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REVIEW

Restoration of threatened species: a noble cause for transgenic trees

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Abstract Some of the first applications of transgenic trees in North America may be for the conservation or restoration of threatened forest trees that have been devastated by fungal pathogens or insect pests. In some cases, where resistance has yet to be found in the natural population of a tree species, incorporating genes from other organisms may offer the only hope for restoration. In others, transgenics may play a role as part of an integrated approach, along with conventional breeding or biocontrol agents. American chestnut (*Castanea dentata*) was wiped out as a canopy species by a fungal disease accidentally introduced into the United States around 1900. Similarly, American elm (*Ulmus americana*) virtually disappeared as a favored street tree from Northeastern U.S. cities after the introduction of the Dutch elm disease fungus in the 1940s. In both cases, progress has been made toward restoration via conventional techniques such as selection and propagation of tolerant cultivars (American elm) or breeding with a related resistant species (American chestnut). Recently, progress has also been made with development of systems for engineering antifungal candidate genes into these "heritage trees." An *Agrobacterium*-leaf disk system has been used to produce transgenic American elm trees engineered with an antimicrobial peptide gene that may enhance resistance to Dutch elm disease. Two gene transfer systems have been developed for American chestnut using *Agrobacterium*-mediated transformation of embryogenic cultures, setting the stage for the first tests of potential antifungal genes for their ability to confer resistance to the chestnut blight fungus. Despite the promise of transgenic approaches for restoration of these heritage trees, a number of technical, environmental, economic, and ethical questions remain to be addressed before such trees can be deployed, and the debate around these questions may be quite different from that associated with transgenic trees developed for other purposes.

Keywords American chestnut · American elm · Transgenic trees

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Introduction

The activities of man, whether by accident or design, have led to the endangerment or outright extinction of hundreds of species of animals and plants. While there are no recorded cases in which a forest tree species has been completely lost due to human activities, in North America at least two woody species (*Franklinia alatamaha* and *Ceratozamea euryphyllidia*) are no longer found in nature, probably due to the impact of man, and several forest tree species either are under severe pressure or have suffered enormous declines during the past century. These latter losses were not due to deliberate activities, but rather to the accidental introduction by man of forest pathogens or insect pests from other regions of the world. In each case, resources have been directed at halting the spread of or finding resistance to the pest or pathogen, but successes have been few and slow in coming.

The very nature of forest trees themselves has made combating these threats a daunting task. Conventional selection and breeding approaches that have allowed development of disease- and pest-resistant crop species are extremely difficult to apply to forest trees, most of which have long juvenile periods before they can be bred, or in some cases, can even be selected for resistance. Because forest trees are undomesticated, out-crossing organisms, homozygous pure lines, the basis of hybrid breeding in crop plants, simply do not exist for them. Selection and breeding programs for a few forest tree species under attack from devastating fungal pathogens have been undertaken with some promising results—but only after decades of difficult work. In addition, given the experience with crops bred for disease-resistance, the ability of plant pathogens to overcome resistance means that new genetic material must constantly be selected for integration into these programs. It remains to be seen whether the relevant pathogens will evolve too quickly for this approach to be realistically applied with forest trees.

Given this background, much attention has been given to the potential for the transgenic technology to greatly accelerate the development of disease- and pest-resistant genotypes of threatened forest trees by directly introducing genes from other organisms, or even synthetic genes that may confer resistance to the pathogen or pest. Compared to conventional breeding approaches, transgenic technology has the advantages of: (1) much more rapid genetic modification than conventional breeding, particularly for forest trees, and (2) transferring only the gene(s) of interest into a tree genotype that is already desirable, rather than transferring whole portions of the genome of another parent, such that any undesirable traits may have to be bred out.

Theoretically, multiple transgenes could also be "pyramided" in to broaden the basis of resistance. Transgenic technology has already been applied with some success to combat insect pests and some diseases of agronomic crops, and some of these engineered crops have been commercial successes in the United States. Notable examples include cotton and corn engineered with modified *Bacillus thuringiensis* endotoxin genes to resist cotton bollworm and European corn borer, respectively, and squash engineered with viral coat protein genes to confer resistance to zucchini yellow mosaic virus and watermelon mosaic virus 2. To date, only one woody plant species genetically engineered for disease resistance has been released in the U.S.

