North Carolina Biotechnology Center
Research Triangle Park, NC
February 12 – 13, 2003

MEETING SUMMARY

Workshop brings together nursery and forest industries, biotechnologists and environmentalists, to identify needs and research opportunities

With sponsorship from the Institute of Forest Biotechnology, USDA CSREES, USDA Forest Service, and Oregon State University, 40 invited scientists, industry professionals, and environmentalists met in Research Triangle Park, North Carolina on February 12-13, 2003 to consider the application of biotechnology to improve trees in urban landscapes through modifying their reproduction, and the related economic, health, environmental, social, and legal dimensions. The agenda and summaries of speaker presentations can be viewed at www.forestbiotech.org.

The meeting explored the opportunities and obstacles associated with the development and marketing of an urban tree with modified flowering from in-depth and diverse perspectives, so that research needs could be prioritized and solutions to problems identified. The meeting focused on ways to modify flowering to: reduce the spread of invasive and weedy species; reduce release of allergenic pollen; and prevent unwanted fruits. It also considered the importance of limited commercial releases of reproductively sterile trees as a means to rigorously test the effectiveness of technologies for containment of genes before commercial releases occur.

Presentations were given by representatives of leading nursery and forest industries, biotechnology researchers, environmental organizations, and USDA APHIS (the agency that is responsible for regulating most plant biotechnology products). Breakout sessions allowed participants to consider research needs and opportunities for progress in detail.

KEY CONCLUSIONS

Improved gene insertion methods are a priority.
Unlike agronomic crops, gene transfer methods need substantial improvements in efficiency to fully meet the needs of the nursery and forestry industries. New, innovative methods are needed to address the diversity of species and genotypes in use, and to enable genetic engineering to create modified versions of potentially invasive and allergenic species. Fortunately, as a result of the genomics revolution in plant biology, there are good prospects for breakthroughs in this area.

Genes need to be tailored for outcomes and tested.
A diversity of genes, breeding strategies, and management methods are needed to meet the great diversity in industry, consumer, and environmental goals. For example, reproductive control that acts late (i.e., does not prevent flowering or fruiting entirely) will be desired for some applications so that important sources of food for urban wildlife are not removed. These need to be evaluated for each major product and tested in field trials. Collaborations among public and private institutions are essential for engaging the needed diversity of expertise, conducting interdisciplinary research, and building stakeholder approval.

Public sector research and variety development is critical.
Because of the long time frame for product development, small market size for individual varieties, high costs of regulatory approval, and modest size of companies that develop and market urban trees, the public sector should play a key role in technology development. Core research and technology transfer needs could be met by creation of a university-based and/or USDA ARS center of excellence that aims to advance platform biotechnologies, particularly gene transfer and flowering control. This center could also produce selected varieties that solve important problems or are of high demonstration value with respect to societal benefit.

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MEETING RATIONALE, STRUCTURE, AND GOALS

Steve Strauss, Oregon State University

This meeting deals primarily with one form of biotechnology, genetic engineering (GE). GE is defined as the use of recombinant DNA methods to modify the genetic qualities of an organism, whether native or foreign genes are modified and inserted. The main trait considered was sexual reproduction, particularly genetic engineering of traits that would be of value to consumers or the environment and also provide important demonstrations of the effectiveness and stability of GE flowering control traits. For example, the spread of potentially invasive tree species might be greatly reduced by introduction of pollen — or fruit-free forms, pollen-free trees would have reduced allergenic properties, and fruitless trees would avoid nuisance litter on streets and near to homes. The release and monitoring of such varieties, over many years and environments, would provide information about the effectiveness of engineered flowering control, and thus whether current technologies are adequate to provide high levels of containment of transgenes from wild populations, where this is desirable. Without commercial releases, it would be very difficult and costly to get high quality data on containment efficacy. Regulatory agencies, and many public interest groups, are likely to demand such information before they will support commercial uses of other forms of GE trees.

However, many obstacles stand in the way of producing and marketing flowering-modified GE trees. There is not a high consumer demand for modified flowering trees providing an impetus for company investment in this area, nor will they produce large revenues for companies. Transformation methods are inadequate for most urban tree species and genotypes. Regulatory issues, public acceptance of transformants, intellectual property costs and uncertainty of success are substantial, impeding long-term investments. A key focus of the meeting was to explore the opportunities and obstacles in depth and from diverse perspectives, so that research needs could be prioritized and solutions to structural problems identified.

BIOLOGICAL RATIONAL, GOALS, ISSUES

The Nursery perspective on the economic and environmental benefits of biotech sterile trees

Keith Warren, J.F. Schmidt Nurseries, Oregon

The nursery industry views seed reproduction of trees as an essential part of its normal business operations. But in some cases, seeds from the plants we sell can become harmful to the environment and costly to our society. Among the array of invasive species troubling the U.S., a small but significant number began as nursery introductions. The nursery industry wants to be responsible about preventing future escapes from cultivation. In addition, seed dispersal of cultivated plants can sometimes be messy or weedy, increasing maintenance costs in cities and landscapes.

