6

Breeding Douglas-fir

Glenn T. Howe, Keith Jayawickrama, Marilyn Cherry
Department of Forest Science
Oregon State University
321 Richardson Hall
Corvallis, Oregon 97331-5752

G. R. Johnson
U.S.D.A. Forest Service
Pacific Northwest Research Station
3200 SW Jefferson Way
Corvallis, Oregon 97331-4401

Nicholas C. Wheeler
21040 Flumerfelt Rd. S.E.
Centralia, Washington 98531

I. ABBREVIATIONS
II. INTRODUCTION
  A. Rationale
  B. Objectives
III. DISTINCTIVE CHARACTERISTICS OF FOREST TREES
  A. Biological Characteristics
  B. Ecological Characteristics
IV. DOUGLAS-FIR: THE SPECIES
  A. Taxonomy
  B. Range
  C. Silvical Characteristics
V. FACTORS THAT INFLUENCE DOUGLAS-FIR BREEDING
  A. Genetics and Life-History
  B. Abiotic Environment
  C. Insects and Disease
  D. Genecology

Plant Breeding Reviews, Volume 27, Edited by Jules Janick
ISBN 0-471-73213-3

I. ABBREVIATIONS

BCMoF, British Columbia Ministry of Forests
CMP, controlled mass pollination
EST, expressed sequence tag
GCA, general combining ability
H^2, broad-sense heritability
h^2, narrow-sense heritability
IETIC, Inland Empire Tree Improvement Cooperative
IFA, Industrial Forestry Association
IUFRO, International Union of Forest Research Organizations

MAS, marker-aided-selection
N, census population size
N_e, effective population size
NWTIC, Northwest Tree Improvement Cooperative
QTL, quantitative trait locus
RAPD, random amplified polymorphic DNA
RFLP, restriction fragment length polymorphism
SCA, specific combining ability
SMP, supplemental mass pollination
SNP, single nucleotide polymorphism
SSR, simple sequence repeat

II. INTRODUCTION

A. Rationale

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] is one of the most important timber species in western North America (Fig. 6.1). In 2002, over 27.8 million m^3 (8 billion board feet) of Douglas-fir lumber was produced in the U.S., and in 1999, it comprised a third of all U.S. log exports (Howard 2001; Anon. 2003). Douglas-fir is planted as an exotic timber species in Europe, New Zealand, Australia, and Chile, and as a Christmas tree throughout the northern U.S. It is the most important North American tree species introduced into Europe, and European forests of Douglas-fir cover 600,000 ha, 350,000 of which are in France (Hermann and Lavender 1999). New Zealand has the largest area of Douglas-fir in the southern hemisphere, where it ranks second in importance to radiata pine (*Pinus radiata*).

Douglas-fir is ideal for structural lumber and veneer—it is among the strongest and stiffest of all North American softwoods, dimensionally stable, and moderately durable (Forest Products Laboratory 1999). Although Douglas-fir is used for producing paper and cardboard, it excels as lumber for heavy-duty construction and veneer for plywood and engineered products (e.g., laminated veneer lumber). The fine-grained appearance of large, slow-growing trees made Douglas-fir a prized softwood for interior use, although these trees are rarely harvested anymore. As Douglas-fir crops shifted from large, old-growth trees from natural stands to smaller trees from planted forests, the use of Douglas-fir in engineered products increased. Douglas-fir trees are easy to establish, fast growing, and mostly free from major insect and disease pests, making them an ideal forest crop.