Papaya (*Carica papaya*), which was engineered with a viral coat protein gene to confer resistance to papaya ring spot virus, has had a dramatic effect on returning the papaya industry to viability in Hawaii (Gonsalves 1998). A second transgenic woody plant, the plum pox virus-resistant plum 'honeysweet', is currently under review for release in the U.S. (Scorza et al. 2005). Outside of China, however, no forest trees genetically engineered for any trait have been released for commercial production. Given the successes with crop species and the problems associated with applying conventional breeding for disease and pest resistance to forest trees, it is possible that one of the first releases of transgenic forest trees in North America will be threatened species engineered for resistance to the pest or pathogen responsible for their current status.

In this paper, we will briefly review the current status of several North American "heritage" forest tree species threatened by pathogens or pests. Then, we will focus on the status of conventional and transgenic research programs aimed at restoring two of the most important North American species, which were devastated by fungal pathogens in the 20th century: American elm and American chestnut. Finally, we will discuss some of the issues of applying transgenic technology specifically to the restoration of these threatened forest species.

Threats to North American "heritage" forest species

Insect pests Insect pests introduced into North America from overseas have caused more damage to our heritage forest species than have home-grown pests. Three introduced insect pests with the potential to eliminate some of our most important North American heritage forest species are the balsam wooly adelgid (*Adelges piceae*), which targets true firs (*Abies* spp.), the hemlock wooly adelgid (*Adelges tsugae*), which infests hemlocks (*Tsuga* spp.), and the emerald ash borer (*Agrilus planipennis*), which attacks species of ash (*Fraxinus* spp.).

The balsam wooly adelgid was introduced into the United States from Europe around 1900. The adelgid attacks fir trees by feeding on fissures within the bark of infested trees, releasing toxins within its saliva. These toxins cause the tree to produce reaction wood, reducing sapwood conductance, causing water stress that eventually kills the tree. The forest species under the most severe threat from the balsam wooly adelgid is Fraser fir (*Abies fraseri*). Since the adelgid was discovered in Fraser fir stands in 1957, the tree has suffered catastrophic mortality throughout most of its natural range (Smith and Nicholas 2000).

The hemlock woolly adelgid, native to Japan, was first seen in Virginia in the 1950s, but was reported in western North America 30 years earlier. The insect feeds on xylem ray parenchyma at the base of needles, leading to desiccation of the needles and death of buds. While western species of hemlock are relatively resistant, both the eastern hemlock (*Tsuga canadensis*) and the less common Carolina hemlock (*Tsuga caroliniana*) are susceptible, and the adelgid has already caused extensive damage and mortality

of these species in the mid-Atlantic region (Small et al. 2005). Fortunately, multiple predators of the hemlock adelgid have been identified. Some of these predators, which are all species of lady beetles, show promise as biocontrol agents (McClure et al. 2001). One of the predator beetles, *Pseudoscymnus tsugae*, also feeds on eggs of the balsam woolly adelgid.

The emerald ash borer was introduced from Asia and was first discovered in Michigan in 2002. The larvae feed on the inner bark of ash trees, disrupting the tree's ability to transport water and nutrients. Since its discovery, the insect has killed millions of ash trees in Michigan, Ohio, and Indiana (MacFarlane and Meyer 2005). A number of parasitoids of the borer have been recently identified in China that may be useful biocontrol agents in North America (Zhang et al. 2005).

Fungal pathogens Most North American forest trees are attacked by pathogens. However, as with insect pests, fungal pathogens accidentally introduced from overseas have caused the most widespread destruction of our heritage trees. Examples of diseases caused by introduced fungal pathogens that have had devastating impact on heritage forest trees include dogwood anthracnose (*Discula destructiva*), which infects flowering dogwood (*Cornus florida*), butternut canker (*Sirococcus-clavigignenti-juglandacearum*), a disease of butternut (*Juglans cinerea*), Dutch elm disease (*Ophiostoma ulmi* and *Ophiostoma novo-ulmi*), which attacks elm (*Ulmus* spp.) trees and chestnut blight (*Cryphonectria parasitica*), a pathogen of chestnuts and chinkapins (*Castanea* spp.).

In addition to these four diseases, we should also include a fifth disease, the destructive potential of which has yet to be determined—sudden oak death (SOD). Although to date, most of what is known about the threat of SOD comes from its impact on populations of western oaks and tanoaks, there are predictions that *Phytophthora ramorum*, the pathogen responsible for sudden oak death (McPherson et al. 2005), could cause massive destruction of oak forests in the eastern United States.