Because of these issues, the nursery industry has been successfully improving its products through conventional breeding and selection to produce low maintenance plants, including seedless varieties. This is biologically possible in some species, but these species are in the minority. The most common approach is to select male plants, which accomplishes the goal of eliminating seeds, but this may add to the pollen load in the air. The majority of species produced by the nursery industry still have the potential to produce seeds, and for these, present techniques are inadequate to create seedless plants.

The nursery industry as a whole does not look to profit from the creation of biotech sterile plants. People are not likely to buy more nursery plants because they are sterile. They add plants to their landscapes because there is room, and they want to add beauty. Sterility is a utilitarian feature that will aid the environment and society more that it will increase the sales of plants for the nursery industry.

The nursery industry is both market driven and market oriented. It constantly strives to improve and differentiate its product. It is much more involved in marketing than other phases of agriculture. New introductions are often patented and trademarked, with royalty payments going to the originator. Marketing campaigns focus on these new introductions. But because of public concern about biotech modified organisms, a partnership of private industry, government, academia, and environmental groups would be most desirable in bringing biotech sterile trees to landscape reality.

The nursery industry has benefited greatly by being the ultimate "green" industry. Our products are beneficial to the environment, and this is recognized by society. We risk being tainted by the looming issue of invasive species. Biotech sterile plants could benefit the nursery industry by creating a solution to the problem of invasiveness and maintain our positive image. In addition, biotech sterile plant could be pollen free; again benefiting society and helping the industry maintain a healthful image.

Ideally, a sterile plant would produce no reproductive seeds, messy fruit, or pollen, but keep the flowers intact. In a few cases where fruit is ornamental, it may be preferable to eliminate the seeds while keeping the fruit, if that is possible. In some cases, flowers are insignificant and elimination them would not be objectionable. It is foreseeable that a sterile plant may possess improved health and flowering display, as seed formation sometimes prevents additional flower production and stresses the energy balance of the plant.

The following urban trees would make good initial targets for the goal of sterility:

Acer campestre (Hedge Maple) — non-native with heavy, messy seed load, sold in relatively small numbers.
Acer x freemanii (Freeman Maple) — native, mostly dioecious, with seedless male clones which produce pollen, sold in large numbers.
Acer ginnala (Amur Maple) — non-native, locally invasive, heavy, messy seed load, sold in relatively small numbers.
Acer platanoides (Norway Maple) — non-native, locally invasive, a very important urban tolerant tree that has become invasive in New England. Sold in large numbers.

Acer rubrum (Red Maple) — native, mostly dioecious, with seedless male clones that produce pollen, sold in large numbers.
Eleagnus angustifolia (Russian Olive) — non-native, frequently invasive, sold in small numbers, but valued because it can grow in climates where other trees cannot survive.
Liquidambar styraciflua (Sweetgum) — native, with large messy fruit balls, sold in moderate numbers.
Malus (Crabapples) — mostly non-native, fruit is ornamental but sometimes quite messy, generally non-invasive but some concern reported.
Pyrus calleryana (Flowering Pear) — non-native, fruit is messy, has been reported invasive in a few locations, sold in large numbers.
Ulmus (Elm) — mostly non-native, seed is messy, invasive potential is unknown as most are new introductions selected for Dutch elm disease resistance, sold in moderately large numbers.

Finally, there are a couple of practical paradoxes to solve regarding biotech sterile urban trees. First, the species that are known to be invasive are so now because of a long history of use. They have already escaped. Sterility will prevent future escapes, but do nothing to stop existing invasions. Prevention of seed formation in a newly introduced potential invader makes the most sense environmentally, as this plant has not yet escaped. But a new nursery introduction sells in small numbers at first and may never become popular, so there is little financial incentive to create sterility. And while the nursery crops represent a huge annual market of 11 billion dollars, these crops are divided into thousands of individual varieties, each comparatively minor in sales. Again, this lessens the financial incentive to work on an individual variety. These paradoxes can only be solved by developing a quick and inexpensive biotech protocol that can create sterility for numerous plants in a cost effective manner. Fortunately, the history of technology is that cost efficiency develops as technology becomes more widely utilized.

Economic and environmental benefits for nursery and horticulture industries

John Keller, Monrovia Nurseries, California

The nursery industry is a large industry with annual sales of $11 billion, comprising 11% of total U.S. crop sales and as a commodity group is ranked fourth overall (1997 Census of Agriculture). The industry is also extremely diverse comprising growers of ornamental plants, fruit and nut trees, vegetable transplants, cut flowers and greens, sod, etc. A large variety of plant material is produced and it is not uncommon for nurseries to produce hundreds, if not thousands, of different plant varieties.

The industry has an insatiable appetite for new plant varieties, and there has been a great rush to bring new plants to market. Often these varieties are brought into production with little or no testing. Nurseries introduce new varieties by working with various people, including professional breeders, government and academic institutions, public gardens, arboreta, and hobbyist breeders. A wide range of plant material is evaluated and the challenge is to sort through all this material and bring the superior ones to market. This evaluation process should include evaluating potential invasiveness.

Invasive species is an important issue facing the nursery industry. Invasive species are a national problem, but solutions should be implemented at the local level. A variety that is invasive in one area of the country is not necessarily invasive in another area having a different set of climatic conditions.

