# The Case for Genetic Engineering of Native and Landscape Trees against Introduced Pests and Diseases

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**Abstract:** *Many important native forest trees and familiar landscape trees of the northern temperate zone have been devastated by introduced pests and diseases. Without human intervention, many of these trees will become extinct or endangered. As trade and travel increase, it is likely that further devastating epidemics will occur. To undo the damage that has been done, we suggest limited, cautious transfer of resistance genes from the original host species in the source region of the pest or disease. The transgenic trees can then be replanted in forests or countryside to resume their original ecological niche. This method could have some advantages over tree-breeding techniques, including introgression. For instance, fewer tree generations would be required and fewer unnecessary genes of the non-native tree species would be introduced. Furthermore, once the technique is perfected it would be possible to separately add resistance genes to local land races of trees, for reintroduction to their original habitats, without relying on intensive and lengthy local introgression programs. Practical problems with identifying and transferring resistance genes do exist, however, and somaclonal errors might lead to genetically engineered trees that do not resemble their parent in growth form. Nevertheless, we believe that, with further work, this approach may offer a preferable alternative to introgression with non-native trees.*

El Caso de la Ingeniería Genética de Árboles Nativos y de Paisaje Contra Plagas y Enfermedades Introducidas

**Resumen:** *Muchos árboles forestales nativos y árboles de paisajes familiares a la zona templada del norte han sido devastados por plagas y enfermedades introducidas. Sin la intervención humana, muchos de estos árboles se extinguirían ni estarían amenazados. A medida que el comercio y los viajes se incrementan, es muy probable que ocurran epidemias devastadoras en el futuro. Para revertir el daño causado, sugerimos la transferencia limitada de genes resistentes de las especies hospederas originales en la región de inicio de una peste o enfermedad. Los árboles transgénicos pueden ser plantados en bosques o en el campo para que reactiven sus nichos ecológicos originales. Este método puede tener algunas ventajas sobre las técnicas de reproducción de árboles, incluyendo la introgresión. Por ejemplo, se requeriría la introducción de menos generaciones de árboles y menos genes innecesarios de las especies de árboles no nativos. Más aún, una vez que la técnica se ha perfeccionado, sería posible agregar de manera separada genes de resistencia a razas locales de árboles, para la reintroducción en sus hábitats originales, sin depender de programas de introgresión local intensiva y prolongada. Sin embargo, existen problemas prácticos de identificación y transferencia de genes de resistencia y algunos errores somato-clonales podrían conducir a la producción de árboles genéticamente modificados que no se parezcan a sus padres en cuanto a la forma del crecimiento. A pesar de todo, creemos que con trabajos a futuro, esta técnica puede ofrecer una alternativa preferencial a la introgresión con árboles no-nativos.*

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## Introduction

In both Europe and North America, common native trees of forests, cities, and the countryside have been devastated by introduced pests and diseases. These include the elms (*Ulmus*) of both Europe and North America and the North American chestnut (*Castanea dentata*). In the case of the chestnut, eastern U.S. forests have lost one of their dominant trees (Bailey 1995).

It is likely that these types of catastrophic pandemics will continue. In North America a repeatedly introduced Cerambycid beetle from Asia (*Anoplophora glabripennis*) threatens to wipe out buckeyes (*Aesculus*) and the maples (*Acer*) that dominate the eastern deciduous and mixed forests (Cavey et al. 1998). In California, evergreen oaks (*Quercus*) and some related species are being killed by a new species of the Oomycete fungus *Phytophthora* thought to have been imported on rhododendrons (Rizzo & Bailey 2000). Although not a native species, *Cupressus sempervirens* has been widely planted in the western Mediterranean region and is being devastated by the introduced fungus *Seridium cardinale*, which probably originated in California (Anselmi & Govi 1996). This fungus has also damaged native stands of *Cupressus macrocarpa* in the United States. A similar case in Europe is the sycamore or plane tree (*Platanus orientalis* and *Platanus hybrida*) and *Ceratocystis fimbriata f. platani*, which causes a fungal disease. The fungus arrived in Mediterranean areas from the United States, probably during World War II (Anselmi & Govi 1996). The disease kills both cultivated and wild trees, whereas the North American sycamore (*Platanus occidentalis*) appears to be naturally resistant. With the additional climatic stress of global warming, tree populations may become even more susceptible to outbreaks of introduced pests and diseases (e.g., McCarty 2001).