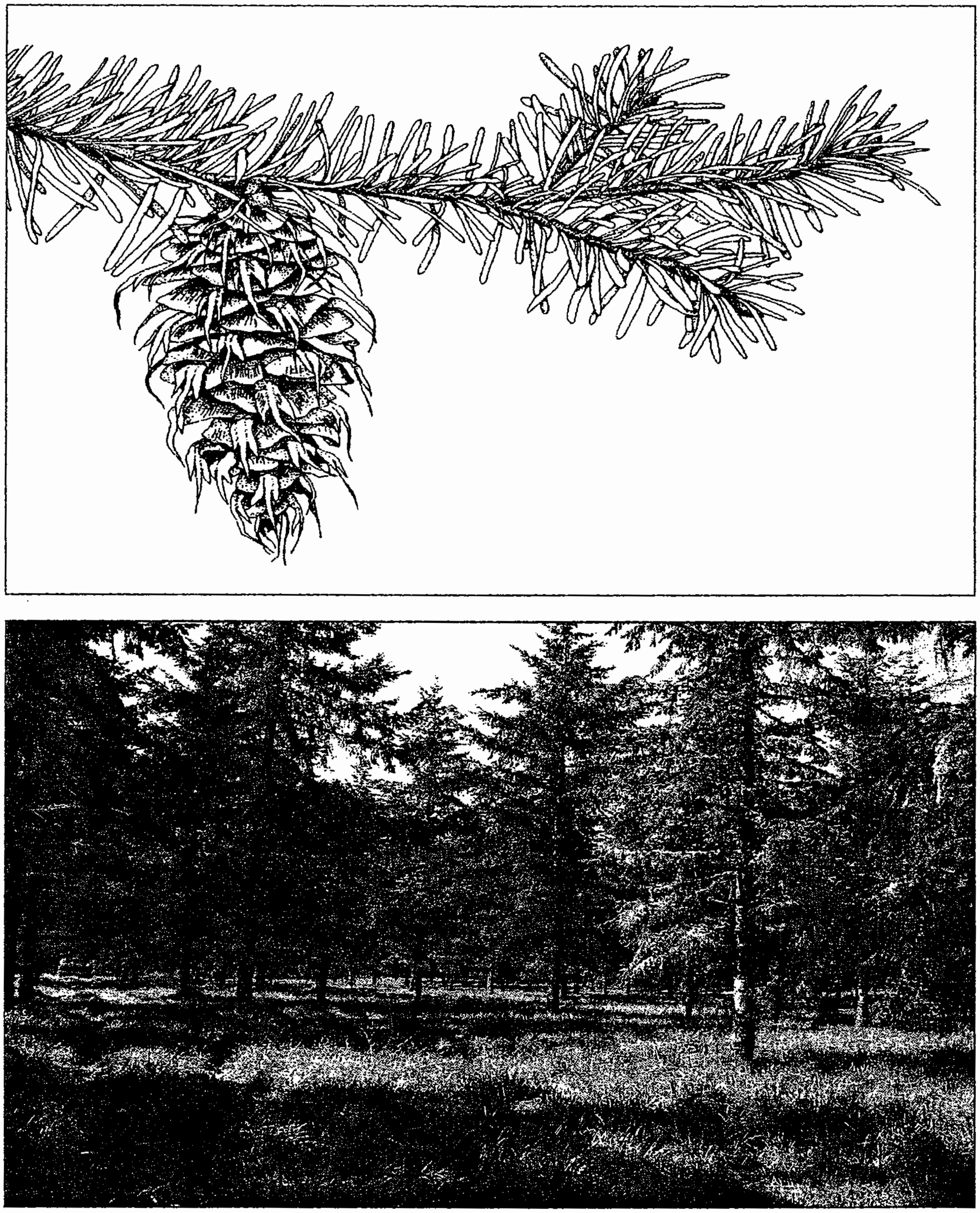

Figure 6.1. Upper panel: Douglas-fir branch with seed cone (drawing by Gretchen Bracher). Lower panel: Mature seed orchard of Douglas-fir (photo by Terrance Ye).

One of the distinctive characteristics of Douglas-fir in western North America (compared to most agronomic crops) is that it is extensively planted among natural stands of the same species. Douglas-fir is an ecologically important, dominant tree species, particularly in the Pacific Northwest (Oregon, Washington, and British Columbia). In the U.S., it grows on nearly 17 million ha, 3 million of which are in plantations (Smith et al. 2001). Another 4.5 million ha of Douglas-fir are found in Canada (Hermann and Lavender 1999). Over much of its range, Douglas-fir is considered a keystone species (Lipow et al. 2003).

