project. The valley was declared a national park in 1985. In Slovakia, an NGO, WOLF, has since 1993 been working to save natural forests that include large predators such as wolves. WOLF is predominantly managed by local tribes, each tribe adopting a mountain range to save the natural forest. Whether practiced in the Australian outback by the Aborigines or in Amazonia by native Indians, these movements have fought, often successfully, unscrupulous exploitation of forest resources by larger interests. Several such movements have, over time, gained sufficient strength and publicity that they have been later adopted into more formal approaches to forest management.

Conclusions and Implications

Prior to the major human settlements and advances tropical forests covered about 17 million km^2 of the earth's surface. Today, less than half of this remains. The forests lie in some of the most economically underdeveloped and heavily populated countries in the world. Consequently even these remnants face extreme pressures due to an increasing demand on the forest resources by the developing economies. It is feared that unless urgent measures are taken to conserve the remaining forest, not only will these forests be lost but there will also be an irreversible loss of the variety and performance of life functions on earth. Awareness of both threats and consequences has stimulated urgent efforts, initiated mostly at the beginning the twentieth century, to develop various approaches to managing forests in a manner that would conserve biological diversity and ecosystem processes. The establishment of protected areas has been central to these efforts, and now about 6.3% of the earth's land surface is under protection. While protected areas have their faults, there is an overriding consensus that they could be the last refugia for several scores of critically endangered species. Besides state-regulated protected areas, several semiformal approaches to managing forest for conservation also exist, such as sacred groves and people-inclusive forest management (e.g., joint forest management and community managed conservation areas). While the reach of these systems has been restricted, they have nevertheless been moderately successful in managing forest for conservation and local benefit in many developing countries. People-led movements have also been a powerful force in lobbying for improved management for conservation and sustainable development in countries such as India and Brazil, and have been precursors to some major conservation movements. It is believed that collectively the various models of conservation, from the very formal protected area networks to the informal but powerful people-led movements, will complement each other to avoid exploitative management in favor of sustainable management.

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Genetic Aspects of Air Pollution and Climate Change

D F Karnosky and R C Thakur, Michigan Technological University, Houghton, MI, USA

Introduction

The first incidences of air pollution impacts on the genetic constitution of forest tree populations were those documented near point sources of sulfur dioxide (SO_2), particulates, and heavy metals. Localized extinction of forests around these point sources was documented by ecologists in the past two centuries. In North America, the most spectacular of these areas were those surrounding ore smelters in Trail, British Columbia, Sudbury, Ontario, and Copper Basin, Tennessee. In Europe, the most dramatic areas included the Black Triangle area (of eastern Germany, Poland, and the then Czechoslovakia) which was largely due to soft coal burning in power plants and numerous situations where industrial facilities were located in valleys such that toxic emissions destroyed vegetation on the surrounding hillsides. With these early pollution problems, large areas of forests have simply been replaced by grasses or other tolerant vegetation.

Around the middle of the twentieth century, another type of air pollution, smog consisting of various photochemical oxidants, including nitrogen oxides (NO_X), ozone (O_3), and peroxyacetyl nitrate (PAN), began to impact forest tree populations. The first documented consequences of photochemical oxidant on forest tree populations occurred in the San Bernardino mountains where sensitive genotypes of ponderosa pine (*Pinus ponderosa*) began to die in large numbers, being replaced in the forest by more smog-tolerant species such as white fir (*Abies concolor*). Ozone downwind of major metropolitan areas has also been implicated in the loss of sensitive individuals in eastern white pine and trembling aspen in the eastern USA. In parts of the highly polluted Ohio Valley region in the eastern USA, sensitive genotypes of eastern white pine were virtually eliminated from the breeding populations between the mid-1950s and the mid-1960s due to deadly combinations of SO_2 and O_3. Since the 1980s, O_3 has been implicated in the loss of hundreds of thousands of pines in the mountains surrounding Mexico City, where O_3 levels are among the highest in the world.

