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SUMMARY. There is a well established and rigorous framework to regulate interstate movement and environmental release of transgenic plants. Three federal agencies, the U.S. Department of Agriculture, the Food and Drug Administration, and the Environmental Protection Agency, are involved in the regulatory process. To date, more than 10,000 field trials have been conducted and approximately 60 different crop-species combinations have been deployed commercially in the U.S. This includes a wide range of crops that have been genetically engineered for a variety of traits. The purpose of this article is to describe the process by which one obtains permission to release transgenic plants for both experimental field trials and for commercial purposes. doi:10.1300/J411v18n01_07 [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <<http://www.HaworthPress.com>> © 2006 by The Haworth Press, Inc. All rights reserved.]

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REGULATORY OVERSIGHT

The United States Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) are the three Federal agencies responsible for regulating the testing, movement, and commercial deployment of transgenic plants and their products. Oversight for these agencies was outlined by the Office of Science and Technology Policy in 1986 through the Coordinated Framework for Regulation of Biotechnology. The USDA, via the Animal and Plant Health Inspection Service, Biotechnology Regulatory Services (APHIS, BRS), has the authority to regulate interstate movement and environmental release of all transgenic plants by virtue of the Plant Protection Act. The EPA, through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), regulates the planting and food and feed use of transgenic plants into which genetic material has been inserted that imparts a pesticidal property (plant-incorporated protectant, PIP). In addition to registering the use of a PIP, the EPA also establishes tolerances, or exemptions from the requirement of a tolerance, through the Federal Food, Drug, and Cosmetic Act (FFDCA). The EPA is also responsible for registering the agrochemicals used on engineered crops. Finally, the FDA is granted authority under the FFDCA to regulate transgenic food and feed crops or products from transgenic crops that may come in contact with food. A description of their monitoring protocol is provided in their Statement of Policy: Foods Derived from New Plant Varieties (1992). Thus, depending on the trait for which a crop has been engineered, more than one Federal agency may be involved in its regulation. More details on the biotechnology regulatory process and its history can be found on the Agricultural Biotechnology page at the APHIS website (<http://www.aphis.usda.gov/brs/index.html>). Below is a brief description of the requirements for each Federal agency involved in regulating transgenic plants. Due to space constraints and their overarching authority, the role played by APHIS is emphasized.

Regulatory Oversight by APHIS

Currently, two mechanisms are available for the importation, interstate movement, and release (field-testing) of genetically engineered (GE) plants. Initially (between June 1986 and April 1993), transgenic materials could be moved or tested only under a "permit." The permit application review process was lengthy (minimum of 180 days) because an Environmental Assessment (EA) was needed for each application. In

May 1997, APHIS introduced its “notification” system for a wide range of plant species, which streamlined the process (i.e., a 10-day review for interstate movement notifications and a 30-day review for notifications requesting environmental release) for organisms and traits with which APHIS had familiarity in managing risk. Transgenic plants requiring more careful scrutiny are still subject to the permitting process (e.g., those plants containing genes: (1) derived from a human pathogen, (2) encoding the production of a pharmaceutical, or (3) whose function is unknown). During their review, applications (for permits and notifications) are forwarded to the appropriate regulatory authority within the affected state(s), which then approves the request, denies it (with justification), or imposes requirements more stringent than those specified by APHIS.

Detailed instructions for filing a notification can be found on the following page of the APHIS website: <http://www.aphis.usda.gov/brs/usergd.html>. Briefly, a notification must contain the following information:

- *Responsible party* (the applicant, who is legally liable for non-compliance);
- *Duration of introduction* (notifications are usually valid for only 12 months, though extensions can be requested; longer periods are allowed, but annual status updates are required);
- *Recipient organisms* (scientific names of species transformed; limited to a single species);
- *List of regulated articles* (including line designation(s); description of gene inserted; expected phenotype; designation of genetic construct(s) used to transform plant(s); and genetic components that went into assembling construct(s), such as promoters, coding sequences, terminators, and selectable markers);
- *Mode of transformation* (e.g., *Agrobacterium tumefaciens*);
- *Introduction* (where the transgenic material will originate, as well as its final destination); and
- *Certification* [statement in which the responsible party certifies that the regulated article will be handled in accordance with the relevant Performance Standards (see below) and regulations set forth in the coordinated framework (7 CFR 340.3)].

