An *Ex Situ* Gene Conservation Plan for Fraser Fir

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Abstract: Fraser fir (*Abies fraseri* (Pursh) Poir.) is an economically and ecologically important conifer species that faces an uncertain future in its natural stands. The exotic balsam woolly adelgid (*Adelges piceae* Ratz.) has decimated Fraser fir populations during the last 50 years, while changing climate conditions threaten to render Fraser fir's current habitat on Southern Appalachian mountaintops unsuitable. We have developed an *ex situ* gene conservation plan to help facilitate the restoration of Fraser fir into its natural stands if such action becomes necessary, and to ensure the continued existence of a genetic resource base for the economically important Christmas tree industry.

This gene conservation plan integrates existing Christmas tree breeding and gene conservation efforts with additional measures to archive Fraser fir genetic resources and to expand the amount of genetic variation included in *ex situ* conservation. It has four main components: **1)** a seed bank representing both major and minor Fraser fir populations, in addition to seeds generated by breeding efforts; **2)** a set of conservation plantings, **3)** tree breeding elements (provenance and progeny tests, seed orchards, and clone banks); and **4)** an archive of genomic DNA. The tree breeding elements already exist, as does a Fraser fir seed bank, which will be expanded.

In its entirety, the gene conservation plan is designed to act as a multiple population breeding system (MPBS) for Fraser fir, with the conservation plantings and the natural stands together serving as the populations upon which long-term tree breeding efforts are based. The MPBS approach involves establishing multiple breeding populations from diverse sources, with the combination of genes from throughout the range of the species ensuring the introduction of varied germplasm into a tree improvement program. Its goal is to generate the genetic variance needed both to adapt to environmental changes and to emphasize economically important quantitative traits in a breeding program.

Keywords: conservation genetics, population genetics, seed bank, tree breeding, *Abies fraseri* (Pursh) Poir.

Fraser fir (*Abies fraseri* (Pursh) Poir.) is endemic to a handful of high ridges in the Southern Appalachians of North Carolina, Tennessee, and Virginia. This economically and ecologically important conifer species faces an uncertain future in its natural stands: An exotic insect has decimated its populations over the last 50 years, and changing climate conditions could render its current habitat unsuitable. A gene conservation plan for Fraser fir would help facilitate the

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restoration of the species to its natural stands if such action becomes necessary, and will ensure the continued existence of a genetic resource base for Christmas tree breeding.