Recently, the scientific community has realized that greenhouse gases of anthropogenic origin are building up in the earth's atmosphere (**Figure 1**) and that these gases are likely contributing to the trapping of heat near the earth's surface. As a result, there is a sharply rising trend in global mean temperatures (**Figure 2**). The increasing temperatures will likely eventually lead to changes in species-richness in a given area and changes in the range of many forest tree species.

In this article, we first discuss genetic aspects of air pollution effects on forests and then examine how the changing climate may impact the genetics of forest trees. Finally, we discuss some of the remaining knowledge gaps and research needs with

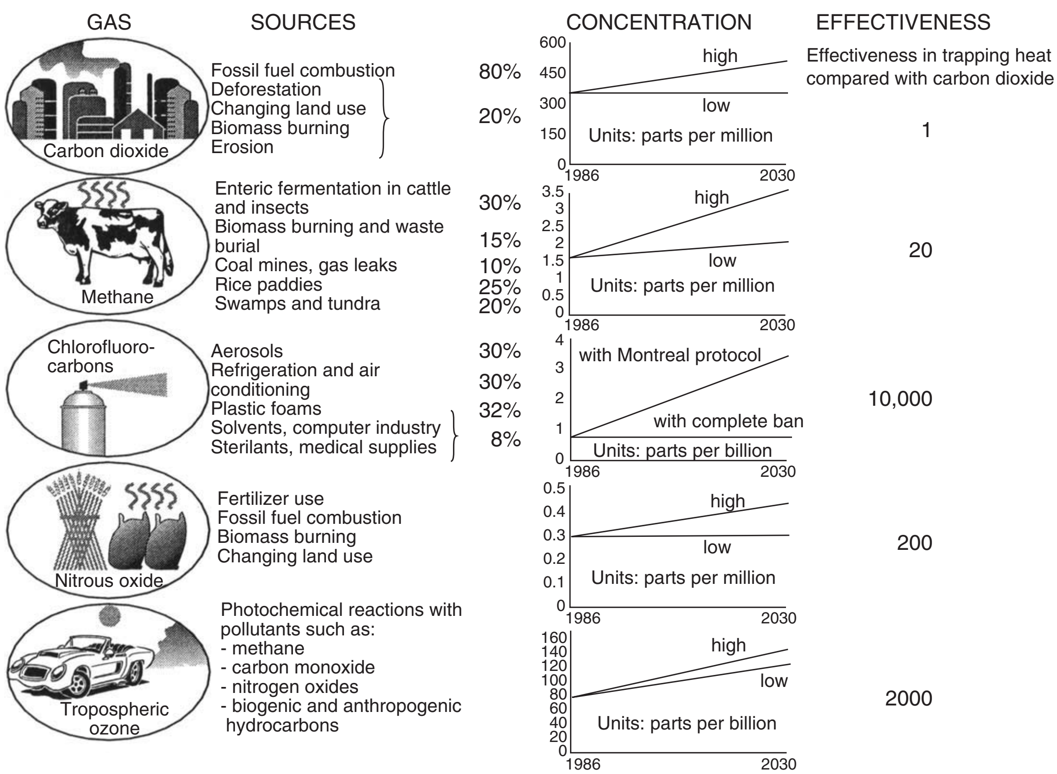

Figure 1 The major anthropogenic greenhouse gases. Reproduced with permission from Karnosky DF, Ceulemans R, Scarascia-Mugnozza GA, and Innes JL (2001) *The Impact of Carbon Dioxide and other Greenhouse Gases on Forest Ecosystems*. New York: CABI. Adapted from Milich (1999) Global *Environmental Change* 9: 179–201.

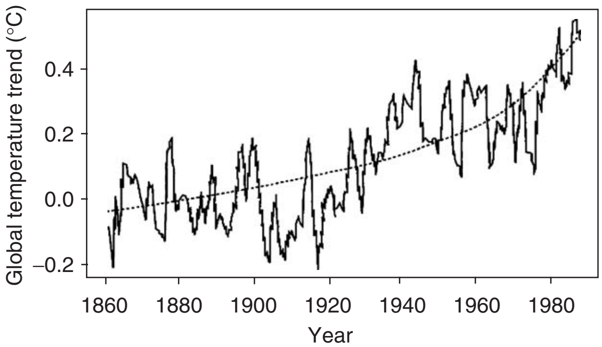

Figure 2 The Intergovernmental Panel on Climate Change (IPCC) global temperature record and a set of predicted temperatures. Reproduced with permission from Karnosky DF, Ceulemans R, Scarascia-Mugnozza GA, and Innes JL (2001) *The Impact of Carbon Dioxide and other Greenhouse Gases on Forest Ecosystems*. New York: CABI. Adapted from Bloomfield (1992) *Climate Change* 21: 1–16.

respect to genetic aspects of air pollution and climate change.