Douglas-fir exhibits high levels of genetic variation for all economic and adaptive traits studied, providing a rich foundation for genetic improvement. Applied tree breeding efforts in Douglas-fir are among the most extensive in the world, with more than 4 million progeny from nearly 34,000 selections growing on almost 1,000 test sites in western North America (Lipow et al. 2003). Douglas-fir tree improvement is also being practiced in New Zealand, Australia, Chile, and Europe. Despite extensive breeding efforts and wide-scale planting of genetically improved Douglas-fir, the public has little knowledge about tree breeding concepts, practices, and implications. Nonetheless, the public often voices strong concerns about the management of Douglas-fir forests.

B. Objectives

In this paper, we describe the (1) key elements that distinguish tree breeding from other types of crop breeding, (2) differences between Douglas-fir breeding and other types of tree breeding, (3) environmental, biological, and sociological factors that make Douglas-fir breeding unique, (4) current state of Douglas-fir breeding, and (5) future of Douglas-fir breeding and research needs. Although Douglas-fir improvement is practiced throughout the world, we will focus on Douglas-fir breeding in western North America (i.e., California, Oregon, Washington, British Columbia, Idaho, and Montana), and the Pacific Northwest in particular—where most of the Douglas-fir breeding is occurring.

III. DISTINCTIVE CHARACTERISTICS OF FOREST TREES

A. Biological Characteristics

The distinctive biological and ecological characteristics of trees present both challenges and opportunities for tree breeders. The biggest challenges are that trees are large and slow to reach reproductive and economic maturity. Douglas-fir trees are infrequently harvested before they are 40 years old, and rotation lengths greater than 70 years are possible. Therefore, genetic selections must be made well before the final crop can be evaluated. Although final selections can be made when the trees are about 10 years old (Johnson et al. 1997), this still makes breeding progress slow. Although the time it takes to reach reproductive maturity is often cited as a constraint, natural stands of coastal Douglas-fir begin producing seed when they are about 7 to 10 years old (Stein and Owston 2002) and it is possible to induce flowering on slightly younger trees. Therefore, the breeding cycle is constrained mostly by the final selection age, rather than by the age to reproductive maturity. In contrast, the long life spans of trees can be an advantage because desirable genotypes can be maintained essentially indefinitely using grafting or other methods of vegetative propagation. Although artificial methods of vegetative propagation are possible, natural reproduction occurs exclusively by seed.

Because trees are large and must be grown for a long time, genetic field tests are both large and expensive, and this is exacerbated by the large number of families or clones that are often tested. A Douglas-fir progeny test might include 150 to 300 families planted in single-tree plots at 3 to 12 sites of 2.5 to 5 hectares each. Because trees occupy so much space, seedlots are infrequently tested in large block-plot experiments. Thus, the yield of single families or orchard seedlots in large production plantations is usually extrapolated from experiments in which these seedlots were actually grown in single-tree-plots or small row-plots. On the other hand, because individual trees can produce thousands of offspring, it is possible to precisely estimate their genetic worth using progeny tests.

Most forest trees have a mixed mating system that is predominantly outcrossing, and inbreeding depression is usually severe (Sorensen 1999). This is one reason why few naturally occurring mutants have been characterized in forest trees. Inbreeding depression and the long generation interval make it difficult or impossible to develop inbred lines, use recurrent backcrossing, or recover recessive mutants in otherwise healthy trees. Therefore, mutational approaches to gene discovery, which have been so valuable in plants such as *Arabidopsis* and tomato, are not practical.

In many agronomic crops, grain yields have been dramatically increased by breeding plants that allocate more of their photosynthate (and perhaps nitrogen) to the reproductive parts of the plant (Sinclair 1998). In contrast, genetic improvement in forest trees mostly comes from increasing vegetative growth, improving resistance to biotic or abiotic stresses, and enhancing wood and stem quality. Despite the challenges, genetic improvement of Douglas-fir is biologically and economically attractive. Although a generation may take 15 years, gains in stem volume of 20 to 30% may be possible in a single generation. Furthermore, Douglas-fir is in the earliest stages of domestication, and tree breeders can draw on a vast resource of untapped genetic variation for future genetic improvement.

