Pergamon

Biomass and Bioenergy Vol. 14, No. 4, pp. 403-414, 1998

Printed in Great Britain

P11: 50961-9534(97)10077-0

0961-9534/98 $19.00 + 0.00

ENVIRONMENTAL EFFECTS OF GENETICALLY ENGINEERED WOODY BIOMASS CROPS

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(Received 10 March 1997; revised !2 May 1997; accepted 2 December 1997)

Abstract The use of genetically engineered crop plants has raised concerns about the risks these crops pose to natural and agricultural ecosystems. The potential environmental hazards of transgenic woody biomass crops is discussed, and based on the biology of these crops and their transgenes, recommend a scientific framework for assessing risk. The potential impacts of transgenes based on both characteristics of the transgenic crop and potential for spread of the transgene to other organisms is considered. It is argued that risk assessment should focus exclusively on the phenotype expected from the transgene within a given plant host and environment, weighing both the costs of foregoing the benefits a transgenic variety can provide and the possibility of adverse environmental effects. Basic principles of population genetics can be used to facilitate prediction of the potential for transgenes to spread and establish in natural ecosystems. For example, transgenes that are expected to have neutral or deleterious effects on tree fitness, including those for lignin modification, reproductive sterility and antibiotic resistance, should be of little environmental concern in most biomass crop systems. In contrast, transgenes that are likely to substantially affect host fitness pose a greater risk, as are plants with transgenes which produce a substance known to disrupt ecological processes. Field experiments to determine population replacement and transgene flow are desirable for testing such predictions; however, the long generation times of tree crops makes such studies prohibitive. It is argued that a combination of demographic data from existing non-transgenic populations, simulation modeling of transgene dispersal, and monitoring field releases can be used to guide current risk assessment and can be used to further scientific knowledge for future assessment.

Keywords-Genetic engineering; herbicide resistance; insect resistance; Bacillus thuringiensis; Populus; hybrid poplar; gene flow; introgression; invasiveness; lignin; reproductive sterility; environmental risk assessment.

1. INTRODUCTION

Gene transfer technologies hold promise as a means to accelerate the genetic improvement of woody biomass crops. For example, genetic engineering is being used to improve resistance to insects and herbicides, and to alter wood chemistry to facilitate pulp production. Of the woody biomass crops, genetic engineering of poplars (Populus spp., including cottonwoods and aspens) is the most advanced. Poplars are considered models for tree genetic engineering because they are amenable to *Agrobacterium* gene transfer, have rapid growth, can be readily cloned, and genes are available that are clearly useful for their management and production. l

The goal of this paper is to provide a scientific framework to assess potential environmental risks associated with transgenic woody biomass crops. The literature pertaining to en vironmental effects of genetically engineered herbaceous crops is briefly reviewed and the relevance of this information to transgenic woody biomass crops is discussed, particularly in regard to how ecological and genetic factors influence risk. Genetic engineering is defined as the isolation, configuration and transfer of genes using recombinant DNA methods. Manipulation of plant genomes via traditional breeding, genome mapping and marker-aided selection is not included.

Introductions of crops into new regions has sometimes led to substantial displacements of native vegetation and the creation of weeds due to gene flow between the crop and wild relatives.2 However, the continued genetic modification of crops through traditional breeding has produced a good record of environmental safety.3 Whether the products of genetic engineering should be treated similarly to the introduction of exotic species or to traditionally bred crop varieties has been much

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debated. Although transgenic plants clearly pose some unique risks because they can contain genes derived from vastly different organisms, transgenes and the phenotypes they impart are typically known in great detail. Therefore, a tiered approach is suggested where only those plants whose transgenes pose significant risks based on knowledge of the phenotypes they impart be required to undergo special evaluation before commercial use. In Section 8 of this paper, scientific principles are suggested for determining which transgenes pose significant risks.

Potential environmental impacts resulting from the use of crop plants with novel genes are described elsewhere.[4–7] These risks are grouped into two categories: those due to properties of the transgenic crop itself, and those resulting from transgenes spreading to other organisms. Included in the first category are transgene properties that enhance the crop's ability to invade native or managed plant communities, or transgene products which impact ecological processes and nontarget organisms. Included in the second category are risks associated with transfer of genes to interfertile wild or feral plant populations, or to distantly related organisms (horizontal gene transfer). It is considered here how well current regulations address environmental risks of transgenic plants, based on the biological factors that influence risk.