Genetic Aspects of Air Pollution

For air pollution to induce natural selection, there must be variation in air pollution sensitivity, the variation must be heritable, and the pollution must be a strong enough selection force to disadvantage sensitive trees severely. According to Anthony Bradshaw, who has studied natural selection in grasses growing in the presence of heavy metals, evolutionary population change takes place in three stages:

- Stage 1: elimination of the most sensitive genotypes
- Stage 2: elimination of all genotypes except the most tolerant (note: elimination of *all* forest tree genotypes, as has occurred in many point source pollutants, results in extinction, not evolution)
- Stage 3: interbreeding of the survivors to give even more resistant genotypes which are then further selected

The rate of selection is dependent on the severity of the pollutant stress, the type of reproduction (sexual or asexual reproduction), and the level of competition between genotypes (the more intense the competition between sensitive and tolerant trees, the faster the effects occur). Finally, air pollution-induced selection can take place at the level of differentiation in survival among individual trees (viability selection) or among pollen grains on a stigmatic surface (gametic selection).

In our laboratory, we have long studied the responses of forest trees to air pollution in an attempt to gain a better understanding of the

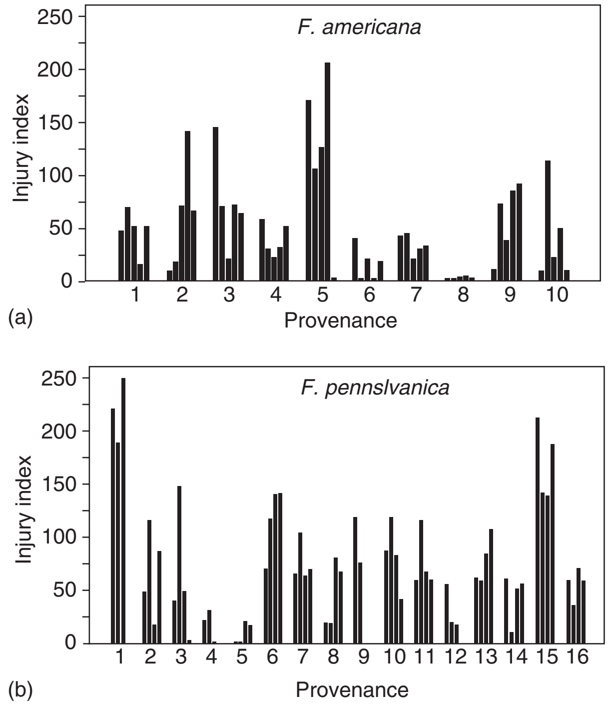

Figure 3 The average injury index for visible foliar injury after exposure of 1-year-old seedlings to 50 pphm ozone for 7.5 h. Each mean shown represents the average of five trees per family. There were either four or five half-sib families for each white ash (*Fraxinus americana*) provenance (geographic location) and either three or four families for each green ash (*F. pennsylvanica*). Reproduced with permission from the 1996 Air Quality Criteria for Ozone and Related Photochemical Oxidants. Washington, DC: US EPA Office of Resources and Development.

