and DDT. The oil I'm working with has been in the ground since Rockefeller set up his first refinery, 100+ years ago. And although DDT hasn't been used since the 1960s in the United States, we still find it in food supplies around the world. It's hard to get it back into the bottle once you let it out. And there are other examples from the world of

[1]Generally regarded as safe.

ecology—rabbits in Australia, innocently released into the environment and it's now one of their biggest problems. I am wondering how to make it possible to recall our mistakes, which is why it makes a lot of sense, not to stop progress but to go very, very slowly. Twenty years in terms of technology innovation, seems like a huge amount of time. We run on a time scale where a long-term investment is 3 months. And that's crazy. We have to be thinking on at least a generational, if not a geological, time scale because that's what we have to live with. How do you put the genie back in the bottle and what do you do when you change your mind?

Miller: We just had a good example. I hope I don't understate it when I say that that's why EPA may be the worst regulatory agency in the history of the world. I gave the example of the ice-minus fiasco[2]. Nobody, I think, is arguing that there shouldn't be any regulation. What we are arguing is that it should make sense, it should be based on science and that the triggers for regulation should be risk-based. I'll give you an example. About 20 to 30 years ago, a new variety of wheat was developed in a number of countries, the United States, the Soviet Union, Germany and so on. This construction contained all the chromosomes, all of the genes of bread wheat plus all of the genes of wild quackgrass or couch grass. So it contained the entire genome of red wheat and tens of thousands of new genes that were introduced who knew how, who knew with what effect. Nobody, least of all, EPA or other regulators or the anti-biotech activists asked whether the introduction of these genes from a wild grass could make the plant more weed-like, more aggressive in the field, nobody asked whether the gene products of any of these tens of thousands of new genes could be toxic or allergenic in any way. And so these went into the field, they went to large-scale, they went to commercial scale, they went into the food supply with no oversight and not a whimper. But now if someone were to move a single gene of couch grass into *Triticum* using recombinant DNA techniques—even a housekeeping enzyme of some sort—it would bring down this massive, debilitating, very expensive, very lengthy regulatory process from EPA and/or USDA and/or FDA, adding tens of millions of dollars to the regulatory process. This is irreconcilable scientifically. It's preposterous. Roger and I are arguing, not for the absence of regulation—that it be rolled back—but that it be rationalized, that it make some scientific sense.

Shelton: I wonder if the previous speakers, Davies and Korban, who commented on that technology, have any questions for the panel members.

Maelor Davies: Thank you. With the *Nicotiana* system—we don't call it tobacco because we are using other species, we are moving away from conventional crops—we hope to facilitate further development of PMPs with a plant that cannot cross-pollinate with the existing related crop, conventional tobacco. It will be different enough to represent a unique plant variety for PMPs. My hope is that, as we do that, if those companies—and we do work with all the companies that have significant activity now in tobacco-based

[2]See page 53

PMP technology—can also advance our situation we may be able to prototype, as it were, a larger-scale commercial PMP in the field setting. In time then, that may enable companies with food-plant-based PMP technologies to advance as well. So, we hope to facilitate the whole thing almost by circumventing this issue for the time being. We'll just have to see how that goes and how that develops.

I hope that I didn't, in any way, cast any negative perspective on USDA. I think USDA-APHIS-BRS is doing what it has to do. It has to go carefully and check out every detail of this whole PMP situation. But what I did point out is that those regulations—such as they are today—are probably not commercially workable. Temporary stringency is necessary to build up a comfort level, and as experience is gained in the field we will look at gene flow. At our center, we are designing specific experiments to look at these issues over the next few years to examine how effective is the mandated containment, what is the gene-flow situation and other such issues. My hope is that eventually things can lighten up significantly because the regulatory system needs to be one that companies can work with economically, commercially successfully, and that growers can afford to implement and safely and routinely comply with, without excessive cost.

Miller: One set of precedents, although they are not perfect for PMPs, are such pharmaceuticals as morphine, codeine and even Metamucil®, which come from plants and with which regulators and industry and consumers have a great deal of experience.

Schuyler Korban: I would echo the comments just made. We do not object to the regulatory processes as long as they are based on sound science and are realistic. Vaccines are produced in eggs or in yeast cells, both of which are part of our food supply. We have to similarly isolate those food crops that are used as vehicles for producing subunit vaccines or other therapeutic proteins. And we heard of some of the ways of reducing or minimizing or essentially eliminating their presence. However, there is no such thing as 100% safety in anything that we deal with. With any of the food or environmental issues that we are trying to resolve, there is no such thing as a 100% guarantee for anything. We can think in terms of minimizing the risk and that's being realistic. Our expectations must be sound, science-based and realistic.

Nelson: I agree that the regulations need to be based on sound science and they need to be proportional. That's why we are suggesting that permit requirements be based on the potential risk if the product were to get into the food supply. And I agree with that no system is 100% perfect. That's why we are asking that safety evaluations be done ahead of time so that we understand what the agencies' actions will be ahead of time, so that we can work with them. We want to avoid another StarLink™ situation, which ended up not being a safety issue but an adulteration issue that caused hundreds of millions of dollars in recall costs and the food safety system took a dent. And we have had huge decline in corn exports because of that exercise and that experience. So, we would like to make sure that it's proportional and know ahead of time what it is we are going to do. We're smart enough to do that. We're smart enough to make that kind of assessment so we aren't scrambling around like a bunch of chickens.

Shelton: Speaking of chickens and eggs and their getting into the food system—if the plants are not going to be grown on the moon, like you said, but if they were grown in the field like tobacco would that be something that GMA would support—the idea of producing PMP's in tobacco?

Nelson: Well it's a non-food crop, but clearly the smoking and chewing tobacco industry has certain concerns about GMOs, I guess. Yeah, our preference would be for non-food crops. But, we know that that is not always going to be economically or biologically feasible. We are asking that if it is grown in a food crop, that the assessment is done and then appropriate controls be put in place to manage it so that we do minimize entry into the food supply.

Shelton: GMA has a tremendous amount of clout. The decisions that you make are really going to affect PMPs in the future. Have you given guidelines in terms of crops that would be acceptable and not acceptable?

Nelson: No we haven't gotten into that detail because we are not experts on particular crops. For the primary food crops—wheat, corn, soy, rice—field tests are limited at this point. We are concerned that management controls be in place. We are seeing that in some of the permit requirements now coming out for such field trials; they do seem to be stronger. They emphasize greater separation, better training, and so on, to make sure that they are contained.

Gregory Jaffe (Center for Science in the Public Interest, Washington, DC): Going back 10 to 15 years, a combination of small and large multinational companies did a lot of the research on the GM plants that eventually were commercialized. In the bio-pharming/vaccine area some very small companies are involved and the multinational pharmaceutical companies are not. Why is that? Why are the big, multinational companies that are producing these kinds of drugs and biologics, not doing research and field trials in this area? Also, will the success of these products need those companies to get involved as partners or to assist in bringing the products to market? I'm sort of curious as to why they haven't become involved. The speakers listed all the benefits of this type of production model, so why aren't they dabbling in this?

Korban: It is surprising, considering the advantages of using plants for developing vaccines, not only from a production standpoint but also from a delivery standpoint. But, the way I understand it is that the vaccine industry is not very big and the margin of profit is not very high. As a result, interest is not strong at this point. However, with the recognition that they need to move away from animal-source contamination of biopharmaceutical products I think you will see expressions of interest, including from Monsanto. The big pharmaceutical companies are not partnering—especially with university researchers—to push this further. If major investment in this technology does not come along, it will fail eventually.

Davies: I would certainly concur with that. We've thought about this for a number of years and we've talked to the industry both on the ag side and the pharma side. Let me hit on a couple of points. First of all, the technologies for expression in plants were initiated mostly by university faculty who then started companies. In other words, the big guys didn't have the technologies. But that isn't the ultimate answer because then you could ask why doesn't big pharma or big ag invest in or acquire them. We were very encouraged early in 2005 to get a visit at our facility by a major pharma company. I'm not at liberty to say who it was, but that company is becoming concerned about the pressure on protein production and is beginning to scout out and explore the plant-based technologies. But why has it taken so long to get to this point? It's a business issue. It has to deal with big ag companies and big pharma having their particular strategies. In the case of big ag, not owning the drugs means that they don't see how they would make sufficient money out of it ultimately. On the big pharma side, they tend to be very conservative and very cautious about even new drug technologies. They are waiting for university spin-off drug companies to prove that their particular vaccine or whatever is a real winner and then they will come in and acquire it. So it has to do with business play and market fit and corporate-strategy fit and similar issues. I think that increasing demands for proteins will eventually change this paradigm and make big pharma pay attention to these alternative platforms.

Tom Reddick (Global Environmental Ethics Council, Clayton MO): Glycosylation was mentioned. I heard that hamster ovaries are used for mammalian cell lines to create proteins that will glycosylate properly. Is that a technical hurdle that lies in the future? Will the FDA balk at approving a plant system for efficacy if it doesn't glycosylate like a hamster?

Davies: Probably yes to all of the above. There are certain modifications to proteins that animal cells do, including glycosylation—attachment of carbohydrates—that plants either don't do or do differently. This is a challenge that the microbial fermentation world has also been somewhat limited by and that's why mammalian systems are used to produce mammalian protein drugs. Some protein drugs will not be suitable for plant-based production. They would be more suitable for mammalian cells in culture or whatever. If it turns out that the non-protein component, the carbohydrate substituent, is not essential for function, the plant-made protein with the necessary biological function but without the substituents might be regarded as a new drug and need to be registered as a new synthetic. We'll see how those things play out in time. We don't know the complete portfolio of products that the PMP companies are looking at, but we certainly get the sense that they are focusing on the ones for which this will not be an issue. Dr. Beachy has alluded to some work that is going on to actually modify the plant so that its production of the substituent groups, like these carbohydrates, would be so-called "humanized." Probably quite a lot remains to be done for that to be successful because differences in the carbohydrates are quite complex.

William Goldner (USDA Small Business Innovation Research Program, Washington, DC): If you produce industrial enzymes or industrial proteins in crop plants are the same issues faced as for pharmaceutical proteins?

Nelson: Yes. We have raised the same concerns with the FDA, not just with reference to PMPs but also plant-made industrial chemicals. The same issues apply, but it would be an adulterant. The safety issues may not be as great, but we have asked that it be looked at.

Miller: Once again, it's useful to look for applicable precedents and a very good one is rape seed. The original rape seed oil was highly toxic. It was used as an industrial lubricant; it couldn't be used as a cooking oil because it was extremely toxic. Plant breeders came up with what is called a "double-low" variety, low in erucic acid and glucosinolates. Both varieties are still grown—one to produce industrial lubricants, one to produce canola oil—and we've established means for keeping them segregated. We've established tolerances for the toxins in food. Again, it's extremely useful and important to look for applicable precedents to figure out where we need to go and what makes sense.

Milt Zaitlin (Cornell University, Ithaca, NY). I've seen in the literature that some plant-based vaccines do elicit antibodies, but why don't other proteins that we eat also elicit antibody formation? I guess people get allergenic responses to wheat proteins and so forth, but what characteristics would lead you to believe that a transgene product would be more immunogenic than normal proteins that we eat?

Korban: You could ask the same question of any vaccine that you take, especially when you are dealing with subunit vaccines. These are antigens that produce specific antibodies. All we are doing when we use plants to produce such an antigen is to provide a vehicle for production. Now you can purify that antigen if you so desire and introduce it intramuscularly. That's what the tobacco system would be used for. Or, you can strictly consume it orally without purification in that plant system.

Zaitlin: I don't think you've answered my question. We're talking about an oral route. We're not talking about injections.

Korban: The way to think about it is that with the antigen administered orally, it's inducing specific antibodies that provide protection. Now, there are other proteins within the plant system that we ingest that are used to provide nutrition and do not induce antibodies except if a protein is allergenic and that's when you have a concern over ingestion of this particular protein. I don't know if I've answered your question correctly.

Elizabeth Hood (Arkansas State University, Jonesboro, AR): At ProdiGene we did some animal experiments to address this issue. There's a distinction between whether or not you make antibodies against a protein that you ingest vs. having an allergic reaction. The rabbits had already raised their own antibodies to orally consumed corn-seed proteins that were naturally there. So, we made our own antibodies for testing on pre-screened rabbits that did not already have these antibodies in their blood serum. There's been a

lot of misuse of the term "immunogenic response" with respect to food based proteins. We make a lot of antibodies to things that we ingest; they are not all allergic reactions. The mechanism of making antibodies against an orally consumed antigen is a normal response to proteins that we ingest. We make antibodies to lots of things that we ingest, we just don't always have allergic reactions to them.

Systems Agriculture: Towards a Sustainable Agricultural and Environmental Policy

Bruce W. Ferguson
Edenspace Systems Corporation
Dulles, VA

Edenspace Systems Corporation seeks to transform industries with innovative plant-based services and products that protect health, increase property values and improve environmental quality. Within the general area of environmental phytotechnologies, the company has specialized in the use of plants to extract minerals from soil and water, with more than three dozen successful contracts completed or ongoing. Current company research seeks to develop multi-trait plants for biosensing, phytoremediation and renewable energy.

The company's 6-year experience as a commercial pioneer in environmental phytotechnology has provided it with an unusual perspective on the intersection of agriculture, biotechnology and the environment. Four of Edenspace's current projects illustrate some of the opportunities and challenges presented by this confluence:

- using ferns to remove arsenic from soils in a Washington, DC, suburb;
- engineering plant biosensors—"phytosensors"—to detect and monitor environmental parameters such as heavy metals;
- engineering plants to produce higher yields of ethanol per acre; and
- forming a new agricultural cooperative to provide additional income to producers who work on environmental projects.

After summarizing these projects, I will list some conclusions and recommendations as to where agriculture, biotech and environment may intersect in the future.

Environmental Phytotechnology Projects

Arsenic Phytoremediation

In 2005, Edenspace expanded a project with the US Army Corps of Engineers to remove arsenic from soils of residential properties in Washington, DC. The project uses Edenspace's edenfern™ phytoremediation plants to extract and concentrate soil arsenic in fern fronds, which may then be harvested for safe disposal.

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In 2004, approximately 2,800 edenfern™ plants were installed in fourteen test plots at three different sites in the Northwest Washington suburb of Spring Valley. The ferns showed excellent growth and arsenic uptake, removing an average of approximately 9 mg/kg of soil arsenic across all sites from starting concentrations that ranged from 16 to 127 mg/kg. Remediation activities were completed with no apparent harm to specimen trees or shrubs, and with little interference to homeowner activities on the properties. Importantly, many of the edenfern™ plants overwintered and showed new growth in spring 2005, a key to reducing costs of arsenic phytoremediation in northern latitudes. Based on these results, in 2005 the project scope was increased significantly, with 10,000 ferns planted on thirty-four plots at twelve residential sites.

The techniques under evaluation in this ongoing project include the planting, cultivation, treatment and harvesting of special ferns that accumulate large quantities of arsenic in their fronds. The edenfern™ has demonstrated that bioconcentration coefficients (ratios of arsenic in the plants to arsenic in the soil or water) greater than 100 promise much lower costs for removing and disposing of this dangerous element. For example, at some sites phytoremediation of arsenic may cost as little as 10% of the cost of excavating and removing contaminated soil. The technique also may be useful at sites where excavation is difficult, such as near valuable landscaping plants or above buried pipes or cables.

The arsenic-extracting capabilities of the edenfern™ were discovered by Dr. Lena Ma of the University of Florida. Edenspace has signed an exclusive license agreement with the university, which has received two patents based on the discovery, to enable cost-effective commercial cleanup of arsenic from soil and water.

Arsenic causes cancer, mutations and birth defects and also has been associated with the development of diabetes. The element was once widely used in insecticides in farming, gardening and ranching, and is used as a component of preservatives in lumber and furniture. In some parts of the world, arsenic occurs naturally in groundwater. In some states, decades after arsenic was introduced into the environment, soil concentrations can be hundreds of times higher than the residential standard. Because of its toxicity to humans, farm animals and household pets, the stability of its compounds in soil and groundwater, its once widespread use, and the lack of cost-effective remediation techniques, arsenic today constitutes a significant environmental health hazard.

The ability of plants such as the edenfern™ to serve as solar-powered pumps and filters for removing environmental contaminants offers numerous potential advantages over other remediation techniques. These advantages include preservation of topsoil, potential recycling of contaminants, joint products or use, and, importantly, lower cost. A cost comparison of different evaluation techniques is provided in Table 1.

Table 1. Costs of alternative environmental remediation techniques (Glass, 1999).

Technique	Cost per cubic yard ($)
Soil Washing	50–150
Soil Flushing	75–210
Acid Leaching/Extraction	150–400
Solidification/Stabilization	75–205
Vitrification	40–600
Thermal Desorption/Treatment	150–500
Excavation/Landfilling	100–500
Phytoremediation	25–100

Phytosensors

State-of-the-art collection of soil, water, plants and air is typically based on a sampling grid. For example, to measure concentrations of contaminants in soil or water, a trained professional, such as an environmental technician or home inspector, collects samples on a 30-foot grid for laboratory analysis using atomic absorption spectrometry, ICP mass spectrometry, *etc*. Including collection, this method is expensive, providing data at a cost of $20 to more than $200 per sample. One problem with a grid-based technique is the low spatial resolution of the data—the large "pixel" size. No information is provided for areas between the grid-sampling points, so that the reported concentrations may not fairly represent the level or extent of contamination at the site as a whole (Demougeot-Renard *et al*., 2004). Thus, a high lead concentration in a house's drip zone does not necessarily indicate high concentrations in a play area or pathway, nor does a low lead concentration in a composite sample indicate that a property has low soil levels of lead in all zones of concern. In addition, different sampling events may result in markedly different assessments due to soil and sampling variability. This problem presents additional expense to customers, who usually are required by regulators to remediate the soil some distance outside the perimeter defined by the "high" sample points, in order to account for uncertainty about contaminant distribution. In addition, areas of contamination may escape detection and treatment, presenting a future liability and health risk.

Of particular interest would be the development of indicator plants to provide rapid, low-cost, in-situ *monitoring of environmental and plant conditions.*

Development of wide-area sampling techniques that provide higher spatial resolution at lower cost and over time is desirable. Of particular interest would be the development of indicator plants to provide rapid, low-cost, *in-situ* monitoring of environmental and

plant conditions. Phytosensors could be easily replicated, solar-powered, and unobtrusive. Phytosensors may be thought of as signal transducers and amplifiers that detect and report on an environmental condition such as the presence of a contaminant or a plant condition such as "hidden hunger." Phytosensors address the "grid problem" by inexpensively providing information with high spatial resolution. Because each plant represents a sample point, information provided can be near-continuous. Such phytosensors could also be used in joint applications such as environmental cleanup, agriculture, and landscaping.

Three elements are essential to successful development of phytosensors:

- an element in the plant, such as a promoter gene, that is sensitive to the target parameter;
- a plant reporter pathway that can provide measurable evidence—such as a color change in leaves or stems—of changes in the target parameter; and
- a plant species that is suitable for the intended use.

Current Edenspace phytosensor projects include using a metal-responsive promoter gene, *BjMTP,* identified by Professor David Salt at Purdue University, linked to different reporters such as green fluorescent protein (GFP) and the anthocyanin gene *B* in Indian mustard, maize and turf grass. The transformed plants may be used to detect the presence of heavy metals such as lead, cadmium and mercury in soil, groundwater and/or landfill leachate. Edenspace is also working with Professor C. Neal Stewart, Jr., at the University of Tennessee to insert an arsenic-responsive promoter from *Shewanella* into the edenfern™. Successful insertion of this construct will create an indicator that is visible under UV light in response to arsenic uptake, potentially showing when arsenic is present in the plants and soil as well as when cleanup has been completed.

Energy Crops

In 2004, ethanol production in the United States attained a record 3.35 billion gallons, up 19% from 2003 (Hillgren, 2005). While it is still a small part of the total US fuel market, representing approximately 2% of the gasoline sold in 2004, the recent rapid increase in oil prices has increased the cost-competitiveness of ethanol and indicates that it can continue to capture market share, particularly if production costs can be decreased. Production of energy, and of ethanol in particular, from plant biomass is especially attractive because of plants' renewable conversion of solar energy and recycling of atmospheric carbon dioxide. In October 2002, the Biomass Technical Advisory Committee established by the Biomass R&D Act of 2000 issued national goals for biobased transportation fuels, calling for a substantial increase in the use of such fuels from 0.147 quads in 2001, or only 0.5% of US transportation fuel consumption, to 20% in 2030 (BRDTAC, 2002a). To achieve these ambitious goals, the Advisory Committee subsequently recommended a comprehensive research plan, the elements of which include increased biomass yields, lower biomass costs, new enzymes and catalysts, multi-trait crops, and environmentally sound biomass production (BRDTAC, 2002b). The overall objective of this plan is to reduce the price of biofuels. In 2004, the Advisory Committee updated its recommended research plan, recommending specifically that the US Departments of Energy and Agriculture (DOE and USDA) significantly increase funding for R&D programs

on cellulosic ethanol (BRDTAC, 2004). In 2005, the US Congress appeared likely to implement this recommendation.

A promising biofuel co-production opportunity exists based on a convergence of goals between biomass production for energy and biomass production for phytoremediation. High biomass yields sought for bioenergy, for example, are also desirable in phytoremediation of metals, metalloids and radionuclides, because the rate of contaminant removal is a function of biomass as well as of bioconcentration. In addition, new ways of disposing of contaminated biomass are sought that minimize landfill burdens. Current treatment methods can remove 60% to 90% of recovered metals from plant biomass (Edenspace, unpublished data), which for some contaminants is insufficient to allow disposal of the treated biomass as non-hazardous waste. Metals are typically sequestered inside cell walls, which are difficult to break down cost-effectively using current techniques. The ability to degrade cell walls with low-cost cellulases could significantly improve the current state of the art in contaminant recovery from phytoremediation crops, allowing treatment of contaminated biomass to reduce the costs and liabilities associated with landfill disposal, facilitate recycling of recovered metals, and produce clean, renewable bioenergy feedstocks.

With DOE funding, Edenspace and its research partners, Drs. Mariam Sticklen and Bruce Dale at Michigan State University, have engineered tobacco (*Nicotiana tabacum*) and maize for (i) constitutive production of an hydrolytic enzyme, endoglucanase, to aid post-harvest hydrolysis of plant biomass to simple sugars that are useful as biofuel feedstocks, (ii) greater biomass, and (iii) delayed flowering, which reduces the likelihood of transfer of the transgenes to non-engineered plants. Cellulase levels of about 1% of the plant dry weight (less than 10 mg of enzyme protein per g) are sufficient to convert essentially all of the cellulose and hemicellulose in ammonia-treated plant matter to fermentable sugars in less than 24 h (Dale *et al.*, 1999). Preliminary results indicate that transgenic corn can produce endoglucanase at levels higher than 9% of total soluble protein, close to this benchmark.

Using such "endoplant" cellulases and biomass augmentation, a primary technical objective is to demonstrate ethanol yields greater than those achievable based on hydrolysis of starch alone.

Using such "endoplant" cellulases and biomass augmentation, a primary technical objective of this project is to demonstrate ethanol yields greater than those achievable based on hydrolysis of starch alone, currently at 2.5 to 2.8 gallons of ethanol per bushel of corn grain, or roughly 450 to 600 gallons of ethanol per crop acre. Efficient hydrolysis of the 6 to 8 tons dry weight of corn stover grown per acre, and of the distillers grain that remains after corn grain is processed with amylase, could more than double current ethanol yields. A second major objective is to reduce biomass pretreatment steps that are now necessary to remove lignin and hemicellulose. Performance against these objectives will be measured in a pilot demonstration scheduled for 2006, when transgenic plants

will be processed, and the yields and costs of ethanol production assessed, at the National Renewable Energy Laboratory's Bioethanol Pilot Plant facility in Golden, Colorado. A parallel project between Edenspace and the US Department of Agriculture for switchgrass began in mid-2005.

By sharing biomass production and post-harvest treatment costs with phytoremediation, another plant-based technology that has similar goals, costs of producing hydrolyzed feedstocks for biofuels could be dramatically reduced—an essential, if not in itself sufficient, step toward increasing the cost-competitiveness of renewable fuels. In addition, production of biofuel feedstocks, cellulase and cellulase-rich plants, and phytoremediation crops can provide farmers with new sources of income at a time when traditional crops are increasingly subject to severe market price and trade pressures. A joint 2003 USDA/DOE analysis of the economic impacts of bioenergy crop production showed the potential to increase farm income by up to $6 billion.

Plantavit Cooperative

In the United States, an estimated 294,000 hazardous waste sites await cleanup, with total costs aggregating $209 billion (USEPA, 2004). These numbers exclude potential cleanup costs on agricultural and many residential properties, as well as the costs of pollution prevention over wide areas. Given the costs of current environmental technologies and current funding levels, the environmental challenges posed by such sites will persist for decades.

Many companies, individuals and government agencies are now using plants to restore and protect the environment.

Capitalizing on the low costs and other advantages of environmental phytotechnologies, many companies, individuals and government agencies are now using plants to restore and protect the environment. Plantavit, a new national agricultural cooperative established in 2004 by Edenspace and farmers in California's San Joaquin Valley, seeks to identify, train and hire its producer members to apply such environmental phytotechnologies in the United States and other countries. Large-scale application of such technologies, both on and off the farm, is expected to achieve significant environmental benefits while at the same time providing a new source of income to farmers. The cooperative's goals are to:

- provide additional sources of nonfarm income to agricultural producers, based on application of producer skills, equipment and other agricultural resources to environmental projects;
- provide professional training to members in agriculturally based environmental techniques and related fields, such as plant sciences and soil sciences;
- increase public recognition of member achievements in environmental activities;
- reduce environmental health risks on farm property; and
- increase farm property values.

Environmental phytotechnologies offer a promising way to address wide-area problems. Grasses, trees and other plants can be used to construct riparian barrier strips to retain runoff. Trees can be planted to sequester atmospheric carbon and remove other contaminants from the air, soil and water. With the use of appropriate techniques, crop plants such as mustard, corn and tobacco can remove arsenic, lead, cadmium, selenium and other contaminants from soil and groundwater. Some of these techniques are already used on farms and ranches by agricultural producers with support from the Conservation Reserve Program, Environmental Quality Incentives Program, the Wetland Reserve Program and the Wildlife Habitat Incentives Program, and other government programs. These techniques are not currently used on the farm, nor is current funding provided for producers to use any of these techniques outside the farm or ranch.

The reliance of environmental phytotechnologies on agricultural techniques provides scale efficiencies similar to those in agriculture: the larger the area, the lower the cost per acre. Because most current phytoremediation sites are less than three acres in size—small for a modern farm—the full cost savings enabled by large-scale phytoremediation have not yet been achieved. For appropriate sites, substantial cost savings may be attained by farmers experienced in modern agricultural techniques, who are also trained in phytoremediation techniques. In particular, the environmental problems that often must be addressed in land development in urban and metro areas may be particularly amenable to environmental phytotechnology techniques employed by adaptive farmers. Edenspace is currently funded by USDA to study the potential environmental markets for agricultural producers under this approach.

Observations and Recommendations

These and similar projects have afforded Edenspace a broad range of experiences, including site characterization and environmental remediation, plant genetic engineering and APHIS field permitting, market research, marketing and sales. Environmental science and regulation, agricultural policy and practice, and plant biochemistry and genetics have all played prominent roles in Edenspace's work. From this experience, we offer the following observations and recommendations.

Change Agricultural Policy from Insulation to Innovation

Current agricultural policy poses high risks for farmers. The 2002 Farm Bill provides nearly $20 billion in farm support annually, primarily intended to slow or stop the erosion of the farm base experienced over the last several decades. However, rapid growth in the federal budget deficit, recent decisions by the World Trade Organization against high tariffs and other farm support, and the ethical issues involved in restricting food imports from developing countries, indicate that, over the long term, agricultural subsidies are not sustainable. The financial supports that allow farmers to ride out temporary rough spots are themselves temporary. When, and not if, these supports disappear, farmers may be left without a means to compete with lower cost food imports, leading to accelerated industry consolidation and the accelerated demise of small farms. One good way to address this problem, successfully achieved in many high-tech US industries, is

to encourage rapid innovation and product development, allowing farmers to compete by offering higher-margin, value-added products rather than commodity products, with life cycles of an increasing number of agricultural products measured in months rather than years or decades.

Promote Systems Agriculture

In most of its projects, Edenspace regards its plants as just one element in the overall solution it provides to a client. In remediating a small arms firing range, for example, Edenspace must sift out and recycle the bullet fragments, as well as phytoextract ionic lead, if site goals are to be met. This integrated perspective on designing plants into customer solutions is an essential element of future agriculture, and provides an important pathway for engineering new high-margin plants to be grown by agricultural producers. I call this approach "systems agriculture"—the engineering of plant traits and agricultural protocols on an integrated basis with other production technologies so as to minimize total costs of end-user products and services. Examples are the integration of new plant traits with new farm techniques and new ethanol production steps in order to minimize the cost of ethanol at the fuel pump, and developing new plant traits that facilitate storage of hypoallergenic vaccines in the syringe. The approach requires that new agricultural products and techniques be developed by considering multiple areas of upstream and downstream production expertise together, on an integrated basis, that are now considered separately. New collaborations will be needed between agriculture and other industries, such as the energy and healthcare industries, that today are found infrequently, if at all. Systems agriculture is likely to lead to the creation of surprisingly good products—such as fuels that are better than ethanol, better than gasoline—that are undiscovered today because of a lack of creativity deriving from the failure to take an integrated design engineering approach.

Create More Receptive Public Opinion

Systems agriculture is dependent on use of biotechnology to create transgenic plants. Traditional breeding methods are simply too imprecise and too slow to achieve the rapid development of products needed to support a competitive US agricultural sector. While the regulation of transgenic plants today imposes increasingly high hurdles for the introduction of new crops, the antecedent cause of the problem isn't regulation but the public perception that too often sees the risks of transgenic plants as outweighing the benefits. Public opinion, in turn, has been shaped by a complicated combination of factors, includ-

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Transgenic plant development is underfunded by the private sector, largely because of the divisiveness of the transgenic crop wars of the last 10 years.

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ing economic competition among countries, substitution competition among industries, product competition among companies, and a host of scientific and nonscientific beliefs. While this will be a complex area to address, a key element is to develop public demand for new transgenic plant products that directly promote human health, provide low-cost energy, or improve property values, rather than simply—though importantly—reduce producer costs with no significant benefit perceived by consumers.

Increase Government R&D Funding

Transgenic plant development is underfunded by the private sector, largely because of the divisiveness of the transgenic crop wars of the last 10 years. To address this market imbalance, the USDA plant biotechnology budget should be at least quadrupled in size. Half of the increase should be apportioned to other government agencies—EPA, NIH, HUD, DOT, *etc*.—that should be instructed to fund crop-plant research related to their missions. USDA should encourage customers to tell farmers what types of plant they'd like farmers to grow. The only "NIH" in government should be the National Institutes of Health.

The United States has one of the strongest agricultural industries in the world, with tremendous natural advantages including fertile soils, a temperate climate, good precipitation, and skilled producers. These advantages are offset in part by another US blessing: a high quality of life that translates to high labor and materials costs relative to many other parts of the world. If we are to continue to realize the benefits of our strong position, we need to pursue systems agriculture and the plethora of new high-margin markets and products that it promises.

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Bruce Ferguson has served as chairman, president and chief executive officer of Edenspace Systems Corporation since co-founding it in 1998. Edenspace is a leading phytotechnology company specializing in environmental uses of plants. Projects include a contract with the US Army Corps of Engineers to remove arsenic from soil, and development of corn and switchgrass varieties for higher ethanol yields.

From 1997 to 1998, Mr. Ferguson was a visiting fellow at George Washington University's Center for International Science and Technology Policy. From 1982 to 1997, he was an executive officer and director at Orbital Sciences Corporation (NYSE:ORB), a space technology company he co-founded in 1982. Prior to his work at Orbital, he was an attorney in the corporate and securities department of the Chicago law firm of Kirkland & Ellis.

In 1981, he received an MBA from Harvard Business School and a JD from Harvard Law School, where he was an editor of the *Harvard Law Review*. He received an AB *magna cum laude* in government from Harvard College and an EdM from the Harvard Graduate School of Education in 1976. He is a member of the Pegasus launch vehicle team that received the 1991 National Medal of Technology, and is a 1999 recipient of the Harvard Business School Alumni Achievement Award.

Plant Transformation Pathways of Energetic Materials (RDX, TNT, DNTs)

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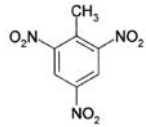
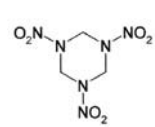
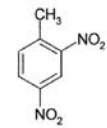
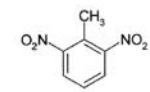
2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are commonly found in surface soil and groundwater at ammunition-production and military-training sites. Approximately 2,000 US Department of Defense facilities are contaminated with explosives both in soil and in groundwater (Medina *et al.*, 2003). Dinitrotoluenes (DNTs), such as 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT), contaminate the sites as by-products of TNT. TNT and DNTs are classified as nitroaromatic explosives having aromatic ring structures, whereas RDX is a nitramine explosive possessing N-nitro groups (Hannink *et al.*, 2002). The explosives are transported to soil and groundwater after open detonation, seepage and/or improper disposal at military and munition-production sites. TNT and DNTs have higher octanol-water partition coefficient (K_{ow}) values than does RDX, suggesting that they are strongly bound to soil organic matter, whereas RDX is mobile as a result of poor sorption. The explosives are not volatile due to their low vapor pressures. Physical and chemical properties of the explosives are shown in Table 1.

Phytoremediation is a promising technology using plants to clean up contaminated soil and groundwater in situ *because of low cost of maintenance and operation, and public acceptance.*

Several studies have reported abiotic methods of treatment of explosives, such as incineration, carbon adsorption, alkaline hydrolysis, and catalytic and advanced oxidation (Garg *et al.*, 1991; Rodgers and Bunce, 2001). Harmful by-products, requiring further treatment, and transport of contaminated soils or groundwater have drawn attention to bioremediation. Phytoremediation is a promising technology using plants to clean up contaminated soil and groundwater *in situ* because of low cost of maintenance and operation, and public acceptance (Schnoor *et al.*, 1995). The fact that plants are able

Table 1. Chemical and physical properties of TNT, RDX, and DNTs.