The nursery industry has an opportunity to use genetic engineering to enhance what we do — improve the success and level of satisfaction of the end consumer while at the same time safeguarding the environment. Genetic engineering has the potential to accelerate the introduction of new plants compared to traditional breeding programs. The industry needs to determine how to positively educate the public on the benefits of genetic engineering. Some of the main benefits are environmental, such as the development of non-invasives, improved pest resistance resulting in fewer pesticide applications in landscapes, and reduction of green waste generation with the development of smaller-stature plants. However, the incredible diversity of the industry poses a problem. Although the total value of nursery crops is very large, the sales of an individual species or variety are relatively small. This makes it difficult to choose a candidate plant for genetic manipulation based on economic return.

Economic and environmental benefits, and related enabling opportunities for forestry and horticulture

Steve Strauss, Oregon State University

Although there might be a large long-term benefit to early induction of flowering to speed breeding, the main interest for GE of flowering in forestry is for sterility to enhance growth and provide containment of exotic and transgenic genotypes. Growth benefits may be substantial in some species, but there is little data on which to predict the extent of benefits, especially in dense stands where flowering is limited. Sterility is generally viewed as being a key enabling technology to allow novel genes such as those for herbicide resistance, pest resistance, bioremediation genes, and novel co-products (e.g., industrial enzymes) to be produced in trees. It may also be desirable for large changes to wood quality that may occur in the future, depending on public acceptance and local environmental considerations. Important environmental benefits from sterile trees would be reduced spread of invasive exotics, and reduced spread of transgenes to wild, or GMO-free certified, forests. The high rate of gene flow from trees creates problems in that imperfectly sterile trees during field testing will release some genes via pollen or seed over very long distances, but this also provides very high dilution of small releases, ensuring an extremely small proportion of transgenic gametes even small distances away from their source.

Allergy reduction benefits of pollenless landscape plants

Tom Ogren, California Arborist
Author, "Allergy-free Gardening"

A virtual epidemic of allergy and asthma is well underway.

- The 1959 edition of the Encyclopedia Britannica states that "between 2 to 5 % of the population suffers from allergies."
- The 1985 World Book states, "approximately 15% of the population has allergies."

- In December 1999, the American College of Allergy, Asthma and Immunology released figures showing that some 38% of Americans now suffered with allergies.

Modern landscapes are stacked with dioecious male cultivars and with monoecious cultivars (asexually propagated clones) that have been modified to maximize male and minimize female parts. Male plants never produce seeds or fruit that could litter sidewalks. Male cultivars are widely used to produce low-maintenance, litter-free landscapes. For the past 20+ years the top-selling street trees have been male cultivars. All of these trees produce large amounts of allergenic airborne pollen. In nature these trees would be sexually balanced. Female flowers would trap large amounts of the pollen, removing it from the air. Wind-pollinated pistillate (female) flowers have a positive (+) electrical charge. Pollen from male flowers has a negative (-) charge, thus the two are mutually attractive. Unfortunately in many modern urban landscapes there are virtually no female trees left.

Pollen is a potent allergen and it is a significant bio-pollutant. Costs to the public from pollen-allergies are staggering and increasing. Pollen exposure is greatest closest to the pollen source. If one lives on a block lined with allergenic trees, one's exposure is many times that of one living on a block lined with insect-pollinated or female-only trees. There are already at least five American cities with some form of pollen control ordinances.

Urban forests have a huge impact on the lives of people. This impact is strongly positive, but with pollen allergies, the effects have a negative component. It is already established that increasing carbon dioxide levels and increasing temperatures result in increased pollen production. It is crucial that we undertake a systematic approach to urban pollen reduction on a national level. Universities could and should take the lead.

There is a real need for pollen-free street trees that are not messy. Urban arborists do not like to plant female trees, even though they are pollen-free, simply because they often shed so much seed, old fruit, seedpods, and other sidewalk litter. A sterile GE produced female tree would be both pollen-free and would also be litter-free.

During recent consulting work for various local county asthma coalitions, they have brought up their concern about the landscaping at their city schools. Often this landscaping relies heavily on male cloned shade trees and these same trees are triggering considerable pollen-allergy and pollen-triggered asthma. Asthma can be a life threatening disease and it is now the most common chronic childhood disease in America. Asthma coalitions are becoming quite popular and common. A simple Google search will bring up hits on over 20,000 coalitions. These coalitions have considerable political clout.

Large wholesale nursery growers insist there is no demand for female or otherwise pollen-free trees. As pollen-control ordinances start to take hold as a popular idea, as a valid way of trying to do something positive about curbing pollen-induced illnesses, the need for pollen-free trees could well suddenly explode. Since large caliber street trees take time to produce, the nursery industry needs to start now to be able to fill the coming demand.

There is also a very real need for pollen-free trees that are not dioecious. Many of our most useful city trees are monoecious, having both sexes on the same tree. With these species genetic engineering could be a real plus, giving the public its beloved alders, oaks, birch, and sugarberry trees, to mention a few, in a pollen-free form. A computer search of the published material on Allergy Free Gardening shows that more than 99% of the considerable press on the subject is positive. If scientists could add to the selection and give us more useful pollen-free street trees, millions of people would benefit and would be appreciative.