potential for air pollution to impact natural selection. It is clear from these studies that there is tremendous variability in responses of forest trees to air pollution and this is manifested as differences between species, provenances, families within provenances, and tree-to-tree within families (**Figure 3**). Furthermore, we and others have shown that variable responses to air pollutants such as heavy metals, SO_2, and O_3, are highly heritable. We have also shown that the differences in responses to air pollution can directly affect the competitive ability of trees as air pollution can dramatically affect growth, as we demonstrated with trembling aspen affected by O_3 in controlled fumigation studies done in open-top chambers (**Figure 4**) and in field plantings under naturally elevated levels of O_3 (**Figure 5**). We have also shown that the effects of air pollution in the stage 1 of natural selection can occur very rapidly, as shown in **Figure 6**, where nearly 50% of the O_3-sensitive trembling aspen (*Populus tremuloides*) were eliminated from highly competitive close-spacing trials in a high-O_3 environment after only 5 years. Similarly, we have

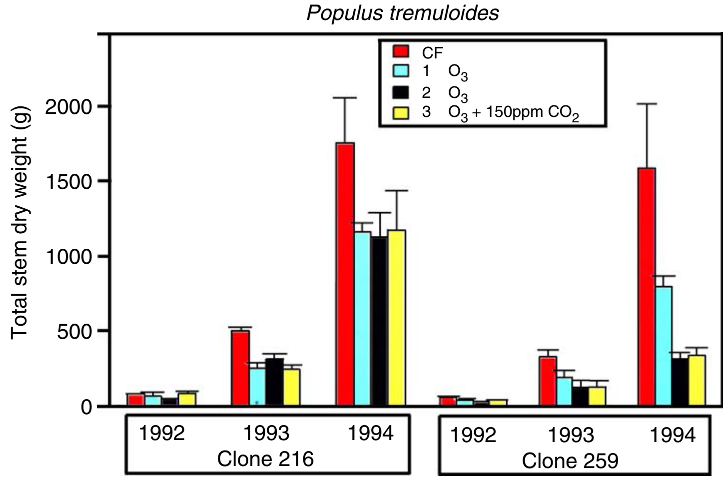

Figure 4 Total stem biomass per tree for an ozone (O_3)-tolerant (clone 216) and an O_3-sensitive (clone 259) trembling aspen (*Populus tremuloides*) clone exposed to charcoal-filtered (CF), ambient O_3 ($1 \times O_3$), twice ambient ($2 \times O_3$), or twice ambient $O_3 + CO_2$ (150 ppm over ambient) for three growing seasons in open-top chambers. The O_3-induced decreases in stem growth for the O_3-sensitive clone are particularly dramatic in year 3.

Figure 5 The effects of ambient air pollution on tree growth can be severe and is often variable due to genetic differences in sensitivity. Here is an example of three southern Wisconsin trembling aspen (*Populus tremuloides*) genotypes varying in ozone (O_3)-sensitivity in this trial in southern New York where ambient O_3 levels were quite high. The two tree plots represent 10-year-old sensitive (left), intermediate (middle), and tolerant (right) genotypes that grew at similar rates under low O_3 exposures.

documented a 10-fold increase in the mortality rate of O_3-sensitive eastern white pine trees (as compared to tolerant genotypes) in southern Wisconsin where ambient O_3 was moderately high. Thus, the evidence for stage 1 of natural selection occurring in natural forests is indisputable.

For forest trees, the final two stages of natural selection induced by air pollution are less well documented, with the exception of those populations surrounding severe point-source pollution where changes in genetic structure of polluted forest stands have been shown via studies of isozyme or molecular markers. For more subtle region-wide pollutants such as O_3, the evidence is less compelling that genetic change has occurred. Indirect evidence of these later stages of selection taking place has been presented by our laboratory in studies of 15 trembling aspen populations from across the USA. In a series of three studies, published in the late 1980s, we showed a strong negative correlation between the amount of visible foliar symptoms induced by O_3 and the maximum daily O_3 averages at the localities where the populations were collected (**Figure 7**). Additional studies are needed to verify