2. CURRENT REGULATION OF TRANSGENIC PLANTS IN THE U.S.

The environmental release of transgenic plants is primarily regulated in the U.S. by the Animal and Plant Health Inspection Service (APHIS), which is part of the Department of Agriculture (USDA), and by the Environmental Protection Agency (EPA).[8] The U.S. Food and Drug Administration (FDA) regulates the safety of food products from transgenic crops, but not the environmental impacts of these crops. APHIS determines the potential for transgenic organisms to become agricultural pests, whereas, the EPA determines the potential environmental effects of those transgenic organisms that are pesticidal or that otherwise might produce environmental toxins.

APHIS's criteria for determining which transgenic plants are potential pests focus on the method of DNA introduction rather than on the characteristics of the modified plant, an approach which has brought scientific criticism.[9,10] Any transgenic plant is considered by APHIS as a potential pest if the donor organism, recipient organism, vector or vector agent is classified as, or suspected of being, a plant pest. Most genetically engineered plants fall under the jurisdiction of APHIS because *Agrobacterium tumefaciens* is widely used to facilitate plant transformation. Also, the regulatory DNA sequences used to control transgene expression are commonly obtained from this and other plant pathogens.

Regardless of whether genetically engineered crops have been unfairly or unscientifically singled out for regulation, APHIS's assessments generally follow the scientifically rational schemes proposed by the National Research Council[11] and National Academy of Science,[12] taking into consideration the biology of the crop and its wild relatives, the nature of the genetic alteration, the environment in which the crop is intended for cultivation, and small scale field test data.[13] Some have criticized APHIS for not requiring sufficient environmental data to be collected during the small scale field trials.[14] Others contend that knowledge has been gained from small scale tests (usually < 10 acres) and there is now a need for large scale tests (e.g. hundreds to thousands of acres) to adequately address environmental issues. Due to their high cost, such tests may only be feasible by incorporating them into the early stages of commercialization.[15]

EPA is primarily concerned with the release of environmental toxins and the potential for toxin genes to spread via hybridization with wild plants.[16] EPA does not currently regulate pesticidal plants produced from sexual crosses, including bridging crosses (use of an intermediate species to transfer genes between two sexually incompatible species), embryo rescue, manipulation of chromosome number and surgical alteration of the plant pistil. This approach exempts poorly characterized hybrids and the importation of exotic germplasm, as occurs during traditional breeding, and discriminates against recombinant DNA techniques that provide new resistance genes whose characteristics have been intensively studied.[17]

However, EPA does not unilaterally regulate all genetically engineered pest-resistant plants. For example, EPA has proposed to exempt from regulation plants with viral coat protein (VCP) transgenes. VCP transgenes can be very effective in protecting plants from viral infections. They do pose a potential hazard in that a second virus could acquire the VCP transgene through transencapsidation, and this acquisition could, in turn, extend that virus's host range. 1','9 The EPA considers the probability of such an event to be very low, and proposes to either exempt all plants with VCP-derived resistance, or those that have a low probability of outcrossing to wild relatives. An important factor in EPA's decision was the great environmental and economic benefits of VCP-derived virus resistance; risks were judged to be small in comparison.

3. HAZARDS ASSOCIATED WITH THE PHENOTYPE OF THE TRANSGENIC CROP

3.1. Increased invasive ability

Emerson defined a weed as "a plant whose virtue has not yet been discovered;- 20 however, in the context of transgenic risk assessment, the concern is over the plant that by nature of its virtue, its vices have been overlooked. The definitions of a weed abound ,21 with the recurrent theme that they are plants which grow in abundance where they are not wanted. The authors concur, and here define weeds to include all plants that displace desired organisms, whether growing in managed or wild habitats.