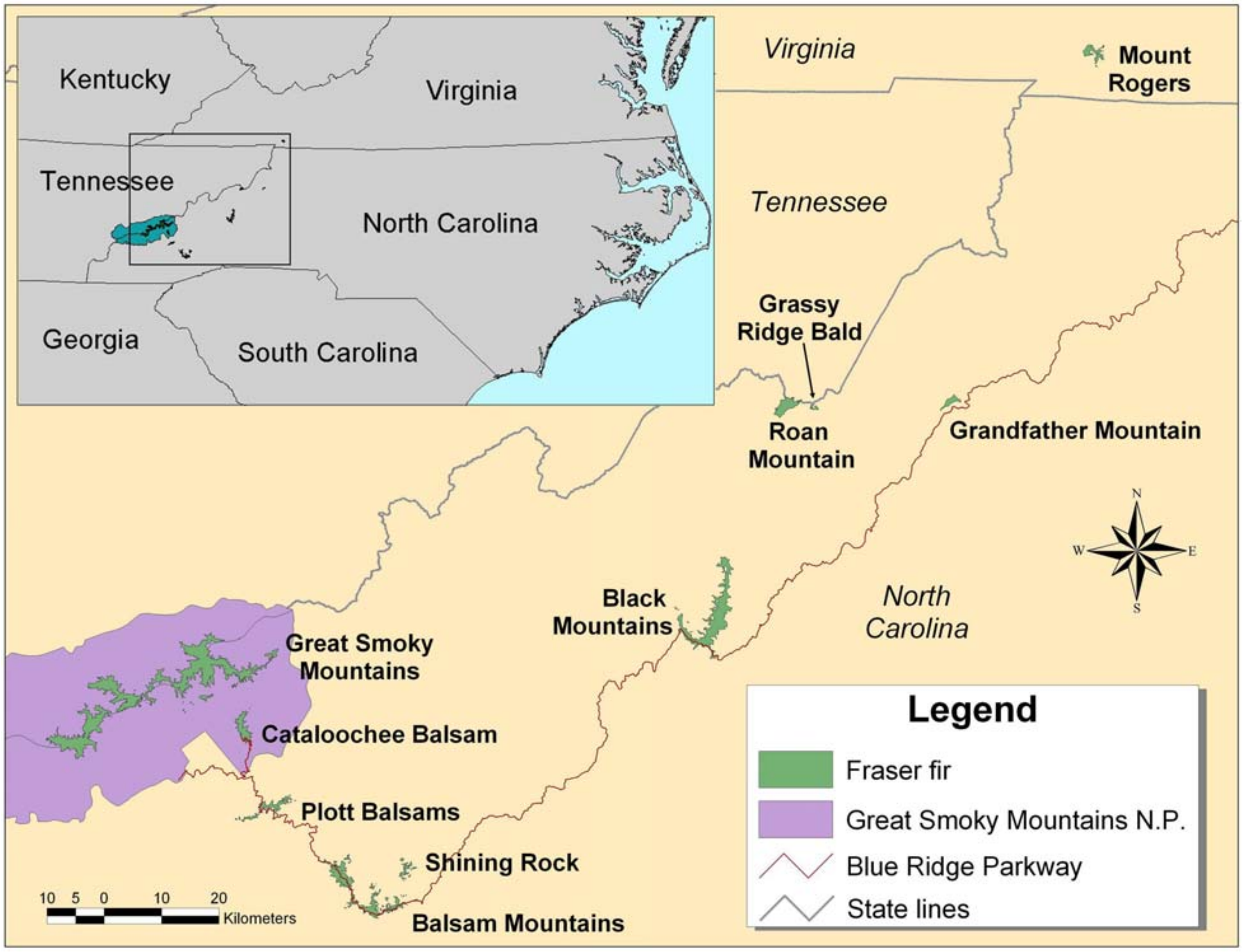

Figure 1. The range of Fraser fir is limited to high peaks in the Southern Appalachians.

Justification for a Fraser fir gene conservation plan

Fraser fir currently exists in six major island-like populations on high ridge systems in the Southern Appalachians: the Great Smoky Mountains in North Carolina and Tennessee; the Black Mountains, the Balsam Mountains, and Grandfather Mountain in North Carolina; Roan Mountain on the Tennessee/North Carolina border; and Mount Rogers in Virginia (Figure 1). Four additional minor populations exist in North Carolina: the Plott Balsams, Cataloochee Balsam, Shining Rock, and Grassy Ridge Bald. The species occurs almost entirely at elevations above 1,300 meters, usually in association with red spruce (*Picea rubens* Sarg.), but Fraser fir becomes the dominant tree species above 1,800 meters (Busing *et al.* 1993; Cain 1935; Whittaker 1956). The populations of Fraser fir are remnants of a boreal forest that extended across much of the Southeast during the peak of the most recent late-Wisconsin glacial period, from 18,000 years to 12,500 years before present (Delcourt and Delcourt 1987; Whitehead 1973; Whitehead 1981).

In addition to its limited distribution, Fraser fir has been severely impacted by human disturbance and the infestation of an exotic insect pest (White 1984). Since the late 19th century, logging and slash fires have dramatically reduced the distribution of Fraser fir and red spruce in the Southern Appalachians (Pyle 1984; Pyle and Schafale 1988; Saunders 1979). More recently, the balsam woolly adelgid (*Adelges piceae* Ratz.), an aphid-like insect from Europe, has inflicted severe mortality on old-growth Fraser fir forest. First detected on Mount Mitchell in 1957, within two decades the adelgid had spread to all Fraser fir populations (Eager 1984). By the 1980s, the amount of mortality among reproductively mature trees ranged from 44 percent on Roan Mountain to 91 percent in the Great Smoky Mountains (Dull *et al.* 1988). Additionally, a recent model (Delcourt and Delcourt 1998) predicts the elimination of Southern Appalachian Fraser fir-red spruce forest with a global mean temperature increase of 3° C caused by greenhouse warming. Fraser fir is listed in North Carolina as an imperiled species, as a species of concern federally, and as a species imperiled and vulnerable to extinction globally (Ameroso and Finnegan 2002). The limited distribution of Fraser fir and the threats to its survival in a natural setting are compelling reasons for the systematic conservation of its gene pool (Nicholas *et al.* 1999).