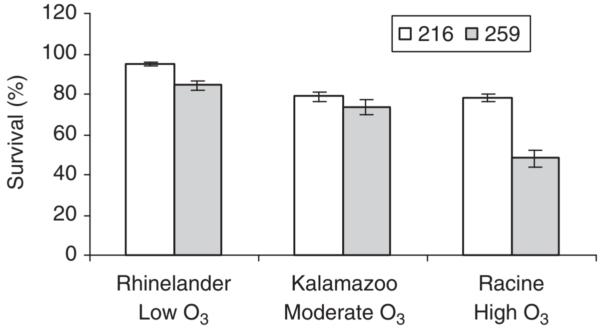

Figure 6 The rapid nature of ambient ozone (O_3) impacts on tree survival in highly competitive (tolerant and sensitive genotypes were intermixed at 0.5×0.5 m spacing) environments across a documented O_3 gradient. Survival at age 5 of two trembling aspen (*Populus tremuloides*) clones differing in O_3-tolerance (clone 216 = O_3-tolerant and clone 259 = O_3-sensitive). The three locations where this experiment was run included Rhinelander, WI = low O_3; Kalamazoo, MI = moderate O_3; and Kenosha, WI = high O_3.

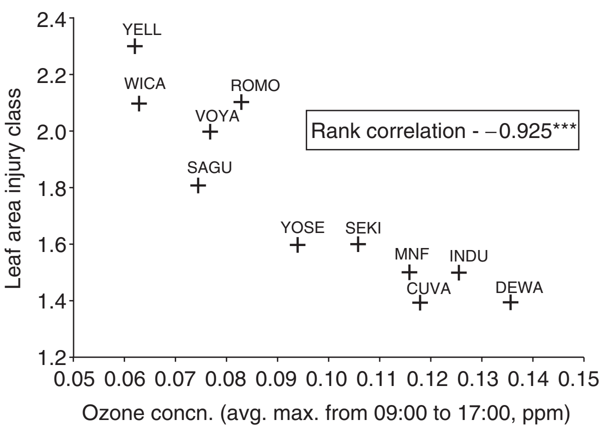

Figure 7 Scatter diagram illustrating the association between one measure of ambient ozone concentrations and one measure of foliar injury after an acute exposure to ozone for several populations of quaking aspen (*Populus tremuloides*). CUVA, Cuyahoga Valley National Recreation Area; DEWA, Delaware Water Gap National Recreation Area; INDU, Indiana Dunes National Lakeshore; MNF, Monongahela National Forest; ROMO, Rocky Mountains National Park; SAGU, Saguaro National Monument; SEKI, Sequoia National Park; VOYA, Voyageurs National Park; WICA, Wind Cave National Park; YELL, Yellowstone National Park; YOSE, Yosemite National Park. Reproduced with permission from Berrang PC, Karnosky DF, and Bennett JP (1991) Natural selection for ozone tolerance in *Populus tremuloides*: An evaluation of nationwide trends. *Canadian Journal of Forest Research* 21: 1091–1097.

that the genetic structure of these populations has changed. An intriguing question remaining is whether or not valuable unique genes or germplasm are being lost with the loss of the sensitive genotypes from the polluted populations.

Genetic Aspects of Climate Change

The rapid nature of the earth's changing climate has raised concerns for the adaptability of forest trees. Long-lived and stationary, forest trees are facing unprecedented changes in the levels of greenhouse gases (especially CO_2; see **Figure 1**) and in the temperature (**Figure 2**). The rapid rates of climatic change anticipated to occur in the near future, coupled with land use changes that impede gene flow, can be expected to disrupt the interplay of adaptation and migration. Implications of these changes for the genetic stability of forest tree populations are not yet fully understood. While ecologists have predicted large changes in the ranges of species over the next 100 years (**Figure 8**), geneticists counter that most tree species have rather large amounts of natural variation and that the changes may be more subtle than those modeled predictions. What is highly likely is that the northward shift of species ranges in the northern hemisphere will proceed with differing rhythms for various species. Species with limited genetic variability to start with, however, such as red pine (*Pinus resinosa*) and red spruce (*Picea rubens*) in North America and silver fir (*Abies alba*) in Europe, will be the first true test of how severe the competitive ability changes may become, how rapidly local populations will be lost, and how great the changes in species ranges may be.

