Genetically modified poplars in context[1]

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Poplars (genus Populus) have emerged as a model organism for forest biotechnology, and genetic modification (GM: asexual gene transfer) is more advanced for this genus than for any other tree. The goal of this paper is to consider the benefits expected from the use of GM poplar trees, and the most significant claims made for environmental harm, by comparing them to impacts and uncertainties that are generally accepted as part of intensive tree culture. We focus on the four traits with greatest commercialization potential in the near term: wood modification, herbicide tolerance, insect resistance, and flowering control. After field trials and selection of the top performing trees, similar to that during conventional poplar breeding, GM poplars appear vigorous and express their new traits reliably. The ecological issues expected from use of GM poplars appear similar in scope to those managed routinely during conventional plantation culture, which includes the use of exotic and hybrid genotypes, short rotations, intensive weed control, fertilization, and density control. The single-gene traits under consideration for commercial use are unlikely to cause a significant expansion in ecological niche, and thus to substantially alter poplar's ability to "invade" wild populations. We conclude that the ecological risks posed by GM poplars are similar in magnitude, though not in detail, to those of routine poplar culture. We also argue that the tangible economic and environmental benefits of GM poplars for some uses warrant their near-term adoption-if coupled with adaptive research and monitoring-so that their economic and ecological benefits, and safety, can be studied on commercially and ecologically relevant scales. We believe that the growing demand for both wood products and ecological services of forests justifies vigorous efforts to increase wood production on land socially zoned for tree agriculture, plantations, or horticulture. This is the key reason for poplar biotechnology: the combination of economic efficiency with reduction of farm and forestry impact on the landscape.

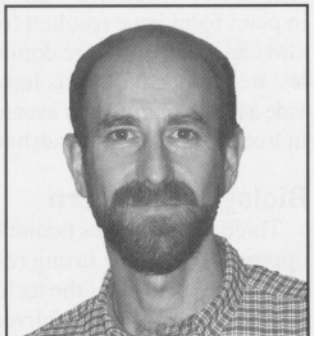
Key words: biotechnology, environmental risk assessment, forestry, genetic engineering, Populus

Le peuplier (du genre Populus) est devenu un organisme modele en biotechnologie foresti&e, et la modification genetique (MG un transfert asexue de gene) est plus avancee pour ce genre que pour tout autre arbre. L'objectif de cet article vise a considerer les benefices attendus de 1'milisation des peupliers MG, et d'etudier les plus importantes pretentions rattachees aux dommages environnementaux, en les comparant aux impacts et aux incertitudes qui sont generalement acceptees en tant qu'elements de la culture intensive d'arbres. Nous nous centrons sur les quartes grands aspects qui ont le plus grand potentiel de commercialisation dans un proche avenir : la modification du bois, la tolerance aux phytocides, la resistance aux insectes et le controle de la floraison. Apres les essais sur le terrain et la selection des arbres les plus performants, comme it se fait au cours de la reproduction conventionnelle de peupliers, les peupliers MG semblent vigoureux et expriment leers nouveaux aspects de faqon fiable. Les enjeux ecologiques attendus suite a 1'utilisation des peupliers MG semblent &re du meme domaine que ceux geres de faqon courante dans le cadre de la culture conventionnelle de plantation, qui comprend 1'milisation de genotypes exotiques et hybrides, de courtes rotations, le controle intensif des mauvaises herbes, la fertilisation et le controle de la densite. Les aspects issus d' un sent gene sous consideration d'utilisation commerciale ne devraient pas entrainer une expansion significative de la niche ecologique, et ainsi modifier substantiellement la capacite du peuplier < d'envahir » des populations sauvages. Nous concluons que les risques ecologiques poses par les peupliers MG sont semblables en terme de grandeur, mais non dans les details, a ceux poses par la culture habituelle des peupliers. Nous invoquons egalement que les benefices tangibles economiques et environnementaux des peupliers MG dans certaines utilisations garantissent leur adoption a court terme-s'ils sont associes a des recherches progressives et a une surveillance-de telle sorte que leers retombees economiques et ecologiques, ainsi que leur innocuite, puissent etre etudiees a des echelles miles tant commercialement qu'ecologiquement. Nous croyons que la demande grandissante a la fois de produits de bois et de services ecologiques a partir des forets justifie les efforts vigoureux d'augmentation de la production de bois sur des terres zonees socialement pour Fagriculture forestiere, des plantations ou 1'horticulture. Il s'agit dun element primordial en biotechnologie des peupliers : la combinaison de Fefficacite economique avec la reduction des impacts agricoles et forestiers sur le paysage.