If permission to move or release transgenic materials is granted, a letter of acknowledgement is sent to the applicant. In that letter, APHIS

specifies the responsible party must comply with a list of Performance Standards, which include the following sections:

- *Shipping and maintenance at destination* (to prevent loss or environmental release of the regulated material);
- *Identification scheme* (to prevent accidental mixing of regulated and non-regulated materials);
- *Termination procedures* (including steps that will be taken to “devitalize” the plant material);
- *Monitoring* (to ensure devitalization was successful and to prevent regulated material from persisting in the environment); and
- *Training* (to ensure that these standards are clearly communicated and adhered to by all employees who have access to the regulated material).

Before transgenic plants can be grown for commercial purposes, a petition for non-regulated status is submitted to APHIS. This is done only after extensive evaluation that typically involves several years of field tests. Instructions for submitting a petition can be found at the APHIS website (<http://www.aphis.usda.gov/brs/petguide.html>). These include requirements for both a complete molecular characterization of the transgenic plants and data on potential environmental impacts. If the petition is approved, the transgenic plant is no longer regulated (i.e., unrestricted distribution and planting is granted (although APHIS could impose restrictions, if needed). Since the Coordinated Framework was established in 1986, more than 10,000 field trials have been conducted in the U.S.; APHIS has granted non-regulated status to approximately 60 different crop-species combinations (a complete listing can be found on the APHIS website). To date, no detrimental effects have been reported. Although 14 tree species have been field-tested, with the exception of papaya, non-regulated status has not yet been granted for any of them.

Function of EPA

The EPA is involved in regulating GE plants when they have been engineered to contain a PIP that imparts resistance to a specific pest. That evaluation considers the extent to which the introduced PIP is toxic to the target pest as well as the potential for unintended exposures through the expression of PIPs in GE plants. The EPA also assesses the potential for targeted pest populations to acquire resistance to the PIP as

a result of its widespread use. All petitions involving plants containing a Bt (*Bacillus thuringiensis*) gene must include a resistance management plan that details steps that will be taken to minimize the risk of insects becoming resistant. EPA's role in regulating GE plants also encompasses: (1) establishing tolerance, or exemptions from the requirement of a tolerance, for the PIP; (2) registering herbicides to which crops are engineered to be tolerant, if the product was not previously registered for use with that species; and (3) assessing the risks associated with new exposures to herbicide residues.

Role of FDA

The FDA regulates human food and animal feed derived from all plant varieties, and holds products obtained from GE plants to the same rigorous safety standards required of all other foods. These efforts can include collecting data on allergenicity, digestibility, and toxicology of the protein encoded by the transgene. Information on safety and regulatory issues related to GE plants can be viewed at the FDA website (<http://www.cfsan.fda.gov/~lrd/biotechm.html>).

Other Considerations

While many other countries are developing their own regulations, U.S. agencies are, to the extent possible, trying to harmonize their regulatory framework with rules being promulgated elsewhere. The existing U.S. structure is also being revised. To aid in the formulation of new regulations for herbaceous perennials, APHIS hosted a public hearing in Baltimore, MD, during January 2003, at which they solicited input from experts in various related fields, representatives from industry and non-governmental organizations (NGOs), and the general public. A similar meeting was held in Washington, DC, in June 2003 to obtain input regarding regulations for woody perennials. In January 2004, APHIS announced its intention to prepare an Environmental Impact Statement (EIS) in which the agency will evaluate its biotechnology regulations and consider several possible changes (<http://www.usda.gov/Newsroom/0033.04.html>). These modifications are likely to include the development of a multi-tiered system to replace the current permit and notification system, along with enhancements to the deregulation process to provide for greater flexibility in approving products as well as long-term monitoring. Any changes to the regulations are expected to

be science- and risk-based, but they are also likely to be influenced by consumer issues, trade considerations, and politics.