In each of these cases, the origin of the pest outbreak appears to be the introduction of geographically alien microorganisms. It appears that the resistance of trees in the source regions evolved over millions of years of exposure to these agents, whereas in the region into which the pest has been introduced there has been no selection for resistance so the trees are killed en masse. In some cases, particularly for the insect pests, natural enemies were left behind as they changed locales, which may also be a contributing factor. Unfortunately, it is likely that the accelerating movement of plants, goods, and people around the world will increase the frequency of tree-disease pandemics, with increasing effect on native forests, cultivated landscapes, and the animals and other organisms that depend on trees for food and habitat (Kennedy 2001; Wingfield et al. 2001).

In the regions affected by these widespread forest diseases, the response has generally been insufficient to prevent damage or restore species. In most cases, selective felling of infected trees has proven too patchy or too late to be effective. However, the use of a hypovirulent strain of the Chestnut blight fungus (*Chryphonectria parasitica*) has apparently diluted the virulent strain and thus reduced disease symptoms in some areas in Europe (Cortesi et al. 1996, 1998). Unfortunately, the American strain of the fungus has so far not been susceptible to this treatment. Attempts to breed resistance into chestnuts from related species (e.g., the east Asian species *Castanea mollissima*) via hybridization may ultimately prove effective, but this work has already taken decades and—depending on how extensive backcrossing and selection is (American Chestnut Foundation 2000)—may result in a species with other significantly altered physiological and morphological properties.

In the case of the European elms, some isolated individuals resistant to *Ophiostoma ulmi* have been located. Because of the trees' long lifespan, however, breeding reliably resistant strains from so few and isolated individuals will take many decades and will itself produce a genetic bottleneck that may expose the resistant trees to other epidemics. Many of the clones of elms that formerly provided a characteristic and attractive appearance to European landscapes show no resistance, so there is almost no prospect of their return as full-sized trees without slow artificial hybridization with other elm strains. Unfortunately, as with chestnut, this may also alter their growth habit and adaptive qualities. We should also consider the possibility that the disease itself may return as a slightly different strain, as apparently happened with elms over the course of nearly 60 years in Europe. When *Ophiostoma novo ulmi* appeared, it killed some elm species and cultivars that were resistant to *O. ulmi* (Brasier & Webber 1987; Namkoong 1991; Smalley & Guries 1993; Brasier 2001). In Italy, extensive tree-selection work on *Cupressus* has produced five clones resistant to *Seridium cardinalem* (Santini et al. 1997), but this involved a considerable loss of genotypic variability.

In the United States, the wild chestnut population has now been devastated by chestnut blight, and there is little prospect of populations returning of their own accord even on a time scale of thousands of years. Strong genetic resistance does not appear to exist in the wild populations of *Castanea dentata*, and the situation appears to be similar to that of American elms (Brasier 2001). The prospects for finding resistant individuals among populations of North American maples or Californian oaks are unknown, but we should consider the possibility that resistance is rare or nonexistent.

Given the widespread mortality that is occurring and will probably continue to occur among temperate and tropical tree populations, how should conservationists respond? One option is to let nature take its course, although in fact this is not "nature" but instead a problem caused by human meddling. Another option is to hope

that either traditional plant-breeding methods, mycology, or entomology can provide naturally resistant strains of trees or biological control options for virulent pathogens or insects. Breeding resistant trees from source populations, although preferable if there are no associated problems, is not a possibility when resistance alleles are rare or absent. Biological control is likely to involve the introduction of new species of fungi and insects with unknown capacities for interacting with flora, despite the study they undergo prior to introduction. Moreover, these new species often fail to become established or provide effective levels of control (Turnbill & Chant 1961).