Mots-cles : biotechnologie, evaluation du risque environnemental, foresterie, genie genetique, Populus

Introduction

Agricultural biotechnology, once broadly praised for its potential to increase crop yields, improve food quality, reduce use of pesticides, and feed a rapidly growing world on a limited land base, is now under siege in much of the world (Gaskell et al. 2000). Although the technology is far less advanced for tree crops, a similar tide of



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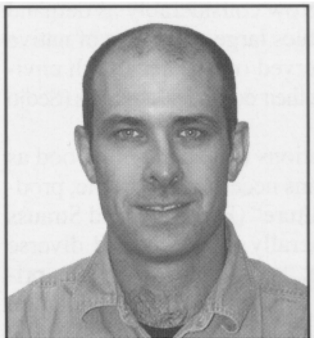

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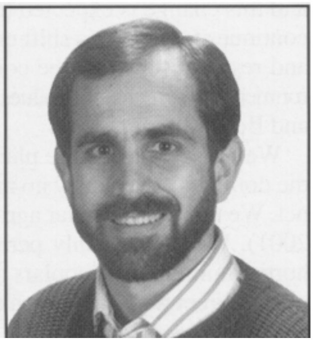

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negative sentiment is evident. A leading certifier of the environmental soundness of forestry products, the Forest Stewardship Council based in Oxaca, Mexico (http://www.fscoax.org/), bans even research with GM[3] trees from certified forests-although they have certified some of the most intensively managed plantations in the world. Two environmental organizations have written reports that demonize GM trees to a large degree (Owusu 1999, Campbell 2000). Why is biotechnology so stigmatized in the agricultural and forestry sectors, despite vigorous endorsement by farmers in the marketplace, and intensive scrutiny and regulation by government agencies? Is there a solid scientific basis for this denigration, or are broader social and political conflicts at work? How can activist groups justify demonizing GM plants categorically when leading scientific panels convened by the U.S. National Academy of Science (National Research Council 2000) and the Ecological Society of America (Tiedje *et al.* 1989) concluded that it is the genes and traits, not the method of genetic modification, that matter?

The goal of this paper is to examine the biological basis of these questions, focusing on poplars as a microcosm (genus Populus, including aspens and cottonwoods, and their hybrids). We first consider the general question of why GM is important for trees, then critically examine the key concerns put forward against GM trees. We argue that the biological risks from use of GM poplars are very similar in magnitude to what is accepted in the routine management of poplar plantations around the world. GM is not a generic panacea or a threat; it is simply a tool that provides new leverage for control of economic and environmental aspects of production. The net effect of any use of GM poplars on environmental aspects of plantation management depends strongly on how the new traits provided by GM are used, not on the process of producing GM plants. Used wisely, we argue that GM traits on the horizon have the clear potential for economic and environmental benefits for tree plantations.

We present this paper as a discussion of the key issues surrounding GM poplars from the viewpoint of those who work with them daily, and have regular contact with farmers and industries who wish to use them. Our perspective is based on a strong conviction that intensive tree plantations play a key role in sustainably meeting society's demand for forest products. Tree plantations are already meeting a large proportion of society's industrial wood requirements (Wernick *et al.* 2000), and this change is expected to grow considerably as demand continues to rise. This shift enables large quantities of native and restored forests to be conserved or managed with environmental and recreation values as their dominant products (Sedjo and Botkin 1997).

We focus on intensive plantations managed with wood as the dominant, though by no means necessarily the sole, product. We term this "poplar agriculture" (Bradshaw and Strauss 2001). We discuss only peripherally the many and diverse horticultural uses of poplars (i.e., "poplar horticulture"), primarily street trees, windbreaks, agroforestry, bioremediation of toxins or agricultural runoff, and biofuels. Intensive, human dominated systems are the only places where GM poplars are under consideration for the foreseeable future.

GM is valuable for tree domestication

Scientists seek to apply GM to trees both because of the availability of traits with obvious economic consequence, but also because GM may allow breeders to transcend some of the major barriers that have traditionally retarded genetic improvement. In contrast to the major agricultural crops, some of which have been domesticated for millennia, trees grown in plantations are only in their first few generations of improvement. The main objects of domestication are:

Increased yield. For fibre plantations, this improvement means more wood that is better suited in quality for its intended uses. For example, even small changes in lignin quality and amount can have dramatic effects on the energy budgets, costs, and environmental pollution from pulp mills and biofuel facilities (Dinus *et al.* 2001).

Altered reproduction. This change could mean advancing or retarding the onset of sexual reproduction, changing a normally outcrossing plant to an inbreeder, producing genotypes that can be readily propagated via vegetative means, prevention of seed release, and avoiding sexual reproduction entirely to improve product quality or quantity, or lessen ecological impacts off-site.

Improved adaptation to intensive culture. This usually means selecting genotypes that take advantage of the improved water, nutrition, and decreased competition of farms and plantations, possess physiological changes that promote per area yields rather than individual plant fitness, and can tolerate the specific kinds of pest proliferation that accompany growth in novel, highly uniform environments (Bradshaw and Strauss 2001).