B. Ecological Characteristics

The ecological characteristics of forest trees also impact breeding programs. Many forest tree crops are planted within their native ranges on sites that are only modestly improved at best. A typical program of "intensive" site improvement might include cultivation and weed control at planting, followed by occasional applications of nitrogen fertilizer and thinning during the rotation. Therefore, medium-term (i.e., rotation length) adaptation to the natural environment is critical. Furthermore, because many forest crops are planted on lands that are expected to remain forested indefinitely, it is important to maintain long-term (i.e., microevolutionary-scale) adaptation to both current and future environments. Therefore, maintenance of genetic diversity and adaptability are important goals in most tree breeding programs.

IV. DOUGLAS-FIR: THE SPECIES

A. Taxonomy

The taxonomy of *Pseudotsuga* is unclear. In North America, two species are well recognized, Douglas-fir and bigcone Douglas-fir [*P. macrocarpa* (Vasey) Mayr] (Hermann 1982). Both species are native to western North America, but their ranges do not overlap. Bigcone Douglas-fir is found only in southwestern California—34 km from the southernmost population of coastal Douglas-fir (Griffin 1964). Not surprisingly, no natural hybrids between these species have been found, although an artificial hybrid was reported by Ching (1959). *Pseudotsuga* populations in Mexico are either included with *P. menziesii* var. *glauca*, or considered additional species (Li and Adams 1989; Gernandt and Liston 1999). A variable number of other species have been described in China, Taiwan, and Japan (Gernandt and Liston 1999). Some DNA-based analyses suggest that Douglas-fir and bigcone Douglas-fir are more closely related to each other than they are to the Asian species (Strauss et al. 1990; Gernandt and Liston 1999), and attempts to hybridize Douglas-fir with the Asian species have been unsuccessful (Silen 1978). Within the Pinaceae, *Pseudotsuga* is considered to have a sister relationship with the genus *Larix* (Gernandt and Liston 1999).

B. Range

Douglas-fir has one of the widest natural ranges of any tree species. It ranges from the Pacific coast to the eastern slopes of the Rockies and from Canada to Mexico (almost 4,500 km), occurring from sea level to over 3,000 m (Hermann and Lavender 1990; Fig. 6.2). Within this area, Douglas-fir grows on over 20 million ha (Hermann and Lavender 1999; Smith et al. 2001).

Two botanical varieties of Douglas-fir are recognized—the coastal variety (var. *menziesii*) and the interior, or Rocky Mountain variety (var. *glauca*)—which are physiologically, morphologically, and chemically distinct (Silen 1978; Hermann and Lavender 1990; Fig. 6.2). Although phenotypic differences are pronounced in the south, the varieties intergrade in areas of contact from the northern half of Oregon northward into central British Columbia (von Rudloff 1972; Sorensen 1979). In contrast, RAPD markers amplified from mitochondrial DNA showed a rather abrupt genetic discontinuity in this same area (Aagaard et al. 1995).

The northern and southern populations of Rocky Mountain Douglas-fir are well separated geographically and almost genetically distinct (Wright et al. 1971; Li and Adams 1989). Based on rangewide patterns of variation in morphology, physiology, isozymes, and terpene chemistry, these northern and southern subgroups may deserve varietal status (Li and Adams 1989).

C. Silvical Characteristics

Coastal Douglas-fir dominates the landscape over much of its range. On the best sites, coastal Douglas-firs can become huge—up to 120 m tall and 5 m in diameter, and may live up to 1,400 years (Silen 1978; Farrar 1995; Hermann and Lavender 1990). The coastal variety is usually an early seral component of forests. That is, large continuous stands tend to regenerate after disturbances such as fire, grow rapidly in full sun, then eventually give way to other species that regenerate and grow better beneath the canopies of mature Douglas-fir. This process, however, often takes hundreds of years.