## The Opportunity Offered by Genetic Engineering

It may be necessary to consider what some environmentalists would regard as unthinkable: genetic engineering to add resistance genes to trees for reintroduction to native forests. This technology is new, particularly for wild trees, and the science on which it is based, tree and plant genomics, is limited. Although genetic engineering is credited with the dramatic rescue of one tree species from disease—Hawaiian papaya (*Carica papaya*) from papaya ringspot potyvirus (PRSV) (Lius et al. 1997)—the majority of diseases threatening forest trees are not viral in origin. In addition, a general set of strategies that appears to be effective for engineering viral resistance, the expression of virally derived transgenes in plants, is not effective for fungal pathogens. No generic technology for overcoming fungal pathogenicity has been described. Thus, is it reasonable to consider that genetic engineering and genomics in trees could provide significant new means for producing resistant trees in the face of devastating diseases? To produce resistant strains, what will be needed, how long will it take, and what will the costs and potential tradeoffs be?

Although there have been many reports of increased fungal disease resistance due to single transgenes in the laboratory, so far there has been only one case of significant field resistance (Gao et al. 2000), and this was for a specific pathogen and crop species. We therefore believe it is wise to assume that restoring forest species via genetic engineering will not result from generically effective "supergenes," but instead it is likely to require the use of resistance genes isolated from their own genomes or from a related tree species in the same genus or family. Such genes, particularly when compared with alternatives like general fungal toxins, would also have the advantage of raising far fewer ecological concerns about their nontarget effects. The problem is how to identify, physically isolate, and transfer such genes in an efficient and physiologically safe manner.

Because of recent advances in identifying common DNA sequence motifs in genes for resistance to diverse kinds of diseases—and in diverse kinds of plant species (Young 2000)—scientists have an excellent idea of what such genes are likely to look like. In fact, as a consequence of highly conserved amino acid motifs in many forms of these genes, it is now possible to isolate them in large quantities in a single day using PCR (polymerase chain reaction) with degenerate primers. But it is also known that these PCR-derived "resistance gene analogs" (RGAs) are so prevalent in the genome (Michelmore & Meyers 2001) that it is necessary to narrow down the search to a small region via traditional genetic mapping or direct biochemical methods first to avoid being overwhelmed with possibilities. The RGAs appear to comprise as much as 2% of all genes in the *Arabidopsis* genome (Michelmore & Meyers 2001).

Because most genes for resistance to obligate pathogens appear to have a role in sensing or signaling pathogen presence to mobilize plant-defense metabolism, it should be possible to isolate genes based on their protein products' biochemical interaction with pathogens. This theoretical possibility has not yet been demonstrated, however, so it may be necessary to rely on some kind of genetic mapping approach, usually involving interspecies hybrids between resistant and susceptible species. This has the advantage of advancing not only genetic engineering, but a conventional backcross approach to resistance breeding as well, as is being pursued in the chestnut; thus, costs for the two alternative approaches are shared.

Under backcross breeding, the hybrid and mapping populations are the actual vehicle for moving resistance genes; under genetic engineering, different kinds of genetic crosses could also be used whose only purpose is to identify resistance genes. This might be the case, for example, when resistance exists in a species that is not fully interfertile with the threatened species or when a chromosomal difference prevents normal segregation in hybrid offspring with the threatened species, complicating mapping and effective backcross-mediated introgression. The genes could still be mapped in intra- or interspecific crosses involving other species where resistance to the pathogen segregates.