Just recently, on the most popular allergy website, www.Pollen.com, it was stated that "a pollen-producing tree in your own yard will expose you to ten times the amount of pollen as would a similar tree planted down the block." It is a viable, growing trend in urban landscaping and the need for allergy-friendly plant materials, especially new and useful shade trees will only increase in the foreseeable future.

TECHNOLOGY AND ENVIRONMENTAL CONSIDERATIONS / RESEARCH NEEDS

State of gene transfer, genetic engineering, and genomics related to flowering

Will Rottmann, ArborGen, LLC

Three general areas of plant biotechnology were reviewed:

- the molecular methods for genetically engineering reproductive sterility,
- transformation and
- *in vitro* regeneration of plants, and field testing.

The theoretical range of flowering phenotypes that can be generated by introducing synthetic genes is quite broad. Reports of pollenless flowers, seedless flowers, and complete reproductive sterility are available in the scientific literature. Modifications of these traits, such as increasing the showiness of flowers or temporary reversion to fertility, have also been described. The methods of making the more sophisticated types of flowering control are still being refined, and require interactions between multiple components that would all need to be transformed into the plant.

Genetic ablation is a stable, well-understood means of preventing the development of specific floral parts, and is probably the method of choice for most forms of genetic containment. The primary requirement is a promoter (segment of DNA containing regulatory information) that is highly specific to the tissue or organs that are to be removed. The promoter is attached to a coding region for a digestive enzyme or other protein that interferes with normal growth of the cell. When this gene is present in the plant, any time that a cell starts to develop in the direction of making a flower (or pollen, etc.) it produces the digestive enzyme and fails to grow. The greatest weakness of this method is that non-specific expression of the ablation gene can cause loss of vegetative growth or premature flower drop.

Suppression, particularly RNA interference (RNAi), is another method that has a lot of experimental support. Introduction of a gene carrying an inverted repeat of a sequence essential for reproductive development will cause the messenger RNA for the reproductive gene to be specifically degraded. The primary requirement for this method is knowledge of the identity and sequence of a target gene or genes – those that must be expressed for development of a fertile flower. Comparative genomics between the species of interest and *Arabidopsis* can provide a selection of educated guesses for suitable target genes, but these must be validated by experimentation. A third category of ways to prevent floral development is repression, a catchall name for the expression of a regulatory protein that changes how genes are turned on or off during development. One method is to over-express a naturally occurring regulatory protein that ordinarily functions to prevent flowering during the juvenile stage of plant growth. Another is to express a mutated form of a flowering regulatory protein that is unable to perform all of its normal functions. These so-called dominant negative mutations interfere with the activity of the non-mutated counterpart so that flowers develop abnormally. Customized, DNA-binding, repressor proteins can be overexpressed to similar effect.

Production of a plant that contains a transgene involves two steps: inserting the gene into a chromosome of a single cell (transformation) and then generating a plant from that cell (organogenesis or somatic embryogenesis). Transformation can be accomplished using a modified form of *Agrobacterium*, a naturally occurring genetic engineer. Alternatively, the DNA can be inserted using a mechanical method such as microparticle bombardment. In this case, DNA-coated metal particles are propelled into the plant tissue, where the DNA dissolves and can become incorporated into the chromosome. The bombarment method is crude, in that many copies of the transgene are often inserted, but it is also readily adapted to many species and tissue types.

Inducing the transformed cells to grow into new plants can be very challenging. A regeneration method should be worked out for the species and cultivar of interest before embarking upon a transformation project. The response to tissue culture conditions varies greatly among species; even individuals from the same species with different genotypes can behave very differently.

Once a potential product is produced that is transgenic, a significant amount of time and money will need to be spent on molecular characterization of the plants and on field tests. USDA/APHIS requires a large amount of experimental data in order to make the decision to allow unregulated release of transgenic plants. Just as important, field demonstrations will be needed to convince potential customers that the tree/shrub behaves as promised. It can be expected that several years of data from multiple sites would be required before approval is given.

Environmental considerations: Threats from invasive plants and how they might be reduced

John Randall, The Nature Conservancy

E.O. Wilson said "On a global basis…the two great destroyers of biodiversity are, first habitat destruction and, second, invasion by exotic species."

The impacts from invasive species can be enormous and far-reaching. These can be described as ecosystem level impacts, and community and population level impacts.

Ecosystem level impacts include disturbance regimes such as displacement of native species, reduction of preferred food by animals and birds and changes in fire cycles. Altered hydrology such as the water table level is another ecosystem impact. Geomorphologic processes such as the extent of erosion and sedimentation can be affected by invasive species. Invasives can alter nutrient cycling and soil or water chemistry impacting the ecosystem.

Community and population level impacts include vegetation structure where invasives can dominate habitats, displacing native species. Community composition and resource competition are affected by invasives since they are competing for available water, light, nutrients and space. There are negative impacts on native animals due to reduction in native species recruitment and promotion of non-native invasive animals. There can be population reductions or eliminations of native species through hybridization with invasive species.

The positive aspect of invasives is the lag phase before they become irreversible. This lag phase can be quite long, as with *Casuarina equisetifolia* in Florida which was not perceived as a problem until after 65 years, to as little as 4 years for *Rubus sp.* in the Galapagos. There exists a window of opportunity to eradicate invasive species just after introduction and detection, but this is most often not utilized due to the fact that public awareness typically does not begin until much later in the process and where control costs steeply rise.