Finally, while working toward commercialization of engineered products, potential petitioners should be mindful that:

- Early consultation with the appropriate regulatory agencies can save a lot of time and needless actions and delays.
- A petition for non-regulated status can include more than one independent line (transformation event).
- Species-gene combinations are evaluated on a case-by-case basis.
- With the appropriate permission, it is possible to use safety data submitted by previous petitioners.
- Gene flow is not considered a risk *a priori*; it must be evaluated to determine whether a perceived risk is genuine.
- Under rare circumstances, it may be possible to commercialize a product derived from a transgenic plant without deregulation by APHIS. However, the developer must still abide by permit and notification requirements and other regulations specified under 7 CFR, section 340. In addition, if the inserted gene encodes a PIP or the plant has any potential food or feed use, the EPA and FDA still remain involved. In general, this alternative approach is unwise and leaves the developer open to significant liability and financial risk.
- Pharmaceutical-producing plants might always be regulated in some way (i.e., they may never be fully deregulated by USDA).

TRANSGENE CONFINEMENT

The National Research Council recently released a report entitled: “Biological Confinement of Genetically Engineered Organisms” (<http://www.nap.edu>). The committee that drafted the report was asked to address the following questions:

- What is the status of various bioconfinement strategies?
- What methods are available and how feasible, effective, and costly are they?
- How can bioconfinement failures be mitigated?
- What methods are available for detecting failures?
- What are the probable ecological consequences of large-scale bioconfinement?

- What new information is needed for addressing any of the above questions?

The report's major conclusions are listed below.

- The effectiveness of a confinement method will vary depending on the organism, environment, and the scale over which it is applied.
- To evaluate efficacy, the genetically engineered organism (GEO) should be compared with its progenitor before release. For trees, it may be necessary to begin field tests before such comparisons are possible.
- Various confinement methods should be tested separately and in combination, in a variety of appropriate environments, and in representative organisms.
- The need for bioconfinement should be considered early in the development of a GEO.
- Non-food crops should be utilized for genes encoding products that need to be kept out of the food supply.
- An integrated confinement system should be based on risk.
- The stringency of confinement should reflect the consequences of GEO escape.
- It is unlikely that any single confinement method will be 100% effective.
- The use of redundant methods will increase the likelihood of accomplishing the desired confinement level.
- Government regulators should consider the potential effects a U.S. confinement failure could have on other nations, and vice versa.
- International cooperation should be sought for managing GEO confinement.
- Social and ethical values should be considered when assessing the stringency needed for confinement.
- Transparency and public participation are needed to develop and implement the most appropriate bioconfinement approach.

The need for bioconfinement in several major crop classes is discussed below. Because the public comment period for a petition to de-regulate herbicide-tolerant creeping bentgrass (*Agrostis stolonifera*) has recently ended, and because there is considerable familiarity with this case, that species will serve as a model for describing work done with an herbaceous perennial. Likewise, considering that poplar (*Populus*)

has been used in more transgenic research than any other tree species, it will be highlighted in the section on woody perennials.

Herbaceous Annuals

In general, herbaceous annual crops are heavily domesticated and have virtually no wild relatives with which they are inter-fertile. In addition, the products harvested (e.g., seed, fruit, pollen) are generally derived from the flowers. Thus, there is neither a need nor a desire to prevent flowering in these species.