Because many of these changes involve traits that would be deleterious to plants under natural selection, domestication via conventional means usually involves the identification of rare alleles and their incorporation into diverse populations via repeated backcrossing. In addition, many domestication genes are recessive in nature, thus requiring inbreeding for their fixation and expression in a homozygous condition (Fehr et al. 1987). For trees, long generation times, inaccessibility of flowers, prevalence of wild populations, and intolerance to inbreeding present strong barriers to domestication via conventional breeding. However, coupled with the knowledge that major changes in plant form have resulted from one or a few genes, and that GM can readily produce dominant forms of domestication alleles, tree biotechnologists have considered that GM may provide a revolutionary new avenue for making large improvements in tree productivity (Bradshaw and Strauss 2001).

Biological Concern

Three major themes dominate the writings of groups that are opposed to, or have strong reservations about, the use of GM trees. The first is that the technology is unreliable and imprecise, and thus the traits delivered are unpredictable and therefore implicitly dangerous. The second is that the new traits imparted to GM trees are likely to cause major perturbations to species that depend on trees or ecosystem processes. The third is that GM "supertrees" or their progeny will be highly invasive, threatening wild forests. We consider each of these by comparison to common practices in poplar culture, practised

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[3] We use the term GM (genetically modified, genetic modification) as equivalent to genetic engineering, or the production of transgenic organisms. GM crops are those which have had one or more genes, or pieces of DNA, added to their genomes via asexual gene transfer (transformation), regardless of the origin or degree of modification of the genes.

Fig. 1. Insect-resistant transgenic poplars in a field trial in the western United States. (A) The trees on the left of the person in the photo contain a BT Cry3a gene flanked by matrix attachment regions (MARS) from tobacco. Nine independent lines are represented, all showing very high levels of insect resistance and nearly uniform growth after two years in the field. On his right is a row with non-transgenic trees of the same clone. (B) Colour infrared aerial photo of part of the stand shown in (A). Transgenic and non-transgenic rows of the same clone alternate throughout the plantation.

according to good standards of environmental stewardship appropriate to plantation systems.

GM trees intended for commercial use are stable and highly predictable

Because there is little control over where transgenes insert into the genome, and because plants have surveillance systems that search for and attempt to suppress foreign DNA and its expression products (Fire 1999), newly produced populations of GM plants show highly variable, and sometimes unstable, levels of transgene expression. In addition, the process of gene transfer and plant regeneration tends to significantly increase the frequency of mutations. This is referred to as somaclonal variation, and can occur in the absence of any gene transfer treatments (Karp 1991). Its extent, and that of unstable transgene expression, is highly variable depending on the plant genotype and the method of gene transfer and regeneration. Because transgenic poplars are expected to be used directly after transformation of an elite genotype, the extent to which newly produced GM trees are unstable or genetically damaged is very important. It is especially important where regulatory acceptance depends on high levels of gene containment. Major breakdowns of transgene expression could have economic, ecological, and legal consequences.

Because rare, unstable transgenic plants have received a great deal of attention in the scientific literature in an effort to understand their causes, there appears to be a common perception that most transgenic plants are unstable. However, in commercial programs very large numbers of transgenic plants are produced and intensively screened for stable transgene expression and normal plant development. This is similar to the large number of crosses that are routinely screened in plant breeding programs, from which only a very small number are selected for further development.

Transgenic poplars produced via *Agrobacterium* transformation appear to suffer little somaclonal variation and instability. In our laboratory, we have produced more than 3200 independent transgenic lines (i.e., transformation events) in 16 hybrid genotypes. We have also observed 557 lines in 26 field trials. We have observed obvious morphological variation in only three lines (0.1 %), all in field trials and all in hybrid cottonwoods. In contrast, the hybrid "aspens" (section Populus: Eckenwalder 1996) we have studied, which have a much shorter transformation process, have been completely devoid of abnormalities. In addition, none of the nearly 1500 transgenic trees we have produced, which contain genes that encode easily observed phenotypes, have shown evidence of gene silencing (Fig. 1). Furthermore, although some tissue culture protocols result in higher levels of somaclonal variation in poplar, stable lines with useful traits have also been selected and successfully propagated (Wang *et al.* 1996).

Although morphological abnormalities and gene silencing appear to be rare in poplars, it is critical that potential commercial GM cultivars be screened over several years and environ ments prior to broad use. Such a practice is routine in commercial breeding, and would certainly be applied where transformation is being added to an established breeding program. It would be of particular importance as genetic modification grows in complexity and increasing numbers of genes, intended to affect several traits and/or genetic mechanisms, are added (Burdon 1999). But it appears that key traits, like engineered sterility for gene containment, can be reliable. However, more detailed, quantitative data on stability are desirable prior to large scale use.

Ecological effects of GM trees are likely comparable to those from conventional plantations

All of the practices of poplar agriculture, as well as those of conventional and organic agriculture, cause dramatic changes in ecological processes when compared to wild ecosystems. For tree plantations, examples include shortening the rotation from several decades to less than 10 years, controlling competing vegetation, and planting evenly spaced trees at high density. The use of highly productive, exotic hybrids constitutes a major departure from the locally adapted trees that might have existed on the site historically. It is into this agricultural context that GM poplars would be introduced and, therefore, this is the context in which they should be considered. It seems likely that a large degree of the conflict surrounding GM in forestry results from divergent views concerning the application of naturalistic versus agricultural management paradigms to plantation forestry-or at least where, and to what extent, each should predominate (Thompson and Strauss 2000). Under a naturalistic paradigm, where preservation of the natural state to the greatest degree possible is the major theme, the departure from nature that GM symbolizes is likely to receive a hostile reception, even if actual ecological effects on stands are small.