	TNT	RDX	2,4-DNT	2,6-DNT
Molecular weight	227.15	222.26	182.14	182.14
Molecular formula	$C_7H_5N_3O_6$	$C_3H_6N_6O_6$	$C_7H_6N_2O_4$	$C_7H_6N_2O_4$
Log K_{ow}	1.6–1.84	0.81–0.87	1.98	1.9–2.10
Solubility in water (mg/L)	100	42	270–273	910
Vapor pressure (mm Hg)	1.99×10^{-4}	$1.0–4.0\times10^{-9}$	1.47×10^{-4}	5.67×10^{-4}
Henry's constant (atm-m^3/mole)	4.57×10^{-7}	1.2×10^{-5}	1.3×10^{-7}	9.26×10^{-8}
Molecular structure				

Data from Yinon and Zitrin (1993), Talmage *et al.* (1999) and HSDB (2000).

to accumulate metals to high concentrations in their tissues is well known (Salt *et al.*, 1998). Phytoremediation research has been conducted on organic pollutants ranging from pesticides, *e.g.* atrazine (Burken and Schnoor, 1997), to industrial pollutants such as trichloroethylene and polycyclic aromatic hydrocarbons (PAH) (Newman *et al.*, 1997; Paquin *et al.*, 2002; Vervaeke *et al.*, 2003). The transformation products of xenobiotics by plants are less toxic than parent compounds. In addition, root exudates are reported to enhance microbial activity for degradation of xenobiotics (Miya and Firestone, 2001; Anderson *et al.*, 1993). The cost for soil remediation is $10 to $100 per cubic meter whereas vegetative cleanup of contaminated soils costs only $0.02 to $1 per cubic meter (Cunningham *et al.* 1995).

This short review summarizes the toxicity of energetic materials (TNT, RDX, and DNTs) and pathways of transformation by plants.

Toxicity of the Explosives

TNT and its degradation products have been reported to be mutagenic and toxic to several organisms. Survival of the midge (*Chironomus tentans)* decreased significantly after exposure to 200 mg/kg of TNT, 1,3,5,-trinitrobenzene (TNB), and 2,4-diamino-6-nitrotoluene (2,4-DANT); and the amphipod (*Hyalella azteca*) was more susceptible to TNT, TNB, and 2,4-DANT than the midge (Steevens *et al.*, 2002). Gogal *et al.* (2002) reported that northern bobwhite quail showed decreases in total red blood-cell counts and plasma protein as dietary TNT intake increased, and they determined a low observed-adverse-effect level of 178 mg of TNT per kg of weight per day. Survival and growth

of two freshwater invertebrates were not affected after a 10-day exposure to 1,000 mg of RDX per kg of sediment (Steevens *et al.*, 2002). The growth and survival of benthic invertebrates, *Neanthes arenaceodentata* and *Leptocheirus plumulosus*, were not affected by exposure up to 1,000 µg RDX per kg dry weight of sediment (Lotufo *et al.*, 2001). Inhibition to growth and reproduction of adult earthworms can occur at less than 95 mg of RDX per kg of artificial soil (Robidoux *et al.*, 2000, 2001), but acute toxicity was not observed up to 756 mg per kg dry soil for RDX.

Gong *et al.* (2002) investigated the influence of RDX on indigenous microbial activities. They measured soil dehydrogenase activity, potential nitrification activity, heterotrophic nitrogen fixation activity, substrate-induced respiration, and basal respiration for 12 weeks. Significant reductions (up to 30% of control) in these parameters were observed in RDX-spiked soil. In the case of a luminescent marine bacterium (*Vibrio fischeri*), the EC_{50} value of RDX (116 mg/L) was above the solubility in water (42 mg/L for RDX) after incubation periods of 90 min (Drzyzga *et al.*, 1995).

Neither 2,4-DNT nor 2,6-DNT were mutagenic with the Ames assay, whereas their hydroxylamine isomers proved to be mutagenic (Padda *et al.*, 2003). Using the uptake response of H4IIE rat hepatoma cell cultures to neutral red, the NR_{50} values were 45 mg/L for 2,4-DNT, 50 mg/L for 2,6-DNT, and 7 mg/L for TNT, suggesting dinitrotoluenes are less cytotoxic than TNT (Mitchell and Burrows, 1995).

TNT was toxic to hybrid poplars at a concentration of 5 mg/L in hydroponic solution (Thompson *et al.*, 1998) and at 50 mg/kg soil there were adverse effects on germination and seedling growth of cress and turnip (Gong *et al.*, 1999). Alfalfa did not grow at 0.55 m*M* (100 mg/kg) 2,4-DNT in soil (Dutta *et al.*, 2003), and lettuce was more sensitive than wheat, mustard, and lentil, indicating that phytotoxic effects of nitroaromatic explosives depend on plant species (Picka and Friedl, 2004). The highest non-observed adverse effect concentrations (NOAEC) for the growth of lettuce were 20 mg/kg for TNT, 2 mg/kg for 2,4-DNT and 10 mg/kg for 2,6-DNT. Hydroponic toxicity of RDX to maize and wheat was estimated to be 21 mg/L RDX, while soybean and sorghum did not show a toxic effect up to 21 mg/L for 30-day exposures (Chen, 1993). RDX was not toxic to hybrid poplars up to 21 mg/L (Thompson, 1997).

Mechanisms of Degradation of Xenobiotics by Plants

Prior to the introduction of xenobiotics to plant cells, they must be taken up through the roots. Several studies reviewed predictive relationships between the uptake rate of a compound and its physical-chemical properties (Briggs *et al.*, 1982; Burken and Schnoor, 1998). Root uptake and translocation of the compounds are related to the logarithm of the octanol-water partition coefficient, log K_{ow}. Root concentration factor (RCF), defined as the the concentration sorbed to the roots divided by the concentration in the aqueous phase, is generally proportional to the log K_{ow} value. The relationship is proposed as follows:

$$\log (RCF–3.0) = 0.65 \log K_{ow} – 1.57 \text{ by Briggs } \textit{et al.} \text{ (1982)}$$
$$\log (RCF–0.82) = 0.77 \log K_{ow} – 1.52 \text{ by Burken and Schnoor (1998)}$$

The transpiration stream concentration factor (TSCF) is calculated as the concentration in the transpiration stream divided by the aqueous concentration. The values of TSCF for various chemicals show Gaussian distribution curves over the range of log K_{ow} values, indicating that hydrophilic compounds (log $K_{ow} < 1.8$) are not able to pass through lipid membranes of roots, whereas hydrophobic compounds (log $K_{ow} > 3.8$) tend to bind strongly to root tissues and are not then translocated to shoots (Dietz and Schnoor, 2001). The relationship between TSCFs and log K_{ow} is proposed as follows:

$$TSCF = 0.784 \exp\{-(\log K_{ow} - 1.78)^2/2.44\} \text{ by Briggs } \textit{et al.} \text{ (1982)}$$
$$TSCF = 0.756 \exp\{-(\log K_{ow} - 2.50)^2/2.58\} \text{ by Burken and Schnoor (1998)}$$

Enzymatic transformation of xenobiotics by plants follows the green-liver model and involves three steps. First, the foreign compounds taken up by plants are transformed by enzymes such as cytochrome P450, carboxylesterases, and peroxidase (Sandermann, 1994). Secondly, the transformed xenobiotics are conjugated with D-glucose, glutathione, or amino acids (Komoba *et al.*, 1995) by enzymes such as glutathione *S*-transferases, glucosyltransferases and malonyltransferases, resulting in either soluble or insoluble products. The third step is storage and compartmentation; the soluble compounds are stored in vacuoles or as cell-wall materials by further processing, and the insoluble compounds are generally assumed to be stored in the cell wall (Schroder and Collins, 2002).

Uptake of the Energetic Materials by Plants

Nitroaromatic explosives showed different uptake and fate in plant systems than nitramine explosives. According to Thompson *et al.* (1998), 95% of the TNT was removed from solution in less than 24 h by hybrid poplar, whereas 71% of the RDX was removed from hydroponic solution in 7 days (Thompson *et al.*, 1999). The uptake of both RDX and TNT from soil was slower than in the hydroponic systems because of decreased bioavailability in soil. Bush beans took up less than 16% of RDX in soil after 60 days; in contrast 60% was removed from solution after 7 days (Harvey *et al.*, 1991).

Over 60% of radioactivity of ^{14}C-RDX taken up by hybrid poplars was found in the leaves after 2 days. In contrast, 78% of radioactivity of ^{14}C-TNT taken up by the poplars remained in the roots after the same exposure time (Thompson *et al.*, 1998), suggesting that RDX is translocated more readily. In addition, an overall low recovery of RDX with no significant mineralization by plants suggested that the final transformation products are volatile compounds (Just and Schnoor, 2000). Recently, poplar nodule cultures were reported to mineralize RDX under sterile conditions (Van Aken *et al.*, 2004).

Regarding DNTs, knowledge of uptake by plants and transformation products is limited compared to information on TNT and RDX. Best *et al.* (2001) applied wetland systems to remove explosives from groundwater at ammunition plants, resulting in average removals of 58% and 61% for 2,4-DNT and 2,6-DNT, respectively, in a 115-day operation at the Volunteer Army Ammunition Plant, Chattanooga, TN. Todd and Lange (1996) observed that 67% of 2,4-DNT from soil was removed in a phytoremediation system using parrot feather (*Myriophyllum brasiliense*). They found 4-amino-2-nitrotoluene (4A2NT) in the

plant tissues after 90 h of treatment prior to 2-amino-4-nitrotoluene (2A4NT) which was detected after 190 h of exposure. However, other transformation products of the DNTs, as well as their fate in plants, are unknown.

Transformation Pathways

2,4,6-trinitrotoluene (TNT)

Subramanian and Shanks (2003) proposed the TNT transformation pathway by plants based on experiments with periwinkle (*Catharanthus roseus*) and parrot feather, as shown in Figure 1.

Two monoamino compounds [2-amino-4,6-dinitrotoluene (2ADNT) and 4-amino-2,6-dinitrotoluene(4ADNT)] have been found as the primary reduction products by plants (Palazzo and Leggett 1986; Thompson *et al*. 1998; Bhadra *et al*., 1999a). Diaminotoluenes (2,4-diamino-6-nitrotoluene and 4,6-diamino-2-nitrotoluene) and azoxy compounds were observed under strong reducing conditions and by the condensation of hydroxylamines, respectively (Pavlostathis *et al*., 1998; Sens *et al*., 1998; Thompson *et al*., 1998).

As for oxidative transformation of TNT in plant systems, Bhadra *et al*. (1999b) isolated six oxidized metabolites such as 2-amino-4,6-dinitorbenzoic acid, 2,4-dinitro-6-hydroxy-benzyl alcohol, 2-*N*-acetoxyamino-4,6-dinitrobenzaldehyde, 2,4-dinitro-6-hydroxytoluene, and two binuclear metabolites from azoxytetranitro toluenes. In addition, they showed that oxidation of TNT by the plant could occur before the reductive transformation. This was based on results where monoamino compounds were added to plants and the oxidized metabolites of TNT were not produced. To date, oxidized metabolites have only been found in parrot feather; they were not detected in *Catharanthus* or *Arabidopsis* (Subramanian, 2004).

2-hydroxylamino-4,6-dinitrotoluene (2HADNT) and 4- hydroxylamino-2,6-dinitrotoluene (4HADNT) were observed following reduction of nitro groups of TNT in non-axenic and aquatic plant systems (Pavlostathis *et al*., 1998; Wang *et al*., 2003). Measurement of hydroxylamines was difficult due to their instability. Wang and Hughes (1998) developed an efficient assay for hydroxylamines by derivatization with acetic anhydride. Recently, these hydroxylamines were shown to be present in axenic hairy roots of *Catharanthus* and axenic *Arabidopsis* seedlings (Subramanian, 2004; Subramanian *et al*., 2005). The hydroxylamines are considered the first transformation products resulting in other metabolites of TNT by reduction, oxidation, conjugation, and polymerization (Subramanian and Shanks, 2003; Wang *et al*., 2003).

The transformed products of TNT are further conjugated and sequestered in plant cells. Over 80% of the TNT label was associated with plant biomass, suggesting that the labeled carbon from TNT was sequestered in the plant tissues (Harvey *et al*., 1991). Thompson *et al*. (1998) showed that 75% of the radioactivity of ^{14}C-TNT was present in unextractable and bound residues in the poplar roots. Bhadra *et al*. (1999a) characterized the four conjugates of TNT metabolites with a 6-carbon moiety by *Catharanthus roseus* and *Myriophyllum aquaticum*. They found that two of them have molecular structures similar

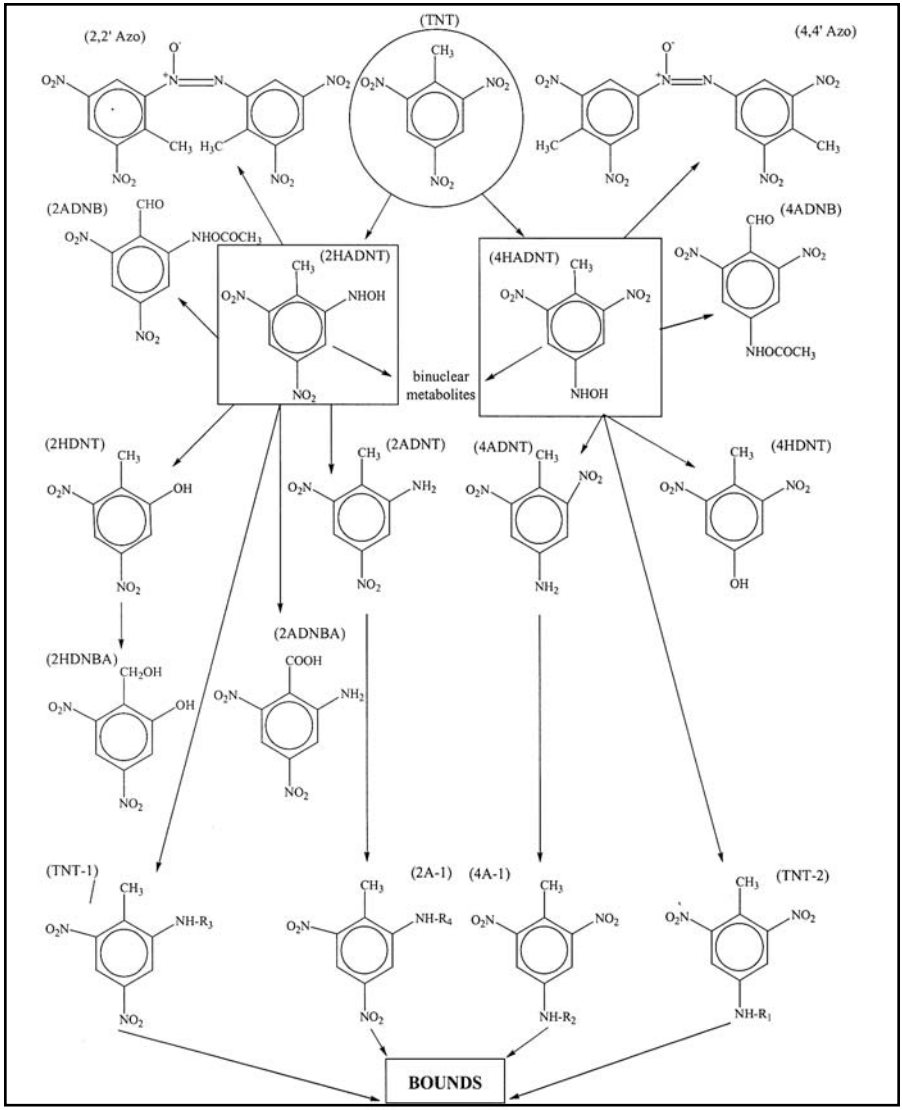


Figure 1. TNT transformation pathway by Subramanian and Shanks (2003). Abbreviations—TNT: 2,4,6-trinitrotoluene; 2ADNT: 2-amino-4,6-dinitrotoluene; 4ADNT: 4-amino-2,6-dinitrotoluene; 2HADNT:2-hydroxylamino-4,6-dinitrotoluene; 4HADNT: 4-hydroxylamino-2,6-dinitrotoluene; 4,4'Azo: 2,2',6,6'-tetranitro-4,4'-azoxytoluene; 2,2'Azo: 4,4',6,6'-tetranitro-2,2'-azoxytoluene; 2HDNT: 2-hydroxy-4,6-dinitrotoluene; 4HDNT: 4-hydroxy-2,6-dinitrotoluene; 2ADNB: 2-*N*-actamido-4,6-dinitrobenzaldehyde; 2ADNB: 4-*N*-actamido-2,6-dinitrobenzaldehyde; 2HDNBA: 2-hydroxy-4,6-dinitrobenzyl alcohol; and 2ADNBA: 2-amino-4,6-dinitrobenzoic acid. TNT-1, TNT-2, 2A-1 and 4A-1 represent conjugates with six carbon sugars (R1, R2, R3, and R4).

to that of 2ADNT (labeled TNT-1 and 2A-1) and the others were similar to 4ADNT (TNT-2 and 4A-2), indicating that the monoamines were precursors to the conjugates. Recent studies have elucidated these TNT conjugates. The conjugates of TNT metabolites by tobacco cell cultures are formed by conjugation of glucose on the hydroxylamine group of either 2HADNT or 4HADNT, and various diglycoside conjugates with gentiobioside or sophoroside forms were identified, including monoglycosides (Vila *et al.*, 2005). In precursor-feeding studies, Subramanian (2004) and Subramanian *et al.* (2005) found evidence for conjugation of monoamines and hydroxylamines with plant sugars.

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Studies on the transformation of RDX by plants are rare, whereas several microbial transformation pathways have been proposed and some established. After being taken up and translocated to leaf tissues, direct photolysis of RDX in the leaves is a feasible fate under natural sunlight. Just and Schnoor (2004) proposed the photodegradation pathway of RDX by reed canary grass, as shown in Figure 2. They identified ring-cleavage products, such as nitrous oxide (N_2O) and 4-nitro-2,4-diazabutanal in leaves under simulated sunlight, including nitrite (NO_2^-) and formaldehyde (CH_2O) in solution. Van Aken *et al.* (2004) proposed three processes for the pathway of degradation of RDX by using poplar tissue cultures and crude extracts from leaves, as shown in Figure 2. First, reduction products such as hexahydro-1-nitroso-1,3-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX) were produced by intact plant cells regardless of light. In the second step, the reduced metabolites were further transformed to formaldehyde and methanol, both in crude extracts and in intact cultures under light. In the final step, light-independent mineralization of one-carbon metabolites by intact plant cultures, but not crude extracts, occurred. Some transformed products may be re-incorporated into plant cells. Formaldehyde may be conjugated by plant enzymes to form compounds like *S*-formyl-glutathione (Just and Schnoor, 2004). Small quantities of CO_2 produced by degradation of RDX by plants may be re-assimilated by photosynthesis (Van Aken *et al.*, 2004).

Dinitrotoluenes (DNTs)

In contrast with bacterial systems, little information is available on the transformation of DNTs by plants. We are aware of only one study: monoamino isomers, 2A4NT and 4A2NT, were reductive transformation products in plants (Todd and Lange, 1996). The bacterial reduction of dinitrotoluenes can take place under aerobic and anaerobic conditions, resulting in the production of monoamines isomers (Hughes *et al.*, 1999). Hydroxylaminotoluenes and dihydroxylaminotoluenes were produced anaerobically in cell cultures of *Clostridium acetobutylicum* (Hughes *et al.*, 1999). Further transformed products, aminohydroxylaminotoluenes and diaminotoluenes, were observed in the cell extracts. *Hydrogenophaga palleronii* and *Burkholderia cepacia* produced oxidative intermediates and mineralized DNTs to CO_2 by mono- or dioxygenases (Nishino *et al.*, 1999). The bacteria converted 2,6-DNT into 3-methyl-4-nitrocatechol with release of nitrite, and then 2-hydroxy-5-nitro-6-oxohepta-2,4,-dienoic acid and 2-hydroxy-5-nitropenta-2,4,-dienoic acid (Nishino *et al.*, 2000).

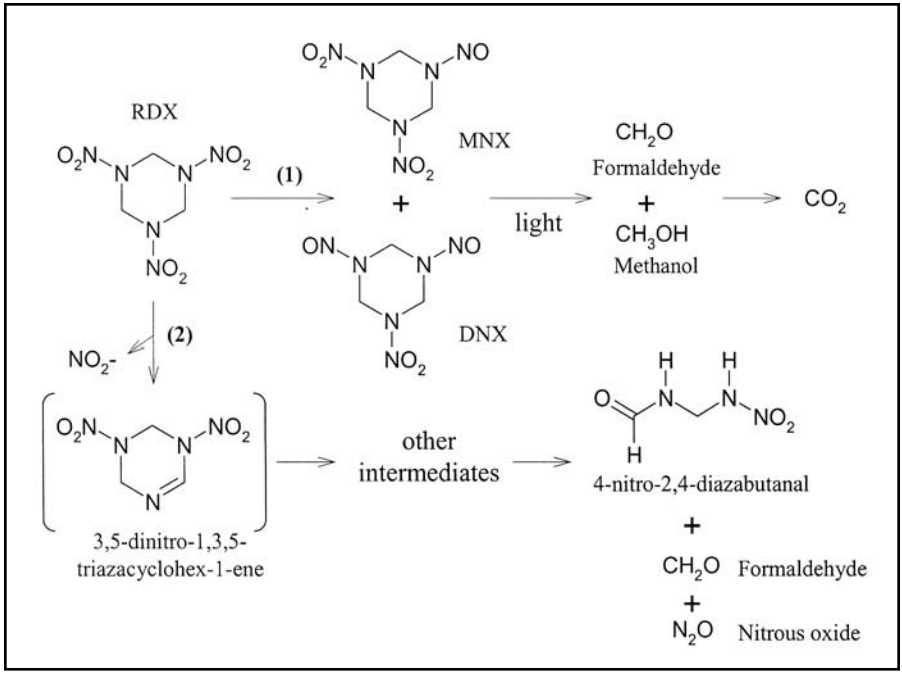


Figure 2. RDX degradation pathways proposed by Van Aken *et al.* (2004) and Just and Schnoor (2004). The bracketed compound was not observed. Abbreviations—RDX: hexahydro-1,3,5-trinitro-1,3,5-triazine; MNX: hexahydro-1-nitroso-1,3-dinitro-1,3,5-triazine; and DNX: hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine.

Transgenic tobacco plants expressing nitroreductases of Enterobacter cloacae *showed enhanced ability to tolerate and remove TNT at high concentration.*

Transgenic Plants and Gene Expression

In the past 6 years there has been significant activity in using genetic approaches to enhance transformation and to reduce phytotoxicity of energetic materials. Genetically modified plants expressing bacterial genes have been developed for phytoremediation. Transgenic tobacco plants expressing nitroreductases of *Enterobacter cloacae* showed enhanced ability to tolerate and remove TNT at high concentration (0.25 m*M*), which is toxic to wild-type tobacco (Hannink *et al.*, 2001). Another transgenic tobacco line expressing pentadrythritol tetranitrate reductase from the bacterium showed better germination and growth in the presence of TNT (0.05 m*M*) than did wild-type plants (Rosser *et al.*, 2001; French

et al., 1999). In addition, these researchers showed enhanced RDX removal in tobacco engineered with an XplA cytochrome P450 from *Rhodococcus rhodochrous* (unpublished results). Clearly, genetic modification with microbial redox enzymes has the potential to enable faster transformation of TNT and RDX and reduced phytotoxicity.

Transcriptomic studies are providing clues to endogenous plant genes involved in transformation. Specific genes such as those for glutathione-*S*-transferases and cytochrome P450 in *Arabidopsis* were proposed by Ekman *et al*. (2003) to be involved in transformation of explosives. They used serial analysis of gene expression (SAGE) to compare 14-day-old *Arabidopsis*, exposed to 15 mg/L of TNT after 24 h, to untreated plants. A glutathione-*S*-transferase was found to be induced up to 27-fold. Among the highly induced genes were those encoding cytochrome P450 (CYP81D11-A-TYPE), an ABC transporter that is known to expend ATP energy to transport hydrophobic molecules into or out of the cytoplasm, and a 12-oxophytodienoate reductase having high homology to nitroreductases of *Enterobacter* sp. (Ekman *et al*., 2003). However, as noted previously, oxidative compounds were not found in *Arabidopsis* (Subramanian, 2004), thus the role of P450s in transformation pathways in *Arabidopsis* is unclear. In microarray experiments, *Arabidopsis* gene expression was monitored after long-term exposure (10 days) to various concentrations of TNT (Mentewab *et al*., 2005). In response to TNT amendment, fifty-two genes were upregulated and forty-seven were downregulated, many of which have cell-defense and detoxification functions. Glutathione-S-transferases and cytochrome P450s were not found to be significantly upregulated in this study. Most of the genes differentially expressed were observed at the higher concentration of TNT amendment (10 μM) and genes expressed at 1 and 10 μM rarely overlapped. They confirmed the gene expressions of pathogenesis-related protein-1 precursor, DNA-binding protein, and ABC transporter-like protein by real-time PCR analysis.

The transcriptome studies provide clues to genes that may be involved in TNT transformation. Upregulation of some of the genes may be the result of a generalized stress response without synthesis of enzymes involved in the TNT phytotransformation pathway or in a reduced phytotoxicity response. Reverse-genetics approaches using the genes identified should enable further clarification of the transcriptome results. In a forward-genetics approach, ten activation-tagged *Arabidopsis* mutant lines showing significantly better germination rates than the wild type on the TNT-amended medium were isolated from 300,000 mutant seeds (Moon *et al*., 2004).

Selection of high-performing native plants, engineering plants with enhanced transformation capabilities, identifying the fate of transformation products in plants, and designing the external variables to operate a more effective phytoremediation process are all dependent on a knowledge base of the genetic structure, enzymatic structure, and biochemical reaction pathways. The genetic approaches discussed here will enable the design of effective strategies for remediation of energetic materials in the future.

Conclusions and Future Directions

Plants can remove contaminants from soil and groundwater, and transform them into less harmful compounds. Based on information on transformation pathways and gene

Research on the post-harvest fate of explosives is required.

expression, further studies on metabolic engineering and genetic modifications may make plants tolerant to higher concentrations of xenobiotics by inducing faster rates of uptake and using less toxic metabolic pathways. In addition, the explosives taken up by plants can be released by action of water—*e.g.* rain and river—and thus may be returned to the environment as hazardous contaminants. Research on further treatments and the post-harvest fate of explosives is required. Information about phytoremediation of dinitrotoluenes is lacking compared to that for TNT and RDX; thus, it also merits further investigation.

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Engineering Forest Trees with Heavy-Metal Resistance Genes for Phytoremediation

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Over the past century, mining, agriculture, manufacturing and urban activities have all contributed to extensive contamination of soil and water with heavy metals. In the United States, mercury is a common pollutant at government production sites, where it is used in energy- and defense-related activities. Thousands of square miles of land, rivers, lakes and estuaries are contaminated with millions of kilograms of mercury. Methylmercury, produced by bacteria in contaminated aquatic areas, is particularly toxic. Because it is quickly biomagnified in the food chain, it can have devastating effects on humans and other animals. Another common pollutant in the United States and worldwide is arsenic, a naturally occurring element widely distributed in the earth's crust. In the environment, arsenic combines with oxygen, chlorine, and sulfur to form inorganic compounds. These extremely toxic metalloids, classified as "group A" human carcinogens, cause skin lesions, lung, kidney and liver cancer, and damage to the nervous system. Conventional procedures for cleaning up heavy-metal-contaminated sites (*i.e.* excavation, dredging, electrolytic extraction, chemical leaching) are all prohibitively expensive and destructive of the natural environment. An alternative to these physical remediation approaches is the use of plants to remove pollutants from soil and water through their root systems, an approach known as phytoremediation. Once extracted, plants may sequester the pollutants in their tissues and/or convert them to less toxic forms. They can accomplish this at a fraction of the cost most physical/chemical methods and without disrupting the environment. Although some plants, known as hyperaccumulators, can take up and sequester normally toxic amounts of heavy-metal pollutants, most of these species accumulate little biomass, and thus are probably not suitable for rapid remediation of extensive areas. An alternative approach is to genetically engineer plants possessing faster growth and greater biomass-accumulation potential with genes allowing them to handle these pollutants. Forest trees, in particular, with their large biomass and penetrating root systems, make excellent candidates for engineering with phytoremediation genes. Such an approach is under development at the University of Georgia, where a number of plant species, including some forest trees, have been engineered with genes from bacteria that have been modified to function efficiently in plants. Our work indicates that forest trees can be engineered to thrive on and detoxify a variety of heavy metals on polluted sites.

Forest trees can be engineered to thrive on and detoxify a variety of heavy metals on polluted sites.

Engineering Trees for Mercury Phytoremediation

Mercury-resistant bacteria express MerA to convert highly toxic mercuric ion, Hg(II), to much less toxic elemental mercury, Hg(0). Following a demonstration that a modified *merA* gene conferred mercuric ion resistance to *Arabidopsis* plants (Rugh *et al.,* 1996), we used embryogenic culture (Merkle *et al.,* 1990) and gene-transfer systems (Wilde *et al.,* 1992) that we had previously developed for the fast-growing forest species yellow-poplar (*Liriodendron tulipifera*) to generate trees expressing a modified *merA* gene (Rugh *et al.,* 1998). Yellow-poplar proembryogenic masses (PEMs) were transformed with three modified *merA* constructs via microprojectile bombardment. Each construct was synthesized to have altered flanking regions with stepwise increases (0%, 9%, and 18% blocks) of modified coding sequence. All of the *merA* constructs that we tested conferred resistance to toxic ionic mercury that had been incorporated into the tissue-culture medium. Yellow-poplar somatic seedlings containing the most modified *merA* gene (*merA18*) germinated and grew vigorously in media containing a normally toxic level (50 μ*M*) of ionic mercury. A mercury volatilization assay indicated that the *merA18* plantlets released elemental mercury at approximately ten times the rate of untransformed control plantlets, indicating that they efficiently reduced mercuric ion to the elemental form.

While our work with yellow-poplar demonstrated the potential to engineer a forest tree with mercury-handling genes, we did not test these trees outside of *in vitro* conditions. Yellow-poplar is not adapted to the wet soils or riparian sites where mercury contamination is a major problem. For this reason, we proceeded to look for another tree species to engineer with mercury-handling genes. *Populus*, a forest-tree genus of the Salicaceae that includes aspens and cottonwoods, is easy to establish and grows quickly. Its high transpiration rate and wide-spreading root system make it ideal to intercept, absorb, degrade and/or detoxify contaminants, while reducing soil erosion (Dix *et al.,* 1997). Many *Populus* species, in particular the cottonwoods, are especially adapted for growth on riparian sites, making them a good choice for establishment on sites requiring remediation. In addition, *Populus* is amenable to *in-vitro* propagation and genetic engineering (*e.g.* Han *et al.,* 2000), making it a suitable target for enhanced phytoremediation ability via transgenic technology. Non-transgenic poplars had already been tested by some groups for phytoremediation applications (Licht, 1990; Newman *et al.,* 1997; Burken and Schnoor, 1997). We used *Agrobacterium*-mediated transformation of leaf explants to generate transgenic eastern cottonwood (*P. deltoides*) trees expressing *merA9* and *merA18* genes. Leaf sections from transgenic plantlets produced adventitious shoots in the presence of 50 μ*M* Hg(II), supplied as $HgCl_2$, which completely inhibited shoot induction from leaf explants of wild-type plantlets. Transgenic shoots cultured in medium containing 25 μ*M*

Transgenic cottonwood plantlets exposed to mercuric ion evolved two to four times the amount of Hg(0) relative to wild-type plantlets.

Hg(II) rooted and showed normal growth, whereas wild-type shoots were killed. When the transgenic cottonwood plantlets were exposed to mercuric ion, they evolved two to four times the amount of Hg(0) relative to wild-type plantlets. Transgenic *merA9* trees tested in a Georgia Piedmont soil contaminated with approximately 400 ppm Hg(II) showed growth indistinguishable from those in uncontaminated soil, while control plantlets were completely defoliated and dead within a week following potting (Che *et al.,* 2003).

Trees of one *merA* eastern cottonwood clone, along with wild-type control trees (approximately 200 trees total) were planted on a mercury-contaminated site that was formerly the location of a hat-making factory in Danbury, CT, in June 2003; *merA* and control trees all grew well. Leaves were sampled from *merA* and control trees in fall 2004, and analysis indicated that those from the *merA* trees contained one-third to one-half the amount of Hg as leaves from the control trees. This result was expected since the *merA* trees produce and volatilize Hg(0), which is then able to leave the plants as a vapor, whereas the control trees are unable to do so.

Methylmercury (MeHg), produced by native bacteria at mercury-contaminated wetland sites, is more toxic than elemental or ionic mercury. Because it is efficiently biomagnified up the food chain, it poses the most immediate threat to animal and human populations. Building on work performed in *Arabidopsis* (Bizily *et al.,* 1999), we produced eastern cottonwood shoots engineered with a bacterial *merB* gene, which converts MeHg to Hg(II). These shoots expressed the MerB protein and demonstrated their resistance to the methylmercury analog phenylmercuric acetate (PMA) by producing longer adventitious roots and higher fresh weights than control shoots cultured on rooting medium supplemented with 2 or 5 μ*M* PMA (Che *et al.*, submitted). However, in order to remove organic mercury from a contaminated site, it is desirable to have trees expressing both the *merA* and *merB* genes, so that organic mercury compounds can ultimately be converted to the least toxic form, elemental mercury. Results with *Arabidopsis* indicated that combining the *merA* gene with the *merB* gene in the same plant can increase the ability to grow on levels of organic mercury up to fifty-fold higher than wild-type plants and up to ten-fold higher than plants engineered with *merB* alone (Bizily *et al.,* 2000). This enhanced resistance to organic mercury is probably due to the fact that plants expressing the *merA* and *merB* genes together are able to transform both organic and ionic mercury to volatile Hg(0). Thus, the goal of our recent research has been to engineer both genes into eastern cottonwood. To accomplish this, we developed a system to re-transform *merA* cottonwood trees with the *merB* gene. Preliminary results comparing the *merA/B* trees to wild-type controls and trees engineered with either *merA* or *merB* alone indicate that these *merA/B* trees can efficiently convert PMA to Hg(0) (Lyyra *et al.*, in preparation).

We plan to engineer these same gene combinations into eastern cottonwood and other trees and test their potential for arsenic remediation.