While more than 90 percent of the extent of Fraser fir stands occur on public lands managed for the continued existence of the species (Dull *et al.* 1988), this is no guarantee that the species will be able to evolve in response to rapid environmental changes. An *ex situ* conservation plan will help ensure much of the genetic material of these populations is preserved, and could allow for the evolution of genes that might allow natural Fraser fir populations to evolve and survive drastic environmental changes.

Goals of Fraser fir *ex situ* gene conservation

Genetic diversity is essential for the long-term survival of species and populations because it provides the raw material for adaptation and evolution, especially when environmental conditions have changed (Eriksson *et al.* 1993; Rajora and Mosseler 2001). A central objective of genetic resource conservation, therefore, is to maintain genetic integrity and natural levels of genetic diversity, and to enhance genetic diversity in populations and species where it has been eroded (Rajora and Mosseler 2001). The final goal of gene conservation, however, should not be the preservation of only *existing* levels and patterns of variation; it should also allow for the evolution of the species or population (Eriksson *et al.* 1993). In other words, gene conservation efforts should aim to increase genetic variation by increasing the probability that new alleles will be saved and that genetic variance in quantitative traits will be increased (Namkoong 1997).

Ex situ conservation strategies are those that conserve plant genetic resources outside the area of natural occurrence and/or in a controlled manner (Yang and Yeh 1992). These methods can include seed, pollen, and tissue banks; clonal orchards; botanical gardens; and arboreta (McIlwrick *et al.* 2000). *Ex situ* approaches may also encompass any collection or planting of material not purposefully established or held to regenerate itself naturally, such as provenance and progeny tests (Yanchuk and Lester 1996). *Ex situ* conservation is particularly important for rare and endangered plant species, even when efforts are underway to preserve existing natural populations, because off-site conservation provides insurance against catastrophic events (Holsinger and Gottlieb 1991). After collection from their natural habitat, plants or their seeds may be propagated elsewhere or stored long-term, with an eventual goal of reintroducing the species into its natural habitat (Brown and Briggs 1991).

In situ conservation of forest gene pools, through networks of protected areas, typically represents a more evolutionary dynamic approach than *ex situ* methods, because the target species or population can maintain its full range of evolutionary and ecological functions and processes, and can track the environmental changes to which it must remain adapted (Rajora and Mosseler 2001). Not surprisingly, therefore, forest geneticists have not suggested that *ex situ* collections replace natural populations. Instead, these methods are often implemented to serve as an insurance policy against catastrophes that might eliminate one or more of the few remaining wild populations of a rare or endangered species (Holsinger and Vitt 1997).

Our *ex situ* gene conservation strategy for Fraser fir has two objectives:

1) Preservation of natural population genetic diversity as a source of genetic material for the restoration or augmentation of Fraser fir populations, should this become necessary.

2) Conservation and recombination of genetic resources for the breeding of an economically important tree species.

There should be no conflict between gene conservation for the maintenance of natural ecological processes and for the utilitarian objectives of plant breeding, because the goals of these two strategies are complementary (Rajora and Mosseler 2001; Yanchuk and Lester 1996). Since Fraser fir is widely grown throughout the Southern Appalachians for the $92 million-per-year fresh-cut Christmas tree market (North Carolina Department of Agriculture and Consumer Services 2003), Christmas tree seed orchards and genetic progeny test sites offer a starting place for *ex situ* gene conservation. At the same time, conservation is useful to tree breeding because it focuses on retaining alleles of low frequency that, while of no current value to breeding programs, may become important in the future (Yanchuk 2001). Because tree breeding requires the preservation of a large genetic base from which to select trees with desirable characteristics (Zobel and Talbert 1984), conserving Fraser fir gene diversity should benefit the Christmas tree industry by providing a genetic source for valuable traits such as growth rate and crown form (McKeand *et al.* 1995).

Characterization of existing Fraser fir genetic resources

To design an effective gene conservation program, it is necessary to first identify the present population structure of the species of interest. This includes assessing the genetic and reproductive status of the targeted populations and species (Rajora and Mosseler 2001), the amount of genetic variation within and among populations, the proportion and distribution of rare alleles, and levels of inbreeding (Li *et al.* 1992). We are using molecular markers, including microsatellites, to achieve these objectives, and are conducting a "gap" analysis to determine which populations are under-sampled in existing Fraser fir tree breeding efforts.