Deployment assumptions

For considering the ecological effects of GM poplars, we make several assumptions about how they would be deployed commercially, at least in the USA. These are:

GM does not force extreme reduction in genetic diversity**.** One or a very few GM clones do not replace a large diversity of clones used in production. This approach will require progressive development, testing, and deployment of GM clones. We believe that few growers will want to risk a large narrowing of genetic base-unless they have already chosen to do so for other reasons.

Steps are taken to minimize transgene spread into the wild. Genes are added at the time of gene transfer that make trees highly sexually infertile, or the trees are grown in an area where they are either virtually unable to spread sexually due to maladaptation, or human control of the environment is sufficiently great that their wild spread is unlikely or inconsequential (e.g., this is likely to be true in heavily populated areas, such as many parts of China and India).

Appropriate transformation technology is employed. Selectable marker and reporter genes used to aid transformation, such as genes for antibiotic resistance, are either not used in transformation, or are deemed safe for use in plantation trees by regulatory authorities.

GM trees are tested adequately, at least as well as those in conventional breeding programs. GM trees enter production forests on a large scale only after a number of years of field trials on several sites, and for a period of time that represents a substantial fraction of their normal rotation length. Knowledge gained overtime regarding the degree of somaclonal variation, transgene instability, and direct transgene effects on tree performance from particular transformation programs will dictate whether testing can be shortened, or should be lengthened, compared to that used during selection of new clones during traditional breeding.

GM trees will be monitored for their production and environmental characteristics. Because of public concerns about their roles in the environment, research will be conducted, and made publicly available, to assess the performance of GM in relation to environmental characteristics. For example, use of insect-resistant trees should include assessments of insect response/adaptation and non-target impacts of concern; use of transgenic sterile trees should assess the degree to which they are effective in minimizing gene flow; and use of lignin-modified trees should include assessment of their general adaptation and vigour under biotic and abiotic stresses.

GM poplars will involve four kinds of traits. For the foreseeable future, GM poplars will include one or more of four traits: herbicide tolerance (HT), wood chemistry or structure modification (WM), insect resistance (IR), and sexual infertility (SI). Each of these is discussed separately below.

Herbicide tolerance

HT is of considerable interest to poplar growers because it appears capable of reducing management costs significantly (Sedjo 1999) by enabling the substitution of less costly, more effective, and more environmentally benign herbicides. Tolerance to the herbicide Roundup® has received the most attention (reviewed in Strauss *et al.* 1997), and is highly effective in poplars (Meilan *et al. 2000),* but several other types of herbicide tolerance have been demonstrated in poplars (Han *et al.* 1996). In many places, superior weed control is expected as a consequence of HT, particularly close to trees where competition for water and nutrients is most intense and tillage or sheltered sprays are least effective. This should result in increased productivity and survival, with substantial economic benefits for growers.

In irrigated plantations, more effective weed control could also result in reduced water consumption, providing economic value and environmental benefits. On sloped land prone to erosion, substitution of herbicide use for tillage should give large reductions in soil erosion and soil compaction; the former may have benefits for aquatic wildlife if the herbicide employed has low toxicity and mobility in soil. Finally, the added flexibility in control can have major benefits. Growers could also wait to see if a significant weed problem develops on a site before choosing to spray, possibly avoiding the application of herbicide treatments (Strauss *et al.* 1997).

Two major environmental concerns are presented by use of HT poplars. First, because HT genes are genetically dominant, if GM-HT trees are sexually fertile, it is highly likely that they will give rise to wild HT progeny via pollen or seed release. Although most such progeny will occur close to plantations, a number could also occur at a long distance because poplars are capable of long-distance pollination. Escape of herbicide tolerant trees could lead to reduced control options for other growers, and may force them to rely on less effective or less environmentally benign herbicides for poplar control. Transgenic sterility is likely to dramatically reduce the problem, both quantitatively and spatially, though is unlikely to provide perfect containment in the immediate future (Mullin and Bertrand 1998).

The second kind of environmental concern of HT trees is the possibility that the trait will promote excessive use of herbicide. This increase could have detrimental effects on water quality, wildlife safety, and biological diversity that depends on weed flora. The extent to which this might occur will be highly grower-, year-, and site-specific. Poplar managers presently have the ability to completely control weeds via tillage, manual cultivation, and sheltered spraying. However, this intensity of weed control is not cost-effective. With HT, economic considerations may permit managers to spray less frequently, waiting until a weed population develops. This might have both economic and biodiversity benefits. However, many intensive plantation programs, or regional governments, choose to manage biodiversity at the landscape scale-by allocating different portions of the land base to different resource priorities (Binkley 1999). In such a system, if HT does lead to more complete, farm-like weed control and production in plantations, but on a limited portion of the landscape, this may have the greatest joint benefits for both biological diversity and production.