Tree breeding and genetic selection have generally involved either plus tree selection followed by progeny testing or provenance testing followed by progeny testing of superior phenotypes. Then, seed orchards have been established and rogued (further selected on the basis of progeny test information) to provide the seed for the next generation. This process has continued with advanced generation selection and breeding in a few commercially important tree species. In all facets of these programs, selection is done based on the conditions prior to selection and for the most part these selections are not done on the basis of predicted pollution and climate scenarios that will be in place during the rotation of the commercial forest. Screening and selection of genotypes suitable for future pollution and climate scenarios are generally thought to be nearly impossible because of the complexity and cost of such programs. Thus, an alternative strategy in which a wider genetic base is maintained in our breeding population is essential for developing future forests. Maintaining large amounts of genetic diversity will increase the probability that adequate adaptability is maintained to meet rapidly changing environmental conditions.

Alternative strategies are also needed to insure that *in situ* and *ex situ* conservation methods such

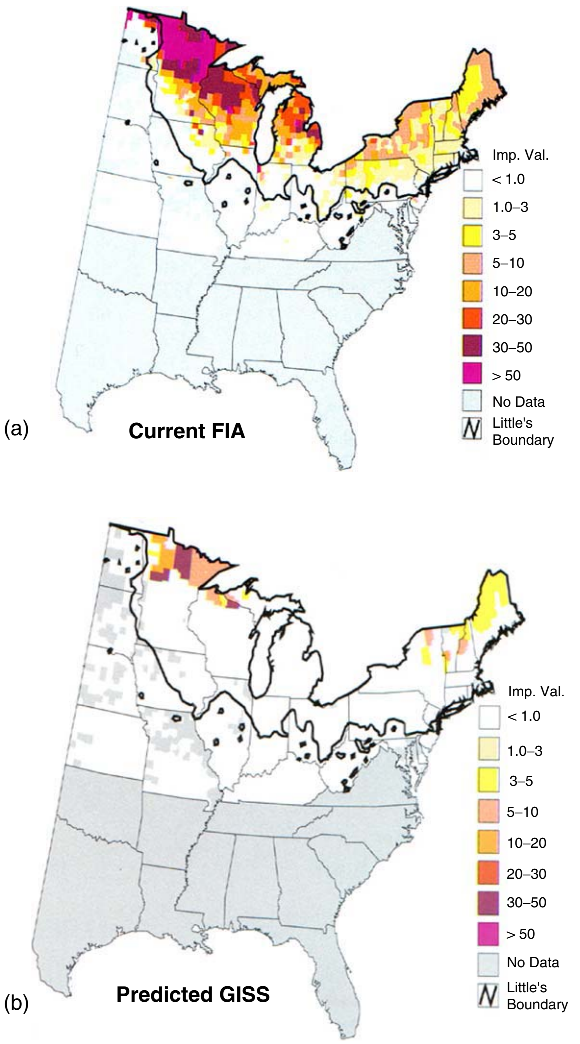

Figure 8 The projected impact of global warming on tree species richness and range is shown in these two figures. (a) Current range and importance values of trembling aspen (*Populus tremuloides*) versus the predicted range and importance value of the same species under a doubled-CO_2 climate (b). Adapted with permission from Iverson LR, Prasad AM, Hale BJ, and Kennedy Sutherland E (1999) *Atlas of Current and Potential Future Distributions of Common Trees of the Eastern United States*. USDA Forest Service Northeastern Research Station General Technical Report NE-265. FIA data are from USDA Forest Service's Forest Inventory and Analysis Program's predicted GISS is the prediction from the Goddard Institute of Space Studies general circulation model scenario.

as gene banks, clone banks, seed zones, or seed collection areas are maintained in several locations such that the changing pollution and/or climate scenarios will not result in the loss of such collections from single vulnerable test sites. Given the past several decades of 'laissez-faire' attitude towards traditional genetic field trials and field conservation efforts, this need to conserve forest genetic resources in multiple amounts may help genetics regain prominence amongst the forestry community.

Knowledge Gaps and Research Needs

Restoration of forests destroyed by severe air pollution (as in the region of the Black Triangle in Europe) remains a great challenge today, and there are currently no methods available to guide these restoration projects to recreate previous genetic diversity and genetic structure. Furthermore, reforestation under today's climate (light, temperatures, phenology, and moisture) and soil conditions (many former severely degraded areas by air pollution still have acidic soils or soils contaminated with heavy metals) may preclude simple replanting with single species. Furthermore, data may not be readily available as to what the stand structure, and genetic diversity were before the areas were affected by air pollution.