There are several organizations that predict new problem species and prioritize existing problem species. Some of these organizations are the USDA Federal Noxious Weed List, California Exotic Pest Plant Council, Invasive Plant Council of New York State, Southeast Exotic Pest Plant Council, and Florida Exotic Pest Plant Council.

Environmental considerations: Non-target organism considerations for use of flowerless, pollenless, fruitless, or seedless landscape plants

Kelly Bender, Urban Wildlife Biologist, Texas Parks and Wildlife

Currently, land is being converted from agriculture or undeveloped land to suburban sprawl at a rate of 9,320 km^2/year (NRCS 2000). This land fragmentation is a major factor in the decline of many species of wildlife. With the increased fragmentation of wildlife habitat, it may be assumed that remaining fragments of land, including land in urban areas, is increasingly important to wildlife. However, landscapes in urban areas are typically human creations, developed according to our values, aesthetical preferences, and activities. Even undeveloped lands in urban areas have been invaded by ornamental plants with marginal wildlife value, exotic plants that spread aggressively into native habitat, and non-native generalist predators such as the house cat. Research by Hunter, Simpson, and Bender (2002) indicates that good urban habitat can increase native bird species, increase ground nesting bird species, reduce introduced bird species, increase insect-eating bird species, and increase over-wintering bird species. Therefore, if one of the things we value is wildlife, we should provide suitable habitat (food, water, and shelter) for native species in our urban lands, whether they are developed or undeveloped. Key elements of wildlife habitat include native plants that wildlife can use for food and shelter, a variety of plant species, structures, and water features, and layers within the plant community (canopy, understory, and groundcover). Plants that are important for wildlife include oak, pine, wild cherry, dogwood, cedar, maple, hackberry, and birch species, but research of specific plant use by all species of wildlife is lacking. Therefore, I suggest the following guidelines when choosing species to modify:

- Consider modification of species that are non-native and are potentially aggressive spreaders.
- Eliminate from consideration species that are native to the area since they most likely provide a benefit to a native wildlife species
- Consider modifications that still allow flower, nectar, and fruit production.
- Consider that seeds are sometimes a very nutritious and beneficial food source to wildlife.

ISSUES FACING COMMERCIAL APPLICATIONS

Intellectual property: Obtaining licenses, patenting varieties / methods

Michael Goldman Esq., Nixon Peabody, New York

The transgenic papaya, which is resistant to papaya ringspot virus, constitutes an important contribution to the papaya industry particularly, and, in general, to the transgenic plant field. After this plant was developed, however, it was determined that it included technology that was covered by others' patents. In order to proceed without incurring liability for patent infringement, it was necessary to determine which patent rights were infringed, to negotiate license agreements, and to insure that the license agreements were properly implemented by the Hawaiian papaya industry. Ultimately, this effort resulted in obtaining licenses from academic and commercial institutions. The need for licenses in order to commercialize the transgenic papaya has important lessons for researchers who produce transgenic plants, entities holding patent rights to basic transgenic plant technology, and organizations who are commercializing transgenic plants.

Some lessons can be summarized from the transgenic papaya example.
Lessons for Researchers

- Recognize that there are patents covering commonly used components and procedures.
- Know that the manufacture, use, or sale of patented materials without a research license is direct patent infringement.
- Use unpatented or easily licensed technology.

Lessons for Patent Holders

- Make technology freely available after filing for a patent.
- Widespread use of technology can lead to adoption as an industry standard.
- Competitors' use of patented technology can provide significant leverage over competitors.
    - Maximize royalty payments
    - Prevent competitors from introducing commercial products
    - Use as trade bait for what you need from the competitors

Lessons for Technology Licensees

- Determine what the patents actually cover.
- Reduce the risk of procuring and paying for unnecessary patent rights by not relying on rumor.
- Control how the licensed technology is made available.

Regulatory approval: Data needs, trade, national and international regulations

John Cordts, USDA APHIS

In 1986 the White House Office of Science and Technology Policy (OSTP) issued the Coordinated Framework for the regulation of biotechnology, which defined the regulatory approach intended to ensure the safety of biotechnology research and products. This Coordinated Framework is consistent with the conclusion of a 1987 report of the National Academy of Sciences that found that the potential risks associated with genetically engineered (GE) organisms should be similar in kind to those associated with traditionally bred organisms. The agencies involved in this regulation include the US Department of Agriculture, the Environmental Protection Agency and the Food and Drug Administration. Depending on their intended uses, products may be regulated by more than one agency.