We believe that it is not possible to predict, categorically, what environmental or production benefits HT will have in poplar plantations. It depends on how it is used, and in what management context. What HT provides is a new control option-an added degree of freedom-so weed-control programs can be designed more effectively with respect to both production and stewardship goals. For example, if a grower wishes to maintain a larger population of non-tree vegetation for soil protection, nitrogen fixation, or wildlife forage, HT provides a means for this to occur while enabling "rescue" of the trees when competition begins to exact an intolerable economic cost.

Wood modification

The uses for wood vary enormously, and many aspects of wood structure and chemistry show useful levels of genetic variation and heritability. Nonetheless, breeders have generally focused on traits of higher priority, such as yield, adaptability, and pest resistance. GM approaches to WM are attractive in that they offer the possibility of altering wood quality in very specific ways, but not encumbering breeding programs by requiring measurement and selection based on an additional, usually complex, trait. The number of ways in which wood can be modified is vast (reviewed in Dean 2001), and growing rapidly as a result of the many genomics programs that are isolating large numbers of genes expressed during wood formation. A number of different approaches have already been tested in transgenic poplars and have given exciting results. Suppression of the gene for CAD (cinnamyl alcohol dehydrogenase), one of the terminal enzymes in the biosynthesis of lignin monomers, causes a modest reduction in lignin content and a change in lignin cross-linking that facilitates Kraft pulping (Baucher *et al.* 1996). This means that less chemical is required and that fibres undergo less damage when extracting lignin from pulp. In a field trial in England, the trees showed no change in their growth and no sign of increased susceptibility to biotic or abiotic stress over four years of growth (W. Boerjan, personal communication). Other exciting results from studies of WM have been reported based on greenhouse tests. Transgenic poplars with suppressed levels of the enzyme 4CL (4-coumarate ligase) showed a large reduction in lignin content and a corresponding large increase in growth rate and cellulose content (Hu *et al.* 1999). These results suggest that the opportunities for GM to modify wood properties are real. In the next few years, many more genes being identified in genomics projects will be tested in transgenic poplars.

Molecular biology clearly has the power to enable the domestication of wood properties, but what are the environmental concerns of doing this? Clearly, we also have the tools to modify wood far beyond its normal ecophysiological limits, potentially producing trees that are structurally unstable and susceptible to pests and abiotic stresses (Dean 2001). However, the goal of domestication is to strike a balance between making plants better producers of materials for human needs and maintaining a sufficient level of adaptability so they can perform adequately in farms and plantations. We are reducing their Darwinian fitness in a directed manner, but do not wish to also make trees that require so many additional life support systems that they present economic and environmental burdens. Adaptability is of particular concern for forest trees, with their long life spans and lower degree of human care, as compared to orchard trees and annual crops.

The concerns for changes in adaptability of trees due to GM of wood or other traits are very similar to those breeders face in conventional breeding. For example, there is abundant genetic variation within and between populations of trees, including poplars, in many traits critical to adaptation. These include traits such as growth rate, timing of bud flush and bud set, and cold hardiness. Modifying populations of trees too rapidly toward an extreme in any of these traits, using genetic differences among trees or among populations, could create forests that are highly susceptible to damage from abiotic or biotic agents. Likewise, the failure to modify adaptive traits based on information from contemporary research trials, in the face of changing climates and environments, natural and anthropogenic, could have similar consequences if breeders uncritically assume that the genetic characteristics of centuries-old forests should be preserved in forests of the future. Breeders and managers, with input from stakeholders, try to balance productivity goals with the need for adaptability and genetic diversity in forests. These decisions are based primarily on results of extensive field trials where both growth rate and evidence of stress tolerance are assessed. Studies of trees with GM wood that is modified would be no different.

Genetic transformation typically results in a population of plants with a range of expression (or suppression) levels for the desired trait. Thus, when we downregulate CAD or other genes with key roles in wood chemistry we can choose trees with different degrees of suppression. Although no deleterious effects have been observed to date, even in highly CAD-suppressed trees, such effects may surface after extensive field testing, or when used in other poplar genotypes. Therefore, a variety of transgenic types should be tested in field trials over a number of years, and selected clones should then be monitored for several years thereafter to ensure that they do not succumb to rare stresses, or promote epidemic buildups of pathogens. This "test and go," adaptive management approach is the rule in conventional tree breeding: desired clones iden tified in research trials are planted on increasing numbers of hectares each year, and those that develop problems, or do not perform well, are discarded and replaced by others over time.