Plants which have the greatest potential to become weeds tend to be those which can rapidly invade disturbed habitats. Weeds tend to have:

1. high reproductive potential (due to high fecundity, long lived seeds, short generation times or vegetative reproduction);
2. adaptations to both short and long range dispersal; and
3. and the ability to compete interspecifically by specialized means, such as rapid choking growth, rosettes or allelochemicals.22

Many weeds have one or several of these characteristics, but whether such characters can predict weediness and invasive ability is much debated .2sz4 The absence of natural enemies may also play a major role in allowing a plant species to proliferate and invade. The large number of plants have become weeds when introduced to regions outside their native range, and the subsequent control of these plants when their native insect hervivores were released as biological control agents demonstrates the effect that herbivores can have on host plant invasiveness. 25

To identify the potential risk of a transgenic plant becoming invasive, it is necessary to examine:

1. the characteristics of the parent species that may promote weediness;
2. the availability of habitat suitable for invasion;
3. the extent to which invasion is of concern;

and 4. the phenotype imparted by the transgene.

With respect to the transgene's effect on phenotype, it is critical to consider whether it will significantly enhance invasiveness or detract from human control. Transgenes which provide a large fitness advantage, perhaps by protecting from herbivory or disease, may enhance invasiveness. Transgenes which promote herbicide resistance may impede current ability to control transgenic crops in agricultural and `wild' habitats.

3.2. Direct impacts of gene products on the environment

Transgene products should be evaluated for their potential to become environmental hazards. Products with pesticidal properties fall into this category and should be tested for non-target effects. It is important to consider the phenology of toxin production in the plant, as well the plant parts where they are produced or stored. Products may enter the environment through the roots, leaf litter, or fitter left behind after harvest. Toxins from the bacterium *Bacillus thuringiensis* (Bt) vars. *kurstaki* and *tenebrionis,* for example, have been genetically engineered into several crops including tomatoes, tobacco, corn, potato and poplar. Tapp and Stotzkyzb found that Bt toxins remain active in soil and are both bound and adsorbed by clay particles, reducing their potential leaching rate. However, the presence of the toxin in active form is not necessarily an environmental hazard because Bt toxins affect only select groups of insects, and must be ingested. Furthermore, the scale and pattern of use may mitigate the effects of Bt on non-target populations. 27

Transgenic crops could provide a means by which to reduce the use of environmentally hazardous pesticides. Although biological pesticides often have narrow host ranges and thus little direct impact on natural enemies, 28 their use is limited as a consequence of high cost and specialized application requirements. Weather can greatly reduce their efficacy and persistence, leading to a need for frequent applications. In contrast, engineered crops could produce these toxins when and where needed. When toxins are produced within plant tissues, non-target organisms are exposed to a much lesser extent than with spray applications because only those organisms which feed on the plant tissues come into contact with the toxin.

Unfortunately, if these crops are planted over extensive acreages, the rate of pest biotype evolution towards resistance may increase, 29,30 potentially rendering all forms of the biopesticide ineffective. Resistance is by no means limited to transgenic plants. It has caused pest control problems for some uses of every major insecticide. Externally applied biopesticides pose some risk of causing resistance in pests, but have a short field life, and have never been used extensively for trees. Transgenic trees are likely to be used on a large scale and to remain planted for several years. The net result of resistance mismanagement could be a loss of the investment in genetic engineering, and a renewal of reliance on chemical insecticides, many of which have a variety of undesirable ecological consequences.

4. HAZARDS ASSOCIATED WITH TRANSGENE SPREAD

The risk of a transgene spreading in the environment depends on the likelihood for outcrossing or horizontal gene transfer, and the phenotype the gene imparts. Because transgenes are stably incorporated into plant chromosomes, they can spread into the gene pools of wild or feral interfertile species through outcrossing.s's',32 The probability of successful outcrossing depends on sexual compatibility, physical proximity and distance of pollen movement both out of and into the transgenic crop.32-35 For some woody biomass crops, such as those in the genus Populus, sexual interspecific compatibilities exist, but introgression can be impeded by hybrid sterility, incompatible flowering phenology and reduced fitness of hybrid progeny (hybrid breakdown).