Interior Douglas-fir reaches maximum heights of only about 49 m on the best sites, and rarely grows older than about 400 years (Hermann and Lavender 1990). On moist sites, it may function as an early seral species, but on warmer, drier sites, it may be a component of the climax forest (Hermann and Lavender 1990). In many parts of the Rocky Mountains, it occurs in mixed stands with other species, rather than in extensive pure stands, as is more common in the coastal region. Compared to coastal Douglas-fir, the Rocky Mountain variety tends to be slower grow-

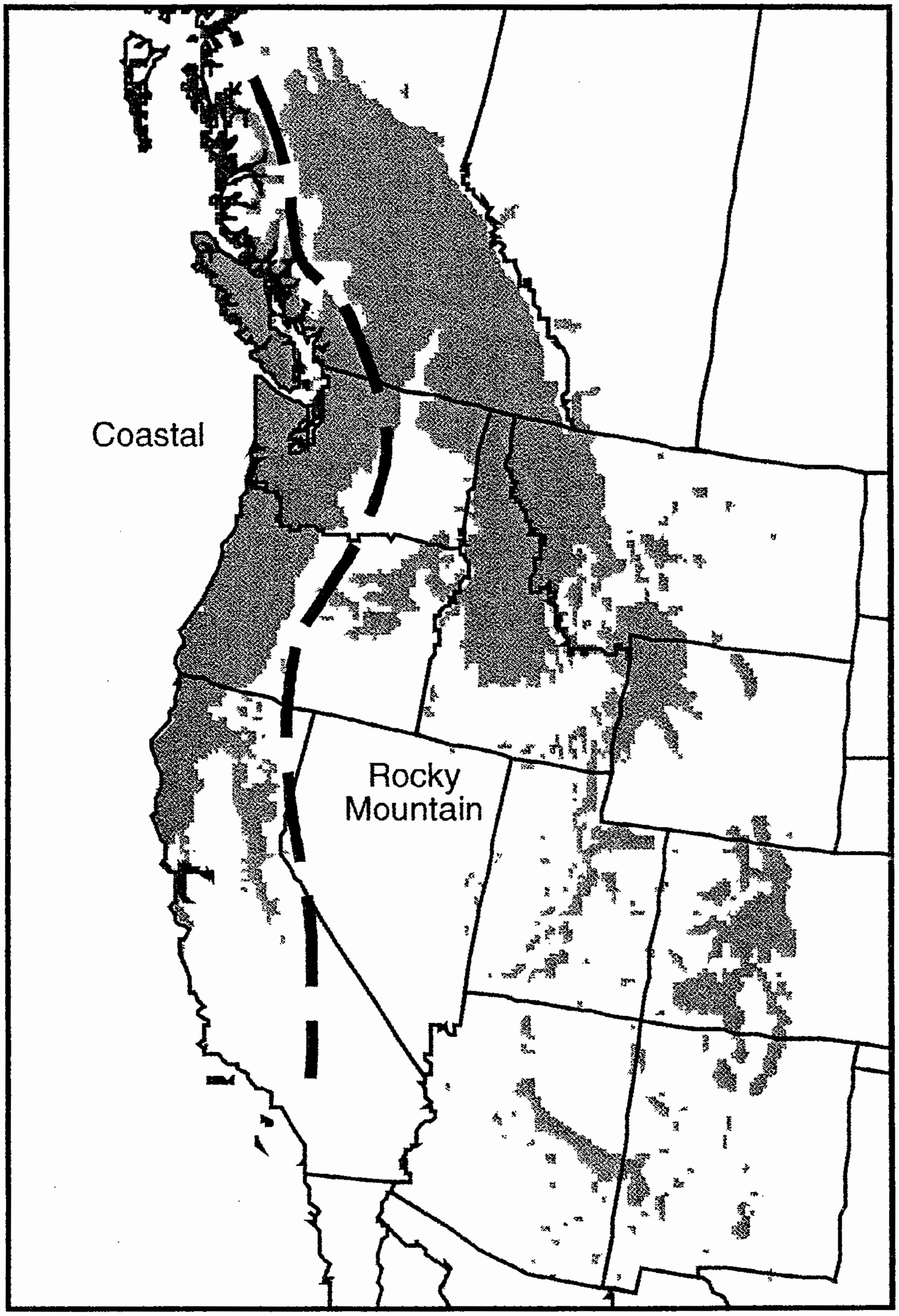

Figure 6.2. Natural range of Douglas-fir (*Pseudotsuga menziesii*) in the U.S. and Canada. The dashed line represents the approximate location of the transition zone between the coastal (var. *menziesii*) and Rocky Mountain (var. *glauca*) varieties. Isolated populations of putative Mexican populations of Douglas-fir are not shown. The range map was downloaded from the USGS web site entitled "Digital Representations of Tree Species Range Maps from *Atlas of United States Trees* by Elbert L. Little, Jr. (and other publications)" (http://climchange.cr.usgs.gov/data/atlas/little/).

ing, more cold hardy, more shade tolerant, and less tolerant of *Rhabdocline* (pathogen, *Rhabdocline pseudotsugae*; Stephan 1973) and Swiss needle cast (pathogen, *Phaeocryptopus gaeumannii*; Ferre 1955; Rohmeder 1956) diseases. It also has shorter needles, bluer foliage, and cone characteristics that distinguish it from the coastal variety.

V. FACTORS THAT INFLUENCE DOUGLAS-FIR BREEDING

A. Genetics and Life-History

Douglas-fir is unique among the Pinaceae in having a diploid chromosome number of 26. The remaining species in the Pinaceae, including other species of *Pseudotsuga*, have a diploid chromosome number of 24 (Doerksen and Ching 1972). As in most other conifers, its nuclear genome is large and complex (3.7×10^{10} bp), chloroplast DNA is inherited paternally, and mitochondrial DNA is inherited maternally (Neale et al. 1986; Marshall and Neale 1992; O'Brien et al. 1996).