Engineering Trees for Arsenic Phytoremediation

Using similar *in-vitro* culture and gene-transfer methods to those we employed with *merA* and *merB*, we engineered a bacterial γ-glutamyl synthetase (γECS) gene into eastern cottonwood. A month after being cultured on the medium supplemented with 800 μ*M* arsenate, leaf sections from γECS transgenic lines remained green and began to develop callus, while the leaf sections from wild-type plantlets showed no evidence of callus and became chlorotic. After 30 days on medium containing 800 μ*M* arsenate, wild-type adventitious shoots did not form roots and their leaves became chlorotic. The γECS shoots appeared similar to those maintained on medium with no arsenate, and adventitious roots began to appear at 21 days after initial culture. The difference between the γECS lines and the wild-type plants in their abilities to produce adventitious roots in medium with 800 μ*M* arsenate was statistically significant (Lima *et al.*, in preparation). Despite the apparent slight enhancement of arsenate resistance conferred to our eastern cottonwood trees by the γECS gene, work with transgenic γECS *Arabidopsis* plants indicated that they removed no more arsenate from the medium than did wild-type control plantlets (R.B. Meagher, unpublished data). Thus, not only does the mechanism of arsenate resistance for γECS plants remain unknown, but it is unlikely that engineering plants with this gene alone will be useful for removing arsenic from contaminated soil or water. Recent research in which the γECS gene was combined with other arsenic-handling genes, such as arsenate reductase, in transgenic *Arabidopsis* plants indicates that some of these multigene approaches have promise (Dhanker *et al.,* 2002). Thus, we plan to engineer these same gene combinations into eastern cottonwood and other trees and test their potential for arsenic remediation.

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Scott Merkle received his BS in biology from the College of William and Mary in 1976 and his MS and PhD in forestry from Virginia Tech in 1978 and 1982, respectively. His graduate training was in forest genetics and tree improvement and in predicting cone and seed yields in southern pine seed orchards using computer models. Dr. Merkle was a postdoctoral associate with Tom Adams at Oregon State University during 1983–1984; his research was on population genetics of Douglas fir using isozyme markers. In 1984, he began postdoctoral work with Claud Brown and Harry Sommer at the Daniel B. Warnell School of Forest Resources at the University of Georgia, focusing on tissue culture and protoplast culture of hardwood forest trees. He joined the University of Georgia faculty in 1987 and is currently a professor in the Warnell School.

His lab has developed embryogenic regeneration systems for over a dozen forest tree species and hybrids, including American chestnut, yellow-poplar, black locust, sweetgum, magnolia and longleaf pine. Merkle has employed these cultures in research involving mass clonal propagation, biomass energy, genetic engineering, artificial seeds and cryopreservation. He has collaborated with Richard Meagher of the UGA Genetics Department to engineer forest trees with heavy-metal-resistance genes for use in phytoremediation.

Panel Discussion and Q&A

Moderator:
William Park
University of Tennessee
Knoxville, TN

Panelists:
Lena Ma
University of Florida

Neal Stewart
University of Tennessee

Steve Rock
Environmental Protection Agency

Lena Ma: Environmental phytotechnologies are being applied to various types of contaminants: organic contaminants, energetic material (mentioned by Jacqueline Shanks), and metals including nickel, cadmium, lead, selenium, arsenic and mercury. Although the last three are not really metals, we still refer to them as such. Phytoremediation of all three types of contaminants is mostly in the demonstration stage; we don't have full-scale applications yet. There are many aspects to environmental phytotechnologies, of which we covered some this morning. One is phytoextraction, which Bruce Ferguson mentioned, and I guess everybody knows about the arsenic hyperaccumulator, the Chinese brake fern (*Pteris vittata*). Over 400 hyperaccumulators have been reported. In addition to phytoextraction, Bruce mentioned phytofiltration, for extraction of contaminants from water. Then there is phytostabilization, which Scott Merkle touched on—the use of poplar trees to control contaminants. Phytovolatilization, also mentioned by Scott, may be applied to remediation of mercury and selenium. One of the issues that we are faced with when we use phytoremediation is how to deal with biomass. You can't really recycle arsenic, but you can recycle valuable metals such as nickel. Furthermore, a phytomining demonstration project is in progress in Canada, using plants to actually mine nickel.

I want to borrow Mr. Rodemeyer's term "speed bumps." Phytoremediation is a relatively new technology and it is not yet well accepted. One "speed bump" is that phytoremediation is a slow process. Using plants, you cannot remediate a site overnight or in a few months; usually you are talking about 5 to 10 years. We need research to speed up the process. Similarly, to demonstrate the efficacy of the technology will take time. And the longer it

takes, the more money it costs. You have to show efficacy through many years and that's not an easy job. And the last thing is biomass. In theory you can use the biomass generated from phytoremediation projects as fuel or to recycle metal. Of course, if it contains arsenic or lead it is less valuable. This area of research needs more attention.

Neal Stewart: I'd like to mention three things. One deals with phytosensing and the second is ecorestoration—a couple of things that we've touched on in this session, but not really delved into—and I'd like to just fill in a few blanks. And then I'll talk a little bit about regulation.

Phytosensing is actually a word that I made up. The idea is that phytosensors are genetically engineered to operate like a check-engine light. We might not know exactly what's there or how much, but we know that something is wrong. That's the reason to pursue phytosensing as a technology. One of the applications is to couple phytosensors with phytoremediation. To have the plant tell you where arsenic is or where TNT is and then—if you have a sensor that is appropriately designed—as it removes the contaminant from the soil, the signal would diminish. A lights-off to lights-on phytosensor would have an inducible promoter fused to GFP or a chromo-protein or another fluorescent protein or bioluminescence; when you don't have the contaminant the lights are off and when you do have a contaminant, the lights are on. RNA interference, going from a lights-on to lights-off situation, will probably work better in the field and we are looking at that now.

Another application for phytosensors is in precision agriculture, which Bruce Ferguson touched on. You can use the sensor to give you information about what's going on in a farmer's field, coupled with GPS. In 10 to 20 years, I think this will be a reality.

A third application is agro-security: plants in the field that can report in real time where there is a disease outbreak, either due to natural infection or an intentional release.

And then the fourth one, which is what Jacqueline Shanks talked about, applies to explosives. She talked mainly about phytoremediation. We are interested in doing in making phytosensors that detect explosives, mainly as a means of landmine sensing—using genetically engineered plants that indicate where landmines are situated, where the lights will go on or will go off, or with a color change in the plant. There are a hundred million landmines in the world and there is no good detection technology, so I think that this humanitarian application is certainly worth pursuing.

I'm glad that Scott Merkle mentioned ecorestoration. Let's say that we could introduce a genetically modified chestnut into the field for chestnut-blight resistance. The current regulatory paradigm is that gene flow from a GM crop to a wild relative, or from a GM crop to its non-transgenic counterpart, is bad. However, this approach would turn that whole notion upside down; we would want and expect gene flow. On the other hand this may be viewed as ecological disruption. Scott mentioned that one in four trees in Appalachia was a chestnut, which grew to large size. With the return of the chestnut, some species will be displaced, because their niche will be retaken by the rightful "king." This will be something to think about.

A lot of people have talked about rationalization of regulations. I think that a more

descriptive term is "reformation." We need to totally rebuild regulations in light of what we know. We have learned a lot in the past 10 to 20 years. For the near term, we have to protect the markets for farmers. That's certainly key. We have to think about using non-food plants as the only way forward in the near term. We have to make sure that these plants have obvious and compelling environmental benefits, and also realize that there are costs for not adopting a beneficial technology. If we don't adopt phytoremediation, for example, and continue to dig up soil and send it to Utah or Idaho—or wherever it goes—that has an environmental cost and is ecologically damaging. For the economists here, we have to think about opportunity costs when we talk about adopting or not adopting.

Steve Rock: I'm not sure everybody noticed, but if you took away the phytoremediation part of the three speakers' presentations you would have seen three different case studies of how to get genetically modified plants into the field. Three different field demonstrations and the process of negotiating the regulatory hurdles and testing them out in the wild where they are loose and able to walk around and shoot spines at people, if that's what they are going to do. I was privileged to work on a couple of those projects, especially the Danbury one. Jack Kozuchowski, the city environmental officer wanted to do something with this piece of land, but no remediation technologies were available. Jack and I talked a lot about toolboxes. Coincidentally, I have an uncle who is Jack and my Uncle Jack's toolbox was an amazing thing for me growing up. He worked at Edwards Air Force Base at the time when they were building experimental airplanes. People would hand him a set of blueprints and tell him to build it. Before he could build the engine, he had to build the tools for it and he had wrenches and screwdrivers that were made for a particular bolt. One of the things he taught me was that when the only tool in your toolbox is a hammer, everything looks like a nail. It's critical that you understand what you need to accomplish and then build the tools and take the project into the world like Bruce Ferguson, Jacqueline Shanks and Scott Merkle and their groups have done.

I sat in on the meetings with the people in Connecticut and the Region 1 regulators were very concerned about taking genetically modified cottonwood trees and planting them in a public area. One by one their objections were raised and were answered, and in a fairly short amount of time we got the plants into the ground. I don't think we lost any growing season to that particular hurdle. There were plenty of other hurdles on that project. But enough interest was raised that we got 3 years of funding to keep looking at it, to check what is transpiring from the plants and to dig up the soil afterwards and analyze effectiveness. So, that is an interesting response to what was said yesterday about how hard it is to get field trials done. You all have done it quite nicely. I would also suggest that people in this group look at the ITRC model. The Interstate Technology Regulatory Council is a group of state regulatory bodies, approximately forty at this point, that have banded together so they don't have to each approve the use of a technology. When they looked at the Edenspace lead-uptake project, for example, and other projects, they were able to say that if this works in New Jersey we don't have to do it again in California and Idaho and everywhere else across the country. It saves a tremendous amount of time from the company standpoint and also from the regulatory side.

For those who are wondering what happened to your European colleagues when the market moved against them for genetically modified food, they have moved into phytoremediation. I just came back from a European Union meeting where a very interesting set of people, Cooperation in the Field of Scientific and Technical (COST) Research. A tremendous number of papers have been written in this group—greenhouse and lab-scale stuff at this point. They haven't figured out how to get outside with it, but a lot of people are working on how to get better accumulators, producing genetic maps of accumulators so we can transfer elite characteristics to poplars and cottonwoods. No pun intended, it's a great growth market that is much, much less scary to the public, and hence to the regulatory bodies, than genetically modified foods.

Scott Merkle: I'm glad Neal brought up the complexity of what is going to happen with chestnut, because discussions are going on now with the American Chestnut Foundation and other groups. Do we really want these trees to be put out to pollinate their wild relatives? It seems like a no-brainer; we want the resistance to spread in the natural population. At the same time, this is not just a forest tree. It's a nut-crop tree and will again be a nut crop tree and eaten by consumers all over the country. Therefore, it's a complex issue and it won't be dull when we get to the point of deciding what we're going to do with transgenic chestnuts.

Bruce Ferguson: In response to Steve's point about funding in Europe for phytoremediation, there has been support at a low level in the United States for very ambitious research on transgenics including phytoremediation. This reflects a fairly low emphasis on the environment in general over the last several years. It's something we need to keep an eye on if we are going to maintain our research lead in this area.

Henry Miller (Hoover Institution, Stanford, CA): We heard some really stunning science today in various stages of development, but it's important to keep in mind the public policy, particularly regulatory obstacles, that many of these products will confront. For example, the chestnut-blight-resistant plants are more stringently regulated by EPA than organophosphate pesticides would be because chemical pesticides are subject to a 10-acre research exemption whereas recombinant plants of any sort are subject to a zero-acre research exemption. So, these products are potentially going to have to be registered as pesticides—not trivial and not particularly helpful, I think.

Stewart: I would like to bring up one point—the back-crossing project that Scott Merkle alluded to. They've crossed American chestnut with Japanese chestnut (*Castanea crenata*), which is a small tree grown a lot as an ornamental. It's not like American chestnut. Although in the back-crossing you remove Japanese-chestnut genes, many will remain in the America chestnut background. I don't see the Japanese chestnut as a pest, but the greatest ecological damage in my opinion, in the United States and worldwide, has resulted from introducing exotics. Another interesting example is insect-resistant trees. In the Appalachians we have a tremendous problem with hemlock woolly adelgid, so we are

introducing a biological control agent. That might seem to be innocuous, but it would make a lot more sense to put a single gene in for resistance. Yesterday, Henry Miller said context, context, context.

Miller: I know, but we're not talking about sense. We're talking about government regulation. Again we go back to the ice-minus *Pseudomonas* where a deletion unit of an innocuous microorganism was considered to be a pesticide.

Stewart: We agree that we need reformation.

Miller: Right, and I agree—but you have to remember there's no incentive for government regulators to alter what they do, particularly if it involves a decrease in their responsibilities and their budgets and their empires. The USDA and EPA—look at their Web-sites—have created enormous empires for regulating products superior to those that they used to regulate with much, much smaller bureaucracies.

Stewart: So, you are saying that it's not exactly a bump in the road.

Miller: Exactly.

Merkle: Henry, thanks for your comments. We had a meeting 2 weeks ago, including two representatives from APHIS and I was really very taken with their positive attitude about wanting to be helpful with the chestnut project. I'm sure we'll find, people at the EPA who will want to work with us on this. I think it will be an interesting situation and I hope we will find a way to work together on it. The APHIS people really impressed me.

Robert Wager (Malaspina University, Naimano, BC): In British Columbia, we have a huge problem with the mountain pine beetle—millions and millions of hectares—and we see nothing natural that's going to knock it down. Is anybody aware of transgenic research to deal with that issue specifically?

William Park: How about Maud? Maud do you know anything about this?

Maud Hinchee (ArborGen, Summerville, SC): I think people in BC are afraid of transgenics and are holding back research in that area.

Daniel McDonald (Phenotype Screening Corporation, Knoxville, TN): I am interested in the biosensor approach. We are developing artificial soil as growth media and we find with *Arabidopsis* that almost any stress turns it purple. So we use that as an indication that our watering is off, or nutrients or lighting or temperature, and it's been very effective. The goal is to keep them green. How specific can you get that? When it turns purple in your case, is it due to a particular stressor versus just broadly stressing the plant?

Ferguson: Our initial results have not been very specific. We usually grow wild-type plants with the transformed plants in the same conditions so that we can see if in fact there is a differential response, which is the way we calibrate right now. Eventually, tissue specificity will be very important—to show a change say in the stems rather than the leaves or produce a variegated type of pattern to indicate a difference from the normal stress response.

Stewart: We work a lot with engineers and protonics people who have drummed into our heads that when we take something into the field, it must have a unique spectral signature. That's going to be the key and it's one reason why we've looked at adding spectral properties to plants that are not there naturally.

Kaye Knowles (Fort Valley State University, Fort Valley, GA): I am a student and I have a question concerning phytoremediation. How do you dispose of the plants that absorb these metals? Take, for example, the fern. What happens after it absorbs arsenic?

Ferguson: We have looked at various approaches. In the United States, the most common is to put the plants in the same landfill as for the untreated soil. The advantage is that a lot less mass is transferred because the plants typically concentrate the contaminants. The disadvantage is you are still moving contamination from point A to point B and eventually the people living near point B will object. The more elegant solution is to process the biomass to extract the contaminant for reuse. That's feasible with lead, but less so with arsenic, which is in less demand in industry but is still used in gallium arsenide chips and to some extent in pesticides. That's the elegant solution. The Japanese are working with us in that area, but it's more expensive so most of our US customers are less interested. It's almost a variation of phytomining—recovering a valuable resource. It's still not easy to do because the metals are often sequestered in the cell walls. The reason we got into the energy side with cellulases was to seek ways of breaking down biomass to extract the metals more easily. We hoped to sell some feedstocks for energy on the side and help pay for the cleanup process, and then realized that there is a $100+ billion market for energy and there is a $10 billion dollar market for cleanup, which is fragmented. So we flipped it around to look at the energy side first. So this is an active area of research. Customers prefer cheap solutions—no surprise—but there are some good long-term paths.

Ma: One of the disadvantages or "speed bumps" for phytoremediation is how to deal with the biomass. Unless we come up with some more cost-effective solution, phytoremediation may require a few more years of research.

Stewart: A question for Scott. With respect to remediation with *merA* and *merB*, the mercury will be blown off into the air. Is that a good thing? Do the regulators like that? It's not something you'd want to have in your office, no doubt; what's the latest skinny on that?

Merkle: Almost every time I give a talk on *merA*, that question comes up. The mercury cycle is similar to the water cycle. There is a global atmospheric pool of mercury vapor, comprising many tons. All bacteria in soil and water containing mercury continuously generate mercury vapor. You might not want to place your grove of *merA* cottonwood trees next to a kindergarten, but at most sites what these trees would produce would be a drop in the bucket compared to what is already being volatilized globally.

Rock: We are measuring the volatilization at the Danbury plant and we can't find it. It's not above background—not measurable.

Tingting Chen (Tennessee State University, Nashville, TN): We have heard a lot of about the technologies and that they are almost ready or already implemented in food or environmental aspects. About the regulations, can we implement a cost-benefit analysis approach? Let's say the chestnut has been wiped out for the last 100 years and the only way to restore it is with genetically engineered plants. Furthermore, insect-resistant plants can now be planted. In China, for example, they are significantly reducing insecticide application. When I was 13 or 14 years old in China, I applied pesticide with no protection at all. In that regard, the benefits from transgenic plants will be very, very significant in terms of reducing pesticide use. With respect to environmental remediation, the current technology is to haul contaminated soils from Connecticut, let's say, to Utah or North Dakota for burial and storage. If you use transgenic plants there might be minor negative aspects, but we can tremendously increase the good aspects. Why are a couple of grains of transgenic corn in tons of non-transgenic corn considered hazardous to human beings? Implementing a cost-benefit approach would help in designing regulations for transgenics.

Park: Thank you very much.

The Application of Biotechnology to Sustainable Forestry

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Author Henry David Thoreau, well known for his romance with the woods near Walden Pond, had great appreciation for the beauty and majesty of forests. But he also was realistic about the practical value of trees: "They warmed me twice—once while I was splitting them, and again when they were on the fire."

Forestry products are the third most valuable commodity after oil and gas.

The challenge of foresters today is to maintain the natural characteristics of forests while meeting society's need for products produced from trees. Forestry products are the third most valuable commodity after oil and gas. Trees supply the bulk of fiber for pulp, paper, packaging and building needs. Some 5,000 products are made from trees. Three billion people depend on wood for fuel. So we must harvest wood. But forests also are an essential component of our ecology. They provide wildlife habitats. They help control erosion. They purify water. They sustain the world's environment by emitting oxygen and sequestering carbon dioxide. And their beauty is unquestioned.

It is, therefore, essential that our forests are managed sustainably for ourselves and future generations. During the twentieth century, wood consumption tripled around the world and continues to grow. The most practical way of preventing this increased demand from further impacting our natural forests is to increase productivity of managed tree plantations.

Silviculture is the agriculture of trees—how to grow them, how to maximize growth and return, and how to manipulate species composition to meet specific objectives. Silvicultural research increased loblolly pine plantation productivity from an average of 10

to 20 tons/acre 40 years ago to 90 tons/acre today on the most productive sites. Advancements in site preparation and selective tree breeding have been the primary contributors to this significant increase. Biotechnology will be another important tool in the sustainable silviculture "tool kit" for stepwise improvements in productivity per acre.

As biotechnology helps to conserve natural forests, it also will give new roles to trees, such as pollution cleanup and restoration of threatened species. It has long been said that people who fail to see the big picture "can't see the forest for the trees." In this case, it is important that we not lose sight of trees' potential by focusing solely on the forest. By improving plantation trees, we can help sustain forests.

How Biotechnology Can Reduce the Impact on Natural Forests

Today, managed tree plantations provide only about a third of the world's need for wood and wood products. The remainder comes from other sources, including natural forests. Clearly, if tree plantations produce more, less will be needed from natural forests.

Genetic research, including biotechnology, holds promise to produce faster-growing trees and to increase the cellulose content of individual trees.

For example, loblolly pine—the major pulp species in the southeast United States—has a rotation of about 25 years. A 5-year reduction in time to harvest would have a tremendous impact over time on total cellulose production per acre. Genetic research, including biotechnology, holds promise to produce faster-growing trees and to increase the cellulose content of individual trees. Eucalyptus, another major source of pulp for paper manufacture, has been manipulated to grow faster through advancements in tree biotechnology.

In the Pacific Northwest, by crossing the eastern cottonwood with the region's indigenous black cottonwood, University of Washington scientists have attained yields five to ten times greater than from trees in the wild. Oregon State University has produced poplar trees capable of reaching 60 feet in height in 6 years.

Biotechnology can also reduce threats to tree health. Research is showing promise in the introduction of traits that confer resistance to pests and pathogens that weaken or kill trees. Improvements through tree biotechnology may also improve weed control, enabling young trees to get a head start over nutrient-robbing competitors. Trees with these traits will improve the competitiveness of the United States forestry industry in international markets for forest products and will improve productivity of lands intended for pulp production.

Other Potential Benefits of Biotechnology

Tree biotechnology promises benefits beyond increased productivity, including:

- Restoration and preservation of heritage trees. Research is underway at several institutions, including the State University of New York, the University of Georgia and the University of Tennessee to develop disease-resistant varieties of important and desirable tree species that are threatened with extinction due to blight. Biotechnology provides the best hope to save and restore species such as American chestnut, American elm, flowering dogwood and various oak species, which have been so important to our culture and the beauty of our cities and woodlands.
- Cleanup of toxic waste and Superfund sites. It may be possible through biotechnology to develop trees capable of absorbing specific toxins from the soil. This has the potential to reduce by millions of dollars the amount of money spent on cleaning up toxic sites. The University of Georgia is among several institutions conducting research with trees for phytoremediation.
- Improvement of water quality. Just as trees can be engineered to absorb toxic metals, they also can be modified to absorb excess nitrogen, which contributes to water pollution and algal blooms in waterways. Rutgers University is pioneering research in this area.
- Biofuels. The US Department of Energy is researching the potential for trees to provide clean, sustainable fuels. One possibility is to convert the cellulose in wood to ethanol as transportation fuel. Biotechnology can play a vital role in producing wood better suited for the production of ethanol, which can reduce our reliance on foreign oil.
- Decreased lignin content for pulping. Biotechnology can reduce the amount of lignin in trees intended for paper manufacture. Lignin—which gives wood its strength—must be removed in the pulping process. Trees that have less lignin or more-extractable lignin are more readily pulped, allowing mills to reduce the chemicals and energy required to purify cellulose (the basis for paper, packaging and many absorbent products) from wood. Thus, pulp mills are expected to better achieve their ambitious environmental objectives while reducing inputs and costs.
- Better lumber. Biotechnology may also produce straighter trees with fewer limbs, resulting in increased production of better-quality lumber.

Continued research in biotechnology may address and solve other issues, such as why some woods resist rot and others do not and why some species are susceptible to insects and others are not. Through this continually expanding knowledge will come advancements that will maximize the value and efficiency of trees.

Techniques Used

While people may think of biotechnology as involving the transfer of genetic material from one species into another, in fact transgenic research is only one of the multiple methods involved. Current applications of tree biotechnology include techniques that identify genes or alleles within a species that contribute important traits. By identifying these genes, researchers can select and breed better genotypes. This work currently is directed

towards improving the growth, health and quality of trees grown in plantations. Methods employed include molecular marker-assisted breeding and the selection and asexual propagation of elite trees. These research areas have been applied relatively recently to the genetic improvement of plantation forestry species, with the most advanced applications practiced in eucalyptus plantations in Latin America and Australasia, and in plantations of Monterey pine in New Zealand, Australia, and Latin America. Further research seeks to apply the technology to additional plantation species such as loblolly pine, spruce and poplar species and hybrids.

ArborGen is focused on faster growth and altered lignin content.

Technologies to improve loblolly pine will rely on the development and commercial application of cost-effective mass propagation techniques (such as ArborGen's ArborGenabled® process) for specially selected elite genotypes. Once these genotypes have been identified, they will be the foundation for the introduction of value-added traits through gene-insertion technology. These genotypes, in addition to being the best of their species, will impart the various benefits discussed above, such as wood-quality improvements, disease and stress tolerance, and bioenergy and bioproduct applications. ArborGen currently is focused on faster growth and altered lignin content.

Field Testing and Deregulation

Several institutions, including ArborGen, have made significant progress in introducing and testing genes that improve wood-volume gains as well as in reducing lignin content. These trees are currently in multiple field tests to determine trait expression and to ensure overall tree performance in plantation conditions. ArborGen has multiple field sites for testing trees in geographies and environments in which industrial forestry is practiced for these species. Some of the trees currently under evaluation will be selected for further product development and future commercial sale.

Commercialization will require that genetically engineered trees go through the regulatory process that has a proven track record for agronomic crops, such as soybean, corn and cotton. The regulatory framework has been successful in its current risk-assessment approach in regulating field tests and commercial deployment. The system under which APHIS has regulated biotechnology since 1987 is effective and protective, as evidenced by the fact that more than 10,000 field trials have been done and more than sixty biotech products have been commercialized without adverse effects on human or environmental health.

This science-based approach allows assessment of risk on a case-by-case basis for a particular trait in a particular crop of interest. This approach is equally applicable for many of the new products under development, including plantation trees. The significant knowledge base that already exists for plantation species must be considered in the regulatory process. The academic community will play a critical role in the trial and testing phases of product development.

The regulatory process should be similar to the coordinated framework currently used for agronomic products, which operates on a case-by-case, trait-by-species basis.

Summary

In summary, tree biotechnology will have many environmental and societal benefits. Faster-growing trees, developed through biotechnology, will contribute significantly to sustainable silviculture by diminishing the demand for wood harvested from old growth and natural forest stands. Many other benefits are possible, including restoration of heritage tree species, such as American chestnut and American elm; cleanup of toxic wastes; nitrogen absorption; biofuels; and lignin modification to improve the production of paper. A robust, science-based regulatory system is essential to bring these improvements to market. The regulatory process should be similar to the coordinated framework currently used for agronomic products, which operates on a case-by-case, trait-by-species basis. Biotechnology will help the forestry industry advance its goals of providing wood products for society while protecting the natural forests that provide beauty and essential ecological benefits.

Maud Hinchee has been Chief Technical Officer for ArborGen LLC since its inception in 2000. ArborGen, a forestry biotechnology company, conducts research and develops products to improve the value, productivity, and sustainability of plantation forests.

Prior to joining ArborGen, Dr. Hinchee was instrumental in the development of transformation technologies for soybean, sugarbeet, potato, and strawberry at Monsanto, and led the introduction of herbicide tolerance, insect tolerance and disease resistance traits into these crops. At Monsanto she also successfully led a business unit aimed at applying biotech traits to specialty crops—alfalfa, sugarcane, forestry species—through collaborative partnerships.

Hinchee is an inventor on five patent applications, and author of over twenty scientific publications. She has organized annual meetings and symposia for the International Union of Forest Research Organizations and the Society of *In Vitro* Biology, has served for four years on the board of the Institute for Forest Biotechnology, and has also been a board member for the Council for Agricultural Science and Technology.

She received her PhD in Plant Morphogenesis from the University of California, Davis, and holds an MS in Botany from the University of Washington, Seattle, and a BS in Botany from Davis.

Understanding Gene Function and Control in Lignin Formation In Wood

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Tremendous effort has been devoted to developing genetically engineered trees, with the emphasis on reducing lignin quantity to improve woodpulp-production efficiency.

Tremendous effort has been devoted to developing genetically engineered trees, with the emphasis on reducing lignin quantity to improve woodpulp-production efficiency. However, lignin chemical reactivity also is a critical barrier to woodpulp production, as lignin removal from wood is either initiated by chemical degradations or—as in most cases—accomplished entirely through chemical reactions. Thus, the current tree-biotechnology emphasis on low lignin quantity must be expanded to include greater lignin reactivity and, ultimately, a combination of low and reactive lignin traits.

Lignin reactivity depends on the frequency of its structural units, guaiacyl (G) and syringyl (S) monolignols. More syringyl monolignol units, or high S/G lignin monomer ratios, are known to induce high lignin reactivity. For more than 50 years, it has been thought that syringyl monolignol biosynthesis in angiosperms occurs via conversion of caffeate to sinapate via ferulate and 5-hydroxyferulate (Figure 1) (Grisebach, 1981; Grand, 1984; Higuchi, 1985). Based on high-performance liquid chromatography/mass spectrometry (HPLC/MS) characterization of products from reactions of microsomal proteins from lignifying stem xylem of sweetgum (*Liquidambar styraciflua*) with a mixture of four potential 5-hydroxylation substrates—ferulate, feruloyl-CoA, coniferaldehyde and coniferyl alcohol—Osakabe *et al.* (1999) discovered that 5-hydroxyferulate was not synthesized. Instead, the exclusive product from this mixed substrate reaction was 5-hydroxyconiferaldehyde, demonstrating for the first time that a coniferaldehyde 5-hydroxylase (CAld5H) is involved in monolignol biosynthesis, and that ferulate 5-hydroxylase (F5H) may not be (Osakabe *et al.*, 1999).

Subsequently, *CAld5H* cDNAs were cloned from aspen and sweetgum. When coniferaldehyde was incubated with a mixture of CAld5H-containing yeast P450 and *E. coli*-expressed caffeate *O*-methyltransferase (COMT), it was converted to sinapaldehyde via 5-hydroxyconiferaldehyde (Osakabe *et al.*, 1999). Thus, CAld5H catalyzes 5-hydroxylation of coniferaldehyde into 5-hydroxyconiferaldehyde, which in turn is methylated by COMT to sinapaldehyde, supporting the idea of a hydroxylation/methylation flux *in vivo* from guaiacyl to syringyl monolignol biosynthesis via coniferaldehyde (Figure 1). Based on HPLC/MS characterization of the kinetic properties of purified recombinant aspen COMT, Li *et al.* (2000) demonstrated that, indeed, COMT is a 5-hydroxyconiferaldehyde *O*-methyltransferase (AldOMT) that catalyzes methylation of 5-hydroxyconiferaldehyde (K_m = 2.6 μ*M*) with some affinity for caffeate (K_m = 75.1 μ*M*) and 5-hydroxyferulate (K_m = 15.0 μ*M*). However, when a mixture of 5-methylation substrates—caffeate, 5-hydroxyferulate and 5-hydroxyconiferaldehyde—was incubated with recombinant COMT (now designated as AldOMT) or soluble proteins from stem xylem, a complete inhibition of caffeate and 5-hydroxyferulate methylation was observed, while the conversion of 5-hydroxyconiferaldehyde into sinapaldehyde (Figure 1) was conserved (Li *et al.*, 2000). Enzyme inhibition kinetics further showed that 5-hydroxyconiferaldehyde is a competitive inhibitor of AldOMT-catalyzed methylation of both 5-hydroxyferulate and caffeate with K_i values of 0.26 and 2.1 μ*M*, respectively, but 5-hydroxyferulate and caffeate are not effective inhibitors of 5-hydroxyconiferaldehyde methylation (Li *et al.*, 2000). Thus, the presence of CAld5H/AldOMT-mediated coniferaldehyde 5-hydroxylation/methylation eliminates the pathway from caffeate to sinapate via ferulate and 5-hydroxyferulate, and CAld5H/AldOMT diverts the guaiacyl pathway from coniferaldehyde to sinapaldehyde via 5-hydroxyconiferaldehyde to initiate syringyl monolignol biosynthesis (Figure 1).

CAD and SAD

The CAld5H/AldOMT pathway together with the long-thought coniferyl alcohol dehydrogenase (CAD) function with sinapaldehyde was once believed to lead to the biosynthesis of syringyl monolignol. However, HPLC/MS-based enzyme functional analyses of aspen xylem protein and *E. coli*-expressed recombinant aspen CAD protein demonstrated that CAD is in fact coniferaldehyde- or guaiacyl-specific (Li *et al.*, 2001). This strongly suggests that a discrete sinapyl alcohol dehydrogenase (SAD) is needed for metabolizing the CAld5H/AldOMT product, sinapaldehyde, into sinapyl alcohol, the syringyl monolignol. This discovery led to the isolation of an SAD cDNA from aspen developing xylem (Li *et al.*, 2001). Like the CAld5H/AldOMT-mediated initiation of the syringyl pathway, SAD protein is widely distributed in angiosperms (Li *et al.*, 2000), but SAD as well as CAld5H and AldOMT proteins and their functions are absent from gymnosperms (Li *et al.*, 2001). These results challenge the traditional model of monolignol biosynthesis and suggest that CAD mediates the reduction of coniferaldehyde into guaiacyl monolignol and that SAD along with CAld5H/AldOMT controls the biosynthesis and utilization of sinapaldehyde for syringyl monolignol.

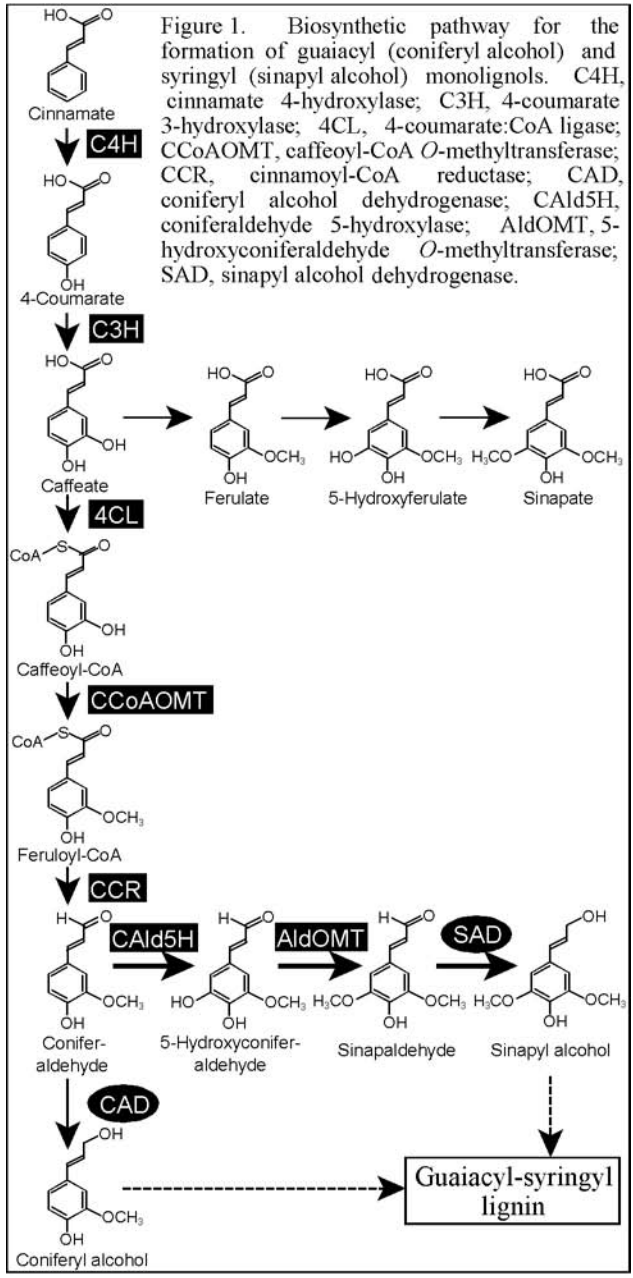

Figure 1. Biosynthetic pathway for the formation of guaiacyl (coniferyl alcohol) and syringyl (sinapyl alcohol) monolignols. C4H, cinnamate 4-hydroxylase; C3H, 4-coumarate 3-hydroxylase; 4CL, 4-coumarate:CoA ligase; CCoAOMT, caffeoyl-CoA *O*-methyltransferase; CCR, cinnamoyl-CoA reductase; CAD, coniferyl alcohol dehydrogenase; CAld5H, coniferaldehyde 5-hydroxylase; AldOMT, 5-hydroxyconiferaldehyde *O*-methyltransferase; SAD, sinapyl alcohol dehydrogenase.