Herbaceous Perennials

Other than cotton, which is intentionally grown as an annual, no transgenic herbaceous perennial crops have yet been commercialized. However, a number of transgenic perennial grasses are being developed at both public and private institutions. These grasses have been engineered for tolerances to herbicides, drought, disease, and cold; enhanced quality (e.g., digestibility, nutritional content); reduced stature; and lignin content. Traits are currently being investigated for creeping bentgrass, bluegrass and fescues, as well as in alfalfa, another herbaceous perennial. As of this writing, petitions requesting the deregulation of two products, glyphosate-tolerant creeping bentgrass and glyphosate-tolerant alfalfa, have been submitted to the USDA. The *CP4 EPSPS* gene, which confers tolerance to glyphosate (the active ingredient in the herbicide Roundup®), has been inserted and expressed in those plants. This gene is identical to that utilized in other crops, such as canola, corn, cotton, and soybean, which have been planted on tens of millions of acres since the commercialization of Roundup Ready® soy in 1995.

Creeping Bentgrass

Research on glyphosate-tolerant creeping bentgrass (GTB) has been conducted since the mid-1990s. This product offers the opportunity to apply glyphosate directly on crops grown either for their seed or as sod and turf, without causing altered performance and growth, or environmental adaptations. Weeds, such as annual bluegrass and rough stalk bluegrass, are extremely difficult to manage within an existing sward of bentgrass. Although mechanical or physical control measures exist, they can be very destructive, especially on a golf course where aesthet-

ics and continuous play are desirable. Furthermore, selective herbicides for such weedy grasses are not available.

In seed-production operations, great care is taken to establish pure stands of bentgrass by first applying pre-emergent herbicides. However, on golf courses weed presence is usually tolerated, so that both unwanted and desired species must be contended with simultaneously, often requiring greater use of pesticides, water, labor, etc. However, if growers were able to control those weeds during the initial production phase, seed purity would increase and golf course superintendents would need to manage only for the creeping bentgrass. Removing the annual bluegrass or rough stalk bluegrass would also eliminate the diseases and insects to which those species are specifically susceptible, which in turn, would significantly reduce reliance on pesticides and potential chemical exposure by farm workers, golfers, area residents, local wildlife, and the environment in general.

Because GTB is not engineered to produce its own pesticides, the USDA is the principal regulatory authority for its oversight. Therefore, prior to commercialization, its developers must petition that agency to deregulate the product so it can be freely transported and planted. In addition, application must be made to the EPA to extend the current registration of specific glyphosate formulations to include their application on GTB. The FDA must also be consulted because creeping bentgrass straw is used as an animal feed.

APHIS has acquired substantial expertise and knowledge about a number of widely planted crops, including cotton, corn, canola, and soybean. However, the specific data required from a petitioner seeking deregulation are based on the plant species transformed and its expressed trait(s). For example, the inserted DNA must be fully characterized and the biology and life history of the plant clearly documented. This information has been agreed upon by USDA-APHIS, the EPA, the Canadian Food Inspection Agency (CFIA), and Health Canada, in a series of meetings beginning in 1998. Such criteria are listed at the USDA website and should be consulted by applicants when determining the studies needed for achieving regulatory clearance for a transgenic crop. By tailoring their requirements to the specific plant and trait under consideration, the agencies can perform a thorough and appropriate EA.

Environmental Assessment

To assess the environmental and non-target safety of GTB, transgenic and non-transgenic creeping bentgrasses have been examined at all

stages of their life cycles to determine if their potential for becoming a plant pest has been affected in any way, as is done with annual plants. These studies compared seed and vegetative establishment, plant growth, flowering, pollen viability and longevity, fecundity, seed germination, seedling vigor, insect resistance, disease susceptibility, and various botanical characteristics. University researchers, along with scientists from The Scotts Company and Monsanto, performed more than 90 individual experiments between 1999 and 2003 at 65 field locations. Those sites represent the northern (or cool), southern (or warm), and transition-zone climates to which turf grasses are adapted. The experimental environments comprise both managed and unmanaged ecosystems, with extreme variations in light, moisture, soil type, nutrition, competition, and temperature. Studies were also conducted to assess the possibility for intra- and inter-specific and inter-generic out-crossing, and the relative fitness of putative hybrids. Finally, herbicide trials were performed to determine the efficacy of glyphosate alternatives for controlling both GTB and hybrids.