Flowering and seed production begin at age 7 to 10 in coastal Douglas-fir, and a little later in the Rocky Mountain variety (Stein and Owston 2002). In mature stands, good seed crops are produced every 2 to 11 years (Stein and Owston 2002). Douglas-fir does not naturally reproduce by vegetative propagation. Pollination occurs in the spring, and mature seeds are shed in late summer or early fall of the same year. Douglas-fir is monoecious and has a mixed mating system (selfing and outcrossing), but it is mostly an outcrosser. Selfing at the mature seed stage is usually less than 10% because of high mortality of the selfed embryos (Sorensen 1999). Inbreeding depression is manifested throughout its life cycle, and selfed progeny rarely become part of the mature, reproductive population (Sorensen 1999). In seedlings and saplings, inbreeding depression in growth traits seems to be linearly related to the inbreeding coefficient (*F*). Under nursery conditions, for example, inbreeding depression in height increased 5 to 6% for each 0.1 increase in *F* from 0.0 to 0.5 (Woods and Stoehr 1993; Sorensen 1997; Woods et al. 2002). Campbell (1979) estimated that a single tree in an old-growth stand is probably selected from 2,000 or more germinated seedlings. Seeds are easily dispersed several hundred feet or more (Isaac 1930; Bever 1954), and because Douglas-fir is wind-pollinated, pollen dispersal is extensive (Adams 1992). This contributes to large effective population sizes, low population differentiation for neutral genetic markers, and low linkage disequilibrium (Neale and Savolainen 2004).

The nutritive tissue (megagametophyte) of Douglas-fir seed is haploid, and genetically identical to the egg cell (i.e., maternal contribution to the embryo). This facilitates genetic analyses using protein (allozyme) or DNA-based molecular markers. The maternal haplotype can be easily measured, and the paternal contribution can be deduced by comparing the genotype of the embryo with the haplotype of the seed megagametophyte. This makes it easy to use open-pollinated or controlled-cross seed to study segregation, construct linkage maps, determine paternity, measure gene flow, and use association genetics to link genes to phenotypes (Adams et al. 1997; Krutovskii et al. 1998; Neale and Savolainen 2004).