Two key requirements of the genetic engineering approach are that a large number of genetic markers must be available for creating a dense genome map and that a sufficiently large group of segregating progeny must be created so that the location of the gene(s) can be determined precisely. High-density maps of resistance genes have been created in poplars (*Populus*) through the use of amplified fragment-length polymorphisms (AFLPs; Cervera et al. 1996). It is likely to continue to be the method of choice for a number of years because it requires no prior knowledge of the genome. A mapping approach also assumes that only one or a few major resistance genes segregate. If resistance turns out to be a polygenic trait (many loci encode partial resistance), it

is unlikely that mapping can be precise enough to physically isolate genes or that a sufficient benefit could be obtained by transfer of one or a few of them. Finally, it also requires that resistance genes be at least partially dominant. Transformation typically inserts single, novel gene copies (although it may do this at multiple locations in the genome) so the gene must confer resistance when hemizygous (no alternative allele present at the locus) or heterozygous. Fortunately, a majority of genes for resistance to obligate plant pathogens show dominance.

Once a small region of the genome, generally one centiMorgan (1% recombination) or less, is identified as containing a resistance gene, it is feasible to consider isolating and transferring it. In a species with a small genome and in which gene transfer is efficient (e.g., poplars), large pieces of the genome that overlap the mapped locus, typically on the order of 100 kb, can be tested directly. This requires cloning of the genome in a bacterial artificial chromosome (BAC) library capable of containing such large fragments and suitable for *Agrobacterium* gene transfer (Hamilton 1997). For other species, however, additional genetic information is desirable to subdivide this large region further so that the task of gene transfer is applied to a small, well-characterized area. To subdivide this area, the BAC clones of interest are first subcloned and studied via mapping or sequencing. Regions that appear promising can then be used to isolate the active forms of these genes from cDNA libraries of expressed genes, or the RGA-containing regions can be subcloned and transferred directly. This resistance gene–isolation method would be particularly useful in species such as conifers that have very large, repetitive genomes. In many cases, however, resistance genes are present in tandem repetitive structures, any member of which could encode the desired resistance. It is therefore desirable to transfer linked groups of genes. Thus, a gene-transfer method that is highly efficient at transfering large fragments may be essential. This strategy also has the advantage of transferring not one but a family of related resistance genes, providing some buffer against pathogen evolutionary change. If more than one locus in the genome contains resistance genes, it is highly desirable to transfer both, which can be done successively or at the same time via cotransformation.

The ultimate test of success is the production of resistant, phenotypically normal transgenic plants. In poplars, which have had more transgenic trees produced and studied in the field than any other tree species (Tzfira et al. 1998), somaclonal variation (unintended phenotypic or genetic abnormality) appears to be small (Strauss et al. 2001). However, these studies have spanned only a few years. It is unclear if somaclonal variation is significant over longer time periods or in other tree species. It is possible that other species will show less tolerance of genomic perturbation than do the developmentally plastic poplars. Researchers and industries circumvent somaclonal variation as an important factor simply by producing large numbers of independently transformed transgenic lines and screening them carefully. Only a portion are considered for in-depth studies or commercial development. To produce a well-adapted wild tree, the production of a large population of transgenic progeny is also important. After the primary transgenic trees flower in the field, natural selection plays the largest role in sorting out the transformants that are most fit. To help produce the levels of genetic variability found in natural tree populations, a single backcrossing between transformants and a range of surviving wild-type individuals from within each local or regional population would also be appropriate. Natural selection in each population reintroduced to the wild would then ensure that resistance genes are favored while substantial genetic variability at other loci is maintained.

## Research Challenges for Effective Genetic Engineering of Resistance in Trees

Unless there is a breakthrough in direct isolation of resistance genes based on their molecular interaction with pathogens, the map-based cloning method described above would be extremely difficult to apply to most trees species today. The key obstacles are the absence of genomics tools and efficient methods of gene transfer. The increasing automation of genome mapping and sequencing, however, may make it possible to create the required genomic tools, at least for a number of keystone angiosperm genera in the temperate zone. Physical maps and associated sequence-tagged markers made in one species would be likely to work throughout many genera. These maps would facilitate the pinpointing of sections of the genome likely to contain resistance genes. In addition, because resistance genes are often clustered on particular chromosomes, the maps would serve as a guidepost, helping to identify the regions most likely to contain useful loci.