USDA/APHIS regulates (1) living organisms (plants, microorganisms, arthropods), that are (2) a product of genetic engineering, and (3) that may pose a plant pest risk. APHIS (the Animal and Plant Health Inspection Service) is responsible for protecting US agriculture from pests and diseases. APHIS regulations are designed to limit the persistence of viable organisms in the environment at the conclusion of a movement or field test of a regulated article. Currently, two mechanisms are available for importation, interstate movement and field testing/release of GE plants. Materials may be moved or tested under "notification" or "permit." Notification is a streamlined system that APHIS uses for organisms and traits with which they have experience in managing the risks. Materials requiring closer evaluation by risk assessors (pharmaceutical-producing plants, those where gene function is unknown, those where gene expression might results in plant disease or production of an infectious entity, etc.) may be planted after approval of an APHIS permit application. After significant evaluation and typically several years of field testing, a petition for non-regulated status of tested plants (and/or their progeny) can be made to APHIS. Requirements for petition submissions are listed on the APHIS website (http://www.aphis.usda.gov/ppq/biotech/ and http://www.aphis.usda.gov/ppq/biotech/petguide.html) and cover requirements for both molecular genetic and environmental characterization data. These submissions are quite comprehensive and, if approved, allow for unregulated planting and distribution of the GE product. Since implementation of the Coordinated Framework in 1986, there have been over 9000 field trials in the US with no reports of significant environmental harm. APHIS has granted non-regulated status to 56 different crops in 13 species. Although field tests have been done with fourteen species of trees, no petitions for non-regulated status have been submitted for a tree species.

EPA's role in the regulation of GE plants applies when a species has been engineered to contain herbicide resistance or a plant-incorporated protectant (PIP) for enhanced resistance to a plant pest. EPA's evaluation considers (1) the fact that the PIP has been introduced to control a pest and therefore has some level of toxicity to a pest and (2) the potentially new exposures provided by the PIP's expression in the modified plant. EPA has also evaluated the risks associated with wide scale use of PIP's selecting for resistance in targeted pest populations. They continue to be actively involved in insect resistance management issues and the responsible use of this pest control technology. EPA's role in herbicide resistant plants includes (1) registering use of the herbicide on a new plant, and (2) considering the risks associated with new exposures to that herbicide's residues.

As part of the Department of Health and Human Services, FDA regulates food and feed derived from new plant varieties and requires that GE foods meet the same rigorous safety standards as is required of all other foods. Guidelines on consultations with FDA on safety and regulatory questions can be located at the referenced website (http://www.cfsan.fda.gov/~lrd/biotechm.html).

Items to consider when moving transgenic plant materials through the evaluation process toward commercialization include the following:

- Plant/gene combinations are evaluated on a case-by-case basis.
- Commercialization is possible without going through the petition process–APHIS anticipates that pharmaceutical producing plants will always be grown under a permit or similar program.
- Gene flow, per se, is not considered a risk–the consequences of gene flow need to be evaluated to determine if there is a real risk.
- A petition for determination of non-regulated status can include more than one GE line.
- Early consultation with the appropriate regulatory agencies can be time saving.

To summarize a variety of international issues and interests surrounding development and commercialization of GE plants, the US and a number of other countries do have comprehensive regulatory systems in place to ensure both the food and environmental safety aspects of biotechnology-derived plants. Many other countries are developing regulations. Significant issues driving new regulatory policies will likely also address consumer issues, trade considerations and politics in addition to scientific evaluations.

Public views of biotechnology in the USA: Lessons from agricultural biotechnology for public acceptance of genetically engineered landscape plants

Tom Hoban, NC State University

The potential benefits of biotechnology will only be realized if society accepts the science and new products as safe and ethical. Consumer acceptance varies for different types of applications and between countries. Public support for applications in new human medicines (85%) is high while applications to increase animal productivity remain low (35%). The benefit of biotechnology perceived by different countries varies greatly. North America leads the acceptance list (66%) with Europe the least accepting (38%). Asian countries vary greatly with 79% acceptance in Indonesia and only 33% acceptance in Japan. Cuba leads the Latin American countries with 79% acceptance and Argentina with 44% acceptance.

Consumer awareness of biotechnology is moderate, but knowledge levels tend to be quite low. American consumers were not aware that they had eaten GM foods (62%). After learning that biotech foods are in stores and they had eaten them, consumers felt more confident that they are safe.

Some lessons from introducing crop biotechnology into society are:

- Preparation is key, not reaction.
- Conduct ongoing social science research.
- Enhance confidence in government and credibility of scientists, transparency.
- Understand and engage thoughtful critics.
- Communicate with consistent and carefully crafted messages using credible sources.
- Show the benefits of the technology, but acknowledge public concerns and the need to carefully manage risks.

Some points to remember:

- Society supports science when it recognizes benefits and views risks as acceptable. Society grants scientists the freedom to operate and provides resources for research.
- “Sound Science” is only one criterion for public policy making. For a growing number of people this alone is not persuasive. Other cultures value other factors.
- Benefits and risks must be carefully evaluated; but few benefits will outweigh serious ethical objections.
- Distributive justice is at the heart of many fears. Pay more attention to ethics and values early in the process.
- Words make a bid difference (“GMO” or “biotech”). Recognize that “Perception is Reality.” Education about the facts will not calm deep-seated anxieties by itself.
- Listen carefully to public before you communicate. People make decisions based on a combination of logic and emotions. Show that you care and act ethically.

Our key responsibility is to ensure that society has enough knowledge and foresight, and continue to confirm safety before products are introduced into the environment.