Douglas-fir is a long-lived tree that undergoes pronounced developmental changes during its life cycle. Compared to juvenile trees, mature trees are competent to flower, have shorter growing seasons (i.e., earlier bud set), slower rates of shoot elongation, less annual height growth, greater plagiotropism, and lower capacities to be propagated via rooted cuttings or tissue cultures (Ritchie and Keeley 1994; Stein and Owston 2002).

B. Abiotic Environment

The natural range of Douglas-fir is large, mountainous, and environmentally diverse, resulting in correspondingly diverse selection pressures and genecological differentiation. The climate of the Douglas-fir region changes dramatically from west to east because of the Pacific Ocean and a series of mostly north-south mountain ranges. Moving from west to east, warm, moist air from the Pacific Ocean rises, cools, and releases moisture as it encounters each of these mountain ranges. These storms mostly arrive during the late fall, winter, and spring. This results in wet conditions along the coast, pronounced rain shadows on the eastern sides of the mountain ranges, and a gradient in precipitation and humidity from west to east. The western part of the region is warmed by the Pacific and protected from the influx of cold, continental air from the interior by the north-south mountain ranges.

As one moves from the maritime climate near the coast to the continental climate in the Rocky Mountains, the sites are generally drier and colder, with shorter frost-free periods (Hermann and Lavender 1990). North-south trends are also observed throughout the region, but they are much less dramatic. The southern areas tend to be drier and warmer, with longer frost-free periods and longer photoperiods in the fall and winter (i.e., when short days promote growth cessation, cold acclimation, and dormancy induction). Throughout the region, additional environmental heterogeneity is imposed by large differences in elevation. The higher elevation sites tend to be colder and wetter, with larger diurnal fluctuations in temperature (Hermann and Lavender 1990).

1. Coastal Environment. Populations along the coast experience a truly maritime climate, characterized by mild wet winters, cool dry summers, and long growing seasons. The climate becomes increasingly continental toward the east, particularly at higher elevations. The major environmental gradients within the region are associated with distance from the ocean (i.e., degree of continentality), latitude, and elevation. The Klamath Mountains in southern Oregon and northern California are particularly hot and dry; whereas more northern areas on Vancouver Island, British Columbia, and the Olympic Peninsula of Washington include temperate rainforests with up to 444 cm (175 inches) of rain a year. Throughout the coastal region, much of the precipitation occurs as winter rain, although snow is prevalent at the higher elevations, particularly in the Cascades and Sierra Nevada (Hermann and Lavender 1990). Despite the high annual rainfall, the summers are remarkably dry, leading to the frequent description of the climate as simply "winter-wet, summer-dry." Droughts in southern Oregon and northern California can last from May until September (Hermann and Lavender 1990).

2. Rocky Mountain Environment. The environment of the Rocky Mountain region can be broken down into northern, central, and southern subregions. The northern subregion has a mild continental climate (except for a dry period in midsummer), the central subregion has a continental climate, and the southern subregion has some of the driest areas and shortest frost-free periods (Hermann and Lavender 1999). Compared to coastal Douglas-fir, the Rocky Mountain variety has a more mosaic distribution with numerous disjunct populations, particularly in the south.

3. Environmental Challenges. The huge diversity of environments that Douglas-fir inhabits presents numerous physiological and developmental challenges. In forest trees, geographic patterns of genetic variation are molded by three major environmental factors—temperature, water availability, and photoperiod—and Douglas-fir is no exception (Morgenstern 1996; Aitken and Hannerz 2001; Howe et al. 2003). Temperature and water availability play two important roles. First, they are environmental constraints that limit survival and growth. Second, they are environmental signals that trees use to adjust their physiology and development to acclimate to unfavorable conditions. Unlike temperature and water, photoperiod acts mainly as an environmental signal. For both varieties, low temperatures are the major limiting factor within the northern part of the range, whereas lack of moisture is the predominant limiting factor in the south (Hermann and Lavender 1990).