Whether or not gene transfer might occur between transgenic plants and phylogenetically divergent organisms, such as bacteria or fungi, can only be speculated on. Bacteria can gain exogenous DNA by three different mechanisms: transformation (the incorporation of naked DNA); transduction (transfer of DNA by way of a phage); and conjugation (genetic transfer mediated through cell-to-cell contact). These mechanisms usually only operate within a species, but may occur between bacterial species, or may lead to gene transfer from a bacterium to a higher organism. 36 If microbes are able to obtain exogenous plant DNA, it would most likely be through transformation, or as a result of parasitic or endophytic interactions. A small fraction of the DNA released from transgenic plants into soil binds to clay particles where it is protected from degradation, 37 yet is available for uptake by bacteria.3g However, if transformation between plants and bacteria does occur in soil, it happens infrequently and has not yet been observed.39

Little direct evidence of gene transfer between plants and microorganisms exists. High molecular weight, exogenous, host plant DNA has been found to regularly occur within spores of the parasitic fungus *Plasmodium brassicae* in the laboratory. However, whether or not this DNA actually gets incorporated into the prokaryotic genome is not clear.40 Other more indirect evidence might include microbial production of complex metabolites identical to those produced by plants. For example, the anticancer compound taxol is produced by trees in the genus *Taxus* and by two different endophytic fungi of *Taxus spp.* 41 And the pathogenic fungus *Gibberella fujikuroi* produces several forms of the phytohormone gibberellin, which appears to be identical to those produced by plants. 42 Horizontal gene transfer may be the most parsimonious explanation for these shared biochemical pathways, although the mechanism and direction of transfer are unknown.

Protein homology does not necessarily mean genetic homology. However, gene homology was found in the case of *Bradyrhizobium japonicum.* This plant pathogenic bacterium has a glutamine synthetase II gene which shows high homology to other plant, but not prokaryotic, glutamine synthetase genes. This gene is probably the result of eukaryote to prokaryote gene transfer. 43

As stated above, the risk of transgene spread in the environment (be it by cross-pollination or horizontal transfer) depends on two things: likelihood of gene transfer and the phenotypic characters imparted by the transgene. Those transgenes which enhance fitness are most likely to increase invasiveness and frequency of recipient species outside of the cropping system. [44,45] Therefore, it is important to take into consideration the biology of the parent crop in environments where transgenic varieties are likely to be used, and the potential effect of transgenes on plant fitness in these same environments. In the Sections 5 and 6, these factors are discussed in the context of risk assessment of genetically engineered hybrid poplar. The poplar is selected as an example because its biology and genetics are largely known, [46--48] a will likely be the first transgenic woody biomass crop to be commercially used in North America, and several of its biological characteristics are similar to other woody biomass crops.

5. APPLICATION OF ENVIRONMENTAL RISK ASSESSMENT TO WOODY BIOMASS CROPS

The biology of woody biomass crops differs from annual crops in several respects that are important to risk assessment. To highlight these differences, the hybrid poplar is compared to yellow crookneck squash and canola, two annual crops for which transgenic varieties have been deregulated (Table 1).

5.1. *Extent of domestication*

Agricultural crops like canola and squash have been under cultivation and selective breeding for centuries to millennia. This domestication process usually leads to the loss of traits that are important to fitness in natural environments. As a result, transgenic varieties of these crops are expected to have a reduced propensity for invasiveness in natural habitats. In comparison, poplars have undergone little selective breeding. Most poplar varieties used in production are hybrids, with the parent trees having been selected from native stands. These varieties (clones) have undergone testing and breeding for production value, but have rarely been subjected to multiple generations of selection. Interspecific hybrids, however, often show reduced fitness (hybrid breakdown) in backcross or F_2 generations. [49] Therefore, crosses between hybrids and wild parental species are expected to show reduced fertility and survival in natural environments. This prediction appears to be borne out by recent surveys of poplar regeneration in the vicinity of flowering hybrid plantations in the Pacific Northwest, where an extremely low frequency of hybrid progeny were observed.

5.2. *Longevity and vegetative regeneration*

Poplar's longevity and capacity for vegetative regeneration potentiate long-term persistence on a site and thus multiple opportunities for reproduction. Even if conditions that promote reproduction are rare, favorable years could allow for pulses of regeneration. In contrast, annual crops like squash or canola can only persist as long as conditions are conducive to establishment, or seeds remain viable in the soil.