Given the rate of advancement in genome technology, and the importance of keystone tree species to forest ecosystems and human economies, it is now in the realm of possibility that complete genome sequences, and/or large expressed sequence databases, might be created for our most important tree genera or families within one to two decades. This would allow RGAs and tightly linked polymorphic genetic markers such as SNPs (single nucleotide polymorphisms) or SSRs (simple sequence repeats) to be directly studied and isolated. It would also allow many resistance genes whose sequences are today unknown to be inferred directly by a combination of map position and sequence comparison to crop and model plant species.

Although a large number of tree genera have been transformed, the frequencies of gene transfer and recovery of transgenic plants are too low in most genera to support the transfer of large DNA fragments and the production of large transgenic populations that will be needed (Brunner et al. 1998; Tzfira et al. 1998). The recovery of transgenic plants is today a highly empirical, species- or genotype-specific enterprise and generally requires the use of accessory genes that are undesirable in the wild (e.g., antibiotic or herbicide resistance genes). But there are signs that this may change radically over the next decade if research continues. New methods are being developed to insert genes that directly promote regeneration of transgenic cells themselves, rather than relying on toxin resistance, and a variety of systems have been developed that can excise permanently any accessory genes employed (e.g., Ebinuma et al. 1997). Thus, the ability to transform trees may become a more effective and generalized technology in the future. This will be critical if large sections of chromosome, and a number of different genes from different loci and alleles, are to be transferred into a number of different genetic backgrounds to maintain the required diversity for success in the wild. But because of the time required to develop transformation systems and test the health of regenerated trees in the field, it is desirable that this work continue in parallel with genomics development. Work on the fundamental interactions between pathogens and resistance genes is perhaps most critical, but most of this is best done in herbaceous plants and model systems, rather than with trees.

We believe that genetic engineering could provide useful options for mitigating the large ecosystem perturbations caused by exotic pests and diseases, but that this capability will require considerably more research before it is practical. The basic elements—from gene mapping to transformation—have been demonstrated, however, suggesting an absence of major obstacles to success. But it is not the only approach, or the only biotechnology approach. Hybridization and introgression may be more effective in many cases, especially when a large number of loci impart resistance or when transformation is difficult. Both introgression and genetic engineering approaches, however, would benefit from improved genome tools for the major tree species. The main advantages of genetic engineering over the introgression approach are the ability to (1) isolate and transfer genes from relatives that cannot be crossed or where crosses cause sterility due to chromosomal incompatibility; (2) insert genes without cotransfer of chromosome sections from related species, which may change adaptability and form; (3) insert genes into juvenile trees without waiting for onset of flowering; and (4) directly insert genes into a variety of genotypes from different areas without the cost and delay of multiple introgression programs.

In addition to the possibility of significant somaclonal variation, the main disadvantage of genetic engineering is the increased information and technical capability required, which presently limits the number of species, genotypes, and genes that it can be applied to.

The extent to which the use of genetic engineering becomes successful will be a direct result of the research effort applied. Given the degree of opposition that genetically engineered crops have met with from many elements of the environmental movement, an unfortunate consequence might be the cessation or curtailment of funding for tree biotechnology research that could save many valued native species from either extinction or near extinction. With the rapid pace of advances in genome research, and the near certainty of continuing pandemics of tree diseases, we believe that developing a background of genomics tools, including the capability for a genetic engineering approach for use when necessary, is an appropriate precautionary strategy.

## Literature Cited

American Chestnut Foundation. 2000. Research and restoration: the backcross method. Bennington, Vermont. Available from http://chestnut.acf.org/r r.htm (accessed April 2001).

Anselmi, N., and G. Govi. 1996. Patologia del legno. Edagricole, Bologna, Italy.

Bailey, R. G. 1995. Descriptions of the ecoregions of the United States. Miscellaneous publication 1391. U.S. Forest Service, Washington, D.C.