Based on the results from these studies, the petitioners have been determined that GTB and its progeny are substantially equivalent to non-transgenic creeping bentgrass.

Stewardship

Seed Production

As part of their usual risk assessment, the USDA, FDA, and EPA do not require a comprehensive stewardship plan. However, stewardship has become an important component of transgenic crop management and should be considered by the developer well in advance of commercialization. Such a plan should address, to the extent possible, that the transgenic plant: (1) remains in the country(s) for which regulatory approval has been obtained, (2) is kept in the environment where first planted, and (3) is used as intended. Thus, while gathering data to support the deregulation of GTB, it has been important to consider the appropriate stewardship practices for all users of the product (i.e., seed and sod producers and golf course managers).

To meet the current demand for creeping bentgrass seed used on golf courses in the U.S., approximately 7,000 acres are farmed annually; about 98% of this acreage is in the Willamette Valley of Oregon. However, to satisfy the anticipated demand by sod farms and golf courses for GTB seed, considerably less acreage, perhaps no more than 3,000 acres,

probably would be needed. This amount of seed could be grown by less than two dozen contract growers, each of whom could be sufficiently isolated geographically (if required) to prevent out-crossing to or from conventional seed farms.

The Scotts Company and Monsanto have worked with growers in Jefferson County, Oregon, and the Oregon Department of Agriculture (ODA) to establish an 11,000-acre Control Area within which only bentgrasses developed through biotechnology would be grown. This Area was approved by the ODA after two public hearings in June 2002, and is located approximately 100 miles east of the Willamette Valley. These two farming regions are also separated by the Cascade Mountain Range. Because the considerable distance and physical barriers will block gene flow to conventional bentgrass fields in Oregon, the risk is managed to help avoid unintentionally exporting GTB to countries in which import approval has not been obtained.

Golf Courses and Sod Production

Creeping bentgrass is a low-stature, fine-textured, soft, dense, carpet-like turf grass that tolerates low mowing. Therefore, frequent watering, optimum fertilization, and disease and soil management practices are needed to prevent competition from other grass or broad-leafed weed species. Even under optimal nutrition and watering regimes, this crop is susceptible to a wide range of diseases, including pink snow mold, brown patch, and dollar spot. Because of its intense cultural requirements, creeping bentgrass is not suitable for planting by homeowners. It is instead used for putting greens, tees and fairways, lawn bowling greens, grass tennis courts, and other specialized applications. Therefore, The Scotts Company and Monsanto intend to market GTB exclusively for the golf-course market through seed and sod production, rather than for residential, industrial, or other recreational applications.

To grow GTB, both sod farms and golf courses will obtain licenses that specify good stewardship management practices. For example, managers on sod farms maintain their growing turf at the same height as its final intended use, between 1/8 to 3/4 inches. Under those conditions, creeping bentgrass is unlikely to flower, pollinate, or set mature seed (Lush, 1988). Therefore, appropriate stewardship will be focused on: (1) implementing precautionary measures that minimize potential seed and stolon scatter or movement via equipment to other courses or farms; (2) maintaining GTB turf at heights that preclude pollination, flowering, and development of mature seed heads; (3) devitalizing stolons and

aerification cores that could enable unintentional vegetative propagation; and (4) controlling GTB volunteers through herbicide applications and monitoring, either after a golf course is renovated or abandoned or when a sod farm has been harvested. These practices should significantly reduce the potential for undesired establishment, reproductive growth, and gene flow to wild relatives. Moreover, mechanical measures and a number of herbicides other than glyphosate are available for removing unintended GTB growth and eliminating environmental risk. However, just as a pesticide applicator takes precautions to ensure an herbicide does not drift to non-target plants, responsible stewardship will mean maintaining transgenic turf grass only where it is intended.