Another concern is the impact of trees with modified wood on ecosystem processes and trophic interactions-particularly nutrient cycles and organisms that feed on trees. These impacts will vary dramatically depending on how wood is modified-generalizations are therefore of little value. For example, lignin-enhanced trees, as might be useful for firewood or bioenergy production via pyrolysis, would be likely to decay more slowly, enhance herbivore resistance, and add to accumulation of soil organic matter. Lignin-reduced trees would be likely to do just the opposite. Context is all-important. In plantations, as in farms, ecological interactions are markedly altered from wild populations, and trophic interactions are greatly simplified by design. The question is not if ecological processes will be altered, but how much, and with what consequences for plantation and landscape sustainability. Plantations may be established with many different kinds of genotypes and species. Each can have numerous differences in plant secondary compound chemistry and lignin quantity and quality, yet these practices are rarely subjected to detailed ecological analyses or regulation. We expect that replacement of conventional plantation trees with congeneric wood-modified GM trees would represent a comparatively modest shift in ecosystem processes, and would hardly warrant heightened scrutiny in this regard.

To take an extreme example, the effects of planting a conifer in a plantation are certain to be radically different from planting a poplar-whether one considers soil, stand physiology, herbivores, or wildlife. The specific changes in wood chemistry imparted by GM will be orders of magnitude less than the vast number of new chemicals that distinguish a pine from an aspen. The differences in GM wood will also be less than that of planting different hybrid poplar genotypes in many cases. Hybrid clones, depending on the species and genotypes of the parents, often differ substantially in many respects important to nutrient cycling and herbivore resistance, including leaf chemistry, bark quality and production, and rate of biomass accumulation (Driebe and Whitham 2000).

Insect resistance

The main avenue currently being sought to impart commercial levels of IR in poplars is the use of endotoxins from *Bacillus thuringiensis* (BT). These toxins have been expressed in poplars that impart strong resistance to lepidopteran caterpillars and leaf beetles, two of the major poplar pests worldwide. Both of these classes of insects can have devastating effects on plantation survival and productivity, necessitating use of pesticides when economically permissible. The main benefits of IR-trees would be improved survival, improved rate of growth, and reduced costs and environmental impacts from pesticide application. Most pesticide sprays go directly into the environment without even contacting the target pests, and thus affect a much wider range of organisms than does expression of a toxin gene within plant tissues.

The main environmental concerns surround how long BTbased resistance could be sustained, particularly in a tree, and whether there might be specific non-target effects that would exceed those of sprayed pesticides or otherwise endanger critical species (Raffa *et al.* 1997). There is little scientific debate about whether major genes for pest resistance will succumb to pest evolution. The main questions involve the time required for insects to overcome resistance genes, and to what degree management practices can forestall this. The same concerns apply when major genes for disease resistance from wild poplars are employed, as has frequently been done. Refugia (proximal trees without BT transgenes) are likely to be used extensively, and required by regulatory agencies such as the U.S. Environmental Protection Agency (EPA), to delay resistance development by insects (National Research Council 2000).

Given the extensive toxicological diversity in the BT gene pool, many additional BT resistance genes could also be identified and employed, perhaps with more than a single kind in each tree. There may, therefore, be enough variation in the BT gene pool to make this form of resistance sustainable, even if no single gene is able to provide durable resistance (Peferoen 1997). For example, it seems reasonable to expect a BT gene to remain fully effective for several 10-year poplar rotations if large refugia are employed or trees are grown near to wild stands (natural refugia) that contain significant insect populations, and the target pest is highly mobile during sexual reproduction (Alstad and Andow 1995). New BT genes could be under test in parallel with deployment of the first generation of insect-resistant trees, then incorporated into newly bred poplar varieties along with the additional transgenes likely to result from continued research. However, there is insufficient knowledge at present to make sound predictions about what kinds of management and research strategies should be implemented. In addition, selection for resistance to one kind of toxin might impart cross-resistance to other, similar toxins; this possibility would need to be tested on a case-by-case basis.

Because of their size and the large number of organisms that use trees as food and habitat (even in plantations), non-target effects from release of pollen, leaves, and other parts are of greater ecological concern than for annual crops. The most prominent organisms that might suffer in the near term from BT expression in trees would be BT-susceptible and poplar-dependent endangered caterpillars or beetles that exist only in a local area where wild poplars are rare, and thus need to feed on plantation trees or their offspring. Apart from these organisms and their taxonomic relatives-and those which are highly dependent on them (e.g., parasitoids)-impacts on other species are expected to be very low as a consequence of the narrow range of biological toxicity of most BT toxins (Peferoen 1997). In contrast, many other IR mechanisms would be expected to have broader impacts, including use of some other transgenes (e.g., broad-spectrum proteinase inhibitors and lectin), and novel or abundant secondary compounds-including those which might be produced by conventionally bred poplar genotypes and hybrids that show high levels of IR (Raffa *et al.* 1997).