The spread of poplars is not limited to seed dispersal. Regeneration may occur from

Table 1. Comparison of attributes of the annual crops canola (*Brassica napus* L.) and yellow crookneck squash (*Cucurbita pepo* L. *ssp. ovifera*) with hybrid cottonwood (*Populus trichocarpa x P. deltoides*)

Attribute	Canola[51,74]	Yellow crookneck squash[51,52,75]	Hybrid cottonwood[76,77]
Interfertile wild relatives?	Yes	Yes	Yes
Extent of domestication[a]	Moderate to high	High	Low
Origin of parent species	India, Mediterranean	Mexico, Latin America	North America
Mode of pollination	Insect	Insect	Wind
Potential for seed dispersal	Low[b]	Low-moderate	High
Breeding system	Self- and outcrossing	Self- and outcrossing	Outcrossing only (dioecious)
Time to reproductive maturity	~45 days	~45 days	4–10 years
Persistence of seeds in soil	> 1 year	> 1 year	< 2 weeks
Vegetative persistence	No	No	Yes
Vegetative propagation	No	No	Yes

[a]Relative degree of genetic separation from wild relatives through breeding. Highly domesticated crops are presumed to have poor survival in uncultivated environments.
[b]Human mediated dispersal can be very high.[78]

shoots or roots, depending on the species and environment. Aspens are well known for their ability to spread via root suckering. Abscised cottonwood shoots may serve as vegetative propagules and disperse by water. 50 Poplar shoots are able to resprout from roots after stems have been cut, providing the basis for the widely used coppice system of regeneration. This capacity can also cause problems for herbicide control, where repeated applications or accompanying mechanical treatments are often required to kill trees. In contrast, annual crops are generally incapable of vegetative regeneration.

5.3. Sexual dispersal

A number of characteristics give poplars the potential for extensive, long distance gene flow. First, poplars are dioecious, and therefore, completely outcrossing. Although canola and squash also outcross, gene flow from plantations is relatively limited in extent because the plants are insect pollinated and small in statute.51,52 Second, in contrast to many agronomic crops, compatible wild or feral relatives are generally common. Wind pollination, and the large height of reproductive trees, favor extensive movement of pollen and seed. Cotton associated with seeds promotes their dispersal by both wind and water. Deployment of reproductively sterile transgenic poplars may be the simplest and most effective means of preventing long distance movement of transgenic seed and pollen. 53 This strategy is not feasible for crops like canola and squash because seed production is necessary for both propagation and obtaining a harvest.

Several other reproductive factors can constrain gene flow from poplar plantations. First, poplars typically do not reach sexual maturity for *ca 4-10* years, which is the majority of a rotation cycle for many intensive biomass systems. In contrast, annual species produce one or more seed crops per year, and thus are capable of a much more rapid population expansion. Second, seeds from poplar are very small and short-lived, and cannot persist in soil seed banks for more than a few weeks. In contrast, long-term seed banks are a major source of recurring weed populations for many annuals. Finally, poplar hybrids can present special barriers to introgression due to interspecific incompatibilities. 54 In sum, although the potential for long distance gene flow exists in poplar, field measurements of sexual dispersal and establishment are necessary because of the many factors that can limit actual rates in the field.

5.4. Environments

To assess the potential impact of transgenic woody biomass crops, it is important to consider the environment in the vicinity of cultivation. For example, to estimate potential impact of engineered herbicide resistance, it is important to know herbicide use patterns in the landscape surrounding the area of cultivation. If the herbicide is rarely used to control weeds outside of areas where the transgenic crop is cultivated, or if other equally effective and environmentally benign herbicides are available, then escape of the resistance gene may be of little concern. Similarly, if the crop or compatible relatives of the crop are not important weeds, or if the herbicide is never used to control these plants, then the presence of resistance in these plants should not pose an agricultural threat.

Another important aspect of the surrounding environment is the availability of suitable habitat for escaped transgenic plants.55 For example, the irrigated hybrid poplar plantations in the eastern region of Oregon and Washington are surrounded by high desert and irrigated potato and alfalfa fields, environments that are not suitable for poplar. Riparian areas that sustain wild populations of poplars are rare. This region contrasts sharply with the western parts of these states, which have high rainfall, poplars are grown without irrigation, and interfertile native poplar populations are widespread. In this region introgression is much more likely to occur and suitable habitats for establishment are common.

6. RISKS OF SOME TRANSGENES UNDER CONSIDERATION FOR COMMERCIAL USE

Several of the transgenes currently being pursued for use in genetic engineering of woody biomass species are briefly evaluated below.