WAYS FORWARD

Public sector / non-profit approaches: A landscape plant breeding and biotechnology center

Steve Strauss, Oregon State University
Harold Pellet, Landscape Plant Development Center, Minnesota

Many factors suggest a predominant role from the public sector will be required to responsibly develop flowering modified trees. Most important are that costs and uncertainties are large and long term, while the market size and value of individual varieties is modest. There are also many uncertainties pertaining to the basic science and technology, regulatory approval, intellectual property licenses, and public acceptance. A novel kind of center that would both conduct applied research, and produce commercial varieties with high demonstration value, was envisioned. It would need to be funded to deal with intellectual property and regulatory issues as well as to conduct research. Because of the complexity and rapid evolution of the technology, a university-based center, with numerous national collaborations to aid in gene identification, transformation, and field-testing, would be desirable.

The Landscape Plant Development Center (LPDC) is a non-profit corporation that was founded in 1990 with a mission of developing superior landscape plants with emphasis on plants that are more tolerant of biological and environmental stresses. Headquarters for the Center are at the Minnesota Landscape Arboretum. The Center has a field research station in Oregon on land that was donated by the J. Frank Schmidt Family Charitable Foundation. The Center accomplishes its research mission as a cooperative approach involving scientists of many different universities, and arboreta in different geographical regions. Through this cooperative approach, the Center has access to and utilizes the plant collections of many arboreta in different regions for its breeding efforts. This provides a broad base of plant germplasm to use in hybridization. First generation progeny are grown at the Oregon station. The segregating second-generation hybrids are distributed to and planted by cooperators in many different geographical locations. In this manner, superior plants well adapted to the climatic conditions of the region in which they are grown can be selected and introduced for landscape use in the respective regions. This cooperative approach provides an effective and efficient method of breeding plants for many different regions.

The first breeding project of the Center, developing small trees of ornamental pears, was initiated in 1991 utilizing the mature *Pyrus* plants growing at the USDA Clonal Repository at Corvallis, Oregon. Crosses were made between plants of ten different species in many combinations. First generation hybrids are growing at Washington State University – Puyallup experiment station. Second generation hybrids are being grown and evaluated by cooperators in many different regions.

Research efforts are also well underway to develop small trees of *Acer* and *Carpinus* and non-vining forms of *Clematis.* Efforts have also been initiated to produce superior small growing forms of shrub genera, to select superior forms of native species and to develop sterile cultivars of exotic species that are valuable landscape plants but that have invasive tendencies.

Much of our research is accomplished as cooperative efforts with other institutions. Our maple and hornbeam breeding are cooperative efforts with Dr. Susan Wiegrefe of the Morton Arboretum. We also have a cooperative arrangement with Dr. Rita Hummel, Washington State University – Puyallup. We provide some of the funding for technicians that are working with Dr. Hummel. They have been growing our original ornamental pear hybrids, some of the maple hybrids, and are now using their greenhouse to propagate many of our hybrid seedlings from other crosses as well. We are also providing grants to Dr. Tom Ranney at North Carolina State University and to Dr. Alan Smith, University of Minnesota to support technical help for cooperative research to develop sterile cultivars of potentially invasive species. Dr. Steve Strauss recently received a small grant to begin a cooperative approach to develop sterile cultivars of Liquidambar (sweertgum) and crabapple. Dr. Herb Aldwinckle, Cornell University and the Center will work with Dr. Strauss on that effort.

In addition to the cooperative efforts in which the Center is providing some funding, we have cooperative efforts with research participants at many different institutions that are involved in evaluating some of our second-generation hybrid populations to select outstanding plants that perform well under the climatic conditions of that region. Plants selected for introduction will be done so as joint plant introductions between the Center and the institution that makes the selection. Cooperators at University of Georgia-Griffin, University of North Carolina, Mountain Horticultural Research Station, Texas A & M, Pennsylvania State University, Iowa State University, Cornell University, Michigan State University, University of Minnesota, and University of Arizona are evaluating seedling populations. This spring, plants propagated from promising individual plants are being sent to those same cooperators in addition to Oklahoma State University, Kansas State University — Wichita, and University of California — Riverside for evaluation under the different geographic conditions.

The Center has a rather large list of "research participants" at many different institutions. These are people involved in research that relates either directly or indirectly to the research of the Center. Although not funded by the Center, their research efforts contribute to achieving goals similar to those of the Center. Some of these are the cooperators that are evaluating our hybrid populations and selections. More will become directly involved, as the Center is able to expand our activities.

Our operating philosophy is that the goal of developing superior landscape plants with emphasis on plants more tolerant of environmental and biological stresses is what is important. In many cases we can leverage the needed physical facilities by entering into cooperative efforts with Universities and/or other organizations that already have the needed facilities but that may have limited manpower. By providing funding to hire additional help we can cooperatively make greater headway that we can by working independently.

The Center's staff includes myself as a volunteer Executive Director, Teri Line, a part time Administrator/research technician and Sarah Doane, manager of our Oregon research station. We are currently recruiting a plant breeder to help carry out the cooperative breeding effort with Cornell University.

Private sector: Biotechnology business approaches

John Pait, CellFor

To outline a mock business plan that supports the production and sales of reproductively modified urban trees, the product must be identified with certain criteria defined. The product must meet a need or solve a problem such as a "pollen-free" tree to alleviate allergy and respiratory problems. The advantages of such an urban tree offer health benefits to urban citizens, it would have the lowest non-target organism effects, female flower structures can be retained, and have aesthetic and maintenance benefits.