Lignin Reduction

Biochemical evidence further demonstrated that, in this principle flux, 4-coumarate:CoA ligase (4CL) may limit total lignin accumulation (Hu *et al.*, 1999). 4CL, an enzyme upstream of coniferaldehdye (Figure 1), has been demonstrated to limit lignin accumulation in various plant species. Transgenic aspen trees with downregulated lignin-specific 4CL, Pt4CL1 (Hu *et al.*, 1999), exhibited up to a 45% reduction in lignin, but this did not alter lignin structure with respect to the S/G ratio, as revealed by lignin thioacidolysis (Hu *et al.*, 1999). Two-dimensional heteronuclear single quantum correlation nuclear magnetic resonance (HSQC NMR) further confirmed that the common lignin structural units are all similarly represented in wild-type and lignin-reduced transgenic trees (Hu *et al.*, 1999). Thus, these data provide strong evidence for the absence of any significant branch pathways at caffeate, the preferred 4CL substrate (Hu *et al.*, 1998), that would otherwise divert caffeate metabolism away from the principal phenolic flux (Figure 1) to result in an abnormal type of lignin. We proposed that, with respect to this principal flux, the result of 4CL downregulation is simply the attenuation of metabolite pools downstream of caffeate, limiting the availability of the normal precursors, the monolignols, for lignin polymerization.

Combinatorial gene manipulation had led to 38% to 52% reductions in stem lignin and to 22% to 64% increases in the lignin S/G ratio.

When antisense *4CL* and sense *CAld5H* genes were simultaneously transferred into aspen via *Agrobacterium*, phenotypically normal transgenic trees expressing each one and both of the transgenes were produced (Li *et al.*, 2003). Forty transgenic aspen lines were obtained, of which 37, 40, and 23% harbored antisense *Pt4CL*, sense *LsCAld5H* and antisense *Pt4CL* + sense *LsCAld5H* gene constructs, respectively, as confirmed by genomic PCR. From each of these three transgenic groups grown in a greenhouse, several trees were randomly selected and harvested at the age of 10 months during the growing season for various characterizations. 4CL-protein levels were drastically reduced in lines harboring only antisense *Pt4CL* transgene, leading to a 70% to 90% reduction in xylem 4CL enzyme activity, and a 30% to 40% reduction in stem lignin (Table 1). No significant effect on the lignin S/G ratio was found (Table 1). Over-expressing the *LsCAld5H* gene alone drastically elevated the xylem CAld5H-protein levels, giving rise to a 2.2-2.8-fold increase in xylem CAld5H enzyme activity. As a result, these transgenics exhibited up to a remarkable 2.5-fold increase in the S/G ratio as compared to the control (Table 1). The single *CAld5H* gene effect had no influence on total lignin accumulation in transgenic trees (Table 1). However, the single-gene effects became additive in transgenics harboring both antisense *Pt4CL* and sense *LsCAld5H* genes. Alterations of 4CL- and CAld5H-protein levels in these trees were consistent with changes of the corresponding enzyme activities: 80% to 90% reduction in 4CL and 60% to 110% increase in CAld5H. This

combinatorial gene manipulation had led to 38% to 52% reductions in stem lignin and to 22% to 64% increases in the lignin S/G ratio (Table 1).

Transgenic trees with reduced lignin exhibited increases in cellulose content.

Transgenic trees with reduced lignin exhibited increases in cellulose content—up to a remarkable 30% increase—was observed in antisense-*Pt4CL*/sense-*LsCAld5H* transgenic line 141, due to a 52% lignin reduction (Table 1). Consistent with the observation reported by Hu *et al.* (1999), the increased cellulose content together with reduced lignin quantity resulted in a cellulose:lignin ratio of 3 to 5 in the transgenic lines, as opposed to 1.9 in the control (Table 1). The relative abundance of the major hemicellulose component, xylan, was essentially unaffected in all transgenic lines, confirming our previous results (Hu *et al.*, 1999).

These transgenics are potentially valuable lignocellulosic substrates for woodpulp production.

Conclusion

Lignin reductions in trees can be achieved by antisense *4CL*, technology and over-expression of sense *CAld5H* results in S/G increases. These effects were independent but additive, with plants expressing both transgenes having less lignin, a higher S/G ratio and more cellulose. These transgenics are potentially

Table 1. Chemical compositions in stem wood of control and transgenic aspen

Plant	Control	21	22	23	25	36	32	84	96	94	102	72	74	141	143
Gene integrated												-4CL	-4CL	-4CL	-4CL
-4CL[a]		-4CL	-4CL	-4CL	-4CL	-4CL						+CAld5H	+CAld5H	+CAld5H	+CAld5H
+CAld5H[b]							+CAld5H	+CAld5H	+CAld5H	+CAld5H	+CAld5H				
Lignin content (%)[c]	22.2 ± 0.8	16.0 ± 0.6	15.3 ± 0.4	14.4 ± 0.5	13.1 ± 0.3	14.9 ± 0.2	22.4 ± 0.5	21.6 ± 0.4	21.1 ± 0.4	20.7± 0.6	19.7± 0.4	13.7± 0.4	12.4± 0.5	10.7± 0.4	13.2± 0.3
Lignin S/G ratio	2.2	2.1	2.0	2.2	2.3	2.1	4.8	4.0	5.5	4.9	3.0	3.6	3.4	2.7	3.3
Cellulose content (%)[d]	41.4±0.4	43.1±0.3	ND	44.8±0.4	47.3±0.5	ND	40.0±0.3	42.6±0.2	44.7±0.1	43.4±0.2	44.3±0.1	49.2±0.3	50.9±0.1	53.3±0.2	ND
Xylan content (%)[e]	15.8±0.2	16.8±0.3	ND	16.1±0.3	16.9±0.4	ND	16.5±0.4	15.3±0.1	15.7±0.5	15.6±0.5	15.2±0.1	15.3±0.3	14.6±0.2	15.4±0.4	ND
Cellulose/lignin ratio	1.9	2.7	ND	3.1	3.6	ND	1.8	1.9	2.1	2.0	2.2	3.6	4.1	5.0	ND

Values are means ± SE of two to three assays of different samples from each line. [a](*-4CL*) and [b](*+CAld5H*) denote antisense *4CL* and sense *CAld5H* transgenes, respectively. [c]Lignin, [d]cellulose, and [e]xylan contents are % of dry wood weight. ND: not determined.

valuable lignocellulosic substrates for woodpulp production. They may not be the ultimate lignocellulosics for bioethanol production, but they are benchmark transgenics and are rich sources of information for understanding cell-wall biosynthesis and thus for further metabolic engineering, allowing the generation of the ultimate raw materials for woodpulp production.

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Dr. Chiang's interests include chemistry, biochemistry and the molecular biology of lignin, cellulose and hemicellulose biosynthesis. His research involves investigating genes, RNAs, and transcription factors associated with the regulation of lignin, cellulose and hemicellulose biosynthesis during wood formation, using oligoarrays to study the expression of genes and regulatory RNAs associated with woody cell-wall formation, and genetic engineering of these regulations to develop superior trees with desirable wood traits for pulp/paper, lumber and energy productions.

Commercialization of a Protein Product from Transgenic Maize

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The concept of plants as factories was launched approximately 15 years ago as a method for bio-manufacturing pharmaceuticals, vaccines and industrial enzymes. The advantages of the plant system include low-cost manufacturing with limited capital investment for growing the biomass, easy scale-up with planting of increased acreage and a system without the threat of animal pathogens whether viral, bacterial or prion.

Proof of Concept

The concept has been tested in a number of plant systems at several biotechnology companies, including: ProdiGene (corn; Hood *et al*., 2003), SemBioSys (safflower; Moloney, 2002), Ventria (rice; Huang, 2004), Medicago (alfalfa; Vezina *et al*., 2002), Biolex (*Lemna*; Gasdaska *et al*., 2003), CropTech (tobacco; Cramer *et al*., 2000), Large Scale Biology (tobacco; Grill *et al*., 2002) and Planet Biotechnology (tobacco; Larrick *et al*., 2001) as well as in university research laboratories. The proof of the concept is embodied in these research efforts. Plant systems can express proteins at high levels, assemble multimeric proteins such as antibodies, and correctly process them, *e.g.* cleave signal sequences and attach glycosylation sequences. Many types of proteins have been expressed in plant systems including those ranging in molecular weight from 5,000 daltons (Zhong *et al*., 1999) to over 200,000 daltons (Lamphear *et al*., 2004). These various examples show the versatility of the system. The subject of this paper—bovine trypsin produced in transgenic maize—illustrates the concept of gene to market, *i.e*., steps in commercialization of a protein product.

This paper illustrates the steps in commercialization of a protein product.

Trypsin is a pancreatic serine protease involved in food digestion. Commercially, it is produced from bovine refuse from slaughterhouses for applications in cell culture and protein processing. Many of the cell cultures are for production of pharmaceutical or vaccine proteins, requiring pharmaceutical-grade manufacturing conditions. With the existing problems arising from hoof and mouth and mad cow (bovine spongiform encephalopathy) diseases, non-animal sources of trypsin are in high demand. Because bovine pancreatic trypsin is now available in maize lines, large-scale production for industrial and pharmaceutical applications can be achieved to meet this demand.

Molecular Biology

In order to express a protein at commercial levels (*i.e.*, as high as possible for the protein and plant host), several molecular and cellular parameters must be considered. The use of codons that are common to the host plant can enhance the translatability of the messenger RNA. From experience, the leading amino acids appear to be the most critical in this regard, presumably because they initiate efficient translation. In the case of bovine trypsin, the native *Bos taurus* gene was fused in frame with the maize-optimized barley alpha-amylase signal sequence (mo-BAASS; Rogers, 1985), providing twenty-five codon-optimized amino acids for translation (Woodard *et al.*, 2003), sufficient in this case to achieve good expression. Other parameters of interest for high expression of foreign genes for protein production include tissue specificity, subcellular localization, germplasm/breeding and protein-specific considerations. For bovine trypsin, tissue specificity was achieved through use of the maize globulin-1 promoter, which is embryo-preferred. Although several subcellular locations were tested, the best location for high protein accumulation was the apoplast (cell wall) (Hood and Woodard, 2002). By far, the greatest challenge with expression of bovine trypsin was regeneration of transgenic plants when the gene for the active enzyme was used. Therefore, the zymogen form of the protein, trypsinogen, was expressed from the holo-gene and transgenic plants, with high expression obtained (Woodard *et al.*, 2003). Thus, optimal expression of trypsin in maize was achieved by expressing the zymogen form of the gene from the embryo-preferred globulin-1 promoter, and targeting the protein to the apoplast using the maize-optimized barley alpha-amylase signal sequence. Using these conditions, the highest first generation seed showed 3.3% of total soluble protein (TSP) by enzyme activity (TRF, Figure 1).

Variation among transgenic events and among multiple plants from single events, *i.e.*, clones, is commonly observed in maize-derived transgenic plants (Hood *et al.*, 2003). Because of this, multiple individual lines from multiple events must be screened for the protein of interest, to choose the best lines to move forward. However, the maize varieties that perform best in tissue culture and transformation are not suitable for field growth. Therefore, lines selected for high first-generation expression are planted in field nurseries to be improved in agronomic characteristics through a breeding program. Breeding into elite inbred germplasm is conducted and resulting lines subsequently selected for high expression of the foreign gene of interest as well as for field performance. Trypsinogen was recovered in bulk seed lots at 58 mg trypsin per kg of seed, or 0.006% of dry weight from fifth-generation elite inbred material (Woodard *et al.*, 2003), or approximately double the first-generation high single seed.

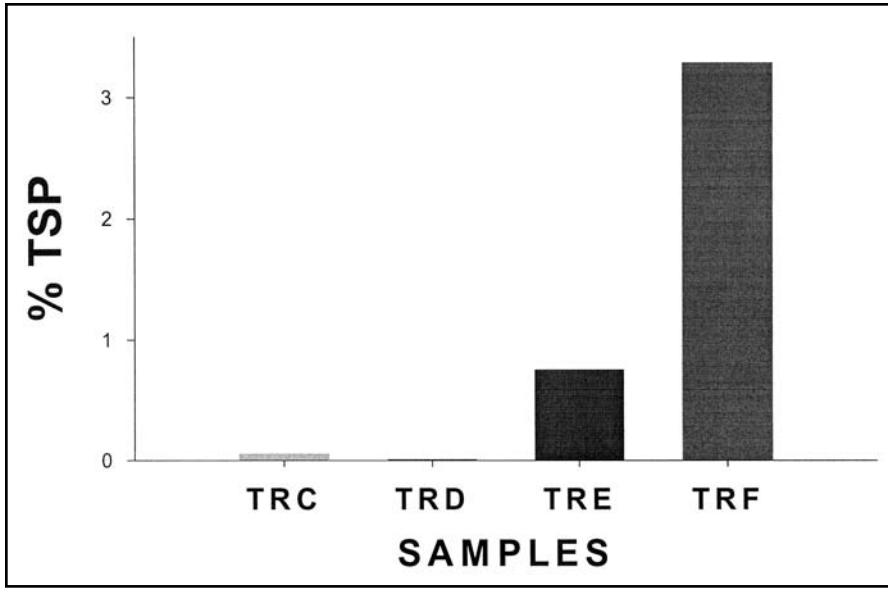

Figure1. Trypsin expression in various transgenic maize lines as a percent of total soluble protein (%TSP) from ground seed. TRC—zymogen form of trypsin expressed from a constitutive promoter and targeted to the cell wall; TRD—mature trypsin expressed from a constitutive promoter and targeted to the cell wall; TRE—zymogen form of trypsin expressed from an endosperm-preferred promoter and targeted to the amyloplast; TRF—zymogen form of trypsin expressed from an embryo-preferred promoter and targeted to the cell wall.

For commercialization of a product from a new production platform, it is crucial to determine the biochemical qualities of the plant-derived protein.

Biochemical Characterization

For commercialization of a product from a new production platform, it is crucial to determine the biochemical qualities of the plant-derived protein, including its activity. Maize-derived trypsin was mostly active when extracted from ground transgenic seed, and exhibited several molecular-weight forms (Figure 2). The largest disappeared upon treatment with enterokinase, suggesting it was the trypsinogen form (Woodard *et al.*, 2003). When each of the remaining high-molecular-weight forms was subjected to N-terminal micro-sequencing, the sequences were the same and matched exactly the sequence of the native bovine trypsin, confirming that the N-terminal signal sequence was correctly cleaved (Figure 2). In addition, these forms were active (Woodard *et al.*, 2003).

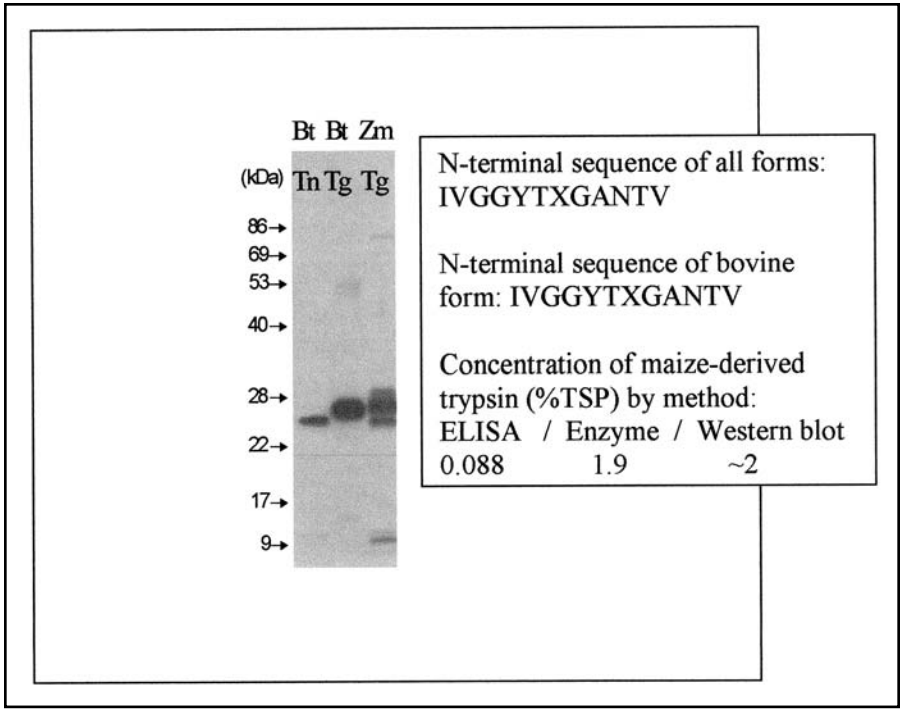

Figure 2. Trypsin characterization. Western blot of maize-derived trypsin. Bt = *Bos taurus*; Zm=*Zea mays*; Tn=trypsin; Tg=trypsinogen. Numbers to the left indicate migration of molecular-weight markers. Box to the right indicates N-terminal sequence of three active forms of maize-derived trypsin and values for trypsin concentration from several averaged samples using three assay methods.

Several methods of analysis were developed to determine the concentration of protein in transgenic maize seed. Enzyme-linked immunosorbent assays (ELISAs) provided disappointing results in that most transgenic lines exhibited very low concentrations of trypsin. However, Western blots suggested that the enzyme was present in substantially higher concentrations than the ELISAs had indicated (Figure 2). Subsequently, enzyme assays were developed to assess the activity of the maize-derived enzyme, and the results agreed closely with the Western blots, suggesting that the trypsin was digesting protein components in the ELISA.

Physical parameters of maize-derived trypsin matched the native protein in all instances with the exception of the molecular weight of extracted protein (Table 1; Figure 2). The differences in molecular weight were apparently due to O-linked glycosylation sequences on the maize-derived protein, and when removed by chemical means, the proteins had the same apparent molecular weight (Woodard *et al.*, 2003). Specific activity, pH optimum, Km and Vmax values were not significantly different between the two sources of enzyme (Table 1).

Table 1. Biochemical characterization of bovine trypsin from maize and native sources.

Parameter	Maize-derived trypsin	Bovine trypsin
Molecular weight (MALDI TOF)	23,297 daltons	23,306 daltons
Specific activity	175 U/mg protein	166 U/mg protein
Glycosylation	O-linked	none
Km	2.7 m*M*	3.2 m*M*
Vmax	0.29	0.30
pH optimum	8.6	8.7

Development of crop-derived proteins into real-world industrial or pharmaceutical products comprises a number of activities.

Product Development

A product is not just the protein of interest in a transgenic plant. It is the protein of interest utilized in a certain market application, each of which creates a different product. Thus, many products can be developed from one protein-expressing plant line. Development of crop-derived proteins into real-world industrial or pharmaceutical products comprises a number of activities including applications testing and market development, a freedom-to-operate assessment, patent protection, a safety assessment, breeding into elite material for best field performance, small-scale production and formulation requirements. All aspects must be developed in a way that meets cost targets.

Applications for trypsin include digestive aids, detergent additives, processing of commercial proteins, commercial cell culture, an active pharmaceutical product, eye-care products and leather processing. The major question for maize-derived trypsin is whether it can substitute for the bovine product in these applications. In several trials, the maize-derived enzyme functioned equally as well as the bovine counterpart (S.L. Woodard, unpublished).

Market development for maize-derived replacement products for existing markets mainly comprises focus on the advantages of a plant-derived protein. First and foremost, the plant-derived enzyme can be utilized in markets that are sensitive to the source of the product and the associated need to avoid animal pathogens. This is particularly true for cell culture, pharmaceutical use, and commercial reagent proteins that are utilized to process pharmaceutical proteins. A second advantage is low cost for markets not sensitive to these cleanliness issues. A third advantage is the ability to scale-up for large market applications for which microbial production systems are less appropriate because of capital requirements.

Freedom to operate (FTO) is a complex issue for plant-derived products—"can I practice my processes and produce my product without running afoul of others' patents?"

(Sweeney, 2002). Determining FTO is a process that requires constant diligence and many licenses, particularly in competitive areas of research and technology development. Whenever new materials are brought into an entity or the entity's materials are shared with others, FTO issues can be raised. And, obviously, not only materials are at stake. Often processes for creating products are patented; for example, transformation protocols and breeding strategies are equally subject to licensing requirements. Diligence in this area with company or university attorneys is critical to success.

Patent protection is essential to successful market entry, particularly if the technology or product can be manufactured by other firms, or the technology of interest makes a product possible when it previously was not available. Proteases in general are difficult to produce in xenogenic systems because of their detrimental effect on native protein content. In 2000, ProdiGene was issued a broad-based patent (USP # 6,087,558) describing the production of proteases in transgenic plants, claiming expression of any protease in any transgenic plant, where the protease is expressed in the zymogen form. This is a fundamental technology, because recovery of transgenic plants expressing high levels of active proteases is nearly impossible unless zymogens are expressed in seed.

Breeding into elite germplasm is essential for field performance that meets production requirements.

Maize transgenic events are generated in material that has poor agronomic characteristics. Thus, **breeding into elite germplasm** is essential for field performance that meets production requirements. This process takes from 2 to 4 years depending on the need to reach yield parity for profit margin and whether year-round nurseries are used for the acceleration of generations. If yield parity with commodity corn is required to meet production-cost requirements, then five to seven generations of crossing into elite inbred germplasm is required with two additional selfed generations before making a hybrid (D. Delaney, personal communication). The germplasm of choice depends upon the field production location, and if more than one zone will be required for crop production, more inbreds will be required for the breeding program. A minimum of two generations per year can be accomplished in midwestern US summer nurseries alternating with Puerto Rican (or other Caribbean island) winter nurseries. However, with year-round nurseries in Hawaii, 2.5 to 3 generations per year can be accomplished. If the cost of the product and immediacy of the market opportunity warrant, the higher cost of year-round nurseries can often be justified, cutting significantly the time involved in development of genetic production material.

One of the major goals of the breeding program, in addition to lines with adequate field performance, is generating lines with commercial levels of expression of the gene of interest. Minimum concentrations of protein in corn meal for different types of products—purified pharmaceuticals, orally delivered vaccines, industrial enzymes or cellulases—are shown in Table 2 (J. Howard, personal communication). For the TrypZean™ product, the minimum concentration of trypsin in dry seed material should be 0.01–0.1% to meet production-

Table 2. Minimum concentrations of foreign protein in genetically modified production material for cost-effective production.

Product	Application requirement	Minimum concentration
For oral delivery of vaccines	~1 mg required for dose	0.1% DW
For purified pharmaceuticals	Less expression-sensitive	0.01% DW
For industrial enzymes	<$200/kg production cost	≥0.1% DW
For cellulase for ethanol	120 g/gallon ethanol	≥3% DW

cost targets because in this lyophilized powder formulation it is considered a cell-culture reagent protein, thus falling between the purified pharmaceutical and industrial enzyme application requirements. As stated above, the concentration of trypsin in fifth-generation breeding material is approximately 0.006% of dry weight, somewhat lower than what will be required for a profitable product. However, because breeding and selection can generate material that expresses the recombinant protein at ten to a hundred times higher than the initial transformant (Hood *et al.*, 2003), continued breeding and selection should yield lines with the required expression level.

During the breeding program, **small-scale production** can be accomplished from hybrid seed samples taken from the breeding material and subsequently grown in field plots. Two goals can be achieved with this material: assessment of the improvement in agronomic character of interim hybrids and small-scale extraction/purification of the protein of interest for applications trials. These two assessments are critical to timely identification of potential problems in the product-development timeline.

The final **formulation** of the protein product is entirely a requirement of the application. For maize-derived trypsin, the product TrypZean™ is a lyophilized powder bottled for laboratory use as a cell-culture-dissociation reagent. For other uses, the formulation might be 1) a stabilized liquid, 2) ground, unextracted corn meal, or 3) ground, defatted corn-germ meal. In all cases, the cost of formulation must be compatible with the sale price of the product.

A product **safety assessment** includes understanding the hazard of the specific protein of interest alone or within the plant material. Once the inherent hazard of the protein has been quantified, one can determine the risk associated with various levels of exposure to it (risk is proportional to hazard multiplied by exposure) (Howard and Donnelly, 2004). While this assessment will not satisfy all regulatory-compliance issues, it is a required component and will assist in establishing confinement measures for regulatory compliance. The field of product safety versus regulatory compliance is in flux and requires constant attention to remain apprised of status.

Production

Production of crop-derived proteins requires scale-up of activities similar to those described in product development. Additionally, it requires a plan for compliance with US Department of Agriculture (USDA) and Food and Drug Administration (FDA) regulations, or

Corn containing bovine trypsin—or any transgene for protein production—should be grown in areas where crop development is efficient and cost-effective.

those of other agencies depending upon the product. The steps involved in production, growing, harvesting and associated activities, processing, and extraction/purification are outlined in Table 3.

Corn containing bovine trypsin—or any transgene for protein production—should be grown in areas where crop development is efficient and cost-effective, *i.e.*, the midwestern corn-belt. Otherwise much of the advantage of using a commodity crop for low-cost production is lost (Howard and Hood, 2005). The total acreage required is a product of seed yield and recombinant protein concentration in the seed. For example, trypsin in the fifth generation was at a concentration of 0.006% of whole-seed dry weight. If the corn produced 150 bushels per acre, equal to 3,750 kg of grain per acre, the yield of trypsin per acre would be 225 g. If the desired production amount is 10 kg for market entry, approximately 45 acres would be required. Confinement to meet USDA regulations requires a buffer zone of 1 mile wide surrounding the field, plus other measures such as delayed planting compared to other corn-production fields in the area. Male sterility can also be utilized, but the trypsin material is not male sterile.

Table 3. Production steps and parameters to be considered during production of trypsin from transgenic maize.

Production step	Considerations for trypsin in corn
Growing	Biological requirements for corn, seasonal and geographic limitations, recombinant protein yield, confinement regulations
Harvesting/ Transportation/Storage	Mechanical issues, time, temperature sensitivity, cost, protein stability in tissue
Tissue processing	Stability in tissue, potential for enrichment, small batches, whole seed, large batches, dry mill for germ, extract oil
Extraction/Purification	Protein stability, biomass quantity versus yield, cGMP for pharma applications, formulations

Corn seed is harvested mechanically when the moisture content is below approximately 20%. Shelling is accomplished best when moisture content is below 15%. Field drying is the most cost-effective method, but harvest should occur before a severe frost, and if necessary the ears can be mechanically dried after collection. Current regulations require dedicated equipment for non-food products from genetically modified crops. Once the grain is harvested, dried and shelled, it can be stored for months to years. Most foreign proteins in genetically modified seed are quite stable in dried grain (Hood *et al.*, 1997; Hood *et al.*, 2003; Lamphear, 2004), and trypsin is no exception (S.L. Woodard, unpublished).

One of the advantages of grain crops for bioproducts is the ability to partition proteins into specific tissue sinks (Figure 3). Trypsin is expressed largely in the embryo of the kernel, with much less present in the endosperm. Because the embryo represents only 10% of the dry weight of the kernel, separating it from the endosperm effectively concentrates the protein ten-fold on a dry-weight basis. Tissue separation can be accomplished with either dry-milling or wet-milling operations. The recombinant protein must be compatible with the temperatures used in either process, and the solutions used in the wet-milling process. In the future, oil will be extracted and sold as a co-product when the germ is isolated,

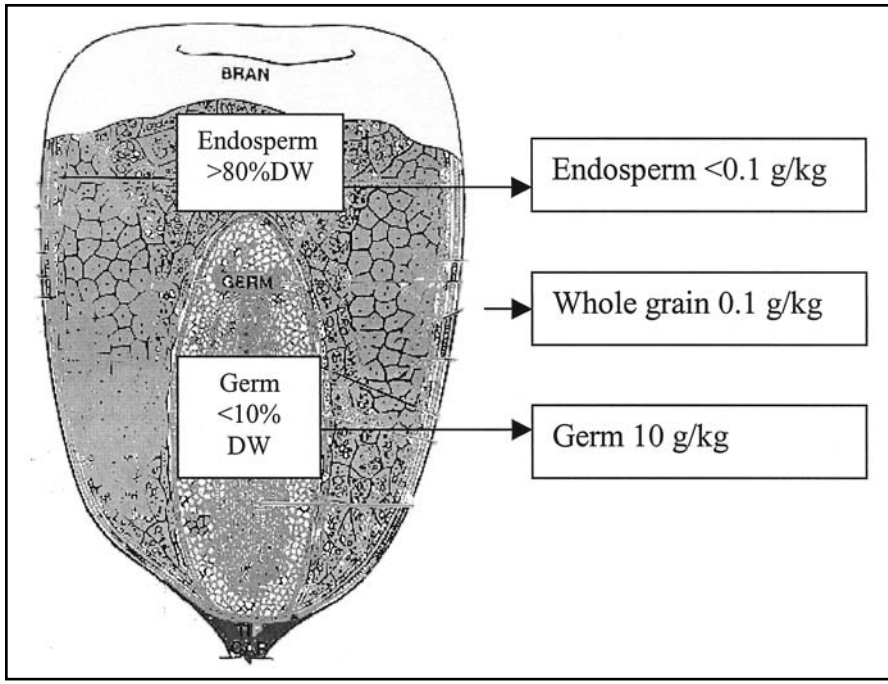

Figure 3. Relative contribution of maize kernel tissues to total seed weight. Numbers to the right compare the concentration of recombinant protein in each tissue based on a whole-seed expression level of 0.01% dry weight.

particularly if the transgenic product is deregulated. Tissue processing is not usually cost effective at less than a million bushels. Therefore, at 7,500 bushels from 45 acres of corn, whole-seed grinding and subsequent extraction will be a more cost-effective production method than seed fractionation for trypsin.

Currently, trypsin is a purified, lyophilized product, TrypZean™ (Sigma Chemical Co., St. Louis, MO). Thus, extraction and purification are necessary for its production and sale. Extraction buffers have been developed to maximize trypsin recovery and minimize extraction of native corn proteins. Subsequent purification protocols maximize recovery and stability balanced with purity. In many cases, 50% or less of the protein is recovered from the starting material, indicating that twice the acreage estimated above would be needed for production.

A lack of public acceptance is the major barrier today to producing biopharmaceutical or bioindustrial products in plants.

Public Acceptance and Sale

A lack of public acceptance is the major barrier today to producing biopharmaceutical or bioindustrial products in plants. Response to this public distrust has driven current regulations to be quite restrictive. The scientific community and the regulatory agencies are striving to gather substantive safety data to support regulations that are based on scientific principles and will protect the public as well as allow this new industry to develop. This topic has been discussed in detail in recent reviews on bioproduction and product safety (Howard and Donnelly, 2004; Howard and Hood, 2005). The critical asset for general public acceptance is whether the consumer sees benefits and whether these perceived benefits outweigh costs and risks. When products with obvious benefits are available to the consumer, public acceptance, science-based regulations and sales will fall into place.

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Elizabeth Hood has had twenty-five years experience in biology. She is currently associate vice chancellor for research and technology transfer at Arkansas State University. She was a program director in molecular and cellular biosciences at the National Science Foundation for the 2003–2004 academic year. Dr. Hood was a leader in forming one of the world's foremost transgenic plant-research groups at ProdiGene, a biotechnology start-up company. Previous to ProdiGene, she was director of the cell biology group for plant production of therapeutic proteins at Pioneer Hi-Bred International.

Hood has over seventy publications and patents to her credit and she has been an advisor for graduate programs at several universities. Currently she holds adjunct positions at Texas A&M University in the Department of Biochemistry and Biophysics and in the Molecular and Environmental Plant Sciences program. Her previous positions were with Utah State University, the Swedish University of Agricultural Sciences, Washington University in St. Louis and Oklahoma State University. She earned her PhD in plant biology from Washington University and an MS in botany from Oklahoma State. Her research interests are in the areas of renewable resources, foreign-gene expression in transgenic plants, plant cell-wall structure and function, and plant cell biology and protein targeting.

Module III
Gene-to-Product Development

Panel Discussion and Q&A

Moderator:
Kim Jensen
University of Tennessee
Knoxville, NY

Panelists:

William Goldner
USDA Small Business Innovation Research
Washington, DC

Roger Conway
USDA Office of Energy Policy and New Uses
Washington, DC

Alex Day
Kentucky Life Sciences Organization
Frankfort, KY

William Goldner: I'll take a few minutes to introduce you to the Specialty Crops Regulatory Initiative—a USDA-sponsored program now getting underway. I believe it's germane to the topics that we've been discussing. It's a program that came to us based on the output of a biotechnology workshop held in November of 2004 that I chaired with Ann Marie Thro, sponsored by the Agricultural Research Service, Cooperative State Research Education and Extension Service and the Animal and Plant Health Inspection Service. The workshop was in response to the frustration that small business and public developers of specialty biotechnology crops have felt in terms of being unable to get their products—the crops they have developed—to market. And as we've seen today, including Beth Hood's representation of what it takes to get a product to market, there are many hoops to go through as a crop developer. And university researchers, ARS researchers and small-business developers of biotechnology crops generally are not prepared to address these issues. It takes a good deal of expertise, involving navigating a regulatory process that is always in some state of evolution with interaction with as many as three federal agencies, the FDA, EPA and APHIS itself. Only a few of the specialty crops have been deregulated and that's really the issue that came up in the workshop—the need for an organization to assist public-sector and small-scale private-sector developers through the regulatory approval process. And there is precedence for this; the orphan-drug program in FDA assists pharmaceutical companies in bringing through the regulatory process drugs that serve only a small population of patients because of disease rarity. Also the USDA's IR-4 program addresses the needs for small-acreage pesticide use and assists companies to develop datasets that meet the regulatory process to gain approval for use on minor crops.