6.1. Herbicide-resistance

Transgenes conferring herbicide resistance have been criticized because they would maintain, if not promote, the use of herbicides and their attendant problems. 56 Others, however, have argued that herbicide-resistance can have many environmental benefits, such as facilitating reduced tillage methods to conserve soil and water, and promoting the use of those herbicides that have low environmental impacts. s7'ss These benefits apply to glyphosate-(active agent of Roundup"') resistance in poplars. Glyphosate is rapidly inactivated in soil and binds tightly to soil matrices, greatly reducing its ability to leach into ground water. It also has very low mammalian and avian toxicity.s9 During the establishment period, poplars are sensitive to many broadleaf herbicides. Therefore, weeds are controlled primarily with tillage, which can be costly, promote soil erosion and is ineffective against weeds growing close to the trees. Such problems would be reduced if glyphosate could be used during the growing season.

Gene flow from transgenic plantations may create problems on some adjacent lands, but the extent of such problems depends on the availability of suitable habitat, and the extent of glyphosate use in these areas, as discussed in Section 5.4.

6.2. Insect resistance

Trees with high levels of insect resistance could produce a number of environmental benefits, most notably, reduced use of more toxic pesticides. [60] Bt toxins are of particular interest for genetic engineering because they have very low mammalian and avian toxicity, are highly host specific, yet are effective against major pests of poplars. Furthermore, Bt-toxin genes function effectively when inserted into plants. Genes for protease inhibitors, scorpion toxins, lectins and cholesterol oxidases have also been studied for used in genetically engineered plants, but only protease inhibitors have as yet been inserted into woody biomass crops.

Although genetically engineered insect-resistant trees have the potential to impact endangered species and ecological cycles, the risks are much lower than for foliar applied chemical or microbial pesticides because only those animals that feed directly on plant material are exposed to any toxins. It has been estimated that < 0.1 % of foliar applied pesticides actually reach targeted insect pests; the rest is released into the environment. [61] If the engineered toxin has high host specificity and poor persistence in the environment (as do Bt toxins), then potential for impacting non-target organisms is very low.

Sustainability of resistance is probably the largest concern associated with genetically engineered pesticidal trees. 29 Several insect species, including the cottonwood leaf beetle, have already developed resistance to Bt under either laboratory or field selection, and many others are probably capable of developing resistance if placed under strong selective pressures. 62,bs It is unclear, however, how insects will respond to the mosaic of stands and genotypes likely to be encountered in biomass crop ecosystems, or whether conscious deployment of varietal mixtures of various kinds might greatly delay resistance development. Large field tests, in combination with experience from pesticide resistance development, laboratory studies, and simulation models are needed to assess whether transgenic insect resistant trees can be sustainably applied to woody biomass systems.

6.3. Lignin chemistry

Genetic modifications to reduce lignin content in wood, or alter its chemistry to enhance extractability, are expected to increase the quality and efficiency of pulping and decrease mill effluents. 64,65 Lignin is a major structural component of cell walls, and provides strength, rigidity and water impermeability. b6 It is expected that substantial reductions in, or modifications of, wood lignin content or quality will reduce tree fitness because lignin is required for structural support and water transport. Transgenes for lignin modification are not likely to spread rapidly into natural populations because they will be selectively disadvantageous.

Transformation with lignin modification genes may lead to unintended side-effects which may have ecological implications. For example, altering enzymes involved in lignin biosynthesis can result in many pleotropic effects, particularly when using gene promoters that do not provide xylem-specific expression. °4 Lignin and tannins have been positively correlated with seed survival in sorghum. 67 Lignin is known to reduce the ability of herbivores to digest plant material, 6s and any alterations might affect feeding and population growth rates of defoliators. Finally, lignin retards litter degradation by microbes and slows decomposition. Modified lignin biomass could therefore affect soil structure and fertility. 69,70

6.4. Reproductive sterility

Transgenes causing reproductive sterility can be used to limit spread of engineered varieties and introgression of transgenes into native populations. Sterile trees may also enhance biomass production because energy will not be diverted to produce pollen or fruit, although this has not yet been demonstrated.