Preliminary results indicate small but significant genetic differences exist among populations, and point toward genetic outlier status for Mount Rogers in Virginia, the most isolated population. These findings are consistent with a study of Fraser fir isozymes, which found that allele frequencies differed slightly but significantly among five of the species' populations, most likely as a result of local, restricted mating and genetic drift (Ross 1988). The isozyme research further determined that Mount Rogers had extreme allele frequencies probably associated with a small effective population size at some point in its history, coupled with isolation and with genetic drift. Additionally, a Christmas tree growth trial using open-pollinated seeds from the same five populations found that trees from low-elevation and more southerly sources grew faster than those from high-elevation and more northerly sources (Jett *et al.* 1993; Li *et al.* 1988). Heritability analysis revealed moderate to strong genetic control of this trait, and indicated that the degree of genetic control varied within different Fraser fir sources (Arnold *et al.* 1994).

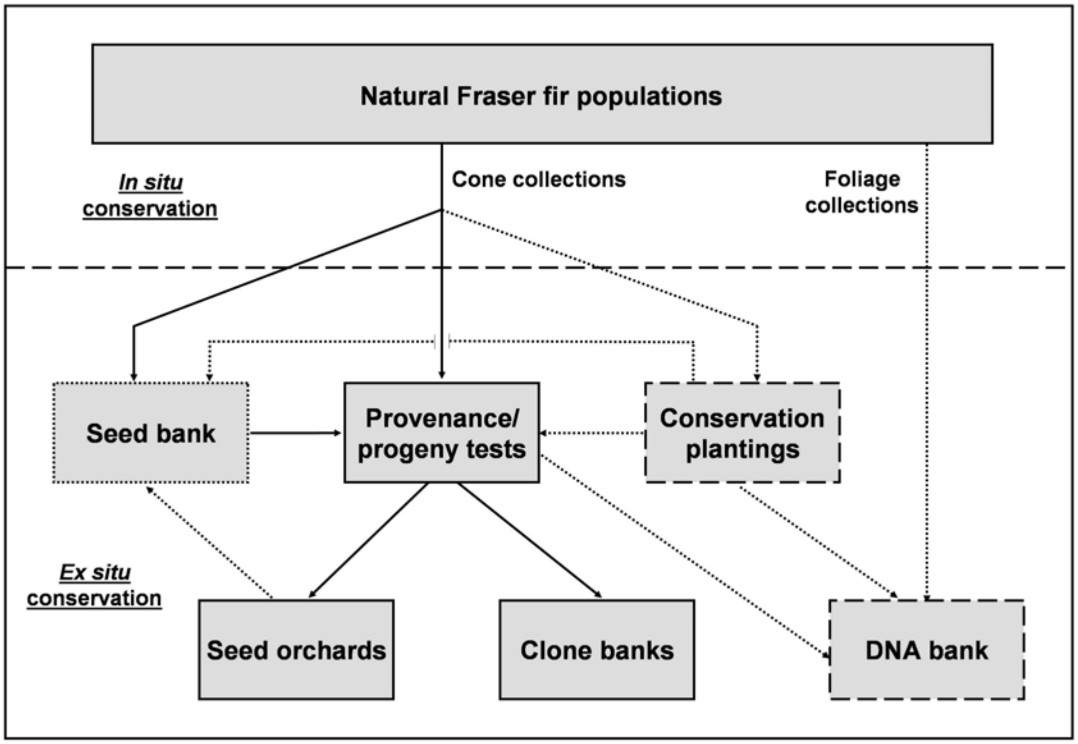

Figure 2: The proposed gene conservation plan for Fraser fir. Boxes with solid borders are existing components, those with dashed lines are new, and the box with dotted lines indicates that the existing seed bank will be expanded. Solid arrows are existing connections and dotted arrows are new.