Roger Beachy and Mike Rodemeyer mentioned issues of management and stewardship that we as scientists are ill-equipped to deal with on a day-to-day basis. So, the concept is to develop the Specialty Crops Regulatory Initiative to identify and prioritize need and public benefit, helping to facilitate the generation of required regulatory safety data for fruits and nuts, vegetables, ornamentals, nursery crops, even forest trees (anything but the four major row crops). This is analogous to what happens in the IR-4 program for specialty pesticides. The idea is to facilitate the process and has nothing to do with the ultimate commercialization or marketing of biotechnology crops or the products from those crops.

The structure mimics that of the IR 4 program in that three committees are proposed: a stakeholder/liaison group, a project-management group and ultimately a headquarters staff. A critical aspect is involvement of and partnership with several different communities: consumer groups, grower groups, distributors, university researchers and technology groups as well as government researchers and administration. The near-term challenges are clear. We must demonstrate an early ability to enable regulatory compliance for a new biotechnology-derived crop. Based on discussions so far, perhaps a non-food crop would be the most suitable target. In fact, a crop from outside the United States—one that would benefit a developing country—could be the target.

A long-term challenge is to make available a broader range of biotechnology crop options for public benefit, meeting economic and environmental needs. We'd welcome input from those involved in crop-development and also in the other aspects such as marketing and commodity groups. We can send you information on the initiative. There is a national planning committee and Beth Hood and I are members; Alan McHughen at the University of California, Riverside, is the national chairman. Also on the committee are representatives of the Agricultural Research Service, APHIS, CSREES, land-grant universities, the private sector and also of some commodity groups. A follow-up workshop will convene in November of 2005 specifically to address the needs and requirements and suggestions of the stakeholders to help us craft and develop this important organization.

Alex Day: I'm with the Kentucky Life Sciences Organization. I also founded a company called Sheltowee LLC. We do life-science business consulting and business development. I want to talk about what I see as a couple of obstacles that we face in bringing products to the market. The biggest obstacle is regulatory. Particularly for the pharmaceutical and health uses of some of these proteins, we haven't been able to convince the regulators to show us the path. When I first became an entrepreneur I was told, "Remember, the pioneer is known as the guy with all the arrows in his back." We can't even seem to find a pioneer who can get all the way through the process and take the arrows so that we know how to get the products through. So, we are dealing with a public-perception problem but also a problem with the regulatory agencies reacting to public perception. Another common problem is early-stage funding. I know that in our state it's very difficult to bring in seed-stage funding. The definition of seed-stage funding has changed dramatically over the past few years. There are very few venture-capital or other sources willing to make seed-stage investments.

Another issue for early-stage products is meshing business with science. I'm more of a business person. I know enough of the science to be dangerous and to understand some of what's discussed in forums like this. The science must be translated into terms that business people are going to understand, providing compelling reasons to put the money in to develop the products. That cross-talk—meshing business and science—is critical to taking products through the development process and bringing them to market. The Kentucky Life Science Organization—a non-profit trade organization—is trying to educate people all the way from the grass-roots level up to politicians as to why these things are important and what we can do to facilitate, to clear a path so that these products can be brought to market.

Roger Conway: I want to discuss the need for a general field theory for commercialization. There has been a lot of frustration in the bioproducts and bioenergy areas. Some people have felt that we've done a lot of research, but haven't accomplished as much as we'd like to. I'd argue that there might be under-investment and other links in a causal chain. You might want to think of it as a pipeline of different links that need to be looked at for commercialization. And, of course, research is important—the plant genomics, the conversion work is very important. We want to lower the cost of production, we want to increase yields—that's an important component—but other things are important too: for example, having life-cycle analyses from cradle to grave to show environmentalists that these products are environmentally beneficial. This is something that is featured in our federal biobased-products-preferred procurement program that my office is responsible for. In addition, having ASTM/ISO-compliant standards is important for demonstrating to potential buyers that your product really works, and in some cases in the bioproduct industry that hasn't been done and needs to be done. We've dealt with the Defense Logistics Agency who are fastidious about the products they buy; when they put a lubricant in a army tank that's going to Iraq they want to be sure it works. These are potentially huge markets, but the need for testing is extremely important. So that's another link.

Another link is in terms of regulatory initiatives. For example, the main reason that the ethanol industry has doubled recently is because of the reformulated gasoline program, which requires oxygenates. In California, MTBE has been replaced with ethanol—as a regulatory initiative—which has virtually doubled the use of ethanol. Other things can be done. For example, Lou Honary at the University of Northern Iowa has developed transformer fluids from soybean oil. There is a problem with fossil-fuel-based transformer fluids containing PCBs. Perhaps EPA could differentiate between bioproducts and fossil-fuel products based on toxicity, biodegradability and flashpoint. We will continue dialog with EPA on these issues.

Another link is product differentiation and commercialization, and the Federal Biobased Products Preferred Procurement Program that we are running is an opportunity for product scale-up because it offers a guaranteed market. It's an opportunity for the private sector to see what's happening in the public sector as we use these products and could greatly expand markets.

Another link is public-sector initiatives such as investment tax credits, which are highly effective, and the USDA CCC Bioenergy Program for example. The latter is responsible for creating the biodiesel industry as it is today. Before the Program, biodiesel consumption was approximately 2 million gallons now it's up to around 60 million gallons. The USDA can take pride in helping to advance that industry.

Financing issues are also important. Especially for rural areas, obtaining capital continues to be a problem. Having some sort of public-private cooperation may be useful. Having access to specialized insurance may help reduce the risk. Once again, tax-credit issues may help.

Finally, education and outreach: science-based outreach to the public sector is necessary, one that explains environmental issues and also explains the performance characteristics of these products.

So, in the past we may have under-invested in some of these links and I'd argue that we need to view this thing in a more holistic fashion. Research is absolutely important, but there are other features that can help get these products to the market.

Allan Bennett (University of California, Davis, CA): For Beth. I was impressed with the seventy-five licenses for your product and I'm wondering if you have a sense of how many of them were actually required for FTO and the final product. Do you have any sense of the financial burden of those licenses on the TrypZean™?

Elizabeth Hood: A lot of the licenses were for our transformation system, promoters, leader sequences, trailer sequences and the selectable marker genes. Probably, some of them were package deals that we were able to bring with us from Pioneer—pass-through licenses, *etc*. I would say that the majority had an impact on the product. The financial burden? I couldn't tell you because I think each set of them had a different percent royalty based on sales in some cases and based on a flat fee in others. Therefore, the more products you develop the less the burden is on any one particular product. It's important to have those licenses so that you can legally market the product. But you don't pursue seventy-five licenses with the objective of only one product. You assume you're going to have a pipeline of products.

Goldner: Do you anticipate any phenotypic effect from lowering the lignin content in the softwood or hardwood trees?

Hinchee: The process of product development is to put the trees in the field and then assess them for all the usual performance characteristics in terms of a breeding program and a clonal development program. Are they stress-tolerant? Are they disease-resistant? Do they perform like the non-transgenic trees with the exception of the reduced lignin trait? Our anticipation is that if we launch a softwood or a hardwood product with reduced lignin that it would be within a range that allows the pulping industry to benefit from that advantage, but doesn't affect any of the other phenotypic characteristics and the health of the plantation can be maintained.

Ralph Hardy (NABC, Ithaca, NY): Will your reduced lignin trees now make it possible for new pulp and paper mills to be built in the United States? It's my understanding that there hasn't been a pulp and paper mill built in the United States for a long time because of the extensive costs of pollution control plus processing. Are you reducing lignin, capital and/or operating costs enough? And can you give us some range of what that might be? Is it a compelling number or a marginal number?

Hinchee: It's analyzed on a mill basis and is premised on the fact that no more mills will be built in the United States. We are talking in terms of saving the US forestry and pulp and paper industries. They are looking for anything to improve efficiency because the profit margin is small and they are facing abundant supplies of wood from Siberia and other places where they are indiscriminately harvesting very old trees. To maintain the industry in the United States they must improve efficiency in a variety of ways. Genetic improvement is actually a no-cost opportunity to improve efficiency without major investment at the mill, apart from normal adjustments of boilers for lignin extraction for the variety feedstocks that come in already. It's using genetics to enhance the survivability of an industry, in my opinion.

Allan Eaglesham (NABC, Ithaca, NY): Following on from Dr. Goldner's question: is it possible to reduce lignin level to zero? And if not, is there potential to reduce it farther than what you've achieved already?

Chiang: The maximum is a 50% lignin reduction. We've produced 200 or 300 transgenic trees with low lignin and never got more than a 50% reduction. I believe that is related to total carbon-sink control regulating the three major cell-wall components: lignin, cellulose and hemicellulose. Low-lignin content has also been found in apple in nature. It was considered to be disease-related, but it's not. It's just a low lignin content, again about 50% lower than normal apple trees.

Jensen: Anybody have a question for our panelists?

Svetlana Oard (Louisiana State University, Baton Rouge, LA): What change would the panelists make in the regulations to expedite the gene-to-production process?

Conway: As a USDA representative, it's really not up to me to give an opinion about the regulations of our sister agencies and of other federal agencies, but I think it's clear that there is potential for some regulatory evolution, using a science-based rational approach. That seems to be the consensus from everybody I've talked to here.

Day: Being the non-government guy, I'd be happy to provide all kinds of advice to FDA. We believe that the best opportunity for plant-based pharmaceuticals is probably going to be generic biologics. Provide us the guidelines for meeting bioequivalency. Those products are being produced in plants already and if we can just get the agency to actually stick a

stake in the ground and say, "This what you need to do," then it would open the door for a lot of plant-based pharmaceuticals.

Goldner: I'm not specifically familiar with some of these regulations. I'd just say, generically, as an economist, that I'd prefer to see something that is economics-based on benefit/cost analysis. We have an ORACBA within the department in one of my sister offices—the Office of Risk Assessment and Cost/Benefit Analysis—which reviews regulatory procedures within the department and is led by an economist. So, I'm going to be provincial and declare that as my generic interest.

Regulating Pharmaceutical Plants: Meeting the Challenge

Cindy Smith
Biotechnology Regulatory Services
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The United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) has regulated genetically engineered (GE) organisms since 1987 and, in 2002, established Biotechnology Regulatory Services (BRS) to place a renewed emphasis and priority on biotechnology. APHIS has authorized more than 10,000 permits and notifications for the introduction of GE organisms and deregulated over sixty products for use, establishing itself as an international leader in the safe regulation of GE products.

APHIS has authorized more than 10,000 permits and notifications for the introduction of GE organisms and deregulated over sixty products for use.

As the science of biotechnology and the landscape in which it operates continue to evolve, APHIS's role in regulating it becomes increasingly challenging. As a regulatory authority of this rapidly growing technology, we must ensure that we protect US agriculture, allow for the safe development of GE organisms, and not unduly inhibit the advancement of the technology. One important challenge is to keep up with the science's technological advances. An increasingly broad array of traits is being engineered into plants as scientists discover more genes from a wider assortment of organisms that might be useful to improve agriculture, protect the environment or benefit consumers. But perhaps one of the most challenging technological trends of the past few years, from a regulatory perspective, has been the use of agricultural crops to produce pharmaceutical compounds and other items not intended for food or feed. One regulatory challenge is to allow the cultivation of these and have effective systems in place that will prevent them from being mixed with other crops, some of which are to be used as food or feed. In addition, pharmaceutical technology has prompted interest from a new range of stakeholders. Since 1987, BRS has issued 110 pharmaceutical and industrial permits in eleven crops; however, less than 350 acres have been grown since 2002.

Another important challenge is the changing social and political landscape. US citizens are becoming increasingly interested in biotechnology and want to play a larger role in government decision-making. In addition, citizens can be skeptical of the government and are willing to take action against government decisions. We have also seen an increase in the activity of public-interest groups who want to represent constituent views of the science and how government should regulate it. One outcome of this is that government agencies have become the target of lawsuits. In addition, biotechnology does not enjoy the same level of acceptance internationally as in the United States, posing an even greater challenge beyond our borders.

Biotechnology Regulatory Services

Since its inception just 3 years ago, BRS has undergone significant reorganization and is better prepared to anticipate and respond to the challenges being brought forth by the evolving nature of biotechnology and the landscape. The newly reorganized BRS goes well beyond a staff of scientists to evaluate permit applications and petitions for deregulation. It includes a Compliance and Inspection Branch, a Communications and Capacity Building Branch, a Regulatory Analysis Branch, an Office of Science, and a forecasting function that help BRS address these challenges and keep pace with the advancing science. In addition, we have developed five priority areas of emphasis that set program direction and provide the foundation for decision-making. These priority areas are the key to BRS's ability to meet the challenges of regulating biotechnology in general, and specifically, plants engineered to produce pharmaceuticals.

The first priority is maintaining rigorous regulation that thoroughly and appropriately evaluates and ensures safety and is supported by strong compliance and enforcement.

The first priority is maintaining rigorous regulation that thoroughly and appropriately evaluates and ensures safety and is supported by strong compliance and enforcement. APHIS regulation relies on a science-based evaluation of risk, which will be even more important in the future. This approach allows us to focus our regulatory efforts on specific areas such as pharmaceutical plants and reduce burdens in areas of lower risk. In 2003, we strengthened permit conditions for pharmaceuticals and industrials resulting in stringent confinement measures and a greater government role. For example, our confinement measures now include increased isolation distances and fallow zones, and restrict the use of the same land to produce pharmaceutical and industrial crops from the production of food or feed crops. APHIS also requires developers of pharmaceutical and industrial crops to have dedicated equipment and storage facilities for those crops. We currently inspect every pharmaceutical and industrial site at least seven times before, during, and after production. In addition, in 2003, APHIS amended its regulations to require that industrials are tested under the permit system.

Environmental Impact Statement

To ensure that our regulations remain effective as the technology advances, APHIS is currently preparing an Environmental Impact Statement (EIS) that will be used in revising its regulations. The updated regulations will leverage the additional authorities of the Plant Protection Act of 2000, significantly broadening APHIS's authority and positioning the USDA to address a broader range of issues, including human health. In the Notice of Intent that was published in January 2004, we stated that we are considering numerous revisions. One change we are considering is the implementation of a multi-tiered, risk- and familiarity-based permitting system to replace the current permitting and notification system. With respect to pharmaceuticals, another change we are considering is a new mechanism for maintaining regulatory oversight after a crop is commercialized. This mechanism would feature increased transparency and efficiency, and a greater role for the states. We are also considering the establishment of safety criteria that might allow for the deregulation of certain pharmaceutical crops.

BRS is also committed to reducing the regulatory burden as appropriate to the risk.

Reducing the Regulatory Burden

BRS is also committed to reducing the regulatory burden as appropriate to the risk. We appreciate that this is especially important for publicly funded researchers and small businesses. In pursuing this goal, we requested feedback on ways we could reduce regulatory burden in our January 2004 Federal Register notice that announced our intent to prepare an environmental impact statement on our proposed regulation changes. In addition, we have held workshops with the Pew Initiative on Food and Biotechnology and with USDA's Agricultural Research Service and Cooperative State Research, Education, and Extension Service to identify possible regulatory barriers and potential solutions. The IR-4 program for pesticide registration for use on minor crops has been proposed as a model that the government might follow to reduce the burden imposed on researchers and small businesses who are developing GE crops. It has also been suggested that alliances might be established between small businesses and university researchers such that the burden on generating required data might be shared, and thereby not be prohibitive to any individual researcher or small company who might seek to develop a GE crop.

Fostering the Technology

In addition to maintaining rigorous regulation, it is critical that we administer a compliance program that is strong enough to ensure the safety of the science while also allowing for the advancement of the technology. This is especially true for higher risk crops, such as those used to produce pharmaceuticals. BRS's Compliance and Inspection Branch is dedicated exclusively to ensuring that researchers maintain compliance through defined procedures that include violation-prevention efforts, risk-based criteria for quality inspec-

tion, standardized inspection and auditing processes, uniform enforcement, and thorough documentation of any compliance infractions. We also make investigation results available for public and stakeholder viewing. Compliance specialists and APHIS inspectors perform targeted inspections and audits of field tests and use established criteria to thoroughly evaluate all potential compliance infractions.

The second priority is ensuring that our regulatory process and decision-making are transparent to stakeholders and the public.

The second priority is ensuring that our regulatory process and decision-making are transparent to stakeholders and the public. Being transparent about our processes, decisions, and activities is critical for building public confidence in the regulatory system. We also understand that it is particularly important to be transparent in regard to pharmaceuticals. We must meet the challenge of fulfilling this objective while also protecting developers' confidential business information. Part of our transparency efforts include following the National Environmental Policy Act (NEPA), which establishes the criteria on when to conduct an environmental assessment (EA). We make available on our Web-site all EAs conducted for pharmaceutical and industrial field tests, or in cases that we do not conduct an EA, we post APHIS's categorical exclusion criteria and all NEPA decision documents. We also announce the EAs in the Federal Register and allow for a comment period. Our Web-site also provides a listing of all pharmaceutical permits along with their current status, accompanying decision documents, total acreage, and any supplemental permit conditions. Though confidential business information (CBI) limits our ability to post all permit applications, we post as much information as we can. In one recent case, we posted permit applications where little or no CBI was claimed and in another recent case, we summarize the non-CBI information from permit applications in our EAs. In addition, even when specific location and size information of field tests is claimed as CBI, we provide the general location and size information. We have also completely redesigned our Web-site and have included a stakeholder registry that allows registered stakeholders to receive updates and other information relevant to selected topics of interest, such as regulation activities, communication and outreach, capacity building, and compliance issues.

In another effort to ensure transparency, in 2004, we held multiple public meetings to discuss issues associated with the BRS proposal to revise regulations. BRS met with twenty-two stakeholder groups and heard a wide range of viewpoints on the proposed revisions and provided clarification on some of our agency's objectives. In the near future, we will be holding similar stakeholder meetings on a monthly basis as we complete the process of developing a Programmatic EIS on our proposed regulatory changes.

Assuring Safety

The third priority is maintaining a science-based system that ensures that the best science is used to support regulatory decision-making and to assure safety. While we work

The third priority is maintaining a science-based system that ensures that the best science is used to support regulatory decision-making and to assure safety.

to achieve this priority, we face the challenge of obtaining all of the available scientific information needed in order to make sound science-based decisions. To help achieve this goal, we have expanded our staff to include a diversified collection of scientific expertise in science fields, such as plant pathology, botany, entomology, ecology, animal science, virology, environmental science, biochemistry and molecular biology. To keep pace with this ever-evolving technology, BRS staff and scientists attend and host meetings and workshops, read literature, and interact with outside scientists, stakeholders, and the public. Additionally, in 2002, BRS established the Office of Science, which works with the research community to identify biosafety research priorities and to communicate biosafety research results for the use of regulators globally. As part of its agenda, the Office of Science addresses scientific issues associated with pharmaceuticals and in August 2004, conducted a workshop on confinement that focused largely on pharmaceutical crops; more than 100 scientists and experts from six countries participated. The Office of Science also helps maintain science as the centerpiece of regulatory decision-making amidst the challenges of diverse political, economic, and personal viewpoints associated with the technology. We also encourage biotechnology research and are looking into the possibility of becoming a funding agency in the future, such that we might target areas of research that we identify as having a pressing need.

The fourth priority is maintaining communication, coordination, and collaboration with the full range of stakeholders.

Communicating with Stakeholders

The fourth priority is maintaining communication, coordination, and collaboration with the full range of stakeholders. BRS works to meet the challenge of recognizing and reaching out to a broad range of stakeholders and interests. In regard to pharmaceuticals, it is particularly important to reach out to a broad diversity of stakeholders that includes not only the biotechnology industry and researchers, but also stakeholders such as in the food industry, commodity groups, public interest groups and the states. For example, we recently met with a food-industry group to discuss additional science-based measures that BRS should consider for two pharmaceutical field tests.

In another example, we work closely with the states on issuing permits, particularly for pharmaceuticals. We provide information to support their decision-making, which may involve adding additional permit conditions to address the state's concerns or, in some cases, providing support such that the state has a full understanding of the science.

The fifth priority is establishing international leadership.

International Leadership

Finally, the fifth priority set forth by BRS is establishing international leadership to ensure that international biotechnology standards are science-based, international regulatory capacity-building is supported, and international implications of domestic policy and regulatory decisions are considered. BRS faces the challenge of providing international leadership to ensure the development of science- and risk-based regulatory systems while maintaining effective working relationships in which we recognize and respect the differences in their systems and they are in turn receptive to our approach and the benefits that it can offer. In addition, we must consider the international implications of any domestic policy decisions that we make and ensure that the policies that we put in place domestically can be applied equally internationally. These important international partnerships are now serving as a starting point for international discussions of the regulation and confinement of pharmaceutical crops.

Through our evolving regulatory structure, dedicated compliance function, focus on science and risk, increased transparency and communication with a broad range of stakeholders, we are focused on these priorities and managing the challenges posed by new trends such as pharmaceutical crops. As the science progresses, we will continue to evaluate the implications of new technologies, enhance our processes and procedures, and develop appropriate regulations to meet the challenges posed by this new science while continuing to safeguard American agriculture, the nation's food supply and the environment.

Cindy Smith currently serves as deputy administrator for the Animal and Plant Health Inspection Services (APHIS) Biotechnology Regulatory Services (BRS). She has been charged with providing leadership to BRS, the newly formed APHIS program that was created as a result of a restructuring of APHIS's biotechnology regulatory functions in June of 2003. BRS is responsible for the regulation of the import, interstate movement and field-testing of transgenic plants, and is currently evaluating the role it will play in the regulation of transgenic animals and arthropods.

Ms. Smith has worked at APHIS since 1979, playing various roles across four APHIS programs: Plant Protection and Quarantine (PPQ); Biotechnology, Biologics and Environmental Protection (BBEP); Wildlife Services(WS); and BRS. Prior to assuming her leadership role with BRS, she most recently served as associate deputy administrator of Wildlife Services, the federal program protecting agriculture, natural resources, property and humans from wildlife damage.

Smith obtained a masters degree in management from the University of Maryland in 2000, and holds a BS in microbiology, also from the University of Maryland (1983).

Liability Prevention and Biotechnology: A Brief History of Successful Industrial Stewardship

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Agricultural biotechnology's "waves" of grain are not amber, but red, white and green. "Green" applications were the first to arrive: the commercial food- and feed-production side of agricultural biotechnology. "Red" refers to plant-made pharmaceutical applications, whereas "white" refers to industrial applications.

Agricultural biotechnology's "waves" of grain are not amber, but red, white and green. "Green" applications were the first to arrive: the commercial food- and feed-production side of agricultural biotechnology. "Red" refers to plant-made pharmaceutical applications, whereas "white" refers to industrial applications of biotechnology (including biofuels, bioremediation and chemical substitutes (*e.g.*, enzymes that can replace chlorine bleach). Each sector has the potential to provide significant benefits to society, if the risks—including adverse economic impacts—can be managed to the satisfaction of key stakeholders, from farm to fork.

This article sums up the regulatory and liability hurdles that stand in the way of launching new products in each of these categories, beginning with lessons learned from green products. A brief review of successes and failures and existing risk-management methods may help overcome legal barriers to entry. Also discussed are novel barriers to entry posed by risks that may not be compensable, including economic losses incurred by other growers.

These liability risks and the market barriers they create are elusive and hard for American innovators to understand fully and manage effectively. Fortunately, industry-stewardship processes have evolved to anticipate and prevent novel liability risks, including the elusive economic-loss risks. Such stewardship standards also avoid regulatory recalls and help to support a sustained pipeline of productive innovation (Abramson and Carrato, 2001). The largest of the biotech-seed companies have a wealth of experience in identity-preserved production. This allows them to draw upon historical data and experience as they strive to develop stewardship systems that meet modern standards and market expectations for genetic purity.

The Product Pipeline and Regulatory Roadmap

The agricultural biotechnology industry can be visualized as a large oak tree, the trunk of which is made up of the top four crops: soy, corn, canola and cotton. Each of these four interwined trunks has its own history of successes and failures, and each has helped to established biotech crops as essential to modern agricultural production. The environmental benefits of these genetically modified (GM) crops—from soil conservation to reduction in insecticide inputs—are now well documented.

Unfortunately, the tree lost entire limbs after the industry invested significant research funds (over $100 million in some cases) getting product lines ready for commercial launch. While some biotech crops have done remarkably well, there are many more innovative products of agricultural biotechnology that advanced to the verge of commercial launch, only to be shelved pending overseas regulatory approval or resolution of consumer concerns. These other crops lie scattered around the base of the oak like branches blown down by a storm. These product lines, like *Bt* potato, could have brought significant benefits to growers, consumers and the environment. To date, however, plans to revive the GM potato, tomato, wheat, beet, flax, barley, lettuce and other abandoned biotech crops are up in the air.

The challenge facing the agricultural biotechnology industry is to learn from past mistakes and to adjust to realities. While trees will grow in the paths of hurricanes, tropical species evolved roots and trunks that bend better than those of oaks. For agricultural biotechnology to succeed, innovators need to foresee the predictable market forces that may prevent product launch, and design business strategies that meet consumer demand without triggering the barriers erected by regulators or other consumers.

Green Biotech: Steadily Growing Despite "ZAP" Attacks and Traceability

Green-biotech products were first out of the door, paving the way for red and white products. The greenest of the green-biotech crops are the Roundup Ready™ and *Bt* families. The vast majority of soybean growers in the United States and Argentina have embraced Roundup Ready™ soybeans, in what is surely one of the fastest adoptions of new agricultural technology in history. Herbicide-tolerant canola has become the dominant option in Canadian and US fields. *Bt* corn and cotton show similar track records of success, reducing pesticide use and demonstrating their food safety despite the skepticism of activists.

These successful launches of multiple varieties of biotech corn, soy, cotton and canola are the fortunate ones, however. Many other equally useful and innovative crops have been

sidelined due to consumer and food-company queasiness over potential loss of market share. Any food company worth its salt will honor even a seemingly small percentage of concerned and vocal (*i.e.*, "squeaky wheel") customers, for perfectly valid business reasons. A business may not be able to justify losing a 5% share of a branded product's market just to use a lower-cost input that benefits the environment (through reduced insecticide use or soil-conservation benefits). While this corporate marketing decision denies the majority of consumers the choice to support environmentally beneficial, lower-cost biotech crops, such is the stark reality of the modern mass-produced marketplace.

This attention to detail led the biotech industry to build its own system for preventing the development of insect resistance, and to present it to the Environmental Protection Agency (EPA), which then imposed conditions on the growers of *Bt* crops to ensure compliance. For identity preservation, the same conscientious companies can adapt segregation practices, developed in seed production, to produce containment measures that are tailored to various types of agricultural biotechnology. These practices may be adjusted to ensure compliance with various levels of "tolerance" of adventitious presence of undesirable genotypes.

The European Union has only recently lifted a 7-year moratorium on regulatory approval of GM crops, under threat of World Trade Organization action by the United States, Canada and Argentina, who have lost billions in exports of grain (corn and soy) since 1998 due to the European Union's anti-biotech policy. This barrier to marketing of new biotech crops has been lifted only in part, for some varieties of biotech crops.

To date, the first big success in biotech crops—Roundup Ready™ soybean—has proven its worth and gained access in major food and feed markets, but not environmental release. Major markets remain closed to many other biotech genetic events, and troubling moves toward traceability-based testing and associated recalls of unapproved biotech genetic events could make these barriers to entry pervasive and persistent. For example, the export barriers to entry forced soybean growers and processors (represented by the American Soybean Association, United Soybean Board and National Oilseeds Processors Association, "ASA-USB-NOPA") to develop a policy (the "eleven-point plan") that dictates a closed-loop identity-preservation (CLIP) standard for varieties lacking regulatory approval in major export markets. As a result, upcoming *Bt* varieties of soybean (produced under license from Monsanto) can be marketed in the United States only if there is a system that meets CLIP's eleven points.

As a result, *Bt* soybean will have to be submitted to major markets for approval (or be grown in a CLIP system) as long as "zero tolerance," and testing to enforce it, are maintained in the European Union. The United States and its grain-exporting allies have yet to achieve a globally recognized tolerance for adventitious presence of GM products in a world where a zero adventitious presence (ZAP) regulatory import standard (enforced via genetic testing and mandatory disclosure laws) is increasingly prevalent. These policies are spreading to other nations that are key trading partners of the United States, with even more extreme standards emerging. For example, China has adopted "zero tolerance" for GM-food labels, which is even more strict than the European Union's standard of 0.9%.

Through a multilateral environmental agreement that became law on September 11, 2003, the Cartagena Protocol on Biosafety is promoting its "precautionary approach" to regulatory approval of biotech crops as a global standard. Paired with this delay-ridden approval process, the European Union has a ZAP standard for a variety that lacks approval. This is not unusual standing alone; the United States also has zero tolerance for unapproved varieties of biotech crops, but does not mandate disclosure or conduct extensive genetic testing (and rule-making is underway to allow some tolerance for adventitious presence where the variety in the pipeline is ultimately intended for food use).

In contrast to the United States, the European Union has attempted to address tolerances in seed purity, only to find opposition to any number other than zero (*i.e.*, less than the limit of testing). The European Union will use testing centers to track each GM event, forcing destruction of food based on traces of any unwanted DNA (*e.g.*, this is currently occurring with one shipment to Ireland that contains Syngenta's Bt10 corn, which carries a gene for antibiotic resistance that is unapproved for importation by European Union nations.

This testing process and "traceability" for GM events could become a global standard if the parties to the Biosafety Protocol impose, at its next meeting in Brazil (March 13–17, 2006), an international requirement that commodity shipments list all biotech genetic events that they "may contain" (using unique identifiers for each event). This law would spread traceability and labeling for GM content bound for food/feed (known as FFTL in industry email loops) to the parties to that protocol (120 and rising). If these nations are even more concerned about biotech crops than the European Union, they are free to impose even stricter standards than the European Union.

The power of ZAP standards for unapproved biotech crops should not be underestimated, since it empowers activists armed with genetic tests to force recalls of US shipments when they reach port. This is the least business-friendly arrangement imaginable, and it appears tailor-made for activists to rig to their tastes, testing only those corporate shippers whose policies displease them. This testing for GM events is now occurring with shipments of corn seed and feed to the European Union and Japan. Two seed shipments to Japan were found to contain Sygnenta's unapproved variety Bt10. The shipment to Ireland may require disposal of over 4,000 tonnes of corn-gluten feed.

In sum, the European Union is not alone in its quest to label GM food and trace biotech crops globally, and the regulatory environment worldwide could be taking a distinct turn toward anti-biotech policies among US trading partners. The best example of this troubling trend may be China, which has its own thriving biotech research industry with hundreds of crops in the pipeline (and millions of GM poplar trees lining its rain-ravaged hillsides). China has bowed to the European Union and imposed a GM-food labeling standard. To complement this standard, China has legalized only commercial production of biotech cotton (non-food) despite pressing food-security needs (and despite reports of growers using pirated GM rice and corn in violation of its laws). These global trends are increasingly raising barriers to importation of GM crops, leaving some markets (like wheat and rice) without the benefits that come from biotech innovation.

Corn and rice are particularly suitable for production of proteins for pharmaceutical applications, given the relative ease of storing, transporting and refining the protein.

"Red" Plant-Made Pharmaceuticals

Red biotechnology—plant-made pharmaceuticals (PMPs)—is necessary, according to pharmaceutical industry analysts, to meet increasing demands for protein-based large-molecule drugs that can be produced only in living organisms. Shortages of certain new large-molecule drugs, which are generally produced in cell cultures, have led biotech companies to explore new production methods. Corn and rice are particularly suitable for production of proteins for pharmaceutical applications, given the relative ease of storing, transporting and refining the protein.

The cost of maintaining compliance with "zero-tolerance contamination requirements" has led some observers to wonder whether "the economic payoffs from growing pharmaceutical plants outweigh the costs associated with the risk of food contamination." (Elbehri, 2004). To weigh those costs in advance of creating a new PMP, the researcher needs to obtain data on identity-preservation costs, third-party oversight of the process, insurance coverage and other known expenses necessary to manage risk.

In general, the costs of insurance and third-party oversight for the production process will be hard to define. To the extent that these costs are part of a successful risk-management strategy, however, estimates should be incorporated into long-range planning. The comparative costs for different regions (with varying risks) should be factored into analysis of the feasibility of marketing and probable return on investment.

White Biotechnology—Industrial Biotech Comes of Age

White biotechnology is defined as the industrial use of GM crops or microorganisms (*e.g.* bacteria, fungi) to create enzymes, proteins and other industrial compounds and materials. Industrial biotechnology is creating compounds that can replace of hazardous chemicals, providing "greening" companies with new options for reducing hazardous waste. In one remarkable instance of industry reaping corporate value and environmental benefit from biodiversity, Diversa of San Diego has taken a gene from a thermophilic bacterium that encodes an enzyme allowing paper-manufacturing companies to avoid using tons of toxic chlorine bleach (Hessler, 2005).

When a plant is used as the source, rather than a microorganism, a "plant made industrial product" (PMIP) results. If the PMIP crop *per se* has significant export or consumer markets, the PMIP may encounter opposition at commercial launch. Concern has been expressed that PMIPS pose the same threat as PMPs, but do not have the same level of regulatory oversight. The PMIP has been cast as a neglected stepsister on issues like pollen drift, creating what appears to be a liability "bullseye." This gap in understanding of pollen containment by regulators represents a threat to the food supply, since PMIPS that

are not approved for use in food and could be declared an "adulterants" by the FDA, or trigger export losses upon detection by European-Union or other overseas officials. These liability risks were pointed out to USDA by the American Soybean Association and the regulatory gaps were closed.

Identity preservation of the crop used to produce a PMIP is necessary to deliver a pure product, and is equally essential to prevent the undesirable release of a PMIP in a manner that could lead to cataclysmic economic impacts.

Nuisance Claims by Neighboring Growers

Post-StarLink™ case law could allow neighboring growers to recover their economic losses from the source company, if they prove actual commingling of GM variety unapproved in the European Union [see, *In re StarLink Corn Products Liability Litigation, Marvin Kramer v. Aventis CropScience USA Holding Inc. (*2002), 212 F. Supp. 2d 828 (U.S. District Court, N.D. Illinois)].