In summary, the regulatory assessment of herbaceous perennials is similar to that of annuals. While collecting research data, it is important to consider the unique characteristics of the transformed plant and the expressed trait. Communication with the appropriate regulatory agencies and academic and industry stakeholders is strongly recommended as early as possible in the product-development process so that the pertinent environmental, non-target, human safety, and stewardship concerns are addressed. Doing so will facilitate the review process and potentially reduce the time spent gaining approval. Websites for U.S. regulators as well as the countries intended for export or environmental release should be consulted for further information; these sites are updated as regulatory requirements change.

Woody Perennials

Although numerous transgenic crops are currently being grown for commercial purposes (see: <http://www.aphis.usda.gov/brs/>), papaya represents the only commercial deployment of a transgenic woody perennial. Its release resulted from a special effort to save an entire industry from the ravages of ubiquitous ringspot virus in Hawaii (Gonsalves, 1998). This unique case has involved virtually no environmental risk because papaya originally had been introduced to Hawaii (meaning there are no inter-fertile wild relatives) and because the Pacific Ocean is an effective physical barrier to transgene escape.

As of now, all other transgenic tree species in the U.S are being grown only for research purposes, for three reasons: (1) existing regulations were written with the perspective of agronomic row crops, which are highly domesticated and have few, if any, wild relatives; (2) these plant systems have ecological issues that are different from annual row crops; and (3) biotechnological techniques have been slower to develop

for trees (mainly the latter). Even though APHIS has never stated that transgene escape will not be allowed, it has made clear that efforts must be taken to mitigate the risk of transgene spread from those species, at least during the early stages of development. Several strategies can be employed to achieve transgene confinement.

With the current state of technology, it is impossible to guarantee absolute sterility for any species. However, from a purely scientific or even a risk-reduction perspective, complete sterility may not always be needed before transgenic trees can or should be grown commercially. This is especially true for riparian species, such as poplar, that can be grown under fertigation in xeric environments, such as east of the Cascade Mountains in the Pacific Northwest. Native poplars are largely absent from this landscape. Given their reproductive isolation and the half-life of pollen under very dry conditions, there may be no justification for preventing the establishment of even fully fertile transgenic trees in that region. Triploid genotypes of poplar are available that have greatly reduced innate fertility; transgenics produced in these clones might safely be grown in areas where no inter-fertile wild relatives are present.

The need for sterility depends on the trait being exploited; the environment within which the transgenics will be grown; the particular species; and various social, political, and ethical considerations. Each case must be analyzed individually. In some situations, the introduced gene imparts a competitive disadvantage; transgenic trees growing outside an intensively managed plantation would be unlikely to survive. Nevertheless, because of the uncertainty, many research groups around the world are exploring techniques to genetically engineer flowering control. For example, one common procedure is to ablate (eliminate) cells by expressing a deleterious gene in a tissue-specific fashion (Mariani et al., 1990). A second engineering method employs dominant negative mutations (DNMs). DNM genes encode mutant proteins that suppress the activity of co-existing wild-type proteins (Espeseth et al., 1993). A third technique involves gene silencing. Recent studies in a variety of eukaryotic organisms have shown that double-stranded RNA (dsRNA) is an inducer of homology-dependent gene silencing; the use of dsRNA to induce silencing is termed RNA interference (RNAi) (Hannon, 2002).

Aside from transgene confinement, reproductive sterility may be engineered for other reasons. First, rapid (juvenile) rates of growth can be maintained. During the early phase of its life, a plant utilizes all of its photosynthate for vegetative growth; after it undergoes the transition to maturity, some carbohydrate is metabolized for reproductive structures.

This diversion of energy causes a reduction in growth rates, particularly in trees (Eis et al., 1965; Tappeiner, 1969). Therefore, the earlier the flowering process is interrupted, the greater the potential benefit.