The questions of whether or not regional air pollutants such as O_3 have subtly affected the genetic structure of forest tree populations, and if rare alleles have been lost as sensitive genotypes are being lost, needs to be further studied. Recollections of previously sampled populations, as have been done in evolutionary studies of grasses, have been recommended to document selection over time. Also, intensive population sampling with newly developed molecular tools such as microsatellites, single nucleotide polymorphisms (SNPs), amplified fragment length polymorphisms, or restriction fragment length polymorphisms, could determine if population changes have occurred. Studies along sharp pollution gradients would also be useful in this argument.

Exploration of ways to induce more variability in genetically disparate species, such as red pine and white fir, could be beneficial in creating new opportunities for these species to adapt to future climate change. These could include studies of interspecific hybridization, intraspecific provenance hybridization, or insertion of stress tolerance genes isolated from other species via genetic engineering.

Understanding how climate change and related changes in greenhouse gases, such as CO_2 and O_3, will impact intra- and interspecific competition will help us better predict impacts of climate change on forest tree populations. Since these changes are rapid (from a historical viewpoint) but still long-term (from a research project duration viewpoint), long-term studies of population dynamics and competition will need to be done under realistic future climate scenarios. The extensive sets of provenance trials established in the twentieth century around the world should also prove useful in addressing questions related to adaptation to warming temperatures.

It is well known that trees grow at different rates and that they are also variable in how they allocate carbon to various above- and below-ground components. This information could be used to make genetic selections for trees to optimize carbon sequestration. Selections of trees with rapid growth rates, strong allocation to root systems, and inherent resistance to decay could be done for various environmental conditions in the major tree-growing regions of the world. Likely, such selections will include use of exotic species, hybrids of local and exotic species, or genetically engineered trees with altered carbon allocation patterns.

In contrast to long-term evolutionary trends for which local populations are well adapted, the rapid change in stresses of anthropogenic origin suggests that genetic management of forests will be essential. Methods to link tree breeding for utility benefits with gene conservation to facilitate sustainable forestry should be given a high priority. The importance of maintaining high levels of genetic diversity in breeding populations and in plantations cannot be overstated.

Recent development in understanding mechanisms of stress tolerance suggest a commonality of oxidative stress from diverse factors such as air pollutants, herbicides, temperature extremes, toxic salts, and drought. This finding may lead to an increased understanding of the antioxidant tolerance mechanisms and, eventually, to the possibility of selecting for stress tolerance. This could be particularly valuable for developing forest trees for unpredictable future stresses.

Air pollution, climate change, forest trees, natural selection, biodiversity, adaptation, trembling aspen, red pine, silver fir, tree breeding.

See also: **Biodiversity**: Plant Diversity in Forests. **Environment**: Environmental Impacts; Impacts of Air Pollution on Forest Ecosystems; Impacts of Elevated CO_2 and Climate Change. **Health and Protection**: Diagnosis, Monitoring and Evaluation. **Site-Specific Silviculture**: Silviculture in Polluted Areas. **Tree Physiology**: Forests, Tree Physiology and Climate.

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Molecular Biology of Forest Trees

R Meilan, Oregon State University, Corvallis, OR, USA

Introduction

Transformation and Regeneration

In order to genetically engineer a plant, one must be able to insert a gene into the genome of an individual plant cell and then cause that cell to differentiate into a whole plant. The former process is referred to as transformation; the latter, regeneration.

The most common way of transforming cells exploits the ability of *Agrobacterium tumefaciens*, the causative agent of a common plant disease known as 'crown gall.' *Agrobacterium* contains a closed-circular piece of double-stranded DNA called the tumor-inducing (Ti) plasmid. During infection, *Agrobacterium* inserts a segment of the Ti plasmid, called T-DNA (transferred DNA), into the plant's nuclear genome. This T-DNA contains genes encoding enzymes that catalyze the synthesis of plant growth regulators (cytokinin and auxin) which