StarLink™ was cited in a recent Canadian court decision denying a class action to organic growers who sought recovery of their economic losses from the marketing of GM canola varieties unapproved in the European Union [*Hoffman v. Monsanto*, 2005 SKQB 225 (2005)]. This action was filed against Monsanto and Bayer Crop Sciences (BCS) seeking to enjoin the marketing of Roundup Ready® wheat, and also adjudicate liability for price impacts to canola based on "contamination" of organic and non-GM canola that could not be exported by Canadian farmers to the European Union. The canola sold by Monsanto and BCS was fully approved in Canada and posed no known health or environmental risks. The *Hoffman* court denied plaintiffs the class action they sought, but hinted at recognition of claims for violations of environmental statutes (if canola is deemed a "pollutant") (http://204.83.249.88/judgments/2005/QB2005/ 2005SKQB225.pdf).

The *Hoffman* court confirmed the basic idea that "pure economic loss" is not recoverable, holding that the facts did not present a situation allowing a claim for recovery of "pure economic loss" (with no "physical injury"), citing various policy reasons (2005 SKQB 225 at ¶ 80). The court also rejected the idea that defendants committed a "negligent undertaking" when they initiated identity preservation to preserve canola exports to Japan, then dropped that program when they received approval in Japan (even though the European market was still closed to any canola that was still commingled). While the plaintiffs still have a nuisance claim the court is willing to entertain, it remains to be seen how that action will play out (*i.e.*, the plaintiffs may run into the same barrier that the US District Court in Eastern Missouri imposed in *Monsanto v. Sample*, if they cannot prove a "physical injury" from actual commingling of the GM canola with their export-bound crops). With two causes of actions surviving, but no class certified, this decision (if not overturned on appeal) could lead to a flurry of individual filings if Saskatchewan's certified organic farmers are willing to take Monsanto and BCS to court.

Secrecy of Field Trials

February 2005 saw another landmark court decision relating to identity preservation of biotech crops. District Court Judge David A. Ezra rendered his final decision ordering

disclosure of PMP and PMIP field trials [*Center for Food Safety v. Veneman, No. 03-CV-621* (D. Haw, filed Nov. 12, 2003)]. This Hawaii federal court ordered representatives of the USDA to hand over to Earthjustice attorneys the precise locations of open-air field tests of PMP crops. This was the first time the federal government was forced to disclose the location of field tests of GM crops. Earthjustice, representing citizen groups Center for Food Safety, Friends of the Earth, Pesticide Action Network North America, and KAHEA (The Hawaiian-Environmental Alliance) filed the lawsuit to compel USDA to review the environmental and public-health impacts of such activities. In August, 2004, district court ordered the disclosure, rejecting the claims by the government and the Biotechnology Industry Organization (BIO) of potential "espionage," "vandalism," and "civil unrest." The Court denied USDA and industry's motion for a stay of disclosure, and the government handed the information to plaintiffs' counsel.

The court has not yet ruled on the public-disclosure issue, however, and plaintiffs cannot reveal the information to the general public. The disclosure should allow plaintiffs to pursue their original objective of seeking environmental reviews that will determine "how close these experiments are to conventional food crops" and ecologically sensitive areas [see Government Forced to Disclose Locations of Test Sites of Biopharmaceutical Crops (USA). http://biotech. dnsalias.net/or/2005/02/3912.shtml].

Identity Preservation 101: The Legal Tools

Where a company marketing a PMIP or PMP will encounter concern about liability risks, including adverse economic impacts from unwanted commingling, it can use identity-preservation measures to reassure domestic food businesses, exporters, and overseas importers. The use of the approved identity-preservation system will ensure that the PMIP is not commingled with food or grain exports.

The processes for identity preservation and seed-purity assurance are rapidly evolving to meet the demands of the market.

Identity preservation of commodity grain crops to meet specialized customer needs has a long and successful history in seed production. The production of seeds generally operates on tolerances for unwanted input of various types, including genetic off-types. Historically, this posed no problem of commercial significance; corn out-crossed freely in commercial production. In today's world, however, any corn that is bound for export must be "channelled" to particular elevators. One stray corn kernel that lacks regulatory approval in an overseas market can lead to destruction of an entire cargo, where the standard for commingling is "zero tolerance" and a trace of an unapproved variety is found (as occurred in 2005 when the Syngenta Bt10 variety was detected in four separate shipments of US-origin corn). As a result, the processes for identity preservation and seed-purity assurance are rapidly evolving to meet the demands of the market.

Industry Standards

Innovation in agricultural biotechnology begins with the novel steps that lead to an invention meriting a patent; however, the path to market requires sound agricultural management. Industry standards for identity preservation provide a biotech-seed company ("the Company") with a standard of care to follow that meets both quality-control and liability-prevention needs. To protect the Company's investment in innovation, the developer of a new application using agricultural biotechnology, such as a PMP or PMIP, should adapt existing standards to create detailed methods for stewardship in the production process. Stewardship methods for the agricultural management of biotech crops vary with the crop and the location of the production process.

The simplest route to maintaining identity preservation is to anticipate the demands of customers and regulatory agencies, develop an industry standard and stick to it. Industry organizations, led by BIO, developed the Confinement Analysis and Critical Control Point (CACCP) concept for molecular farming applications including ProdiGene's corn-produced vaccine for piglets, and PMIPs (Phillips, 2004).

A sound model for identity preservation of PMPs and PMIPs was generated by the Canadian Food Inspection Agency (CFIA, 2004). After reviewing various methods for identity preservation, the CFIA recommended, in its January 2004 report, eleven elements of confinement systems for "molecular farming crops" (*i.e.* PMPs and PMIPs). This paper is patterned after the CACCP system developed by BIO, and it mandates supervision by a third party, preferably regulators. One item that is missing, in comparison to the ASA/USB/NOPA "eleven point plan" is the express assumption of liability for system failure attributable to the biotech-seed company.

Identity preservation methods have been developed in consultation with growers and grain handlers. The ASA/USB/NOPA CLIP process has been used since 1998 to protect US-export flows of soybeans to the European Union and other major markets, while allowing limited releases of new biotech soybeans. The ASA/USB/NOPA "eleven point plan" for the CLIP process requires the biotech-seed company to assume liability for system failure. This generally precludes the company from using contractual clauses that unfairly shift to growers all the risk of commingling. Properly and fairly operated, such systems for identity preservation will continue to provide grower and biotech-seed companies with protection from liability lawsuits.

Similarly, the National Corn Growers has developed the "Know Before You Grow" process for identity preservation of corn-gluten feed that is bound for export. While exports of whole corn to the European Union have been foreclosed since 1997 by the commingling of GM events that lacked regulatory approval, recent efforts to comply with the European Union's new traceability directives (effective 4/18/04) have succeeded in keeping the $400 million per year in corn-gluten feed flowing to the European Union from the United States.

Identity-preservation methodology has been developed through trial and error, as major life-sciences companies developed their stewardship programs in consultation with growers associations. The ASA/USB/NOPA CLIP process has been applied for the

production of DuPont's high oleic soybean, which is used in specialized biodegradable lubricant applications. This same eleven-point plan was previously presented in 1998 to AgrEvo USA, the corporate predecessor of Aventis Crop Sciences, Inc., which adopted it for a Liberty Link® soybean stewardship program (which soybean was not, however, marketed in the United States, in contrast to the high oleic cultivar).

Under the CLIP system, the first level of concern involves the terms of the contract for sale of the biotech seed. The contract with the grower of the specialty PMP or PMIP crop should have conditions similar to those for certified seed production or federally permitted field trials, including regular inspections and scientifically defensible minimum isolation distances from neighboring crops. The contract should also guarantee the grower a premium adequate to cover the costs of preventing commingling with other crops (field isolation and inspection requirements can be costly for the grower). Only contracted growers should be allowed to grow the specialty crop.

The second level concerns planning to coordinate the harvest process. Growers need training to ensure that combines and transport vehicles do not cause commingling and are cleaned out to industry standards. The Company needs to identify elevators where there is willingness to accept the identity-preserved production while keeping it completely separate from all other commodities. These elevators should be confined to particular regions, not widely scattered throughout the farm belt. Coordination of inspections between the elevator and the field inspectors will allow the midseason yield estimates to be matched to the actual delivery, to ensure that the entire crop is delivered and not diverted to other uses.

Thirdly, the CLIP system requires that the Company contract with a third party to certify the process. There are seed-certifying agencies that have conducted such audits for decades [*e.g.*, the Association of Official Seed Certifying Agencies (http://www.aosca.org) and newer specialized operations such as Novecta (http://www.novecta.com) and the United States Department of Agriculture's Process Verified Program (http://processverified.usda.gov)].

Lastly, the CLIP system requires that the Company agrees to assume—and not attempt to disclaim or limit—the legal and financial liability that arises from negligence or other breaches. Under the ASA/USB/NOPA CLIP system, the focus is upon crops that may lead to lost trade with overseas soybean export markets. Commingling of an unapproved variety, in particular a PMIP or PMP variety that is not approved for any food or feed use at any tolerance, can lead to cataclysmic economic loss in major export crops.

These systems have not worked perfectly, as the StarLink™ corn recall and ProdiGene commingling incidents illustrate. In the StarLink™ episode, Aventis Crop Sciences had been warned of the potentially "cataclysmic" economic impacts that existing US precedent pointed toward (Censky, 1999). Despite this warning, Aventis sold StarLink™ corn with inadequate stewardship. Along the same lines, tiny ProdiGene's sprouting corn volunteers contaminated a soybean field, despite a USDA inspector's warning. This commingling incident ended at one elevator, but still required an interest-free loan from USDA to maintain a viable business.

Detecting Gaps in Regulation and Stewardship

The practice of identifying and managing risks adequately is not a perfect process, and troublesome gaps have emerged in regulation of particular aspects of biotechnology. One example in USDA regulation involves setback distances to preclude pollen drift from PMP plants. This glaring gap in regulatory oversight was apparent to anyone comparing the planting distances in the regulations, and it was quickly corrected through the timely intervention of the American Soybean Association. Had this gap not been corrected, the overview of a "bullseye" made a target for plaintiffs' attorneys that exposed a failure to exercise "due care" in the segregation of crops.

This "biotech bullseye" episode provides a "near miss" in liability law, which helped to prevent serious commingling with PMPs. It also provides confirmation that the existing stewardship practices of the most responsible seed companies (Monsanto, DuPont, *etc*.) were more alert to the risks of out-crossing from PMP plants than were USDA regulators. The seed industry had already determined through testing that a 1-mile planting distance is required to maintain a very low tolerance for out-crossing in corn production. These seed companies foresaw the need for stewardship standards that track potential liability risks, including those that might elude a busy regulatory agency.

This does not mean that USDA is incompetent in terms of managing risk, but merely highlights the need to supplement regulatory review with industry oversight. It is a basic principle of product-liability law that even the most proactive regulatory authority can only set a "one size fits all" minimum, based on limited knowledge of the product, while the alert company is expected to know its product and foresee its hazards with more precision.

ISO 9000 and ISO 14000 are process standards that can be used to identify customer needs and product hazards, and to implement processes to prevent both product-related (under ISO 9000) and environmental liability (under ISO 14000). Biotech-seed companies may use these standards or a modified system that is not certified by an ISO registrar but still provides necessary oversight that regulation cannot impart.

The US Supreme Court recently affirmed the role of industry in post-market surveillance for product risks. In *Bates v. Dow* (2005), the Court put all pesticide manufacturers on notice of the power of tort law to keep them alert to new risks that emerge over time, which may elude the detection of regulators. This landmark decision expands the horizon of biotech-seed-company liability for certain EPA-registered crops, and creates a feedback loop, as described by Justice Stevens, requiring companies to adjust their practices to avoid the adverse event that triggered the state's tort law.

Companies that do not quickly react to feedback can find their markets disappearing due to consumer opposition. In the PMP setting, this occurred in the ProdiGene case; failure to remove volunteer PMP corn from a soybean field led to a $3 million loss of the elevator's commingled contents. Subsequently, the biotech industry implemented additional safeguards and regulatory agencies reviewed inspection policies.

However, policy positions of major players in the chain of commerce shifted to a more anti-biotechnology position after StarLink™ (with help from the ProdiGene-PMP commingling event). The Grocery Manufacturers of America (GMA) began insisting on

abandoning any use of food crops for PMP production, whereas grower and grain associations merely insisted on the use of well tested closed-loop identity preservation. GMA's position was not irrational or poorly considered; its board is made up of CEOs whose judgment is well informed. This position reflected a loss of trust from a critical group of customers—the primary buyers of "green" biotechnology products had lost faith in the ability of the makers of "white" and "red" products to maintain necessary segregation in production. Winning back trust, once lost, is much more difficult than the effort required not to breach that trust in the first instance.

There are hazards from food that are heightened by anti-biotech regulations that deny consumers access to use of the best available technologies for controlling carcinogenic mycotoxins in their staple food supply..

Will a Liability Backlash Follow the European Union's Precautionary Approach?

Two potential backlashes are created by the European Union's ZAP approach to GM crops. First and foremost, there are hazards from food that are heightened by anti-biotech regulations that deny consumers access to use of the best available technologies for controlling carcinogenic mycotoxins in their staple food supply. These toxins, which can be better controlled with use of *Bt* corn, are not a hazard of significance in the United States or the European Union (which have resources to detect it and avoid exposure). However, they pose a significant risk to the health of mothers and children in Africa, Central America and Mexico where less-varied diets lead to higher corn consumption in farming communities.

The European Union has implemented both GM labeling and ZAP regulatory approval by invoking the "precautionary principle," an approach to regulatory approval that would consider the presence of antibiotic-resistance genes in corn-gluten feed cause for concern and, paradoxically, would mandate the destruction of said feed. This would apply even if the destroyed feed would be healthier than the alternative due to lower mycotoxin levels.

Although liability laws dictate the use of the best available technology to avoid feeding carcinogens to children and pregnant women, the regulatory environment instituted by the European Union to meet "collective preferences" operates to ban any trace of this best available technology—setting up a future where liability could apply even if the company in question were to assert as a defense in court, "The European Union made me do it." The law in the European Union and the United States may provide a presumption of reasonable behavior based on regulatory requirements, but it can be overcome by a tort theory stating that there was a risk that required a warning or could be implemented at minimal cost (in the case of biotech crops, the cost of using them could be lower).

The anti-biotech activists who run the show in Germany have succeeded in creating their own innovative approach to liability, which helps to close the door to any grower who might attempt to use the "best available technology" for mycotoxin-insecticide reduction (the European Union has approved some varieties of *Bt* corn). German growers of biotech crops are discouraged by a GM liability law that forces them to avoid any commingling with other growers, who may have agreed to supply non-GM products.

The concept of protecting the non-GM grower's contractual promise of "zero" is also emerging in the negotiations of the Biosafety Protocol's liability regime, which is now underway with hopes of producing a text by September 11, 2007, for approval by the parties to the Biosafety Protocol. Activists have suggested innovations in liability law that would:

- reverse the burden of proof to the GM-crop grower or grain exporter,
- protect the economic loss of the non-GM grower (who may be harboring a reservoir of "biodiversity" in his choice of seed), and
- place the ultimate blame on the company that developed the biotech seed, since the GM event that "harmed" the non-GM grower can be traced.

The seed of anti-biotech liability law now sprouting in Germany could have a broad dispersal if the parties to the Biosafety Protocol do not come to their senses and reject such standards.

With member states free to impose their own liability regimes like this one, and balk at European Union-wide regulatory approval, the European Union's misguided anti-biotech-innovation policy could take decades—or an entire century—to reverse, even if it is shown without dispute to have caused well documented harm to human health and the environment. To ensure that the costs of the European Union's precautionary approach and ZAP testing are counted, foundations that care about neglected populations of people (*e.g.*, African refugees who are denied food aid based on traces of locally unapproved GM events) and neglected plants (*e.g.*, wild and indigenous soybean, rice and other crops) need to stand up to the challenge of tracking the harm that can be caused by misguided regulation.

While the US federal regulatory system is streamlined and efficiently operating to manage risks, there are emerging roles for states, counties and cities to react to authorities that seek to protect economic interests and social, cultural or indigenous concerns.

State Law Barriers and Tools for Marketing Biotech Crops

While the US federal regulatory system is streamlined and efficiently operating to manage risks, there are emerging roles for states, counties and cities to react to authorities that seek to protect economic interests and social, cultural or indigenous concerns. These local

authorities may be reacting to international opposition that limits markets from particular states. In response to California's rice industry, a bill was passed in 2000, Assembly Bill 2622, that licensed all rice for its economic impacts—specifically addressing the threat that biotech rice poses to export markets; Arkansas passed a similar law in March 11, 2005, that gives the Arkansas State Plant Board the power to regulate "commercial impact" of rice commingling—without specifically mentioning biotech rice (HB 2574, 3/11/05).

For example, Iowa has passed a law precluding any county from declaring itself GM free (a reaction, no doubt, to the three California counties that went "GM free" in 2004). Iowa grower Bill Horan just reported that production of a PMP will finally be allowed to occur in Iowa, at a military base over a mile from other corn production. The Iowa experiment now underway will provide more data on the cost-effectiveness of growing pharmaceutical corn in the nation's largest corn-producing state.

The story of Ventria's rice provides a narrative that touches upon the entire range of regulatory and liability issues facing new PMPs launched in the United States, and the key stakeholders in the commercial launch of a PMP. Ventria started out trying for state-level regulatory approval in California. While the California Rice Commission approved Ventria's application, leaving it up to the state Department of Agriculture to decide, the announcement that a public hearing would be held resulted in Ventria's choosing to relocate its operations to Missouri, giving up on California approval.

Missouri proved inhospitable to Ventria as well, as major food producers who use Missouri and Arkansas rice expressed concern about commingling. Press reports indicated that a counterseasonal South American site may be next in line, or a small plot in North Carolina.

If the agricultural biotechnology industry is going to meet customer expectations for segregation of the green, white and red, it may need to work with growers to create districts that establish identity preservation as a matter of civic law.

One little known legal aspect of the Ventria story is the unused Missouri "grower district" statute that was enacted in late 2004 (effective January 1, 2005) with Ventria in mind (but not used by Ventria) to line up growers in a dedicated region (a solution that might have reassured rice interests opposed to Ventria's plans). A future PMP- or PMIP-production system can avail itself of this tool, however. This concept of a grower district appears in a new Missouri statute that was passed in 2004, around the time frame when Ventria moved its operations from California to Missouri in early 2005. If the agricultural biotechnology industry is going to meet customer expectations for segregation of the green, white and red, it may need to work with growers to create districts that establish identity preservation as a matter of civic law.

Other novel agricultural technologies have arrived on the scene with similar segregation issues. Canola only recently became an edible food product by innovations adjusting rapeseed's nutritional profile. The canola industry uses a variety of production methods to maintain the necessary segregation, including grower districts in Idaho and Washington.

Another angle on PMP production that is under review is simply to go underground in the farm belt. This allows production to occur close to existing processing centers in the midwest (*e.g.* Sigma Aldrich in St. Louis, MO), while avoiding the controversy and exposure to eco-terrorism that can occur aboveground. Like any innovative step, however, this option will have to prove its economic worth before innovators will adopt it.

Conclusion

Despite past successes, and the knowledge gained from failures and near misses, the road to future commercial success in agricultural biotechnology remains as full of hidden economic hazards as the road to the Baghdad airport. The European Union and its like-minded trading partners will continue to hold their ZAP standards over the heads of grain exporters and will increasingly drive innovation in agricultural biotechnology into contained, closed-loop production systems. Like a game of three-dimensional chess, biotech crops will face three levels of regulatory oversight, starting with federal approvals, but with more requirements emerging at the state level (even in counties and/or cities), and overseas. For many biotech crops, international approvals that may be required prior to market launch in the United States (for soybean, rice, wheat and other primarily export-bound crops). As a result, US agricultural biotechnology operations will need to maintain perfect divisions between the green, white and red sectors—as neatly divided in the fields of America as the stripes in the Italian or Irish flags, which do not blur their colors together.

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From 2000 to 2002, he chaired the Agricultural Management Committee for the American Bar Association's Section on Environment, Energy and Resources (ABA SEER) and is vice-chair for that Section's Environmental Litigation and Toxic Torts Committee. He has represented the American Soybean Association on liability prevention programs for agricultural biotechnology since 1998, and has advised various clients in the food-production and grain-handling industries on liability risks arising from biotech crops (including the Cartagena Protocol on Biosafety). He is the founding chair of the International Biotechnology Regulation Roundtable hosted by ABA SEER, Croplife America and the Council for Agricultural Science and Technology (summarized at http://www.cast-science.org/roundtable.htm).

Mr. Redick has been honored several times for his *pro bono* work in political asylum appeals and guardianship petitions for abused and abandoned children.

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Biological Confinement of GEOs: Opportunities for Reducing Environmental Risks?

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With the introductions of genetically engineered organisms (GEOs) into the environment over the past 20 years, there has been a growing number of questions and concerns about consequences for natural and managed ecosystems. An emerging body of research evidence shows that GEOs are viable and capable of reproduction with wild relatives in natural ecosystems. There is also evidence that transgenes can move from one domesticated variety to another. Also, given the fact that future GEOs may feature traits that have the potential to significantly modify the ecological niche that these organisms occupy, there has been interest in developing methods and approaches to confine certain GEOs and their transgenes to specifically designated areas. Of the various methods available to confine GEOs, those that are biological in nature are of particular interest. These include induced sterility and related methods—approaches that, in some cases, have been applied to non-engineered organisms such as shellfish and crop plants.

The US Department of Agriculture requested the National Academies to review and evaluate biological methods of confinement for GEOs.

In 2000, the federal government completed an interagency review of its regulatory oversight of biotechnology products. This review revealed that ensuring confinement could become a regulatory requirement for approval of some GEOs. In 2001, the US Department of Agriculture requested the National Academies to review and evaluate biological methods of confinement for GEOs and report on their application in confining transgenic crop plants, shellfish, trees, grasses, fish, microbes, insects and other organisms. This paper summarizes that report (*Biological Confinement of Genetically Engineered Organisms*) with the hope that biological confinement methods for current and future GEOs will be given adequate consideration as mechanisms to reduce environmental risk.

The expert committee convened by the National Academies was asked to address a set of six questions:

- What is the status of scientific understanding about various biological confinement methods for genetically engineered organisms?
- What methods are available, and how feasible, effective, and costly are they?
- What do we know about when and why methods fail, and what can be done to mitigate such failures?
- When these methods are used in large-scale applications, what detection and culling procedures can be used if the biological confinement methods have failed? What is the cost-effectiveness of these procedures?
- What are the probable ecological consequences of large-scale use of biological confinement methods on wild populations, biological communities and landscapes?
- What new data and knowledge are required for addressing any of these important questions?

The report is organized into six chapters. The introductory chapter provides definitions and historical context for GEO confinement. The second chapter addresses the questions of when and why biological confinement ("bioconfinement") should be considered. Chapters 3, 4 and 5 are the heart of the report: bioconfinement methods for plants, animals, and microbes are analyzed and reviewed. The final chapter explores biological and operational opportunities and constraints for bioconfinement, examines the potential for confinement failure and mitigation, and looks to the future in terms of unanswered research questions and needs that should be addressed in order for bioconfinement methods for GEOs to be successful.

Definition of Bioconfinement

There is a fundamental assumption in the report that the movement of GEOs or their genetic material might need to be restricted to designated areas, which in turn creates the need for confinement. Three types of confinement are defined—physical, physicochemical and biological—each involving barriers that prevent the survival, spread, or reproduction of the organism in the natural environment. Bioconfinement refers to the methods that utilize biological mechanisms to achieve confinement.

Many, if not most, GEOs will not require bioconfinement.

When and Why to Consider Bioconfinement?

The report repeatedly acknowledges that many, if not most, GEOs will not require bioconfinement. Each should be determined on a case-by-case basis that takes into consideration the risk associated with the escape of the GEO or its transgenes. The most commonly known environmental risk is that of GE crop alleles being sexually transferred to wild

relatives and conferring new traits that result in increased weediness. Other risks include effects of a GEO on non-target species (including humans). These range from potential food-safety concerns associated with crop plants engineered with genes expressing novel proteins not intended for the food chain to GE animals out-competing or driving related and locally rare taxa into extinction.

The report notes that researchers and GEO developers need to consider the role of preventative action; in other words, are there options that might eliminate or mitigate the need for bioconfinement or that may also prevent failure of a given confinement effort? Such an assessment often presents a greater set of options in contrast to the often higher expense of remedial action. The question of "How much bioconfinement is enough?" must also be asked; an appropriate risk assessment, exploring the need for stringency and redundancy, will be influenced by the risks posed by the organism, its biology, the transgenes and other factors.

The consequences of failure of bioconfinement are varied and are difficult to determine in advance. Potential consequences include negative ecological impacts at local to regional scales, and political impacts in instances where bioconfinement failure results in significant impacts for human or environmental health, followed by public outcry or concerns that the failure was due to negligence or inadequate regulatory oversight.

Plants

Many approaches are possible for the bioconfinement of plants, due in large part to the diversity of reproductive strategies they employ. Of the methods discussed in the report, a few are based on existing agronomic and horticultural practices. Other methods are newly developed and untested or are merely working hypotheses. An evaluation of the strengths and weaknesses is included in the discussion of each method. As a whole, the methods typically target sexual and/or vegetative reproduction.

The report also evaluates these methods for their effectiveness at different special and temporal scales, given that they are the equivalent to natural mechanisms of reproductive isolation that act to maintain species barriers. In nearly every instance, bioconfinement of reduced numbers of GE plants planted in smaller regions for shorter periods of time is likely to be more successful than efforts with larger numbers planted over larger areas and when confinement needs to be maintained for longer periods of time.

These issues become more important as genetic engineering is applied to new types of plants, including long-lived species such as trees. Furthermore, new types of GE traits are being developed including traits that produce medicinal and chemical precursors for such products as vaccines and natural rubber. Our ability to combine multiple GE traits that make plants hardier and more prolific also presents potential environmental risks that might warrant bioconfinement.

Another concern involves the level of public acceptance of sterile seeds in staple food crops.

Overall, the outlook for confining plants is positive. When recommended methods work as planned, there is no reason to expect environmental problems. However, the report acknowledges that nearly all of the methods have limitations, mostly in the areas of availability and reliability. This is particularly true for long-lived, clonal plants. Another concern that might impact the use of bioconfinement involves the level of public acceptance of sterile seeds in staple food crops. This issue was raised some years ago with the public perception of "terminator" technology that a company considered using to protect their technology and investment.

Animals

For the discussion of bioconfinement of animals, the report limited its focus to insects and aquatic species, in large part because these two categories are active areas in current GE research and development efforts. Consequently, they are likely to be among the early GE products considered for commercialization. Finally, the potential for negative environmental effects from confinement failure is much higher relative to terrestrial livestock species. Of the methods evaluated, the best understood systems are those applied to fish and shellfish species, due to the success of aquacultural programs employed around the world. These methods focus on disrupting sexual reproduction by a trio of approaches:

- sterilization through induction of triploidy;
- combination of triploidy with monosex lines; and
- interspecific hybrids alone or with triploidy.

In most instances, successful bioconfinement relies on the ecological characteristics of the GEO and the production site to reduce escape.

The best developed methods for animals involve the induction of sterility. However, no method is 100% effective, and the success of any method relies heavily on effective screening for failures prior to the release of the GE animals. Some early data involving GE salmon reveal that a high level of screening for triploidy appeared to be cost-effective. As for transgenic methods of confinement, the report acknowledges that they are at very early stages of research, so much needs to be understood before such approaches become commercially viable. One lesson from current aquaculture is that it is very difficult to monitor for failures after commercial release. Overall, animals pose some unique challenges for confinement, but given the level of experience gained from aquaculture, our understanding of bioconfinement methods may be more advanced for GE animals than for the other taxa discussed in the report.

Considerable caution is warranted since relatively little is known of the ecology and evolution of GE microbes.

Microorganisms

The use of GE microbes offers significant potential benefits, given that viruses, bacteria, and fungi are pathogens of insects and of a variety of other pests. Microbes are also capable of

degrading certain pollutants. With genetic engineering, this capability could be extended to a broad list of toxins and pollutants in the environment. However, considerable caution is warranted since relatively little is known of the ecology and evolution of GE microbes. Furthermore, since reproduction is asexual in bacteria and often clonal in viruses, the methods developed for inducing sterility in plant and animals are not appropriate for many microbial species.

There are two major categories of bioconfinement methods. One focuses on "fitness reduction" for microbes. With "phenotypic handicapping," the energy costs of expressing the GE trait causes a loss in competitive ability relative to naturally occurring microbes in the environment. The success of this confinement effort depends on the persistence of the GE-microbe population under such a handicap. However, a limitation with this method is the fact that microbes rapidly reproduce and can mutate, so subsequent generations may be more adapted to the environmental conditions and may be capable of coexistence.

The second category, "suicide" genes, is oriented to confining the GE microbes in the wild. The mechanism involves the GEO carrying a suicide gene that is repressed while the microbe is "working"—for example metabolizing a pollutant in a lake—and is activated when the microbe is no longer metabolizing the chemical in question, resulting in programmed death.

One reality regarding GE microbes must be acknowleged. It is virtually impossible at this time to completely eliminate specific genotypes (GE genotypes for this discussion) in natural populations of microbes. This needs to be considered when deciding whether a GE microbe should be released into the environment.

Biological and Operational Considerations

The bioconfinement methods characterized for plants, animals, and microbes share three features:

- all methods have strengths and weaknesses;
- all vary in efficacy depending on circumstances; and
- no method will achieve 100% confinement.

As noted earlier, in many cases GEOs will not require bioconfinement. For all three taxa the efficacy of bioconfinement will depend on the organism, the environment, and the temporal and spatial scales over which the organism is introduced.

Report Recommendations

Given these shared features of bioconfinement methods, the report provides a number of recommendations (in italics):

- *Evaluation of the need for bioconfinement should be considered for each GEO separately.*

The report emphasizes making biosafety a primary goal from the start of developing any new GEO. This will be an efficient and effective way to prevent safety failures.

- *The need for bioconfinement should be evaluated in the early stages of development of a GEO or its products.*

Because methods can fail, and because it is unlikely that 100% confinement will be achieved by a single method, it is thought that redundancy in confinement methodology will decrease the probability of failing to attain the desired result. It is also understood that the spatial or temporal scale of a GEO field release can influence the potential for confinement failure. The appropriate confinement option will depend on scale. Therefore,

- *Bioconfinement techniques should be assessed with reference to the temporal and spatial scales of field release.*

The question of "How much bioconfinement is enough?" is challenging to answer with most GEOs, but with a systematic risk assessment and management approach,

- *An adequate level of bioconfinement should be defined early in the development of a GEO, after considering worst-case scenarios and the probability of their occurrence.*

Following the decision to develop a new GEO, after a risk assessment, and the research to validate the assessment,

- *An integrated confinement system (ICS) approach should be used in deployment of the GEO.*

The ICS includes a number of features familiar to those who use best-management practices in the workplace. The recommended ICS approach includes

- Commitment to confinement by senior decision-makers
- Establishment of a written plan for redundant confinement measures to be implemented, including documentation, monitoring, and remediation
- Training of employees
- Dedication of permanent staff to maintain continuity
- Use of good management practices for applying confinement measures
- Periodic audits by an independent entity to ensure that all elements are in place and working well
- Periodic internal review and adjustment to permit adaptive management of the system in light of lessons learned
- Reporting to an appropriate regulatory body

Looking Ahead

Much of the report focuses on the front end of the process where determining whether, what kind and how much bioconfinement is needed. There is also the need to follow up once a bioconfinement strategy has been deployed with a GEO. Given the relative inexperience we have with GEOs and the deployment of confinement methods in the environment, the efficacy of the confinement system must be monitored. However, this is where our current knowledge is lacking and where our needs are perhaps greatest.

- *Easily identifiable markers, sampling strategies, and other methods should be developed to facilitate environmental monitoring of GEOs.*

Current lack of quality data and science is the single most significant factor limiting our ability to assess effective bioconfinement methods.

The fact is, the current lack of quality data and science is the single most significant factor limiting our ability to assess effective bioconfinement methods. Methods need to be tested in a variety of appropriate environments and in representative genotypes of the GEO under consideration. In order to implement effective bioconfinement of GEOs, the report recommends support for additional scientific research that

- *Characterizes the potential ecological risks and consequences of a failure of bioconfinement*
- *Develops reliable, safe, and environmentally sound bioconfinement methods, especially for GEOs used in pharmaceutical production*
- *Designs methods for accurate assessment of the efficacy of bioconfinement*
- *Integrates the economic, legal, ethical, and social factors that might influence the application and regulation of specific methods*
- *Models the dispersal biology of organisms targeted for genetic engineering and release, where sufficient information does not exist.*

The objectives for this and any other research on bioconfinement are to minimize the risk or damage to human and environmental health. The success of these efforts will do much to bolster public confidence in the continued growth, development, and opportunities presented by biotechnology.

Further Reading

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Kim Waddell is executive director of the American Vineyard foundation, an educational entitiy that supports research in viticulture and enology for the US wine industry. Before coming to AVF and Napa, Dr. Waddell spent 4 years at the National Academies in Washington, DC, as director for a series of research and policy studies on agricultural biotechnology, pesticides, Pierce's disease, and related subjects.

An ecologist by training, he completed his undergraduate degree in agroecology at the University of California at Santa Cruz in 1990 and his PhD in biological sciences at the University of South Carolina in 1996. He then did postdoctoral research in entomology and taught at the University of Maryland, College Park.

Prior to these academic pursuits, Waddell spent 15 years as a waiter, wine steward, and wine buyer for a number of restaurants throughout the Caribbean and in the San Francisco bay area. He still enjoys good food and wine and is excited to be living on a small farm in the Napa wine country.

Panel Discussion and Q&A

Moderator:
Randy Weckman
University of Kentucky
Lexington, KY

Panelists:
Thomas Hoban
North Carolina State University
Raleigh, NC

Canice Nolan
European Commission to the United States
Washington, DC

Allan Bennett
University of California
Davis, CA

Thomas Hoban: It's fun for me to come back here and talk to you again. I've been on a number of NABC programs over the years and have had some of the papers published. I am back this time as a disinterested observer because I'm really not involved in biotech much anymore. I've moved on to bigger and better things.

So, let me reflect on some of the issues I see coming along. First of all, I'll give you a few main points. The major issue is that this is about food, it isn't about farms. Food is a very emotional and ethical subject for most people. People react with their guts, they don't react with their minds. This is not about sound science, again this is about ethics, confidence and trust. The second main point is that American arrogance and aggression in this area—as in many other areas—has alienated most other countries. We're now seen as using poor countries as pawns. We have no standing with the world when it comes to food because we are seen as barbarians when it comes to our own food lifestyles, certainly relative to Europe. So the European Union, with their cautious approach, is winning the hearts and minds of many other parts of the world right now because by contrast we look pretty callous. Again, this is all about confidence and trust. The data are in. I've seen some of it. People no longer trust the government in the United States. It's clear that people especially fear large corporations. I've been teaching a lot with under-30-year-olds. Young people are convinced that the most serious threats to their lifestyles and to their future are large corporations. They are not afraid of NGOs and things.