One major handicap for scientists working in the area of flowering control in trees is the long juvenile period. Understanding the genes involved in the regulation of floral development will not only expedite sterility research, but also potentially speed the progress to be gained through conventional breeding, by accelerating the onset of flowering. Moreover, delayed flowering is itself a possible confinement strategy, especially when trees are grown under short-rotation intensive culture. Thus, a great deal of effort is being made to identify genes that regulate the transition from vegetative to reproductive phase in a tree's life cycle.

Addressing the Concerns of NGOs

Several NGOs are opposed to the commercial deployment of genetically engineered plants, including trees. One of their primary concerns is that the spread of particular transgenes might increase the modified plants' competitiveness and, therefore, their invasiveness. Another issue is that farmers might lose their ability to use existing pest-control measures. For example, organic fruit and vegetable growers can now exogenously apply purified Bt toxin (which, in fact, is produced commercially using genetic engineering tools), and still maintain their "organic" rating. It is feared that through widespread use of Bt toxins, insect populations will develop resistance to that management tool, rendering it ineffective. Finally, there are people who are philosophically opposed to the idea of genetic engineering (e.g., it's "wrong" or "unnatural").

Any developments that arise from genetic engineering must be viewed in the proper context (in contrast to current practices). For example, companies now plant a single genotype (i.e., clone) of hybrid poplar on thousands of contiguous acres of land. Because those non-transgenic trees have been vegetatively propagated, all of their cells, including their pollen, contain exactly the same DNA. When this synchronous population starts flowering, it produces a monotypic pollen supply. The resulting pollen cloud can be carried considerable distances by the wind and can ultimately affect the genetic diversity of inter-fertile wild trees. Engineering flowering control alone (not coupled with any other trait) would prevent this gene flow from occurring. Under circumstances where it is important to protect sensitive genetic stocks (not just for

trees), engineered flowering control alone may be desirable. A second example pertains to herbicide usage. Some growers currently use environmentally detrimental compounds to control competing vegetation. To obviate this need, we have already engineered trees to tolerate an herbicide that is not volatile, carcinogenic, teratogenic, or mutagenic. In addition, this less injurious compound is soil-inactivated and biodegradable. Having such herbicide-tolerant trees not only can encourage the use of a more “environmentally friendly” product, but also reduce the overall reliance on agrochemicals.

Many public concerns surround the ecological ramifications of complete sterility. For example, insects or mammals may rely on pollen or seed as a food source. However, this argument is not so applicable to a species such as poplar, which is wind-pollinated and does not produce nectar or support a large number of insect or vertebrate pollinators. Furthermore, poplar seeds are very small, virtually devoid of endosperm, and have a short life span. Thus, they are not considered to provide a significant source of food for wildlife. However, for species whose pollen is a valuable food supply, it may be necessary to engineer those plants to produce pollen that, although nutritionally unaltered, is infertile. The intention is not to supplant wild trees with genetically engineered sterile versions; the goal is to plant trees that have been domesticated through genetic engineering into intensively managed plantations, on previously disturbed sites, to satisfy society’s increasing demand for renewable resources. This paradigm will help protect native and old-growth forest from being harvested.

On the other hand, trees that never produce any pollen could be beneficial under certain circumstances. Planting pollen-less stock in some urban settings would reduce the pollen loads from species that are highly allergenic to a large proportion of the human population [e.g., *Cryptomeria japonica* and birch (*Betula*)]. It may also be desirable, from the perspective of a homeowner, to engineer street trees that are incapable of producing nuisance reproductive structures [e.g., sweetgum (*Liquidambar styraciflua*) and *Ginkgo biloba*]. This conundrum highlights the need to consider each crop-trait combination on a case-by-case basis.