PROPOSED *EX SITU* GENE CONSERVATION PLAN FOR FRASER FIR

Our proposed *ex situ* gene conservation plan for Fraser fir integrates existing Christmas tree breeding and gene conservation efforts with additional measures to archive Fraser fir genetic resources and to expand the amount of genetic variation included in *ex situ* conservation (Figure 2). The components of this gene conservation plan represent integrated phases of a continuum for plant genetic resource management (Bretting and Duvick 1997). In its entirety, the *ex situ* gene conservation plan is designed to act as a multiple population breeding system (Eriksson *et al.* 1993; Namkoong 1997; Yanchuk 2001). Such a system should generate the sizeable genetic variance needed to adapt to future changes in environmental conditions and in the economic value of quantitative traits (Eriksson *et al.* 1993). A multiple population breeding system

(MPBS) emphasizes interpopulation diversity within an array of populations both in the traits targeted for improvement and in environmental adaptabilities. It involves establishing multiple breeding populations from diverse sources, thereby ensuring the introduction of varied germplasm into breeding. It is a dynamic process in which gene conservation is more than gene preservation; it can be coincidental with or supported by long-term breeding, and can eventually create greater genetic variability than what originally existed (Erikkson *et al.* 1993).

In the gene conservation plan proposed here, a set of conservation plantings could serve – together with natural Fraser fir stands – as the populations upon which the tree breeding efforts are based. Once established, trees in the conservation plantings will be allowed to reproduce, creating new allele combinations that could be of ecological or economic importance. The trees in these populations could serve as a source of seeds for future Christmas tree breeding efforts, while the continued development of interpopulational variation should assure the presence of diversity to meet changing environmental and market conditions.

Sampling

If an *ex situ* approach is to succeed in conserving a plant species' genetic diversity, it must involve a sampling regime that is representative and encompasses the full genetic diversity of the plant's natural populations (Frankham *et al.* 2002; Templeton 1991), especially genetic characteristics involved in a plant's specialization to a habitat (Huenneke 1991). A gene conservation program should attempt to preserve the maximum among-population variation, with the goal of preserving evolutionary potential rather than individual genes (McIlwrick *et al.* 2000). While it would ideally include at least one example of each alternative allele from each locus (Chapman 1984), this is probably neither practical nor achievable.

There is a strong diminishing return associated with increasing the sampling effort in a population: Increasing the sample size by an order of magnitude will net, on average, only a single additional allele (Holsinger and Gottlieb 1991). Brown and Marshall (1995) determined that a sample of 59 unrelated gametes from a population is sufficient to capture at least one copy of 95 percent of the alleles that occur at a frequency greater than 5 percent. This, they note, can be accomplished in a fully outbreeding species by collecting seeds from 30 randomly chosen individuals in the population, although they suggest a precautionary sample of 50.

The overall objective of our sampling efforts, therefore, is to ensure the collection of viable seeds from at least 50 individual trees in each population. This figure is only a baseline, however, because the collection from each population should be stratified by microhabitat to maximize the chances of sampling unique genotypes (Brown and Briggs 1991; Holsinger and Gottlieb 1991). We propose ensuring that samples from each population are stratified by elevation, because growth trials have demonstrated that Fraser firs from different elevations are genetically different for important growth and morphological traits (Jett *et al.* 1993; Li *et al.* 1988).

1) Fraser fir seed bank

Seed banks represent a reasonably simple, efficient, and cost-effective approach to the *ex situ* conservation of plant genes (Given 1994; Maunder *et al.* 2004; McIlwrick *et al.* 2000). They are especially suited for the preservation of conifer seeds, which can easily be placed in long-term storage at temperatures between -20 ºC and 5 ºC (Rajora and Mosseler 2001).

Table 1: Number of seed lots (half-sib families) sampled during the 1994 range-wide Fraser fir cone collection effort, by population, and the number of seed lots with germination rates greater than or equal to 20 percent.

Population	Subpopulation	Total seed lots	Seed lots with ≥ 20% germination
Balsam Mountains		73	7
Black Mountains		124	72
Cataloochee Balsam		12	7
Grandfather Mountain		90	77
Great Smoky Mountains	Andrews Bald	6	2
	Clingmans Dome/ Mount Buckley	51	26
	Mount Collins	11	1
	Mount LeConte	7	5
	Mount Sterling	9	3
	Newfound Gap/ Indian Gap	9	2
	Silers Bald	3	1
Mount Rogers		37	25
Plott Balsams		23	6
Roan Mountain	Roan Mountain	66	59
	Grassy Ridge	0	0
Shining Rock		0	0
Total		**521**	**293**

A Fraser fir range-wide cone collection conducted in 1994 will form the foundation of a seed bank for the species (Table 1). That collection harvested 521 open-pollinated seed lots from the major Fraser fir populations during an exceptional cone production year (McKeand *et al.* 1995). While cones were collected from a range of elevations on 17 mountains across the six major populations, we will undertake additional cone collections to:

- **a)** Resample populations, including the Balsam Mountains and the Plott Balsams, that had many low-germination seed lots;
- **b)** More thoroughly represent populations, such as Cataloochee Balsam and the higher elevations of Mount Rogers, which were not adequately sampled in 1994;
- **c)** Sample minor populations (Shining Rock and the Grassy Ridge Bald outlier near Roan Mountain) and remote subpopulations in the Great Smoky Mountains where collections were not undertaken in 1994;
- **d)** Ensure that the seed bank contains adequate representation of different elevational microhabitats (5,000-5,500 feet, 5,500-6,000 feet, and 6,000-6,500 feet); and
- **e)** Take more samples from populations shown by our molecular marker work to have high allelic richness and/or a disproportionate number of "private" alleles.

Given (1994) recommends including at least 50 seeds from each of 50 to 100 seed lots per population in the seed bank, representing a total of 2,500 to 5,000 seeds per population. We should be able to far exceed this number because an average mature Fraser fir may produce 22,000-30,000 viable seeds in a year (unpublished N.C. Division of Forest Resources data). We will divide each seed lot, making part available for research usage and periodic viability assessment, while setting aside a portion for long-term storage, possibly at the USDA Agricultural Research Service's National Center for Genetic Resources Preservation. Since each represents a single open-pollinated full-sib family, seed lots are highly useful for Christmas tree provenance and progeny tests. Conversely, seeds produced in seed orchards and conservation banks could be placed in the seed bank for future tree improvement efforts or for other Fraser fir population genetics studies (Figure 2).

One minor drawback in the use of seed banks is that differential seed lot survival in storage, as well as different rates of germination and seedling development among seed lots, could result in artificial selection and the loss of conserved genetic diversity (McIlwrick *et al.* 2000). This could be addressed by periodic collections of new seed, perhaps every 10-15 years.

2) Components of Fraser fir breeding efforts

The components of tree-breeding research efforts, including provenance and progeny tests, seed orchards, and clone banks, can house a considerable portion of a species' *ex situ* genetic resource (Lipow *et al.* 2002). Provenance and progeny tests are also important because they expose diverse genotypes to uniform environments, allowing greater resolution of economically important genetic variation due to minimized local environmental variation (Yanchuk and Lester 1996).

Fraser fir seeds from the 1994 range-wide collection were used to establish the 2000 NC State provenance/progeny test series, which is assessing genetic differences for growth and Christmas tree quality traits among all six major populations (Frampton 1998; McKeand *et al.* 1995). While most of the trees in this trial will be harvested, cuttings have been taken from select trees for incorporation into a clone bank and Christmas tree seed orchard. These, and earlier established seed orchards and clone banks, represent an invaluable archive of Fraser fir genetic diversity. For example, the N.C. Division of Forest Resources Rattlesnake Knob Clone Bank contains elite and genetic conservation selections from the 1983 Fraser fir geographic variation study. The clone bank encompasses selections from 90 open-pollinated families (10 families from each of nine seed sources with differing elevation classes), from the Balsam Mountains, the Great Smoky Mountains, Mount Mitchell in the Black Mountains population, Roan Mountain, and Mount Rogers.

Components of a tree breeding program could also contribute genetic material to the Fraser fir seed bank and genomic DNA bank (Figure 2).

3) Fraser fir conservation stands

Ex situ conservation stands are populations outside a species' area of natural occurrence that have been propagated from known or unknown stock, or have resulted from the regeneration of propagated trees. They can have multiple purposes, including conservation, research, recreation, aesthetics, education, and wildlife habitat. Breeding, seedling establishment and vegetative propagation may be uncontrolled or partly controlled (McIlwrick *et al.* 2000). In these stands, the genetic composition of the target species is allowed to adapt to the prevailing environmental conditions. Because this is a dynamic process, the genetic resources are generally conserved, and stands subjected to unique selection pressures are expected to develop in different directions genetically (Graudal *et al.* 1995).