Market considerations would include which species to use; market size, segments, distribution channels, and penetration; technical feasibility of pollen prevention constructs and propagation platform. The statistics of annual US nursery market for volume and sales reveals that the largest market size for nursery trees is in oaks, pines and hollies, with the unit price for each tree being $3.62 for pines, $8.85 for hollies, and $32.05 for oaks. Both oaks and pines are considerable contributors of pollen. If price drives the product selection, then oak should be used as the target product. Note that these statistics include all types of oaks, not just one species.

The business model should include:

- Advance acceptance
    - Focus groups in target markets
    - Gain advocates from health and urban forestry groups
    - Contingent commitments in likely markets: lowest and highest pollen cities
- Manage risk and reward the risk takers
    - Spread risk: form consortium among those who bear development and regulatory burdens
    - Finance: public funds for regulatory testing and equity funds for technology development
    - Maintain long term monitoring in test areas
- Branding and perceptions

When defining the pollen-free oak value chain, likely consortium partners and their value capture and sharing would include anti-pollen construct owners (5%), transformation experts (30%), propagators (40%), wholesale and retail nurseries (20%), landscape contractors (5%), and cities, offices and homes (delivers full value). From this information, the market penetration and incremental sales to the value chain can be calculated as:

25% incremental Value: $20 MM	Penetration rate (%) & Sales ($)		
Value Chain	5%	10%	15%
Construct owner	$ 50,000	$100,000	$ 150,000
Transformation expert	$300,000	$600,000	$ 900,000
Propagator	$400,000	$800,000	$1,100,000
Nurseries	$200,000	$400,000	$ 600,000
Landscaper	$ 50,000	$100,000	$ 150,000

From this mock business model some observations can be gleaned. Given the substantial investment required for anti-pollen constructs, transformation and propagation, a single species will not provide adequate revenue; other species should be phased in. Fair value capture to elements in the chain will require simulation, negotiation and patience. In the longer term, gene stacking may be acceptable in the market. Employ the best science guided by the highest integrity. Proceed very, very carefully with the public; first impressions last a lifetime.

KEY LEARNINGS

Tree flowering and reproduction control are important research topics in forestry and horticulture. Basic studies in this area enable scientific progress in forest ecology, molecular biology, physiology and population genetics of tree species. Applications of existing knowledge can help to accelerate some kinds of tree breeding. New knowledge, and technical advances in gene transfer and control of gene expression, are needed for (a) reduction of pollen loads and associated allergy problems; (b) control of invasive species; (c) ecologically acceptable means for control of gene flow from genetically modified varieties; and (d) reduction of "nuisance fruit problems" associated with some popular tree species in urban and suburban settings.

The Institute of Forest Biotechnology is in a position to work with other interested parties to develop a strategy for better defining and realizing the benefits of research on flowering and reproduction control in tree species. The strategy will need to address the problems imposed by inadequate R&D funding support for three critical platform technologies: 1) genetic transformation, 2) regeneration of transformed tissues, and 3) acceleration of flowering onset. Due to the diversity of species and varieties cultivated by the nursery industry, effective transformation methods are a particularly large impediment to developing genetically engineered urban trees. In addition, most laboratories do not have the necessary capabilities and resources to develop, acquire, or propagate transformed lines, and those that do lack the resources to subsidize the research of others. Similar platform technology limitations in other fields of science have been overcome by creating and supporting mechanisms such as user facilities, centers of excellence (e.g., a transformation research and service center), and material exchange programs. Consideration and respect for intellectual property related to transformation and tissue culture will be critical to success.

There appears to be good potential to align forestry and horticulture interests in support of a strategy to accelerate research on flowering and reproduction control in tree species. The cultures, capabilities, and information needs of forestry and horticulture are different but complementary. Basic biological research on model tree species would be useful to both sectors, and there are opportunities for collaboration on applied research on human health and allergenicity; ecological concerns of invasive species and genetic containment; and economic issues related to regulation, intellectual property rights and market feasibility.

Flowering control technologies need to be diversified so they can be tailored to the highly diverse needs of urban environments. As cities and suburbs grow, and wild habitat is diminished, urban plants provide increasingly important habitats and food resources for wildlife. Tree fruits and flowers, even pollen cones or catkins, may be important food sources. To minimize food reduction by backyard and park trees that support wildlife, it will be desirable to have late-acting reproductive control mechanisms (e.g., pollen or seed germination lethality). However, for city street or home-proximate trees, fruit removal may be most desirable to reduce nuisance litter and improve tree vigor.

The connection between male and new exotic plants to increased levels of allergies and asthma is strong in some locations, and interesting but not compelling in others. Epidemiological and modeling research is needed to understand how changes in pollen production of planted trees could alter pollen counts and the incidence of allergies and asthma locally (homes, localities) and regionally (different species and climatic conditions) over time. One possibility is to establish large-scale, non-transgenic, pollen-free plantings in a few selected cities where the reduced allergy result could be demonstrated. In other situations, biotechnology might be the only means of achieving reduced allergenicity.

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