To answer one criticism stated earlier about transgenic crops, several steps can be taken to minimize the risk of insects becoming resistant to Bt toxins. As mentioned previously, the EPA requires that petitions involving plants containing a Bt gene must incorporate a resistance management plan, which details the process to be followed in reducing the possibility of such a resistance developing to the PIP. These plans often

include the establishment of refugia, which are adjacent areas on which only non-transgenic plants of the same species are grown. Because insects cannot become resistant unless they are exposed to the toxin; the greater the exposure, the greater the chance of resistance development. Therefore, insects feeding on non-transgenic plants will remain susceptible to the toxin and mate with those that may become resistant by feeding on transgenic plants, thus maintaining a susceptible population.

Another strategy is to engineer plants with two Bt toxin genes, each of which being effective against the target insect via a different mechanism. The likelihood that insects will become resistant to both toxins is the product of the individual probabilities of them becoming resistant to each toxin. Furthermore, the non-target insects are much more likely to be exposed to exogenously applied Bt toxin (as is practiced by organic growers) than to a toxin produced inside plant cells (i.e., the insect actually has to eat some of the transgenic plant in order to become exposed). Again, we must put this issue in the proper context; the alternative to a PIP is the use of broad-spectrum insecticides that kill all insects, both targeted and beneficial.

Finally, there are great risks associated with doing nothing. With the recent increase in international trade agreements but a simultaneous lack of funding for Federal agencies charged with enforcing import laws, the potential for environmental catastrophes has risen tremendously. Given this, circumstances can occur under which it would be desirable to spread a transgene into the wild in order to contain a newly introduced environmental threat. For example, the Emerald Ash Borer (EAB, *Agrilus planipennis* Fairmaire) is an aggressive exotic pest recently arrived from Asia that is attacking and killing all the North American ash (*Fraxinus* spp.) trees it invades. First identified in Michigan in 2002, the EAB has since been detected in 13 counties in Michigan, two counties in Ohio, and one in Maryland (from transported nursery stock), as well as in Windsor, Ontario (Haack et al., 2002). To prevent the spread of the EAB, these areas are under quarantine to restrict the transportation of ash trees, branches, nursery stock, logs, and firewood. This pest is fatal to all trees it attacks, no means are available for its control, and evidence suggests that, unchecked, the EAB will spread throughout North America. Currently, in efforts to prevent its continued migration, when an infestation is found, all ash trees within a half-mile radius are fallen and incinerated. However, if nothing else is done to eradicate this highly destructive pest, American ash species may go the way of American chestnut and elm. Genetic engineering may be the only effective tool for managing the threat of EAB.

THE NEED FOR FIELD TRIALS

Transgenic plants should be field-tested for several reasons. The first is proof of concept—it must be shown that the inserted gene is having the expected effect. *Agrobacterium tumefaciens* is widely used to insert genes into plant cells, but these insertions occur at random locations within the genome. The DNA surrounding the site of insertion influences the efficiency with which the transgene is expressed (i.e., “positional effects”). In addition, transgenes can be differentially expressed under various conditions (e.g., Callahan et al., 2000). Thus, it is prerequisite to screen independent lines (plants derived from cells that have undergone separate gene insertion events) to verify that the transgene is being expressed at commercially useful levels. It may also be necessary to conduct field trials to determine if there is sufficient value so that end-users are willing to pay a biotechnology premium (licensing fee) for use of a genetically modified plant. Finally, long-term field studies are needed to detect somaclonal variation and assess the stability of transgene expression (Meilan et al., 2002, 2004).

INTELLECTUAL PROPERTY CONSIDERATIONS

Other than directly isolating and cloning a gene of interest, it is virtually impossible to obtain genetic material for plant transformation without signing a Material Transfer Agreement (MTA). Standard agreements are now widely used, and usually specify that biological material (constructs, bacteria, plants) containing the genetic material covered by the agreement cannot be shared with a third party without the expressed written permission of the provider. University researchers who use transgenic plants only for research purposes may be able to obtain an exemption from paying license fees. However, even those scientists are well advised to keep intellectual property (IP) issues in mind when configuring their transformation vectors, especially if there is any chance that the resulting transgenic plants could enter a commercial stream.

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