One mechanism for engineering sterility is to use transgenes that produce cell toxins programmed to destroy reproductive cells. Such transgenes may raise concerns from regulators, particularly those toxin genes derived from pathogens. However, such transgenes are often modified to disarm the toxin. For example, the *Diphtheria* toxin A chain is unable to enter cells when the B chain has been deleted. Thus, toxin A is only effective against the cell in which it is produced and should be safe if released into the environment (reviewed in Strauss *et a1.53).*

7. METHODS OF RISK ASSESSMENT FOR WOODY BIOMASS CROPS

The biology of woody biomass crops constrains experimental studies of transgene movement and effects on invasiveness. In particular, the long time required for trees to reach reproductive maturity causes a substantial time lag before data can be generated on transgene movement in seeds and pollen. Also, once experimental trees become reproductive, it is nearly impossible to guarantee high levels of gene containment because of the extreme mobility of seeds and pollen, and the proximity of wild interfertile relatives. One way to circumvent these limitations is to use a risk assessment approach that combines retrospective, demographic studies and simulation modeling (ef Ref.71).

We are using morphological and molecular markers to study movement of genes from plantations of *P. trichocarpa x P. deltoides* hybrids into natural *P. trichocarpa* populations in the Pacific Northwest. This study is providing data on dispersal of hybrid genes, and on relative fertility and fitness of hybrids as compared to native trees. A computer model based on remote sensing databases and published information on poplar habitat is being developed to define the distribution of potential habitat in space and time. When combined with simulated gene dispersal data (based on empirical studies), this model will be used to predict how different transgenes and environmental treatments (such as the use of glyphosate) might influence the rate and extent of transgene spread. The product will be a risk assessment of transgene movement. Sensitivity analysis of the model should allow the evaluation of which factors are most critical to prediction, identify the levels of sterility that might be needed for containment, and identify the ecological and demographic parameters which warrant further empirical study.

8. GUIDELINES FOR ENVIRONMENTAL RISK ASSESSMENT

It would take an enormous investment of time and resources to intensively study all risks for all genes. Such a policy would effectively preclude the use of transgenes in `minor crops'. For example, the protocol proposed by Rissler and Mellon, [72] which requires empirical assessments of the competitiveness and reproductive rates for transgenic varieties, would exclude non-sterile woody biomass crops due to their long juvenile period. Given the large potential benefits of transgenic varieties, it is believed that a more reasonable set of biological principles is needed to help evaluate risks. Based on these principles, in combination with the simulation models described above, it should be possible to adequately predict, monitor and manage transgene risks during research and commercialization.

Three basic tenets underlie the principles proposed. First, risk assessments should focus on the phenotypes expected from knowledge of specific transgenes, and not on how genes were delivered or whether they were derived from intra- or inter-generic transfers. This position has been supported in many scientific assessments of biotechnology. [4,11,12] Thus, transgenes *per se* should not be generically regarded as having inherent ecological risks, nor does safety for one transgene imply safety for another: it is the gene product, whose properties are typically known in great detail, and the effect of this product on the biology of the recipient crop species, that requires consideration.

Second, although the technology of genetic engineering is so new that little risk assessment data is available for basing the evaluations, in formation gained from traditional genetic technologies can be utilized. This older technology has produced many unique gene combinations through hybridization and selection, and many of the traits bred for are the same as those being pursued through genetic engineering (e.g. disease and insect resistance). Furthermore, the mechanisms of gene dispersal in the environment will be the same.

Third, although transgenic plants with impaired performance will be generated as a consequence of the gene transfer process, they are expected to be identified and removed during further selection and breeding. They are caused by somaclonal effects and occasional disruption of native genes. These kinds of transgenics do not pose any greater ecological risk than do products of conventional breeding, which often have low fitness as a result of wide crossing, recombination and growth in novel environments.

The principles described are modified from those developed by S. Strauss (Oregon State University), M. Gordon (University of Washington), D. Robison (Syracuse University) and J. Turnbull (Electric Power Research Institute) in conjunction with a panel considering a report by B. Haissig on risks of genetically engineering biomass crops.73

8.1. Transgenes are of significant environmental concern when, based on the known function of their gene products, they are likely to substantially enhance fitness of their hosts or associated organisms, or promote their own spread

Such genes include those which provide new mechanisms for resistance to important herbivores or pathogens in natural or managed populations, and which provide resistance to herbicides important for weed control in managed populations. These genes are refered to as *potentially advantageous transgenes.* These genes should be released in a controlled manner when necessary for research, and on a broad scale only in areas where the risks of release have been assessed and considered acceptable (see below). Mobile genetic elements such as transposons could have a neutral or deleterious effect on fitness yet still spread in genomes; therefore, they also should be deployed cautiously.