The dogwood anthracnose fungus, thought to be of exotic origin, rapidly kills dogwood trees. Mortality has exceeded 90% in some forest types (Holzmueller et al. 2006). Hybridization of *C. florida* with the resistant *Cornus kousa* has resulted in some resistant cultivars with flowering characteristics similar to those of flowering dogwood. Some evidence indicates that the differential resistance to the fungus among *Cornus* species may be due to differences in chitinase isozymes (Cardwell and McDaniel 1998).

The fungus that causes butternut canker, thought to be of exotic origin, was described as a new species in 1979. While it is a pathogen of several species, it only kills butternut trees. Currently, butternut is threatened by the fungus throughout its natural range (Michler et al. 2006). Various options for developing resistance to the disease in butternut are discussed in Michler et al. (2006), including breeding within the species, hybrid breeding, and genetic engineering.

Special case 1: American elm and Dutch elm disease

American elm was once arguably the most popular street tree in the eastern United States. Its typical open-grown form, characterized by a trunk that divided close to the ground into a few erect stems that arched and terminated in numerous slender, drooping branches forming a vase-shaped crown, made it a favorite shade tree in yards, parks, and university campuses (Harlow et al. 1996). In the 1930s, Dutch elm disease (DED), caused by the fungus *Ophiostoma ulmi*, which had already devastated elms in Europe, was accidentally introduced into the US, probably on imported elm veneer logs. The fungus is vectored by European elm bark beetle (*Scolytus multistriatus*) and the native elm bark beetle (*Hylurgopinus rufipes*). More recently, new strains of the fungus have emerged with sufficiently different cultural and molecular characters to warrant their designation as a new species, *Ophiostoma novo-ulmi*. Consequently, millions of American elms shading our streets and parks in the northeastern United States were lost to DED (Hubbes 1999).

Conventional selection and breeding approaches to developing American elms with resistance to DED have been underway for decades. The USDA has produced several hybrid cultivars (e.g., 'Pioneer', 'Homestead') between American elm and European and Asian elms that show good DED resistance, although their forms are variable. Straight selection of DED-resistant American elms by inoculation screening by USDA has also resulted in at least two DED-resistant pure American elm cultivars ('Valley Forge' and 'New Harmony') that have been released to nurseries (http://www.usna.usda.gov/Newintro/american.html). An independent screening and selection program administered by the Elm Research Institute (http://www.libertyelm.com) has resulted in the production of the "Liberty Elm," actually a collection of six American elm cultivars that have been extensively screened for DED resistance.

Given the facts that elms were among the first trees from which adventitious shoots were produced (Gautheret 1940) and that propagation of elms via axillary shoot multiplication has long been practiced (e.g., McCown and McCown 1987), it would seem that progress in engineering elms with antifungal gene candidates would be rapid. However, it was not until relatively recently that significant progress has been made with systems capable of regenerating plantlets

via adventitious shoots or somatic embryos. Both of these routes for in vitro propagation of elms have been pursued as part of restoration efforts, and good progress has been made particularly in Europe.

Reports indicating the ability to regenerate adventitious shoots from elm leaf explants (Bolyard et al. 1991; Bolyard 1994; Fenning et al. 1993; George and Tripepi 1994; Kapaun and Cheng 1997) were promising, as this route offered the possibility of engineering fungal resistance genes into elm species via leaf-disk transformation. More recently, another potentially useful route for elm transformation has opened, with the publication of reports of somatic embryogenesis from immature zygotic embryos of *Ulmus minor* and *Ulmus glabra* (Corredoira et al. 2002; 2003b), and from leaves of mature *Ulmus minor* trees cultured on a medium with kinetin (Conde et al. 2004).

The first successful transformation work with elms was performed in Europe. The English elm (*Ulmus procera*) was transformed by infecting proliferating shoot cultures with a wild-type strain of *Agrobacterium*, resulting in tumors from which transformed shoots were regenerated (Fenning et al. 1996), and by Ri-plasmid-mediated transformation of internodal segments, after which dwarf shoots were regenerated from hairy roots (Gartland et al. 2001). Gartland et al. (2000) regenerated phenotypically normal transgenic English elm plants after co-cultivation of internodal stem segments from shoot-tip cultures with *Agrobacterium*.