Another important consideration is that, regardless of what scientists and technologists say, organic food has become the gold standard against which everything else is judged. And much of their market appeal is that they can put right on the bag—even though FDA was supposed to write rules about this but didn't—"non-GMO." "Buy this product,

spend the extra dollar, because it doesn't have GMOs in it." That's what we are seeing. I hope you all paid a lot of attention to Mark Nelson from the Grocery Manufacturers of America (GMA), because the big food companies have all bought organic lines. Kraft owns Boca. General Mills has Cascadia Farms. Kellogg's has Morning Star Farms. On and on. They are going to play both sides of the aisle and they are ready to turn their businesses around and aim in that direction as they see the market develop.

And as a sociologist, this is an interesting example of what we call cultural lag. And this is where the material culture, the science, the technology, is far outpacing our nonmaterial culture. I'd say we don't have the knowledge, the attitudes, the regulatory or policy systems in place to deal with these technologies, and we're only talking about plants. The big challenges are going to be in human genomics, human genetics, and NABC just sticks its head in the ground when it doesn't even look at those issues. The fact that you've excluded animals from this discussion is really interesting because that's the one that's going to blow up in everybody's face.

Let me get a little more specific. I've looked at a very good study from Rutgers. I had a chance to review all the Council for Biotechnology Information tracking data, which I'm not going to talk about in detail, but there are some real clear trends in the United States. One of the most disturbing things is that the vast majority of people in the US still don't have a clue that products in the supermarket contain genetically engineered ingredients. They are still saying, "Oh no, I don't eat that." It's still a common fallacy. And as people find out, they are finding out because they are learning about the negative side. They're not learning about it in any positive way. And, increasingly, the data that aren't usually shared show that there is increasing concern over risk among US consumers. And that has increased over time. The food industry itself—in terms of the state of social acceptance—is about to pull the plug. You need to pay close attention to what GMA says about PMPs. And you aren't even listening to the food retailers and the restaurant chains because all they've done is told the manufacturers, "Keep that junk out of our food." You know the whole system is blocked at that end and there's no sign that anything is going to come along that's going to spring that open. A food retailer will still be very, very susceptible. And the bottom line, as I think we are finding, is there are no benefits for any of these stakeholders, including consumers. There is a perception of possible risk and so, in many ways, it's very rational to reject the technology. With Europe, the interesting thing there is the data showed that things were actually looking a little better, then along came the WTO lawsuit to the front page of the paper, and the Europeans dug in deeper than in the past.

Where would I lay the blame for all this? I would lay it at the step of the Bush administration. The main indicator of that is they never bothered appointing a full-time FDA commissioner. It was just not important enough. We've got Les Crawford in there, nice man, strong industry advocate, strong lobbyist for industry, but they never bothered getting a confirmation on a full-time FDA commissioner. That just shows you where the priorities are. The WTO case was brought 2 weeks after the misplaced Iraq invasion that we are still trying to dig out from under. And the headlines in the European press were in terms of retaliation against the French for not backing us in Iraq. One thing I did

support: towards the end of the Clinton/Gore administration, FDA hearings were held on the future of food and regulations. The one thing that everybody agreed on, from Greenpeace to BIO to everybody in the middle, was that there ought to be mandatory premarket notification of FDA. Well, Les Crawford, in his great style, said, "You know, we don't really need to do that because we can trust all the companies. They're going to come in to us voluntarily." And that shows you the mindset of the administration.

As I mentioned, there were supposed to be labels that would prevent misleading promotion of products as being "non-GMO": but, we never did it. The one that is going to come back and hit the hardest, though, is this continual push to try to convince people that meat and milk from cloned animals is substantially equivalent. The public knows that is not true. The scientists can't even say it's true. Everybody knows it took 300 mistakes to make Dolly and then she died early. Nobody wants to eat meat or milk from such animals. What I've seen is just pretty much characteristic of this administration: the wrong decisions at the wrong time at every possible opportunity.

Finally, how about a few implications for the universities? I think there is still a lot that we can do. What this industry sorely needs is something that people may actually want, something that food consumers may actually demand. If you go back to some of the horticultural crops and produce, some of the flavor-saver constructs, and deliver something to the consumers that they actually like, you might even make supermarkets receptive. I think universities need to quit being so hung up on sound science. There are many, many other ways of knowing. And when it comes to food there are many, many other things that are much more important to people than sound science.

I've published a review of a film made my Deborah Garcia, *The Future of Food*. It's an unabashed attack. She's likening herself to Michael Moore. It summarizes all the concerns that people have, and I think that's one of the things that universities need to do more of. You need to start inviting and engaging the critics. You need to listen to someone else besides the biotech industry. And I know—coming from a land grant university—that the people with the money, it's tempting to listen to them. But I really think that we need to do a much better job as universities of being honest brokers and asking the tough questions and coming up with answers that may or may not be politically correct.

Canice Nolan (European Commission to the United States, Washington, DC): My mission is not to convert you to the European ideal. My objectives here in the United States are to listen, to inform and to promote the dialog. In the area of GM, dialog is something that has been missing for several years, mainly due to the fact that a WTO case is going on and our instruction in the past has always been, "When there is a court case going on, you speak through the lawyers, you don't actually speak to the other parties." This is important. Before coming here, I was responsible for six years for pesticide legislation in Europe. As a scientist, I learned very quickly that you don't play political games. You don't play with journalists because they are professionals and they will eat you alive. I'm a scientist and I prefer to stick to the science. I learned how valuable that was in the area of pesticides, because there are many people who don't care what the risk assessment is, they just want it banned anyway. I heard reference to "zero zealots" this morning. I've also

heard these people referred to as the taliban: "tali ban this and ban that and ban the other," and it doesn't matter what's out there. Now, with respect to GMOs, you have to see it in the larger context. We had food scares in Europe, mad cows, dioxins and so on in the 90s, which threw the whole food supply system into doubt. At the end of the 90s there was a period of reflection that included GM. During that time we set up the European Food Safety Authority. We brought in a huge amount of new legislation on food safety. Also in the area of GM we brought in new rules, new scientific requirements, scientific assessments, rules on labeling, on traceability, and things had started moving again in the sense that requests were moving through the system. We had a new commission at the end of last year, and one of their first actions was to stop and say, "Well, GM: yes the commission is pushing it. We've analyzed case by case, we've seen where things are safe, we will go ahead and make positive proposals." But the member states are still not on board. So, there was a period of reflection in February, 2005, in the commission and it was agreed that the system we have in place is a good, correct system, so let's continue with it as is. And we have continued pushing forward proposals. You might say everything is okay now, everything is moving; well, in fact, everything is not okay. Everything is *not* moving as we would like and there are a few reasons for this. One of them is that the real problem is not with the regulators, the problem is with consumers. And the politicians see that consumers don't want this. The supermarkets see that the consumers don't want this. And they're not buying; they're not sourcing. The food processors are not sourcing where there is a risk of GM and, until the consumers are convinced, I'm not sure that the markets will allow GM to go forward in Europe. It doesn't matter what we as regulators say. It's the market that will rule the game. And the politicians are just being responsive to the wishes, if you like, of the electorate. The GMO case is almost seen as big industry forcing unwanted food down people's throats and the commission sees itself almost in a position of being a tool of industry in forcing this down people's throats because the laws say that it is safe that you have to put it out there. I'd always thought that the American system was that the consumer is king and what the consumer wants the consumer should get and if the consumer doesn't like this, well the consumer doesn't have to have it. In the field of GM, I have the impression of the industry saying this is good, take it, buy it, eat it, it'll do you good, and we have to get around that disconnect if we are going to make progress in this area.

Allan Bennett (University of California, Davis, CA): One of the themes that has gone through this meeting is the opportunity for a greater diversity of agbiotech products. Also a notion that was mentioned by Roger Beachy has come up again: the opportunity for new players, including the public sector, as developers and providers of new GE crops. A few of us believe that the markets would react differently today if, in fact, there were additional players in this field and additional products representing broader diversity. Clearly, some high barriers are working against this objective of diversity of products and of players. The irrationalized regulatory environment and its smothering effect on development and deployment of new phyto-technologies has been addressed over and over at this meeting. The bad news that I want to present today is that you also need to

navigate a thicket of patents before you can even get to these regulatory hurdles. So, my role is to shine a little bit of light on the intellectual property environment that is also impacting what projects can advance and what institutions can effectively move along the path to product development.

Interestingly, these two areas—intellectual property and regulatory approvals—are not unrelated. The regulatory environment imposes high cost on the ultimate deployment of a product and, in industries where there are high regulatory hurdles, intellectual property becomes extremely important simply because no one is prepared, or willing, to invest the cost required for regulatory approval unless they have exclusivity to market the product. And so, in this sense, intellectual property is very important in fueling innovation and it is very important fueling innovation in this sector. Having said that, intellectual property—if the landscape becomes too complex—can also inhibit innovation, and that's the situation that we are dealing with here; it's clearly important, but has developed into a complex landscape. So, what does this intellectual property landscape look like? We've studied it a little bit. It turns out there are sixteen to twenty thousand patents in the crop biotechnology sector, depending on what and how you count. That's an interesting number given the very narrow base of products that we have in this arena. It's clearly not for lack of innovation and innovative technologies that have been developed. These cover the so-called enabling technologies such as transformation methods, selectable markers and promoters. In an ideal world these enabling technologies would be very broadly available, licensed on a non-exclusive basis to enable a wide diversity of players but—unlike medical biotechnology, where in fact that did occur—it didn't happen in agbiotech. Therefore, many of the enabling technologies are very narrowly owned and strategically deployed rather than supporting broad innovation. There is an initiative called BIOS, Biological Innovation for an Open Society, in Australia, which is attempting to invent new enabling technologies and make them broadly available free of charge. This group of sixteen to twenty-odd thousand patents also covers the trait technologies—genes that encode specific pharmaceutical proteins or traits such as disease resistance, which is the arena in which exclusive access is critically important. Elizabeth Hood indicated that somewhere in the neighborhood of seventy-five licenses were needed to encompass all of the technologies for the production of trypsin, very similar to the seventy-odd technologies required in the production of golden rice.

To address some of these issues, twenty-eight universities and not-for-profit research centers have joined forces to create the Public Intellectual Property Resource for Agriculture (PIPRA). In this area, there is strength in numbers. Fully 25% of crop-biotech patents represent inventions that were made in the public sector, and this is about a 10-fold higher proportion than in any other technology sector. So PIPRA, as a collective organization, represents a very broad and significant portfolio of intellectual property. What is it doing? It's deploying a unified database of all the public-sector intellectual property, helping organizations and individual researchers evaluate freedom to operate around specific technologies, developing transformation vectors that have maximum freedom to operate and it's working towards a collective management—bundles of intellectual property for multiple institutions—to try to reduce these transaction costs. PIPRA has

a vision and this vision is related to something we heard yesterday: to be able to partner with an organization such as the Specialty Crops Regulatory Initiative (SCRI), and jointly navigate both the intellectual property and the regulatory environments that will deliver the benefits of biotechnology to a broader base of crops and to consumers.

Bruce Ferguson (Edenspace, Chantilly, VA): My first question is for Tom Redick. Given this evolving standard of strict liability now that is being faced by seed producers and sellers and others in the agricultural chain, has any effect been observed yet on insurance rates or availability of insurance? Private insurance?

Tom Redick: This is a rapidly evolving situation. Domestically we've had troublesome memoranda from our main brokers who appear to be having lunch with activists in their spare time, talking about how these risks are so great, and that we need to discontinue coverage on various aspects of production. And these insurers will actually pull all of my growers' pearls, just in the name of biotech. They did the same thing with mold coverage. They said because of mold problems we're not covering any losses from water damage. So the insurance industry is reacting. What we've done on the growers side is we've calmed them down, domestically. Internationally there's a company called Swiss Re. It's a big re-insurer and they're a big troublemaker. I think they're having lunch with activists too and they sent an entire CD of material of what I would call inflammatory presentations to the biosafety meeting and handed it out and didn't consult with industry in advance. So I do think there's an insurance that's available and, as I noted, physical injury has been recognized by the courts, from commingling, and that goes right into the basic standard insurance policy and gives coverage. That's why, when the insurers tried to take it away, we really complained quite bitterly and said, "You already cover this and you're not going to take it away." But yeah, it's something I'd love to see, frankly, more Europeans getting involved and just maintaining the level of insurance coverage that already exists so that they don't pull it away. I think the activist goal is: eliminate all insurance and you eliminate the industry.

Ferguson: A quick follow up question for Cindy. Has there been any contingency planning or other activity looking at how the existing framework of government agriculture insurance might be supplemented or extended to backstop farmers and others in this industry in the event that private insurance becomes much more expensive or unavailable?

Cindy Smith: I want to make sure I captured your question. The question is: is the government, from a regulatory perspective, looking at the question of liability insurance?

Audience Member: My understanding is there is a pretty broad framework of crop-support insurance for instance for farmers, but I'm not certain, I haven't looked at whether it applies to farmers who have economic harm from inadvertently having a little bit of DNA in crops sold to Europe. I see some headshake no, so I'm assuming that perhaps it doesn't extend that broadly. I come from another industry where the government did

step in to provide liability backstop and I don't think it's a really directly comparable industry—the space launch industry—except to the extent that there were some hazards in commercial space launches that were very low risk yet could not be insured because they were so hard to calculate. The example often given was a commercial rocket landing on New York City; very low probability but hard to insure against. So, just as a reference, the government has stepped in—both in that and the nuclear power industry—to provide some creative frameworks for liability protection, for the participants in the industry. I just wondered if your office, or anybody else in the USDA, is looking at that whole area as another means of encouraging innovation in this sector?

Smith: This is a little separate from my area, but relevant for other parts of USDA. Probably what I can offer most relevant to the question is what we've done. We've had some of this kind of dialog at USDA as compliance issues have arisen, particularly with the ProdiGene issue. At the time, we were questioning their ability to assume the loss that was going to be associated with their lack of compliance, and made a decision, which was later criticized, to have the department put up the initial money to ensure that the company could eventually directly assume the loss rather than have that loss spill over to farmers or others. So, the way we have addressed this to date has been to look at each individual situation and, with respect to what we've been regulating, and make every effort to hold the company directly accountable for lack of compliance. We've had some other situations too. Fortunately we've had a lot of success in getting companies to agree to address those issues. But clearly there's a larger question here that is probably a whole other discussion that should be considered and include other parts of USDA and the insurance industry.

Henry Miller (Hoover Institution, Stanford, CA): One concept that is dramatically underrepresented here is that the universe of recombinant DNA-modified organisms, whether you call them GMOs or GEOs or GEMs, is not a meaningful scientific category. It's not amenable to generalizations about risk or safety and, if you doubt that, look at the lists of topics at the Keystone meetings and the Gordon conferences and they don't focus on recombinant enzymology or recombinant bioenergetics. That has important implications because regulators like Cindy Smith saying that her agency has a scientific approach and it's dedicated to science, doesn't make it so. You labor within an irrational scope of what comes in overall to your system. And in science you don't get to be scientific for just a little bit of the process. You have a process, as does EPA, where the degree of government scrutiny is *inversely* proportional to the degree of risk. You are regulating a superior technology more stringently. And you are very congenial and you are very collegial but it doesn't make your scheme any more rational and it doesn't make the obstacles that you put in the path of research in industry and in academia any less.

PART V

Banquet and Luncheon Presentations

The Nature of Change: Towards Sensible Regulation of Transgenic Crops Based on Lessons from Plant Breeding, Biotechnology and Genomics

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> *...in a vast number of cases, we cannot recognize...the wild parent-stocks of the plants which have been longest cultivated in our flower and kitchen-gardens.... breeders could never have expected or even have wished to have produced the result which ensued.* —Charles Darwin (1859)

With these words in his *Origin of Species*, Darwin made clear the power of selection and plant breeding to alter the appearance and usefulness of crop plants. The selection of plants with improved agronomic traits, along with improved agricultural technology, have been key factors in maintaining agricultural productivity during exponential growth of the global population over the past two centuries (Evans, 1998).

With the advent of genetic engineering, transfer of DNA between species became possible, thus vastly increasing the power of genetic modification. At the same time, genetic engineering captured the public's attention in a way that more conventional plant breeding techniques never did. Genetic modification has come to be feared in its own right, particularly in Europe and in several developing countries. The result is an onerous patchwork of regulatory systems around the world. The regulatory requirements all too often mirror concerns voiced by groups opposed to the technology, and thus focus on the DNA of the transgene and its accompanying vector sequences, and any possible changes in the DNA around the transgene-insertion site, rather than on the trait itself. In consequence, the cost of regulation can run into the tens of millions of dollars per transgenic event. As such, the regulatory environment is actively preventing the marketing of dozens of transgenic crops, while contributing little, if anything, to public and environmental safety.

The cost of regulation can run into the tens of millions of dollars per transgenic event. As such, the regulatory environment is actively preventing the marketing of dozens of transgenic crops.

Whether this DNA-centric regulation is warranted depends on the extent of DNA-based changes that differ between the engineering process and traditional plant breeding. The prevailing wisdom has been that plant breeding primarily depended on pre-existing variation, and thus need not cause novel DNA changes. Furthermore, the nature and extent of the DNA-level variation within a crop have been poorly quantified until now, although there have been indications in the literature that the plant-breeding process itself is mutagenic, that plant genomes are fluid and dynamic, and that there is a large amount of DNA-level variation.

Rasmussen and Phillips (1997) provided one of the first insights that the plant-breeding process is mutagenic when they concluded that barley breeders had achieved more progress from breeding and selection than could be explained by the amount of genetic variation originally present in the parents.

Are DNA Insertions Dangerous?

> *Traditional breeding is based on sexual reproduction between like organisms. The transferred genes are similar to genes in the cell they join.... In contrast, bioengineers isolate a gene from one type of organism and splice it haphazardly into the DNA of a dissimilar species, disrupting its natural sequence.* —Alliance for Biointegrity (http://www.bio-integrity.org/health-risks/ health-risks-ge-foods.htm)

It has long been known that DNA content can change during tissue culture, in the neighborhood of 10% per culture cycle (*e.g.*, De Paepe *et al.*, 1982). More recently, retrotransposon amplification has been implicated in tissue-culture-induced DNA changes (Jiang *et al.*, 2003).

Yet, DNA content changes in the absence of tissue culture. The literature contains many suggestions that plant genomes are highly variable. One early indication was the discovery that maize inbreds differ in the number of rDNA copies, ranging from a low of 5,000 in "W23" to 23,000 copies in "Illinois Reverse High Protein" (Phillips, 1978). Total DNA content varies also within crop varieties. For example, soybean genotypes differ from each other by as much as 12% in DNA content (Graham *et al.,* 1994). For red pepper, the difference goes up to 25%, (Mukherjee and Sharma, 1990) and for maize, 42% (Rayburn *et al.*, 1989)! It is clear from these results that plants can endure substantial changes to their DNA without ill effect. In the case of soybean, the 12% DNA is equivalent to almost 106 million bp. Hence, an extra 322 bp of vector sequences in something like Roundup Ready© soybean cannot make any significant difference.

Furthermore, it must be emphasized that these changes in DNA content do not necessarily represent ancient events, but rather are the consequence of modern breeding attempts. The previously mentioned case of variation in soybean DNA amount is probably derived from adaptations to growing seasons at different latitudes (Graham *et al.*, 1994); a similar relationship is found between the length of the growing season and the DNA content of maize (Bullock and Rayburn, 1991).

It is possible for DNA content to change within one generation. The most extreme example described is that of genotypes of flax that have heritable changes in plant size depending on the fertility of the soil (Durrant, 1962). These changes are caused by loss (up to 6%) or gain (up to 10%) of DNA content in the weeks following seed germination (Evans *et al.*, 1966). Smaller changes, unaccompanied by dramatic differences in the phenotype, possibly occur all the time but go undetected. For example, DNA content in tall fescue differs between plants germinated at 10°C rather than 30°C (Ceccarelli *et al.*, 1997), and reflects gain or loss of ~30% in copy number of different retrotransposons.

The major component that accounts for variability in genome size is the presence of retrotransposon elements, which are a major constituent of plant genomes (Bennetzen, 1998). Again, the question remains whether retrotransposon movement took place in the ancient past or continues on to the present. Biologically, it would be difficult to explain why retrotransposition was once common, then came to a stop. In fact, the presence of retrotransposon sequences in expressed sequence tag (EST) databases (Kuhl *et al.*, 2004; Neumann, *et al.,* 2003; Echenique *et al.,* 2002) suggests that some retrotransposons are active to this day.

Rapid genomic change is also evident upon polyploidization. DNA segments have been shown to appear and disappear within a generation following hybrid formation in *Brassica* (Song *et al.,* 1995), wheat (Liu *et al.*, 1998 a, b), tobacco (Skalická *et al.*, 2005) and arabidopsis (Pontes *et al.*, 2004; Madlung *et al.,* 2005).

Collectively, these data strongly imply that plant genomes are quite able to endure insertions and excisions of DNA without ill effects.

Collectively, these data strongly imply that plant genomes are quite able to endure insertions and excisions of DNA without ill effects. It cannot be concluded that "disruption of natural sequences" is dangerous.

What About Insertional Mutagenesis?

> *A foreign gene could, for example, be inserted in the middle of an existing gene that instructs a plant to shut off production of a toxin in its fruit. The foreign gene could disrupt the functioning of this existing gene, causing the plant to produce abnormal levels of the toxin in its fruit. This phenomenon is known as "insertional mutagenesis"—unpredictable changes resulting from the position in which a new gene is inserted. — Rachel's Environment & Health News* (http://www. rachel. org/bulletin/bulletin.cfm?Issue_ID=1931&bulletin_ID=48)

> *In most cases of plant modification, DNA insertion takes place at random, unpredictable loci. Such random insertion may lead to unintentional changes in gene expression.* — OECD Report of Task Force for the Safety of Novel Foods and Feeds (2000) [C(2000)86/ADD1]

Insertional mutagenesis differs from the previous topic in that the new DNA inserts itself into another gene or its regulatory sequences, rather than into the intergenic space. To evaluate the safety of insertional mutagenesis, it must be placed in context of transposable elements jumping in and out of genes, where they "can alter gene expression or serve as sites of chromosome breakage or rearrangement," (Wessler, 2001) just like transgenes, and usually without ill effects to the plants or those who consume them.

It must be noted that all crop plants go through a period of field trials before being released commercially. These trials ensure that no unexpected or undesirable effects from the breeding process—conventional or engineered—are present in the final product.

Is Horizontal Gene Transfer Unique to Transgenics?

> *Unlike traditional crop or animal breeding, genetic engineering enables scientists to cross genes from bacteria, viruses, and even humans into plants and animals. Never before have scientists been able to break the species barrier.* — The True Food Network (http://www.truefoodnow.org/ home_whatis.html)

Actually, plant breeders have been transferring genes between related species and related genera for decades. However, it is true that scientists had not crossed the species barrier in terms of gene transfer between kingdoms until the advent of genetic engineering technology. Nevertheless, it must be acknowledged that DNA from unrelated species is transferred and incorporated into plant genomes. For example, plantain bananas contain the entire genome of the banana streak virus, rice contains DNA from the rice tungro bacilliform virus, and tomato has DNA from the tobacco vein clearing virus (Harper *et al.*, 2002). In fact, these authors concluded the following: "It appears that integration of viral sequences is widespread in the plant kingdom and has been occurring for a long period of time." Genes from the bacterium, *Agrobacterium rhizogenes*, have been found incorporated into the genome of some tobacco species (Aoki and Syono, 1999; Ashby *et al.*, 1997), while DNA from unrelated higher plants has been found to be transferred between their mitochondria, and, from there, to their nuclei (Bergthorsson *et al.*, 2003, 2004).

The true extent of horizontal gene transfer will become clear as more plant genomes are sequenced. In the interim, it is fair to say that, although not a common phenomenon, horizontal gene transfer does take place, at least on an evolutionary time scale, and does not appear to pose any hazards to recipient plants.

The Impact of New Genes in a Genome

> *Gene expression is subject to a regulatory network of a complexity that is only just being realized.*

> *[Genetic engineering] assumes that genes act as isolated units within a system. This is simply not true....Genes inserted at random into the genome means* [sic] *are outside of these regulatory control—they are unregulated. GE goes against the current understanding of the complex nature of the genome.*
>
> *…the often forcible insertion of DNA into a tightly controlled genetic regulatory network is likely to produce unintended effects.* —Greenpeace (http://www.greenpeace.org/international/campaigns/genetic-engineering/failings-of-ge)

The new argument being made is that genes are controlled by a regulatory expression web in which no gene is independent. A new gene or a gene in the wrong place can upset this regulatory web. Yet, as discussed previously, genes and DNA sequences move into and between chromosomes. As additional examples, genes are known to have moved from the chloroplast to the nucleus (Cummings *et al.*, 2003). *In situ* fluorescent hybridization has shown how DNA elements move from one genome to another in a tetraploid wheat (Belyayev *et al.,* 2000).

Furthermore, this "new" interpretation that gene expression is regulated by a fragile network of interdependent genes is based on the traditional concept that all members of the same species have the same genes in the same location. However, sequencing homologous DNA sequences from various maize inbreds is revealing a different reality: different individuals within the same species do not even have to have the same number of genes! Fu and Dooner (2002) first discovered this phenomenon. Since then, the finding has been extended to other maize sequences (Brunner *et al.*, 2005; Song and Messing, 2003). In hindsight, this result is not altogether surprising, as it has been known for years that cytoplasmic male sterility in a variety of plants results from the creation of novel genes in the mitochondrion, along with novel fertility restorer genes in the nucleus (Schnable and Wise, 1998). Nevertheless, the point is that to the extent to which these regulatory networks exist, they are sufficiently robust so as not to be affected significantly by the presence/absence or location of single genes or DNA sequences.

Antibiotic Resistance Genes

> *Scientists are concerned that by flooding the environment with antibiotic tolerance genes, these genes will be taken up by disease-causing bacteria, which would then become uncontrollable by antibiotics.* —http://www.sare.org/sanet-mg/archives/html-home/19-html/0256.html

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The concern that resistance can be passed from a transgenic plant to a pathogen is misguided.

The concern that resistance can be passed from a transgenic plant to a pathogen is misguided. As background, transgenic plants can have two antibiotic resistance (AR) genes in them. One is used to distinguish transgenic from non-transgenic cells. These AR genes are modified to be expressed by plant cells, but not by bacteria. Even if they were expressed by bacteria, it turns out that the specific AR genes used during the genetic engineering process of crop plants are already ubiquitous. The gene for kanamycin resistance is an example. It has been calculated that the average human has 1,000,000,000,000 kanamycin-resistant bacteria living in her/his gut, and eats an additional 1.2 million such bacteria each day (Flavell *et al.,* 1992). The bottom line is that while it might be remotely possible to transfer an AR gene from a plant to a pathogen, it is infinitely more probable that such a transfer would take place from the multitudes of AR genes already present in the environment.

The second type of AR gene used is associated with gene-gun-mediated transformation to keep the plasmid in the bacterium, usually by conferring resistance to ampicillin or tetracycline. In contrast to the plant markers, these are expressed in bacteria. However, they are also ubiquitous. For example, 90% of stool samples from Mexico contain ampicillin-resistant *E. coli* (Calva *et al.*, 1996). Fifty percent of the *E. coli* from the average person in France are ampicillin resistant. Using the estimate of 500 g/feces/person/day, and the presence of between 1 million and 1 billion *E. coli* cells per g of feces, half of which are resistant to ampicillin, each French person liberates somewhere between 250 million to 2.5 billion copies of the ampicillin-resistance gene each day (Berche, 1998). Genes for tetracycline resistance are present in many soils. For example, a recent study from Denmark found tetracycline-resistance genes in 10% to 80% of sampled farm soils, and in all samples after enrichment with manure, using a detection limit of 10^2 to 10^3 copies of the gene per g of soil (Agerso *et al.,* 2004). Finally, 6% of wild rodent feces contain tetracycline-resistant bacteria (Hauschild *et al.* 2003).

As of now, transfer of an AR gene from a plant to a pathogen has not been documented under real-world conditions. Nevertheless, the point is that if it were to happen it would not matter, due to the number of resistance genes already in the environment. Thus, efforts to produce engineered plants without AR genes unnecessarily complicates the engineering process, without gaining any safety benefits.

Regulatory Implications

The basis for a phenotypic-trait-based regulatory system, as opposed to a DNA-based system, has been laid out in a series of papers (Strauss, 2003a, b; Bradford *et al.*, 2005). The premise is that examining changes at the DNA level will most likely result in unnecessary expense and not contribute towards environmental or health safety.

Risk Categories

The first step in moving towards a trait-based regulatory system is recognizing that transgenes can be placed into low-, medium-, or high-risk categories based on their function.

Low Risk

The vast majority of transgenes would probably be in this category, and require little or even no oversight. Examples of transgenic crops in this category would include those:

- When the transgenic trait is functionally equivalent to one obtained by breeding
- When the transgenic trait is "domesticating," that is, it lessens fitness in the wild
- No novel biochemical or enzymatic functions are imparted.

Medium Risk

- Plant-made pharmaceuticals/plant-made industrial products (PMPs/PMIPs) of low animal/environmental toxicity
- Resistance traits that require stewardship for their protection.

High Risk

- PMPs/PMIPs with documented ability to cause harm in the environment or upon ingestion
- Plants used for bioremediation that accumulate heavy metals or other toxins

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Once a trait produced by a transgene is deemed to be innocuous, additional transgenics produced with the same transgene should not have to go through the entire regulatory process.

Avoid Event-Specific Regulation

Once a trait produced by a transgene is deemed to be innocuous, additional transgenics produced with the same transgene should not have to go through the entire regulatory process, if at all, particularly if the same transgene is introduced into the same crop. Putatively, *de novo* regulation of each transgenic event precludes unintended effects between the transgene and the recipient genetic background. Yet, the current regulatory climate is such that once a given transgenic event is approved, it in turn is backcrossed into hundreds if not thousands of different varieties, thus virtually ensuring the transgene will end up in various genetic backgrounds anyway. If anything, the widespread use of transgenes backcrossed into different genetic backgrounds is living proof that background effects, if they exist, are not important enough to regulate.

Adventitious Presence

It has long been recognized that zero tolerance is virtually impossible to achieve, be it in food products or in seed. Nevertheless, the continued and stringent regulation of transgenic products has given the public the distinct impression that these are dangerous, to

the point that tolerances for the adventitious presence of transgenes and their products are far more strict than the tolerances for the presence of contaminants. For example, certified seed is allowed to have a low level of foreign matter and seeds from other varieties or even other crops, and some types of weeds. A case in point: certified canola seed may legally have two seeds from other crops per 50 g. It can also have fifty weed seeds per 50 g, though none can be of a noxious weed, and only two can be of objectionable weeds. Also, there can be ninety diseased seeds per lb. Furthermore, one of every 500 canola plants in the seed field can be an off type or from another variety. It is unreasonable to expect transgenic seed to be present at lower levels than within these tolerances.

Likewise, the Food and Drug Administration/Office of Regulatory Affairs (FDA/ORA) filth standards allow limited amounts of insect parts and rodent waste in food. As examples see CPG 7104.02, Sec 578.200 and CPG 7114.29, Sec 585.890 for cornmeal (permits less than one whole insect, or fewer than fifty insect fragments, or fewer than two rodent hairs, or less than one fragment of rodent excreta per 50 g) and for tomato paste (permits twenty-nine fly eggs, or fourteen fly eggs plus on maggot, or fewer than two maggots per 100 g), respectively. As another example, under the *Codex Alimenatarius* (3.2.2.1) international standards, white rice can have impurities of animal origin (including dead insects) of 0.1% m/m maximum. There is something totally irrational about allowing 0.1% dead insects in white rice, but panicking if trace amounts of a transgenic protein were to appear in the same rice.

The adventitious presence of transgenes and their products should not trigger regulatory action as long as they are not present in quantities that exceed the standards currently in place for certified seed, for the FDA/ORA filth standards, or for the Codex Alimentarius.

The adventitious presence of transgenes and their products should not trigger regulatory action as long as they are not present in quantities that exceed the standards currently in place for certified seed, for the FDA/ORA filth standards, or for the *Codex Alimentarius.* Another criterion that may be used is that the adventitious presence of transgenes and their products fits within FDA recommendations as to whether trace ingredients must be labeled. The following is from http://www.cfsan.fda.gov/~dms/flg-4.html, on the need to label:

> *…depends on whether the trace ingredient is present in a significant amount and has a function in the finished food. If a substance is an incidental additive and has no function or technical effect in the finished product, then it need not be declared on the label.*

Thus, if the adventitious presence would not trigger the FDA-labeling requirement, such adventitious presence should not be regulated. Under these criteria, low-level presence of transgenes and their products in foods should be exempted from regulations. In addition, there should be allowances for adventitious presence that are based on risks of specific classes of genes, and not on method (GE or not), with the classes discussed above. These should include the unlimited presence of specific selectable marker and reporter genes, and vector DNA sequences.

Additional Issues

Currently, genetically engineered *Arabidopsis* spp. are exempt from interstate movement restrictions under 7 CFR part 340, as are *E. coli* K-12, *Saccharomyces cerevisiae* and *Bacillus subtilis*. Note that these same organisms are currently regulatory exempt from NIH guidelines as per Appendix C, while *Arabidopsis* is exempt provided that it does not meet the criteria in Section III-E-2-b or other sections of the NIH Guidelines:

> *Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (*e.g., Rhizobium *spp. and* Agrobacterium *spp.).*

The key here is that NIH views *Agrobacterium* as low risk, whereas APHIS regulates interstate transport of all *Agrobacterium* strains, even when they have been disarmed and are no longer pathogenic. Accordingly, all interstate movement restrictions of transgenic organisms that are of low to moderate risk as defined above, or that could not establish in the environment without substantial human aid, need to be lifted. This would greatly facilitate research and breeding with GE materials, and regulatory effort could then be focused on the more important issue of environmental releases, not contained shipments. Exemptions from regulation should include:

- All disarmed *Agrobacterium* strains not containing T-DNA
- All low-risk transgenic plants as defined above (as seed, in soil, or *in vitro*).

Summary

Plant genomes are variable and dynamic, constantly changing in response to breeding efforts and even to environmental conditions. They are buffered against the change that

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Ultimately, it is the trait imparted by the transgene that matters, and as such, it is the trait that should be the focus of regulatory efforts, should these be warranted.