BT expression in tree tissues is likely to have small but significant impacts on decomposing organisms, particularly those insects closely related to the target pests and thus susceptible to the BT toxin (Saxena *et al.* 1999). However, the specificity of its toxicity, and the rapid breakdown of proteins in the environment (compared to the ubiquitous anti-herbivore plant secondary compounds, including lignins) should make these effects relatively minor (Donegan *et al.* 1996, National Research Council 2000). The impacts would be likely to be far less than from plantation establishment itself (compared to either a wild forest or agricultural use), or from planting of a different species or clones-as discussed above with respect to

WM. The effects are also likely to be smaller than the significant variation in soil characteristics that would be imparted by different fertilization regimes, planting densities, methods of weed control, and clones. Nonetheless, monitoring of BT impacts on soil is likely to be required by the EPA.

If sexually fertile trees are deployed, trees that establish in difficult-to-identify places far from plantations can also have non-target effects, and complicate resistance management strategies. The use of trees with high levels of sexual infertility is therefore desirable to minimize these difficult-to-measure effects (Raffa *et al. 1997).*

Infertile flowers

Producing poplars without flowers, or with infertile flowers, is highly desirable because it would reduce ecological complications of gene flow, possibly improve vegetative growth rates, and reduce the production of large amounts of allergenic pollen (Strauss *et al. 1995,* Skinner *et al.* 2000). The environmental concerns over use of SI poplars are few. Because poplars are wind-pollinated they do not produce nectar or support a large number of insect or vertebrate pollinators. The seeds are very small, have a short life span, and are virtually devoid of endosperm. They therefore do not appear to provide a significant source of food for wildlife. Also, if the sterility mechanism is imperfect and some sterility genes are released into wild populations, impacts are likely to be local and short-lived because fertility is a major component of fitness, and trees with sterility genes would likely be at a significant disadvantage.

There are diverse means for inducing SI, but the most common form is to over-express some type of molecule that is deleterious to cells, and do it exclusively in flowers. The "toxins" that have been employed for this purpose are diverse (Skinner *et al.* 2000), but usually lead to rapid and early death of the tissues such that very little active toxin is likely to be present in remaining cells should organisms feed on it. In addition, as plant cells die from external damage or programmed cell death, which happens continuously during their life cycle, they produce a number of natural cell toxins that impede microbial activity. Thus, the cell-lysing toxins expressed in floral meristems for sterility would not be likely to raise significant new ecological concerns above the highly "toxic" ecological chemistry that already exists in trees. The most popular form of sterility employs an RNAse, a common enzyme in plants and animals, isolated from a bacterium.

GM is unlikely to produce "supertrees" that can invade wild ecosystems

Although many people recognize that genetic modifications will alter plantation practices to one degree or another, so long as most ecological impacts stay within plantations, they usually do not present great concerns. The most frightening prospect is that GM trees might invade wild populations like a kudzu vine or zebra mussel, reducing biological diversity and displacing wild, unmodified relatives. The report on GM trees popularized by the Worldwide Fund for Nature dramatized this risk in the press release that accompanied their report where they cited the "threat to the world's forests" (http://www.panda.org/resources/publications/forest/gm-overview.html). Some reports on the ecological threats of exotic organisms have propagated this scientifically specious model (Wolfenbarger and Phifer 2000). However, whereas invasive exotic organisms represent the coordinated interaction and evolution of thousands of genes in a new environment, usually devoid of its pests and pathogen complex, transgenic organisms result from one or a few intensively studied genes that encode highly specific traits (OTA *1988).*

This does not mean that single genes do not sometimes impart important competitive traits, or that transgenics will not have some potential for spread or effect on wild populations. Conventional breeding already produces substantial, though rarely documented, impacts on interfertile relatives of crop and tree species through their extensive gene exchanges (Ellstrand *et al. 1999).* However, the universe of ecological possibility for transgenics is vastly more restricted than that of the true "superweeds" to which they have been cavalierly compared.

Herbicide tolerance obviously cannot produce an invasive tree in a wild population where no herbicides are used, but if dispersed widely can create difficulties in control of trees where single herbicides are important means of poplar control. Although they have no potential to invade wild populations, it is advisable, in the spirit of good citizenship and environmental stewardship, to limit the spread of HT trees in the environment via engineered sterility or other fertility-reducing mechanisms. Because there are many herbicides capable of killing poplars, occasional spread of HT trees via vegetative propagation or incomplete sterility present no serious issues for management or invasion.

Wood structure is likely to be under balancing selection in wild populations such that changes to suit short-rotation culture for wood products are expected to reduce fitness in longlived wild trees (James *et al.* 1998). For example, ligninreduced trees could be structurally compromised, and may be more prone to herbivore damage or fungal disease, so that productivity, fecundity, and life span might be shortened relative to wild trees. Wood-modified trees, therefore, may not require a method of gene flow restriction, as suggested for insect- and herbicide-tolerant trees. These genes should be effectively eliminated from the wild via natural selection (assuming these populations are not genetically swamped due to small size).