We propose establishing a series of Fraser fir conservation stands using plants cultivated from the Fraser fir seed bank. The exact number of conservation stands will rely in part on the logistics of locating appropriate sites, and in part on the measures of population divergence that result from our molecular marker analyses. Each conservation stand could include roughly five trees from 10 to 20 open-pollinated half-sib families (seed lots) from each natural Fraser fir population. The total number of trees in each stand, therefore, might range from 800 to 1,000. Stands this large should contain enough trees to avoid the inbreeding and genetic drift problems to which all *ex situ* populations are vulnerable (Maunder *et al.* 2004). To reduce long-term genetic erosion in a wind-pollinated species like Fraser fir, Graudal *et al.* (1995) recommend including in a conservation stand at least 150, and preferably more than 500, interbreeding individuals from at least 25 randomly chosen half-sib families. They additionally suggest that such stands eventually be expanded to 1,500 or more individuals through regeneration on adjacent areas, since 500 individuals may not be enough to conserve rare alleles (Graudal *et al.* 1995).

Duplicating each conservation stand will impose extra costs in both time and resources. The replication of conservation stands is necessary, however, to minimize the risk of loss due to unforeseen external events, such as wind throw (Eriksson *et al.* 1993; Graudal *et al.* 1995). At the same time, the number of stands should be limited to allow for their proper monitoring and management (Graudal *et al.* 1995).

These Fraser fir conservation stands, together with the natural stands, represent the breeding units advocated in Eriksson *et al.*'s (1993) multiple population breeding system (MPBS). This system emphasizes interpopulation diversity within an array of populations, both in the traits targeted for improvement and in environmental adaptabilities. Under this system, in which multiple populations are kept as separate entities, between-population diversity is expected to increase, and ultimately as many alleles will be saved as will be lost if selection for diversity among populations is effective (Namkoong 1997). Diversity among populations can be enhanced by excluding intermating among populations and by selecting for divergent characteristic. This kind of controlled evolution is no different than breeding for economic objectives in which multiple selection regimes are employed for either economic or ecological reasons (Eriksson *et al.* 1993). At the same time, Holsinger and Vitt (1997) stipulate an important *caveat*: Even with careful management of conservation stands, inadvertent selection may lead to genetic changes that increase adaptation to the "captive" environment and decrease adaptation to the native environment.

The selection of sites for Fraser fir conservation stands will not be a simple matter. These sites will have to be located above roughly 1,000 meters in elevation on sites free of Phytophthora root rot and from the potential for infestation by balsam woolly adelgid, although the stands could be configured to allow spraying with insecticide to present BWA establishment. To avoid contamination from external sources of pollen, they need to be at least 500 meters away from Fraser fir seed orchards and any other mature Fraser firs (Graudal *et al.* 1995).

Monitoring and maintenance of the stands will be critical, especially immediately following their establishment (Given 1994). During the first few years, all the conservation stands will require seasonal inspection, including at least one visit annually by an expert who can identify insect or plant pathology problems. Five or six years after establishment, the sites should be monitored annually. Any problems, such as infestation by balsam woolly adelgid or other pests, should be addressed immediately. When the trees reach maturity, after about 20 years, cones can be collected for addition to the seed bank, for Christmas tree breeding research, and for any necessary restoration of degraded natural stands (Figure 2).

4) Fraser fir genomic DNA bank

Archives of genomic DNA extracted from trees can provide an efficient and space-saving method for the indefinite storage and conservation of genetic material (Rajora and Mosseler 2001). This DNA could become helpful in any eventual efforts to genetically transform Fraser fir to confer resistance to important pests and pathogens, such as balsam woolly adelgid or Phytophthora root rot.

We propose extracting DNA from needles collected from Fraser fir natural stands, conservation plantings, and provenance and progeny tests (Figure 2). The DNA will be stored at -80ºC, with half of each sample available for population genetics studies and for genetic engineering research, and half retained for long-term archiving.

CONCLUSION

Eriksson *et al.* (1993) note that elaborate *ex situ* conservation methods are practical for only a limited number of commercially important species, as well as for ecological keystone species. We believe that Fraser fir qualifies on both counts. Our proposed *ex situ* gene conservation plan for Fraser fir aims to incorporate existing tree breeding resources with the expansion of an existing seed bank and the development of conservation stands to ensure that genetic resources are available for the restoration of extirpated or degraded natural stands, and for efforts to breed better trees for the economically important Christmas tree industry. The plan should not only conserve existing genetic diversity in Fraser fir, but should cultivate the development of the genetic variance needed to adapt to changing environmental stresses and market demands.

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