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small additions or deletions of DNA can cause. They are buffered against differences in genic content, which probably explains why polyploidy is prevalent in higher plants.

Against this background, it is ludicrous to treat transgenes and their associated DNA changes as inherently dangerous. Ultimately, it is the trait imparted by the transgene that matters, and as such, it is the trait that should be the focus of regulatory efforts, should these be warranted. For most traits, their risk to health and the environment is low enough as to preclude the need for regulatory oversight.

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Creating the Proper Environment for Acceptance of Agricultural Biotechnology

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This paper consists of four sections. First, it describes the Center for Science in the Public Interest (CSPI) and its Biotechnology Project. Second, it discusses the current status of agricultural biotechnology in the United States, future trends for the technology and some of the controversy that surrounds it. Third, the paper discusses the current status and issues surrounding "biopharming," a major topic at this conference. The paper concludes with a discussion of what is needed for broader acceptance of agricultural biotechnology, not just in the United States but also abroad.

The Center for Science in the Public Interest

CSPI is a nonprofit consumer-advocacy organization that has focused on improving the safety and nutritional quality of our food supply. It seeks to promote health through educating the public about nutrition and alcohol; it represents citizens' interests before legislative, regulatory, and judicial bodies; and it works to ensure that advances in science are used for the public good. Its primary focus is on the United States, although it does have a satellite office in Canada. International activities involve food-safety and labeling issues, such as the *Codex Alimentarius* and the Trans-Atlantic Consumer Dialogue.

CSPI is primarily supported by the almost 900,000 member-subscribers to its *Nutrition Action Healthletter.* CSPI receives no funding from industry or the federal government; some funding comes from independent philanthropic foundations.

The Biotechnology Project

In 2001, CSPI began an advocacy project on agricultural biotechnology, the goals of which include to accurately identify risks and benefits of biotechnology, to ensure that the US regulatory system is up to the task of preventing significant risk, and to keep the public informed about the facts surrounding agricultural biotechnology.

In 2001, CSPI stated that "the genetically engineered foods that are currently on the market are safe" to eat.

CSPI's biotechnology positions are based upon current evidence about the risks and benefits of biotechnology, not upon an ideological viewpoint that agricultural biotechnology is inherently good or bad. In 2001, based on its review of currently available evidence, CSPI stated that "the genetically engineered foods that are currently on the market are safe" to eat and that environmental risks associated with those crops are manageable (CSPI, 2001). Also, CSPI has stated on numerous occasions that currently engineered crops grown in the United States are yielding benefits to farmers and the environment by increasing yields and reducing the use of insecticides (CSPI, 2001; Jacobson, 2001a, 2001b; Jaffe, 2001). CSPI publicly acknowledges these beneficial applications and wants to ensure that they will continue to be realized. CSPI has been disappointed that other crops that could provide similar environmental benefits, such as Monsanto's NewLeaf ™ potato, have not been planted by farmers due to fear of a consumer backlash and a loss of market for the crop.

Of course, CSPI has also acknowledged that agricultural biotechnology has real risks that need to be assessed and addressed before products from genetically engineered (GE) crops are marketed. From the consumer's point of view, the key question about biotech foods is "Are they safe?" (Jaffe, 2004a). Thus, before a biotech food is marketed, there needs to be a determination that the engineered protein is not an allergen, that there is no toxic effect from the engineered crop, and that there is no other unintended effect from the genetic transformation (NRC, 2000, 2004; CSPI, 2001). Environmental risks are also possible from engineered crops. There is the potential for harm to non-target species, or the spread of the introduced gene and its characteristics to wild relatives of the transformed crop, or the development of pesticide resistance in insects or weeds (NRC, 2000; CSPI, 2001). Each possible environmental consequence needs to be thoroughly evaluated and adequately addressed before any biotech crop is released to the environment (Jaffe, 2004a).

Current Status of Agricultural Biotechnology and Future Potential Applications

In many ways, the past 10 years have been extremely successful for the biotechnology industry. Several blockbuster products were marketed in the 1990s, including soybeans, corn, cotton, and canola that are herbicide-tolerant and corn and cotton that produce their own insecticide that kills specific pests. Those GE crops have been widely adopted by farmers in the United States and, to a varying extent, in seventeen other countries around the globe. Over eight million farmers grew 200 million acres of GE crops in 2004 (ISAAA, 2005). From 1996 to 2004, the global acreage of transgenic crops increased 47-fold, from 4.2 million acres to approximately 200 million acres (ISAAA, 2005). In the

United States, 36.5 million acres of GE corn (45% of all corn) and 63.5 million acres of GE soybeans (85% of all soybeans) were grown in 2004 (USDA, 2004).

Those herbicide-tolerant and insect-resistant crops—biotechnology's "first generation"—have been found to be safe to humans and the environment in the United States. They have also provided benefits to farmers and the environment by increasing yields, reducing the use of insecticides or increasing farmer income.

Although the biotechnology industry's initial inventions have been quite successful, the introduction of new products with different traits has slowed considerably. In February, 2005, CSPI released a study, *Withering on the Vine: Will Agricultural Biotech's Promises Bear Fruit?* (Jaffe, 2005). That study analyzed publicly available data from federal regulatory agencies to determine whether the number of new commercial products has been increasing, decreasing or remained steady.

The study found that sixty-two biotech crops completed the Food and Drug Administration's (FDA) voluntary consultation process between 1995 and 2004 (Figure 1). In the first 5 years (1995 through 1999), forty-seven of those crops (an average of 9.4 per year) completed the regulatory process, whereas only fifteen crops (an average of three per year) completed the process in the next five years (2000 through 2004). Thus, the number of products per year completing the regulatory process plunged by 68% between 1995–1999 and 2000–2004. More than 75% of all biotech crops that have completed the FDA regulatory process did so between 1995 and 1999.

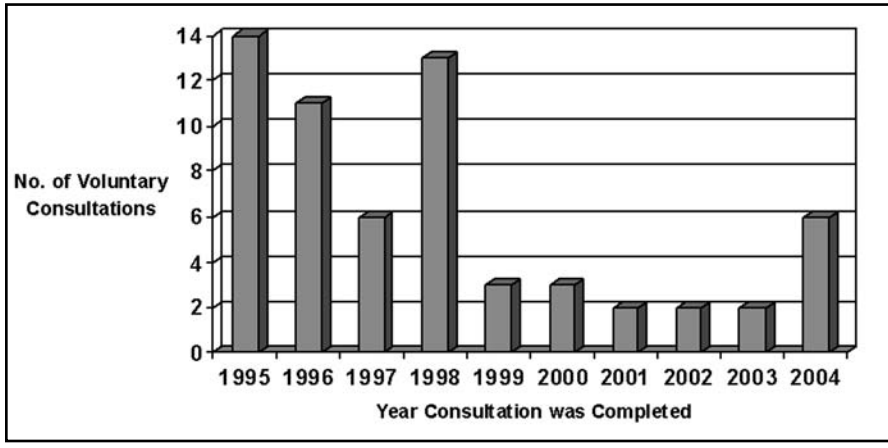

Figure 1. Genetically engineered crops completing FDA's voluntary consultation process (FDA, 2005).

Similarly, publicly available data about the granting of petitions for non-regulated status by the Animal and Plant Health Inspections Service (APHIS) of the United Stated Department of Agriculture (USDA) show a decreasing trend starting in 2000. From 1994 through 2004 (11 years), APHIS deregulated sixty-two biotech crops so that they could

be grown commercially without APHIS oversight. Forty-nine of those approvals occurred between 1994 and 1999 (an average of 8.2 per year) while only thirteen of those approvals occurred between 2000 and 2004 (an average of 2.6 per year) (Figure 2). Thus, APHIS approved almost four times as many crops from 1994 through 1999 than from 2000 through 2004. Clearly, the pipeline for new biotech crops has shrunk considerably, and few new products have become available for commercialization in recent years.

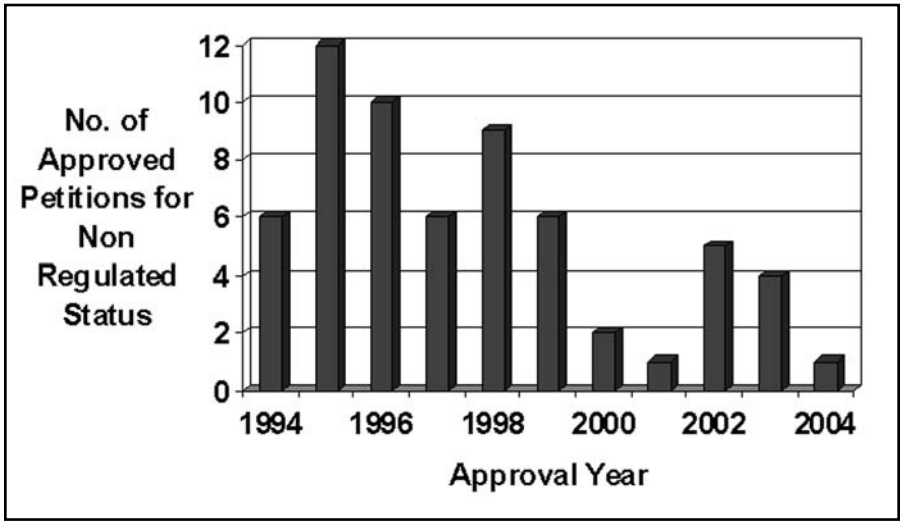

Figure 2. Genetically engineered crop petitions approved by USDA for non-regulated status (APHIS, 2005a)

The CSPI study also found that the GE crops that completed the regulatory process starting in 2000 tended to be variations of existing products with established and proven genes, rather than innovative applications of the technology. For example, of the fifteen consultations at FDA between 2000 and 2004, five involved Monsanto's placing in corn, wheat, creeping bent grass, canola, and sugar beet the same gene for resistance to the herbicide glufosinate ammonium (Roundup®) that was previously engineered into soybean and cotton and reviewed by FDA in 1995. Three applications of the fifteen involved engineering corn, rice, and cotton with a different gene (for phosphinothricin acetyltransferase) conferring herbicide tolerance that several companies had previously engineered into other crops that completed the FDA consultation process in the 1990s. The remaining seven GE products involved engineering corn and cotton with various *cry* genes from *Bacillus thuringiensis* that confer insect resistance. Although some of those applications could be considered "new" because they used *cry* genes not previously approved to address different plant pests, the *Bt* technology had been reviewed by FDA in consultations that go as far back as 1995. Therefore, in the past 5 years, the industry has not marketed a single new agronomic, nutritional, or other trait.

The CSPI study also looked at length of time to complete the regulatory reviews of engineered crops at FDA and APHIS, which it concluded has significantly increased between 2000 and 2004. For the sixty-two voluntary consultation reviews conducted by FDA, the submissions from 1995 through 1999 averaged 6.4 months for completion whereas the submissions from 2000 to 2004 averaged 13.9 months (Jaffe, 2005). Similarly, at APHIS granting a petition for non-regulated status took an average completion time of 5.9 months from 1994 to 1999, but an average of 13.6 months from 2000 to 2004 (Jaffe, 2005). Thus, it took the federal government twice as long to review biotech crops from 2000 to 2004 than it did in the 1990s, yet those products had no apparent novel considerations that might justify the longer reviews.

While the pipeline has slowed, international controversy over current engineered crops has continued. Whereas most governments and many distinguished scientists have found that those crops are safe, some people continue to be concerned with their safety to humans and/or the environment. Similarly, many opponents of genetic engineering do not believe that the current crops have any benefits, not just to consumers, but to farmers or the environment. Also, people throughout the world have called for the labeling of those crops and products from them, and many governments have imposed such labeling and traceability requirements (USDA, 2005).

The controversy over genetic engineering will only increase with the next generation of products.

The controversy over genetic engineering will only increase with the next generation of products. The biotechnology industry and university researchers in the United States and abroad have been inserting a wide range of engineered traits into many different organisms. While research on drought or salt tolerance may reduce the controversy over genetic engineering if they benefit small-scale farmers in developing countries, GE wheat and rice will likely increase the international controversy. Those applications are particularly controversial because those crops are grown primarily for human food needs, whereas the currently grown engineered corn and soybeans are primarily used for animal feed (Foreman, 2005). Similarly, applications of genetic engineering to animals to make faster growing salmon or improved cattle will be extremely controversial as they raise both safety and ethical issues (NRC, 2002a; Foreman, 2005). Finally, engineering plants to make pharmaceuticals ("biopharming") or industrial compounds is particularly worrisome when food crops are employed because no one wants to eat corn flakes with a pharmaceutical in them.

It is clear that those future applications of biotechnology may result in more controversy than the current crops. Already, the possibility that the next generation of products might come to market has sparked an increase in state legislation to hinder or prevent commercialization of those products. In the 2003–2004 legislative session, the Northern Plains states (Montana, North Dakota, and South Dakota) introduced legislation to curb the introduction of GE wheat, while Michigan, California and Alaska introduced

legislation to put limits on transgenic fish (Pew, 2005). In addition, Hawaii and Texas have introduced legislation limiting production of pharmaceuticals using food crops (Pew, 2005). Although the 2005–2006 legislative session has only just started, both Hawaii and Oregon have already introduced legislation on pharma crops. Thus, it is more important than ever to do whatever possible to ensure acceptance of those crops when they reach the marketplace.

Biopharming

Introduction

In the last couple of years, the biotechnology industry has engaged in genetically engineering plants to produce pharmaceuticals, industrial compounds, and other novel proteins ("biopharming" or "pharma crops") (Jaffe, 2004b). Products that manufacturers hope to market commercially include insulin from safflower, human serum albumin (used as blood volume replacement during shock, serious burns, and surgery) from corn, hepatitis B vaccine from tobacco, cholera and Norwalk virus vaccines in potatoes, and lactoferrin (a human protein that protects against infections) in rice.

For the 2004 growing season, USDA, which regulates the planting of pharma crops, received twenty applications to grow them in ten states. (Jaffe, 2004b). For the 2005 growing season, they received eighteen applications to grow them in seven states (APHIS, 2005a). Those applications involve the engineering of six different crops—corn, tobacco, safflower, barley, rice, and indian mustard—with corn, tobacco and rice constituting the majority of the applications (Figure 3).

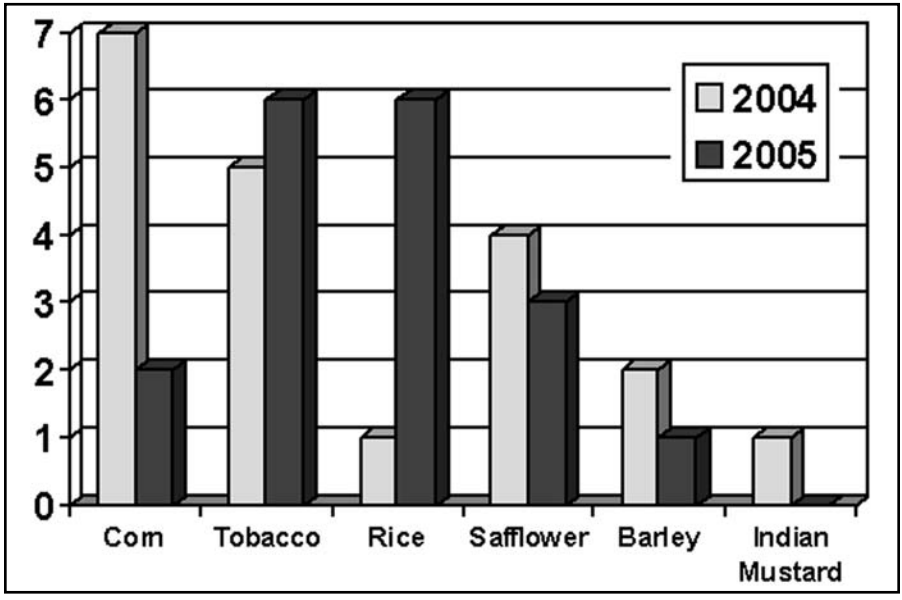

Figure 3. Biopharming permit applications for 2004 and 2005, by crop (APHIS, 2005b).

Although those applications of the technology have the potential to provide consumer benefits, if misused they could harm consumers or the environment. In fact, many scientists and other stakeholders believe that the risks from pharma crops are significantly greater than those from engineered crops grown for food purposes. The National Research Council stated the following in its report entitled *Environmental Effects of Transgenic Plants* about the potential risks of biopharming (NRC, 2002b):

> *Some of the coming applications of biotechnology may involve the issuing of plants to produce pharmaceutical products, biologics, fuels, and other substances not intended for human food use. The introduction of such transgenes poses the potential for environmentally associated risks of a* ***wholly different order*** *than those associated with existing transgenic crops. If such a transgene moves into food crops, either through pollen transfer or physical contamination, there could be* ***serious human safety risk.*** *If such a transgene moves into a wild relative, there could be widespread environmental dissemination of the pharmaceutical substance or other nonfood substances that* ***could have impacts on wildlife as well as microbial populations.*** (emphasis added)

While biopharming raises both environmental and food-safety issues, the controversy surrounding those crops has centered on the concern that they might inadvertently enter the food supply, causing either recalls of food products or rejection by international trading partners. That concern has caused industry stakeholders who normally support agricultural biotechnology to become advocates either against biopharming or for more stringent regulations. The Food Products Association has stated that it "has grave concerns about the use of bioengineered food and feed plants to produce non-food products" and that (FPA, 2003):

> *…given a voice during the early development of this promising technology, [FPA] would not have supported the use of food crops for the production of plant made pharmaceuticals.*

Similarly, the Grocery Manufacturers of America stated (GMA, 2003b):

> *The current US regulatory framework does not inspire confidence among our collective members that these drug and chemical crops will remain isolated and confined and not contaminate the food supply.*

In fact, it is as likely that an industry stakeholder will object to the planting of a pharma crop as one generally opposed to agricultural biotechnology. When the biopharming company Ventria Bioscience attempted to plant rice engineered to produce a pharmaceutical, Anheuser-Busch objected and was able to use its market power to alter where and under what conditions that rice would be grown (Bennett, 2005). Similarly, Agragen's announced intention to grow flax engineered to produce albumin in North Dakota, resulted in industry stakeholders such as AmeriFlax expressing opposition out of fear that, even without a contamination incident, their international markets for conventional flax will be jeopardized (Associated Press, 2005). Thus, it is clear that biopharming using

food crops is radically changing the debate surrounding agricultural biotechnology so that stakeholders who either supported or would support certain applications of genetic engineering, don't support biopharming in food crops.

Regulation of Biopharming and the USDA

A rigorous and robust regulatory system for ensuring that biopharm crops are safe for humans and the environment would do the following:[1]

- **Allow the planting of pharma crops only if the government issues a permit.** The regulatory system should put in place mandatory permitting requirements that must be complied with before the growing of any pharma crop. The permitting process should be transparent and allow public participation before the issuance of the permit.
- **Issue a permit only after a thorough environmental assessment of the potential risks from growing the pharma crop.** Before a permit is issued, the government should conduct a thorough environmental assessment of the potential effects of growing the pharma crop, including the effects from flow of the introduced gene and the effects of the transgenic protein on species other than humans.
- **Issue permits that require strict biological and physical confinement measures.** All permits should contain enforceable conditions requiring state-of-the-art confinement procedures. Those mandatory permit conditions should include isolation distances, geographic restrictions (such as not growing GE corn in parts of the country where commodity corn is grown), physical barriers (such as fences or greenhouses), the use of distinguishable varieties of the crop, biological confinement (such as male sterility), and so forth. The permit should also require extensive segregation and identity-preservation procedures that ensure that none of the harvested materials can commingle with crops destined for human or animal consumption. When using a food crop, the permit should have several redundant levels of confinement, even at the field-trial level.
- **Require regular inspections of the pharmaceutical-producing crop by the regulatory agencies.** As part of its regulation of pharma crops, both USDA and FDA should conduct regular, unannounced inspections of all facilities involved in the production of the pharmaceutical, from the laboratory to the farm to the manufacturing plant. Some of those inspections should occur after the crops have been harvested to prevent volunteer plants in future seasons. In addition, USDA and FDA should inspect neighboring fields and crops to confirm that containment has been achieved.
- **Require that if a pharmaceutical is produced in a food crop, there should be a mandatory pre-market food-safety approval process by FDA's Center for Food**

[1]The remainder of this article focuses on federal regulation of biopharming. It does not discuss state or local regulations, which could play a major role in overseeing the risks associated with pharma crops.

Safety and Applied Nutrition. Although confinement measures need to be strictly adhered to, they will never result in 100% containment over the long term. Thus, before any pharmaceutical is grown commercially in a food crop, FDA should conduct a thorough food-safety analysis to ensure that human exposure to the transgenic crop in the food supply would not result in any health risks. If additional legal authority is needed to implement this requirement, FDA and USDA should seek it from Congress.

Such a regulatory system would be able to protect human health and the environment, provide consumers confidence that their concerns are being adequately addressed, and lead to general acceptance of biopharming applications that are found safe. Unfortunately, the regulatory system for biopharming in the United States does not meet those minimum requirements.

The USDA regulates biopharming using its biotechnology regulations established under the authority of the Plant Pest Act (7 CFR 340). Under those regulations, a permit must be issued before any biopharm plant can be released into the environment. Applicants submit an application and USDA conducts some risk analysis of the proposed planting. USDA then issues a permit with specific confinement conditions and conducts inspections during the release to verify compliance.

The USDA permitting system for biopharming is not as rigorous, transparent, or protective as is needed to ensure safety for humans and the environment.

Unfortunately, the USDA permitting system for biopharming is not as rigorous, transparent, or protective as is needed to ensure safety for humans and the environment. First, it lacks transparency and the ability for the public to participate in many of the regulatory decisions. The non-confidential portion of the applications for biopharming permits are not made available to the public nor is any information about the general location or size of the release. Also, when the permit is issued, it is not made available to the public. In addition, the public is not informed about how many inspections are to be made at a particular site or the results of those inspections. Finally, there is no opportunity for public comment before the issuance of many biopharming permits. The public is given an opportunity to comment on a proposed permit only if an Environmental Impact Statement or Environmental Assessment is performed under the National Environmental Policy Act, which occurs in only a small minority of biopharming permits. In contrast, for every other engineered crop, before a petition for non-regulated status is granted (which is generally the last step before commercialization), the public is given the opportunity to comment on the regulatory decision.

Due to the lack of transparency in USDA's regulation of biopharming, it is difficult to assess whether or not their permitting system adequately protects the environment. The National Research Council reviewed some of the environmental assessments for transgenic food crops and found that they were not thorough and did not address broad ecological issues (NRC, 2002b). Some of the documents that have been released by USDA on their assessment of environmental issues surrounding biopharming have been extensive while others were extremely cursory. Thus, it is fair to state that USDA's environmental assessments for biopharming do not always thoroughly analyze gene flow, effects on non-target species, or any broad ecological effects of the transgenic plant.

Based on the documents released to the public about the permit conditions imposed on biopharming (USDA guidance as well as proposed supplemental conditions), the USDA does not require strict biological and physical confinement measures using state-of-the-art technologies. USDA primarily employs geographic and temporal separations and has not required biological confinement measures (*e.g.* male sterility or chloroplast transformation) nor geographic restrictions (such as not growing pharma corn in corn-belt states). Only by using all available confinement measures in a redundant fashion can both human and environmental health be safeguarded from biopharm crops.

Finally, although USDA has the legal authority to address agricultural and environmental issues surrounding biopharming, they have no Congressional mandate to address food-safety concerns. Under the Plant Protection Act, which USDA uses to promulgate its biotechnology regulations, there is no authority to safeguard the food supply. For this reason, USDA's permitting process does not involve any food-safety assessment of a pharma crop before it is released into the environment. USDA's assessment process does not determine whether the gene product will be harmful to humans if it enters the food supply. At the same time, FDA does not conduct any food-safety assessments of pharma crops. Thus, there is an extremely large gap in the federal government's regulation of biopharming where no agency assesses and addresses the food-safety risks of pharma crops.

The Need for FDA to Regulate Biopharming and Safeguard the Food Supply

The Federal Food Drug and Cosmetic Act regulates anything that is intended to be used as food or feed. However, a pharmaceutical corn plant or one producing avidin, for example, is not intended by the developer to be used as food or feed. Thus, those products are neither food additives, nor would they be subject to FDA's voluntary notification process (or FDA's proposed mandatory notification rule). FDA has limited authority over those products unless they show up in food. At that stage, FDA could consider foods containing the pharmaceutical compound (or industrial chemical) adulterated, and remove them from the market. The burden would be on FDA, however, to prove adulteration.

The current system is not the best way to ensure a safe food supply in view of the fact that contamination by pharma crops is inevitable. A possible solution to this problem would be for Congress to require a mandatory FDA approval process for all GE crops, both those intended for food use and pharma crops not intended for the food supply. Under that approval system, no GE food crop could be commercialized without a food-safety approval by FDA. For pharma crops to be commercialized, FDA would either need to

approve the crop as safe to eat or set a safe tolerance for the non-food substance. Then, if that GE crop entered the food supply, eating the engineered substance would be safe as long it was below the tolerance level; consumers would have no need to fear that they are eating unsafe food. In addition, the rigor of the food-safety assessment conducted by FDA should be proportionate to the physical and biological confinement of the crop. If the pharmaceutical crop is grown at a location far from other like plants, only a limited food-safety assessment might be required because the likelihood of contamination would be extremely small. If pharmaceutical corn is grown in Iowa, however, then a complete food-safety analysis might be warranted.

Providing FDA with mandatory authority to review the safety of pharma crops before they are released into the environment is not a far-fetched idea.

Providing FDA with mandatory authority to review the safety of pharma crops before they are released into the environment is not a far-fetched idea. As far back as 2002, a group of industry representatives at the Grain Quality Workshop concluded the following (Maier, 2002):

> *[We] urge the FDA that when future commercialization approvals of genetically modified grains and oilseeds for non-food and feed purposes are considered, these approvals also meet food safety requirements because inadvertent traces of these genetically modified grains and oilseeds will be detected in food and feed.*

The Grocery Manufacturers of America (2003a) also stated that pharma crops should not be grown:

> *…unless FDA has concluded that any release of the nonfood product into the food supply will be safe and that it will have no adverse effect on human health.*

Other countries have also included food-safety assessments for biopharming. In Canada, if a food or feed crop is used for biopharming (Canadian Food Inspection Agency, 2000):

> *…the developer must submit exposure and hazard data for human and livestock health effects assessment [by Health Canada].*

Finally, in the 107th Congress, Senator Richard Durbin from Illinois introduced the Genetically Engineered Foods Act (S. 2546). That bill would require all GE food crops to have a mandatory premarket approval before commercialization, including pharma crops. Therefore, many stakeholders agree that there are significant risks to the food supply from pharma crops and that a regulatory agency, such as FDA, needs to play a mandatory role in ensuring that those crops do not cause harm to humans.

The Road Forward for Acceptance of Agricultural Biotechnology

With the current state of affairs and the many controversial new applications on the horizon, agricultural biotechnology is unlikely to obtain broader societal acceptance in the near future. This will be particularly true for applications of the technology such as biopharming.

To create the proper environment for greater acceptance of agricultural biotechnology products, there should be the following:

- a strong, but not stifling, regulatory system that manages the potential risks of products using scientific risk assessments and state of the art technology;
- a regulatory system that is transparent and participatory;
- independent risk-assessment research that informs the public and regulators about the potential risks of particular applications and how to manage those risks;
- applications of the technology that provide direct benefits to consumers, both in developed and developing countries;
- broader access to the technology through the free licensing of intellectual-property rights to public-sector and developing-country researchers making products for the public good;
- involvement of the public early on in the development of products so that controversial and/or risky applications can be avoided.

The regulatory system must be transparent and participatory if it is to engender trust among consumers.

Agricultural biotechnology is one of the many tools available to move agriculture forward in the twenty-first century. It can provide beneficial products, including pharmaceuticals. To properly utilize biotechnology, however, the regulatory system must ensure that products are safe for humans and the environment. That system must be transparent and participatory if it is to engender trust among consumers. Only then will there be an environment in which consumers will embrace safe applications of biotechnology.

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He was appointed in 2003 to Secretary of Agriculture Veneman's Advisory Committee on Agricultural Biotechnology and 21st Century Agriculture and in November, 2004, to the Food and Drug Administration's Veterinary Medicine Advisory Committee. He is also a member of the governing Bureau for the International Assessment of Agriculture Science and Technology for Development and the Advisory Board for the International Society for Biosafety Research.

Jaffe earned his BA from Wesleyan University in biology and government and then received a law degree from Harvard Law School.

The Importance of Stewardship in Agricultural Biotechnology

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An important issue that concerns us at BIO is that the biotechnology industry be good stewards. We expect that this will be important for a long time to come. Good stewardship relates to regulatory policy and—contrary to the philosophy that we need less regulation—at BIO we understand the role that regulatory policy plays and we embrace it. It is the backbone for all that we do to ensure biotechnology's success.

At BIO we understand the role that regulatory policy plays and we embrace it.

BIO is a trade association representing all facets of biotechnology. We have over 1,100 member companies—90% of which are small entrepreneurial entities—academic institutions and state centers here in the United States. We have members in all fifty states and in thirty-four nations, and we are involved in R&D across all of the sectors, including food and agriculture, healthcare and industrial manufacturing. Our 2005 annual meeting in Philadelphia had close to 19,000 attendees, a record number indicating how this technology is growing in importance.

It can be hard to tell where food and agriculture ends and healthcare and industrial aspects begin, particularly in terms of plant-made pharmaceuticals (PMPs) and industrial products (PMIPs). It is appropriate, therefore, for BIO to examine all facets of biotechnology, particularly in terms of synergisms across these sectors.

2005 marked the tenth anniversary of commercial planting of biotech crops. This, the most rapidly adopted technology in the history of agriculture, now plays an extremely important role for soybean, cotton, corn and canola, representing well over two thirds of all of the varieties that are being planted. 2005 also marked the cumulative planting of one billion acres of biotech crops around the world, the achievement of which all of us who are part of this industry may be proud.

Ten years ago, we focused on agronomic traits. We have begun to move into quality traits and are now developing plants as factories for synthesis of pharmaceuticals and industrial products and materials—the third wave.

The United States has evolved an elaborate regulatory system that we refer to as the Coordinated Regulatory Framework. There is a lot of history here, going back to the late 1980s. It is a science- and risk-based regulatory system and is transparent, and we are working with the regulatory agencies—USDA, EPA and FDA—to ensure that it will become more transparent in the future. The biotech industry has always embraced strong regulatory policy and oversight; we cannot overstate how important this is to us in terms of promoting consumer confidence.

In 2004, BIO released a containment analysis and critical control point (CACCP) plan for PMP and PMIP production.

Stewardship Areas

Within BIO, we have a robust training program and an active group is laying out principles for development and confinement of PMPs and PMIPs. And late in 2004, BIO released a containment analysis and critical control point (CACCP) plan for PMP and PMIP production. These areas constitute a strong stewardship program to help ensure that we are meeting all federal requirements.

Compliance Training

A couple of years ago, the author met with the NABC board to discuss aspects of training deemed mutually important. We have now developed educational workshops dealing with compliance aspects affecting genetically engineered (GE) corn, cotton and soybeans. These workshops will be offered in conjunction with professional society meetings and conferences such as those organized by NABC. Not only do we want those in our industry to participate in these training courses, they will be offered also to universities and federal research agencies to help ensure that all abide by the federal requirements and understand the legal implications involved in conducting field trials with GE crops.

We are planning to provide accreditation as part of the incentive to participate in these training programs.

Furthermore, we are planning to provide accreditation as part of the incentive to participate in these training programs. We are optimistic that we will be able to offer continuing education credits (CECs). We hope to begin offering classes and workshops in the fall of 2005 in conjunction with professional society meetings and be fully operation throughout 2006 and beyond.

What will be involved? The courses will cover notification and permitting procedures, compliance and enforcement, transport and storage, trial-site management, harvest disposition and post-harvest management. An important aspect is auditing and verification, particularly by third parties, also requirements to be met with pesticidal products with regards to Environmental Use Permits. One-day workshops are envisioned; in some cases a half-day or two-thirds of a day may suffice.

CACCP

Within the PMP/PMIP arena, we have been working on drawing up principles for development and confinement. A reference document that we published looks at two areas. One is the principle for controlled exposures to PMPs and PMIPs, and the other describes development practices for PMPs and PMIPs, which examines confinement systems that control exposure and cross-pollination, confirmation of confinement and the use of identity preservation systems. In 2004, we finished the second phase of this project; the principles document was reduced down to a confinement analysis and critical control points (CACCP) approach to PMP and PMIP production. This terminology resonates with people in the food industry who understand the hazard analysis and critical control point (HACCP) system (which relates to risk analysis and food safety) which has many elements in common with risk assessment and management of PMPs and PMIPs.

The CACCP system entails seven principles: how the critical control points are determined, how limits are established, how the process is monitored (a very important aspect), how corrective actions are to be initiated, how verification procedures are establish, and record keeping and documentation. Whether in industry, at a university or within a federal research agency, all of these principles apply.

Commitment of top management is essential. Prerequisites include GMPs and other good Q&A protocols, facility standards, supplier control, cleaning and sanitation, *etc.* Of primary importance are education and training, and as our compliance training programs evolve, we will include modules covering the CACCP system, for example. Participants in the training courses will return to their universities, companies or research agencies and develop institutional standard operating procedures to fit specific home-base needs.

For biotechnology to continue to evolve, commitment to good stewardship on the part of the industrial sector will be essential, together with embracement of federal regulatory policies.

Summary

Stewardship is an extremely important aspect of the development of biotechnology, one that BIO's membership takes very seriously. Our goals include the highest standards of performance, to demonstrate transparency, openness and commitment to regulatory compliance. For biotechnology to continue to evolve, commitment to good stewardship

on the part of the industrial sector will be essential, together with embracement of federal regulatory policies.

More information is available at http://www.bio.org or from the author at mphillips@bio.org.

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Prior to joining BIO in 1999, Dr. Phillips was the executive director of the Board on Agriculture and Natural Resources at the National Academy of Sciences. Before working for the Academy, he was director of the W.K. Kellogg Foundation program for Food, Agricultural and Natural Resource Issues for the 21st Century. He was also director of the Food and Agriculture program of the Office of Technology Assessment (OTA) of the United States Congress. Prior to his OTA service, he was a faculty member at Purdue University and was a senior staff member in the Secretary's office at the USDA.

Phillips has an MS and PhD in food and agricultural policy from Ohio State and Purdue Universities, respectively. He has authored and supervised numerous studies and reports on food and agriculture with a special focus on agricultural biotechnology.

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