The BT IR gene, as a novel, insect-taxon-specific toxin, has the most potential of the genes discussed to promote the fitness of poplar trees, and thus to increase their competitiveness in the wild. To reduce the chances for ecological impacts of all kinds as much as feasible, it would be desirable to incorporate fertility-reducing genes into poplar together with insect resistance genes. However, if the BT gene were to impart fitness benefits, they are likely to be modest for several reasons. First, only specific groups of herbivores of the many that feed on poplars are affected by the BT gene. Second, outbreaks of defoliating insects appear to be much rarer in the wild than in clonal plantations, and it is unclear to what degree defoliating insects limit natural populations (National Research Council 2000). Finally, as a novel, constitutively expressed protein (as it has been used to date), BT is likely to cause a drain on fitness in nitrogen-limited wild populations, an effect that may not be detectable in plantations (Bergelson and Purrington 1996).

A pest-resistance gene is unlikely to be able to change the general niche of a species, as it does not alter its basic ecophysiological behaviour. For example, many cottonwoods are likely to remain virtually restricted to riparian zones as a result of their poor stomata] control (e.g., Furukawa *et al.*

1990). Likewise, their requirement for moist, competition-free sites for seedling establishment will persist as a result from their small seeds and shade-intolerance (Braatne *et al.* 1996). Poplar is therefore highly unlikely to invade new plant communities even if it is made resistant to one of its major herbivores.

Should the BT gene unexpectedly become common in populations over a very long time period due to some combination of stronger-than-expected natural selection and the breakdown of engineered sterility mechanisms, the consequences are still not expected to be large or lasting. Many insect populations seem to contain resistance alleles in their gene pools at substantial frequencies (including poplar leaf beetles: Bauer 1995); the benefits of BT for insect resistance would therefore diminish rapidly as BT resistance becomes common among insects (Gould 1998). By way of comparison, this might not be the case for an exotic IR poplar clone with a distinct secondary compound composition. This phenotype might promote durable fitness in its wild progeny.

Genes that impart infertility have inherently domesticating effects; they reduce the ability of poplar to spread and compete in the wild. The modest increase of vegetative vigour that sterile trees might possess is likely to be dwarfed by the extraordinary heterotic vigour shown by many poplar hybrids-also capable of vegetative spread. Thus, the theoretical enhanced capacity for spread of infertile GM trees should be very similar to that of conventionally bred trees.

Moving Forward: Flowering Control

Sterility technology makes a great deal of sense for minimizing transgene movement from plantations and increasing wood production, and the loss of flowers and seeds would appear to be without biodiversity issues of consequence in poplar agriculture (discussed above). However, a major issue for deployment of trees that are expected to be incapable of producing seeds or pollen will be the degree of certainty that will be required before a tree is deemed sufficiently "sterile" for a commercial use. Sterility has already been demonstrated in the lab in various ways and forms, and many new genes are being isolated from poplar that are likely to provide new options. Some of these genes might be combined to produce a redundant, extremely stable form of sterility (Strauss *et al.* 1995) if high regulatory costs do not effectively preclude use of accessory genes.

Nonetheless, demonstrating sterility in the field, and over a sufficient number of years and environments to convince skeptics that it is working "adequately," may be challenging. However, if regulators could agree to a plan for monitoring, and if all the major parties agreed that minor breakdowns in sterility during this adaptive research phase do not present significant environmental harms, then sterility is ready to be used in large field trials today-perhaps leading directly to commercial uses if stable phenotypes are observed. The advantages would be advancement of the productivity benefits that GM trees can bring, and the ability to study the effectiveness of sterility mechanisms much more extensively than could be possible from small-scale, short-term research plots alone. Whether such social agreement to move forward with this trait which reduces plantation impact for conventionally bred as well as GM poplars-is unclear. The key issues are likely to be biopolitical opposition from activist organizations that are philosophically opposed to GM crops, rather than questions of biological safety.

Conclusion

We have argued the following points:

- GM is an updated version of the familiar practice of crop domestication, yet scientifically invigorated by vast amounts of new information on genome sequence and function, and the new capacity to directly use that information via recombinant DNA and gene transfer technologies. Though not a replacement for conventional breeding, which will continue to be the major avenue for improving complex physiological traits such as adaptability and yield, GM may be of particular value to forestry because of the serious limitations to breeding imposed by long generation times of trees.
- GM poplars are sufficiently valuable and reliable for commercial use, as demonstrated by nearly 10 years of field trials. If standard testing regimes are followed, similar to those in conventional breeding, it does not appear to be difficult to produce healthy, reliable GM poplars.
- Many ecological criticisms of GM trees appear to be seriously overstated. Upon closer examination, the ecological concerns of GM poplars are very similar in magnitude to those facing breeders and managers on a routine basis. If GM poplars are used wisely, in a way that promotes their sustainable value, they should be able to improve both the efficiency and environmentally beneficial aspects of intensive plantation systems.
- The growing demand for both wood products and ecological services of forests justifies vigorous efforts to increase wood production on land socially zoned for tree agriculture, plantations, or horticulture. This is the motivation for GM trees: the marriage of economic efficiency with reduction of the impact of plantations across the landscape.

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