Brasier, C. M. 2001. Rapid evolution of introduced plant pathogens via interspecific hybridisation. BioScience **51:**123–133.

Brasier, C. M., and J. F. Webber. 1987. Recent advances in Dutch elm disease research: host, pathogen and vector. Pages 166–179 in D. Patch, editor. Advances in practical arboriculture. Forest community bulletin 65, Her Majesty's Stationery Office, London.

Brunner, A. M., R. Mohamed, R. Meilan, L. A. Sheppard, W. H. Rottmann, and S. H. Strauss. 1998. Genetic engineering of sexual sterility in shade trees. Journal of Arboriculture **24:**263–273.

Cavey, J. F., E. R. Hoebeke, et al. 1998. A new exotic threat to North American hardwood forests: an Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (*Coleoptera:Cerambycidae*). Proceedings of the Entomological Society of Washington **100:**373.

Cervera, M. T., J. Gusmao, M. Steenackers, J. Peleman, V. Storme, A. Vanden-Broeck, M. Van Montagu, and W. Boerjan. 1996. Identification of AFLP molecular markers for resistance against Melampsora larici-populina in *Populus*. Theoretical and Applied Genetics **93:** 733–737.

Cortesi, P., M. G. Milgroom, and M. Bisiach. 1996. Distribution and diversity of vegetative compatibility types in subpopulations of *Cryphonectria parasitica* in Italy. Mycological Research **100:**1087–1093.

Cortesi, P., D. Rigling, and U. Heiniger. 1998. Comparison of vegetative compatibility types in Italian and Swiss subpopulations of *Cryphonectria parasitica*. European Journal of Forest Pathology **28:** 167–176.

Ebinuma, H., K. Sugita, E. Matsunaga, and M. Yamakado. 1997. Selection of marker-free transgenic plants using the isopentyl transferase gene. Proceedings of the National Academy of Sciences **94:**2117–2121.

Gao, A. G., S. M. Hakimi, C. A. Mittanck, Y. Wu, B. M. Woerner, D. M. Stark, D. M. Shah, J. Liang, and C. M. T. Rommens. 2000. Fungal pathogen protection in potato by expression of a plant defensive peptide. Nature Biotechnology **18:**1307–1310.

Hamilton, C. M. 1997. A binary-BAC system for plant transformation with high-molecular-weight DNA. Gene **200:**107–116.

Kennedy, D. 2001. Black carp and sick cows. Science **13:**169.

Lius, S., R. M. Manshardt, M. M. Fitch, J. L Slightom, J. C. Sanford, and D. Gonsalves. 1997. Pathogen-derived resistance provides papaya with effective protection against papaya ringspot virus. Molecular Breeding **3:**161–168.

McCarty, J. P. 2001. Ecological consequences of recent climate changes. Conservation Biology **15:**320–331.

Michelmore, R., and B. C. Meyers. 2001. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Research **8:**1113–1130.

Namkoong, G. 1991. Maintaining genetic diversity in breeding for resistance in forest trees. Annual Review of Phytopathology **29:**325–342.

Rizzo, D., and P. Bailey. 2000. UC Davis expert identifies oak-tree killer and warns public to use caution. Available at http://www-news.ucdavis.edu/newsreleases/07.00/news oak fungus.html (accessed 31 July 2000). UC researchers announce results that could complicate measures to halt spread of Sudden Oak Death. Available at http://www.cnr.berkeley.edu/?db=public news&id=27 (accessed 10 January 2001).