In a recent review of the application of biotechnology to deal with Dutch elm disease, Gartland et al. (2005) indicated that English elm plantlets transformed with antifungal genes have been produced and these are currently being tested for their ability to resist the Dutch elm disease fungus. Recently, Newhouse et al. 2006 published details on a leaf piece transformation system for American elm. This system has been used to produce transgenic American elm trees with a gene encoding a cationic antimicrobial peptide called ESF39. Antimicrobial peptides are produced by most organisms as part of their pathogen defense systems and these peptides often have broad-spectrum antibacterial, antifungal, antiviral, and antiprotozoan properties (Hancock and Diamond 2000). Synthetic derivatives of these "natural" peptides have been shown to be efficient antimicrobial agents against many plant and animal pathogens (Schwab et al. 1999; Osusky et al. 2000; Tossi et al. 2000; Gura 2001; Rajasekaran et al. 2001; Ballweber et al. 2002).

The ESF39 peptide was designed to resemble the secondary structure of magainins (Zaoff 1987), but it contains a unique amino acid sequence designed to be quickly digested in mammalian digestive system and to have insignificant activity on plant and animal cells, while effectively inhibiting the growth of selected plant pathogens (Powell et al. 1995, 2000; Powell and Maynard 1997). Several designs of constitutively expressed cationic antimicrobial peptides have been shown to enhance pathogen resistance in transgenic poplar (Liang et al. 2002; Mentag et al. 2003) and apple (Norelli et al. 1998). In juvenile transgenic American elms expressing ESF39, reduced vascular staining of *O. novo-ulmi*-inoculated tissues and absence of the pathogen in these tissues were the first indications of enhanced DED resistance (Newhouse 2005). Small scale field tests of these transgenic American elms, in which their performance will be compared to that of wild-type trees and one of the Liberty elm clones have recently been established.

Special case 2: American chestnut and chestnut blight

The most famous case of an introduced pathogen causing the devastation of a native forest tree is that of the American chestnut (*Castanea dentata*) and chestnut blight. American chestnut once dominated the Appalachian forests of the eastern United States, where it was a major timber and nut-producing tree. Its straight, rot-resistant trunks were used for poles, pilings, posts, shingles, railroad ties, and furniture. Its bark was an important source of tannins for the leather industry and its nuts provided nutrition to wildlife as well as people (Anagnostakis 1987). The chestnut blight fungus, accidentally introduced from Asia on Japanese chestnut trees, began attacking American chestnut trees around 1900. By 1950, the fungus had invaded most of American chestnut's natural range, killing millions of trees. Today, the tree can be found mainly as an understory shrub, due to its ability to resprout from stumps (Burnham 1988).

Attempts to restore the species to the forest have included (1) searching for natural blight resistance in surviving American chestnut trees, (2) hybridizing American chestnut with blight-resistant Asian chestnuts, (3) inducing mutations using gamma irradiation, and (4) using hypovirulent strains of the blight fungus as biocontrol agents (Griffin 2000). With regard to the application of hypovirulence for biocontrol, it should be noted that transgenic hypovirulent strains of the fungus, engineered to facilitate the spread of the hypovirus across vegetative compatibility groups, are currently being tested in the field (Nuss 2005).

While early attempts by the USDA to generate resistant hybrid trees were largely unsuccessful, in the 1980s, the American Chestnut Foundation (TACF) began a new backcross breeding program [based on hybrids between American and blight-resistant Chinese chestnut (*Castanea mollissima*)] that is currently at the point of establishing BC_3F_2 orchards for production of seedlings that should resemble American chestnut in form and other aspects

while possessing levels of blight resistance that approach that of Chinese chestnut (Hebard 2005).

Similar to the case with elms, the first in vitro propagation work and first successful transformation of *Castanea* were accomplished in Europe. Decades of research on in vitro propagation of chestnut have been performed by the Viéitez Lab in Spain, and the most recent summary of this work as well as other chestnut research can be found in Viéitez and Merkle (2004). Viéitez (1995) successfully regenerated several plantlets of *C. sativa* × *C. crenata* hybrids via somatic embryogenesis using zygotic embryos as explants. Similarly, Saur and Wilhelm (2005) regenerated some plantlets from embryogenic cultures of pure *C. sativa*, initiated from ovaries, ovules and immature zygotic embryos.