8.2. Transgenes that impair fitness, and thus inhibit their own spread, should pose little environmental risk

Genes of value in managed plantations may impair fitness of trees in natural environments. Examples discussed above include genes that impair lignin production and reproductive fertility. These genes are refered to as *putative domestication transgenes.* However, large plantations of both transgenic and conventionally domesticated trees could overwhelm adaptedness of small, native plant populations if outcrossing occurs readily, and therefore transgenic trees should be deployed carefully in such instances.

8.3. Transgenes are of reduced concern when they are expected to have a neutral effect on tree fitness

For example, commonly used transgenes such as those encoding reporter enzymes (e.g. /3-glucuronidase and luciferase) and selectable markers such as the antibiotic resistance gene neomycin phosphotransferase, are unlikely to have a significant effect on fitness of woody biomass crops or interfertile species. This class of transgenes is refered to as *putative neutral transgenes.*

8.4. Risk assessments of the potential rate and degree of transgene spread should be based on estimates for specific environments

Transgenes that pose significant concerns in one species or environment may be of little concern in another; generalities regarding risk for classes of transgenes are therefore unsound. Herbicide resistance genes, for example, could be a serious concern in an environment where a specific herbicide is relied on for control of the host species, but of little concern in environments where the herbicide is used rarely, for species whose abundance is not controlled by the specific herbicide in question, or where effective alternative means of control are available.

8.5. Transgenic trees are of significant environmental concern when they produce a substance that can seriously disrupt ecological processes, or affect human and animal health

Transgene products could have direct undesirable effects on ecosystems, even if they have little effect on tree fitness. For example, plants with pesticidal proteins or other toxins should be analyzed for effects on non-target organisms and the evolution of resistant biotypes. On the other hand, as discussed above, selectable marker and reporter genes appear unlikely to affect trophic interactions, nutrient cycling or other ecosystem processes. However, more information on the probability that such genes may be transferred to microbes, and the role these genes may play in microbial ecology is desirable.

8.6. For those transgenes that may reduce the environmental impact of current biomass production practices, risk assessment should take these benefits into consideration as well as the possibilities for adverse environmental effects

All of the genes discussed in Section *6* above have the potential for significant environmental, as well as, production benefits. For example, genetically engineered insect-resistance in woody biomass crops could greatly reduce the use of those pesticides which are known to have detrimental effects on people and the environment. The main disadvantage to engineered insect resistance is that pests may develop resistance to the engineered toxins. However, such resistance is also a major problem with applied pesticides. Other potential problems with insect resistance include increased invasiveness and gene spread in the environment. The potential impact of such events should be weighed against the environmental benefits associated with reducing pesticide use.

The high cost of detailed, multiple year and site experiments to carefully document possible adverse effects of transgenes on natural environments could effectively preclude their commercial use, and thus also forego the environmental benefits they may provide. Instead, such studies might be a condition for initial permitting of commercial deployment.

9. CONCLUSIONS

We believe that the evaluation process for deploying transgenic plants needs more structure and guidance from science, which the present guidelines are intended to help provide. Structure is needed in the evaluation process not only to make it more effective, but to streamline the application process and make clear to applicants the kinds of information needed for sound risk assessment. The complexity of woody biomass crop systems and as sociated wild populations precludes simple answers about environmental risks of transgenic varieties. Research is needed to assess the fate of those transgenes expected to have significant impacts on tree fitness or ecosystems, especially large scale field tests and associated simulation models. However, transgenes expected to reduce plant fitness or to have little effect on ecosystems do not require such detailed studies. Through a combination of experiments, simulations, monitoring and engineered sterility for high risk geneenvironment combinations, it is expected that it will be possible for transgenic varieties to be tested on a large scale and put into commercial production within a few years.

Acknowledgements-The authors thank the Tree Genetic Engineering Research Cooperative at Oregon State University for financial support, and the late Conrad Wessela and the Starker family for supporting Amy Brunner. They also thank Dan Robison (Syracuse University) and Gerald Tuskan (Oak Ridge National Laboratory) for their helpful comments on an earlier draft.

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