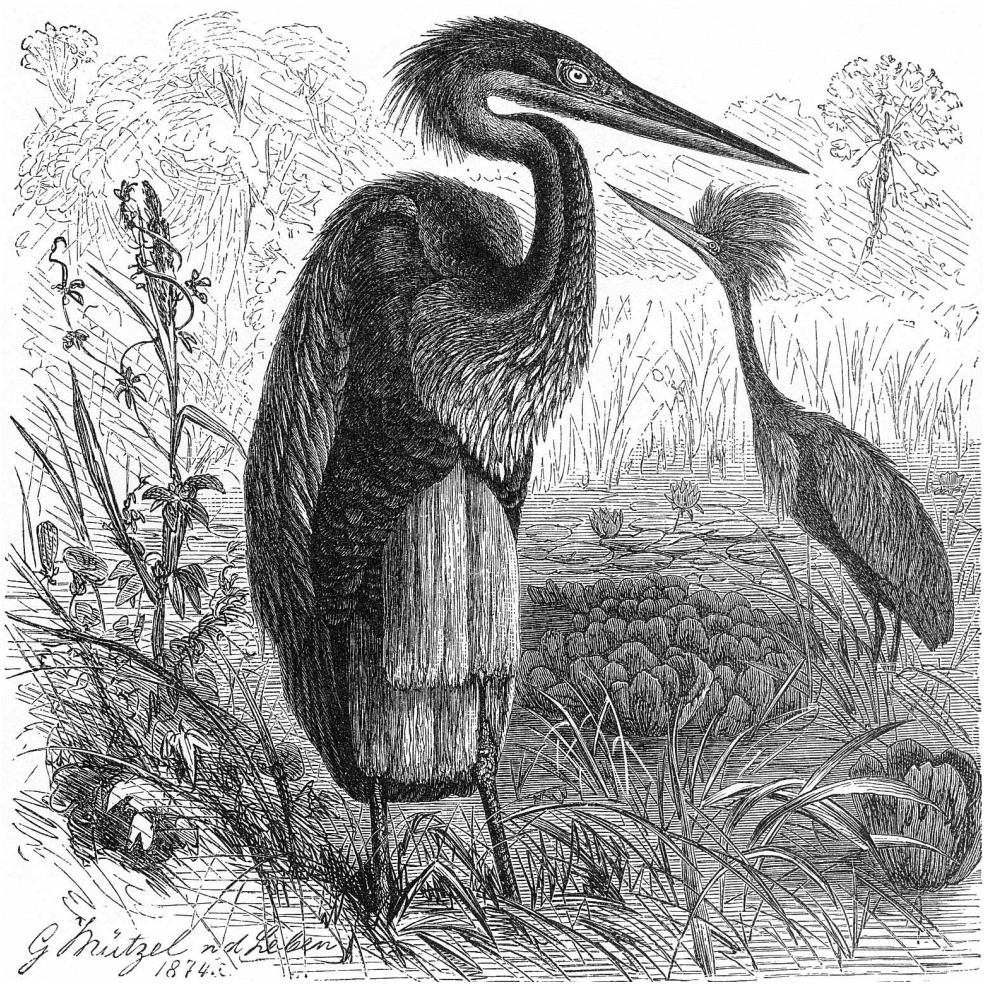
Santini, A., N. Casini, V. Di Lonardo, and P. Raddi. 1997. Canker resistance stability of some *Cupressus sempervirens* clones to *Seiridium cardinale*. Journal of Genetics & Breeding **51:**269–277.

Smalley, E., and R. P. Guries. 1993. Breeding elms for resistance to DED. Annual Review of Phytopathology **31:**325–352.

Strauss, S. H., S. DiFazio, and R. Meilan. 2001. Genetically modified poplars in context. Forestry Chronicle **77(2):**1–9.

Turnbill, A. L., and D. A. Chant. 1961. The practice and theory of biological control of insects in Canada. Canadian Journal of Zoology **39:**697–753.

Tzfira, T., A. Zucker, and A. Altman 1998. Forest-tree biotechnology: genetic transformation and its application to future forests. Trends in Biotechnology **16:**439–445.

Wingfield, M. J., B. Slippers, J. Roux, and B. D. Wingfield. 2001. Worldwide movement of exotic forest fungi, especially in the tropics and the southern hemisphere. BioScience **51:**134–140.

Young, N. D. 2000. The genetic architecture of resistance. Current Opinion in Plant Biology **3:**285–290.