A promising report by Corredoira et al. (2003a) indicated that embryogenic cultures could be initiated from seedling leaf explants of *C. sativa*, and that proliferation of new embryos continued via direct repetitive embryogenesis or via callus derived from somatic embryo cotyledons. If leaves from mature trees can also be used to initiate embryogenic cultures, this would allow elite European chestnut genotypes to be propagated via this route. Corredoira et al. (2004) achieved a transformation frequency of 25% and regenerated stably transformed European chestnut trees by co-cultivation of these leaf-derived embryogenic cultures with *Agrobacterium*, although plantlet regeneration frequencies were low.

In vitro propagation work with American chestnut lagged for several years, but is now making more rapid progress. While propagation of American chestnut via axillary multiplication has been described (Read and Szendrak 1995; Xing et al. 1997), its efficiency has been variable. However, several years of work developing an embryogenic regeneration system for the tree (Merkle et al. 1991; Carraway and Merkle 1997; Xing et al. 1999; Robichaud et al. 2004) are finally beginning to pay off, and some embryogenic lines can now be manipulated in suspension culture to produce hundreds of somatic seedlings (Andrade and Merkle 2005). Gene transfer work with American chestnut also lagged, while the embryogenic regeneration system needed to provide target material for transformation was under development. Carraway et al. (1994) used biolistics to produce the first stably transformed embryogenic cultures of American chestnut, but were unable to regenerate transgenic plants from the cultures.

More recently, Andrade et al. (2005) used the improved suspension culture system described earlier to regenerate over 100 transgenic American chestnut somatic seedlings from multiple genotypes after *Agrobacterium*-mediated transformation of embryogenic cultures. During this same period, the first American chestnut plantlets engineered with a potential antifungal gene were produced when an oxalate oxidase (*OxO*) gene was transferred via *Agrobacterium*-mediated transformation of embryogenic cultures (Polin et al. 2006). The *OxO* gene, which is from wheat, was previously shown to confer resistance to the poplar pathogen, *Septoria musiva*, when engineered into *Populus* × *euroamericana* (Liang et al. 2001). The oxalate oxidase enzyme encoded by the gene breaks down oxalic acid. Because *C. parasitica* infection involves the killing of tissue with oxalic acid, as is the case with *S. musiva*, the overexpression of this gene in chestnut stem tissues may confer resistance to the blight fungus. The transformation/regeneration systems now available for American chestnut will make it possible to rapidly engineer additional antifungal gene candidates into the tree for screening.

Do transgenic heritage trees warrant special consideration?

To date, much of the debate around the development and deployment of transgenic forest trees has paralleled the debate around transgenic food crops, with a few exceptions. For example, as with agronomic crops, concerns have been raised with regard to the potential for generating "weedy" genotypes, the development of insect pest biotypes resistant to toxins such as the *Bacillus thuringiensis* (*B. t.*) endotoxin expressed in trees engineered with *B. t.* genes, and the impact of such products on non-target organisms. One area of even higher concern with transgenic trees than with transgenic crops is the potential for transgenic escape to wild relatives. While most crop plants grown in North America lack wild relatives that could be fertilized by pollen carrying transgenes, forest trees are undomesticated. Thus, plantations of the top commercial trees likely to be genetically engineered for commercial release in North America (e.g., southern pines, Douglas-fir, spruces) all would be surrounded by wild relatives. This situation has led to the generally accepted assumption that any genetically engineered trees released to be grown in North America will need to be sterile or have some mechanism by which flowering could be controlled. Major research efforts are underway to decipher the molecular mechanisms controlling the development of reproductive structures in trees, as well as strategies to disrupt these mechanisms for the production of sterile transgenic trees.

Finally, one aspect of transgenic crops that has not generally been raised with transgenic forest trees is the potential for the products of the transgenes to enter the human food chain. This is surprising, given the numbers of foods, cosmetics, and pharmaceuticals that contain chemicals or fiber derived from forest trees, but it does emphasize the importance of public perception in the debate around transgenic trees.

The application of transgenic technology to restore "heritage" trees in general, and American elm and American chestnut in particular, forces those of us working with these species, those formulating policy, and even those generally opposed to the release of transgenic trees, to consider the deployment of transgenics from a different perspective from the one described above. Of course, one obvious difference between the threatened "heritage" trees like American chestnut and American elm and commercial species is the fact that the stated goal of engineering the threatened trees is restoration rather than commercial profit. Generally, the deployment of these disease-resistant trees would be perceived as aiding the restoration of species or even entire ecosystems. Thus, there is the general perception that this work and its products are for the public good.

On a more technical level, probably the most striking difference when considering our threatened "heritage" trees is the fact that, rather than restricting spread of the transgenes from the engineered trees to wild relatives, many of the stakeholders involved believe that crossing of the resistance genes into wild populations should actually be a *goal* of these programs, as it presumably would lead to establishment of resistance genes in these populations, accelerating the restoration of these species. This is certainly the case with American chestnut, where planning is underway not only to release fertile transgenic trees, but to begin a program of crossing with non-engineered genotypes to begin spreading the resistance genes more quickly. The problem with potential "weediness" of trees expressing the resistance transgenes is also one that does not appear to have been raised as it has been with transgenic commercial species.

This is a particularly interesting conundrum with respect to American chestnut, which was well-known as a proficient competitor on dry ridges in the Appalachians. Would a blight-resistant American chestnut spread more rapidly than is desirable or grow in areas where it is not wanted? Or would its ability to establish itself overwhelmingly be viewed as a great advantage for a heritage tree restoration program? Before the arrival of the fungus in North America, blight obviously played no role in the natural ecology of the species or in limiting its distribution, so one would not expect that blight-resistant chestnuts would be any more "weedy" than chestnuts were before the blight. Of course, these questions can be applied to any resistant American chestnut tree, whether derived from traditional breeding programs or through biotechnology.

Finally, the prospect of transgenic, blight-resistant American chestnut offers another almost unique challenge, as it is one of a very small group of native North American forest trees (along with pecan and black walnut) with the potential to produce a commercial nut crop. Thus, unlike almost any other North American forest tree that might be genetically engineered, this species can certainly be considered to produce food likely to be widely consumed by humans as well as wildlife. Those of us working on engineering antifungal genes into the species are well aware of this problem. As part of our planning, we are, therefore, selecting transgenes that are also used in crop species, as well as attempting to restrict the transgene expression to vegetative tissues or regulating expression in other ways.

With regard to regulated expression, several regulated gene promoters are available from forest trees, including PAL promoters from poplar (Gray-Mitsumune et al. 1999), poplar wound-inducible promoters (Clarke et al. 1994; Hollick and Gordon 1995), and American chestnut vascular promoters (Connors et al. 2001, 2002). In fact, the ESF39 antimicrobial peptide gene, which has been engineered into American elm, as mentioned earlier, is driven by one of these American chestnut vascular promoters (Newhouse et al. 2006). Similar promoters are also available from herbaceous plants. Polin et al. (2006) showed that a soybean vascular promoter from the *VspB* gene (Mason et al. 1993; Sadka et al. 1994) can drive expression of oxalate oxidase in transgenic American chestnut shoots.

Another consequence of the commercial nut-producing potential of American chestnut that should be kept in mind is that we should expect the products of a genetic engineering program with American chestnut to not only fall under regulation by USDA-APHIS, but the FDA as well.

Finally, as we have indicated in this paper, the application of transgenics to help restore threatened forest species is just one tool to help accomplish this goal. Some believe that the other approaches have shown sufficient progress that we need not go through the expense and potential risks of generating transgenic trees to accomplish the same goal. This may be especially true of stakeholders who have invested considerable time, energy, and funds in conventional approaches that appear to be close to paying off. However, we are well aware of the constant battle that plant pathologists and geneticists must wage to stay ahead of pathogens and pests, which can evolve resistance much more quickly than we can select and breed resistant trees. Thus, it would be wise to begin considering approaches for integrating the advances of conventional selection and breeding programs with transgenics to be able to combine the strengths of both approaches as needed.

While those of us working with transgenic "heritage" trees are optimistic about their potential to help restore these species, we are not naïve enough to think that all will agree that this approach can safely make a real contribution to returning these trees to our forests, parks, and streets. However, now that the science is close to being in place to begin testing resistance genes in our "heritage" trees, these concerns can be experimentally addressed, and it is time for

a sustained dialog, involving all potential stakeholders, on eventual release and deployment of this special class of transgenic trees, to begin.

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