Cold temperatures cause damage and death from spring and fall frosts, heavy snow loads, ice storms, and winter exposure (Duffield 1956; Silen 1978). Trees are particularly susceptible to cold after they deacclimate and begin growing in the spring, and before they become fully cold hardy in the fall (Howe et al. 2003). In western Washington, for example, a major cold spell in November 1955 killed or damaged millions of Douglas-fir trees, leaving trees with forked tops and crooked stems that can still be seen today (Duffield 1956). Despite the importance of fall frosts, damage from spring frosts is predicted to be two to three times greater, at least for coastal Douglas-fir (Timmis et al. 1994). Because bud flush occurs progressively later in the spring as trees age (Irgens-Moller 1967b), the potential for spring frost damage should also decrease as trees become older. Although spring and fall frosts are an important cause of damage and death, low temperatures in the middle of winter are rarely a problem because the trees are generally fully dormant and cold hardy at this time. Because spring and fall frosts limit the duration of seasonal growth, the length of the frost-free growing season is a particularly important environmental constraint.

Summer droughts, which are pronounced in many portions of the range, also damage and kill trees (Silen 1978). Because trees are more susceptible to droughts when they are actively elongating, trees from drier climates tend to set bud earlier in the summer, have shorter growing seasons, and less annual height growth. Trees that have stopped elongating and set a terminal bud in response to dry summer conditions occasionally begin growing again (second flush) if water becomes available later in the growing season. High temperatures are also a problem because they contribute to evapotranspiration and drought stress.

Temperature, moisture, and photoperiod also act as important environmental signals that help trees acclimate to adverse conditions (Howe et al. 1999, 2003). Short days and low night temperatures promote bud set, cold acclimation, and winter dormancy in the fall. Temperatures just below freezing induce the second stage of cold acclimation, temperatures just above freezing promote dormancy release by fulfilling the chilling requirement, and warm temperatures promote cold deacclimation and bud flush in the spring. Moisture stress may also act as an environmental signal because it promotes growth cessation and bud set, which enhance drought hardiness (Griffin and Ching 1977).

C. Insects and Disease

Although insects and disease cause economic losses, no insect or disease pests are serious enough to warrant the development of large resistance breeding programs. Swiss needle cast (*Phaeocryptopus gaeumannii*) is a problem in some areas along the Oregon Coast. The root and heart rots caused by *Armillaria ostoyae* and *Phellinus weirii* can also cause problems, particularly in young stands. Insects attack Douglas-fir forests, but major problems occur only during infrequent outbreaks. The Douglas-fir tussock moth (*Orgyia pseudotsugata*) and the western spruce budworm (*Choristoneura fumiferana*), for example, can cause serious defoliation in some areas and years (Hermann and Lavender 1990). Cone and seed insects can destroy seed orchard crops, but they can be controlled with insecticides.

D. Genecology

Genecology is the study of genetic differences in relation to the environment. As discussed above, the key environmental factors to consider are temperature, water, and photoperiod. Because gradients in these environmental factors are associated with location variables (e.g., elevation, latitude, and longitude), genetic differences have been mostly described in relation to these easily measured variables. Nonetheless, location variables are imperfect surrogates for the underlying climatic variables that drive patterns of genetic variation. Mathematical models are now available to predict the climate at any location from geographic and topographic information. Thus, there is an increasing trend toward describing fine-scale patterns of genetic variation in relation to the predicted climate, rather than to location variables alone (Balduman et al. 1999).

The genecology of Douglas-fir has been studied at many scales—using wide ranging provenance tests (Wright et al. 1971) to studies of variation within a single watershed (Campbell 1979). A hierarchical approach is often used to describe genetic variation in forest trees. Variation in Douglas-fir may be partitioned among varieties, provenances-within-varieties, populations-within-provenances, and individuals-within-populations. We use “seed source” as a synonym for “provenance” to denote the original geographic source of seed, pollen, or propagules. Furthermore, the geographic area described by the term “provenance” (or “seed source”) is assumed to contain multiple populations. In this section, we describe the patterns of genetic variation seen in Douglas-fir, starting at the species level, and then progressing to finer scales. An understanding of these patterns can be used to help design effective breeding programs and evaluate the potential effects of climate change.

1. Adaptive Traits. Studies of genecology focus on “adaptive traits”—traits believed to be under strong natural selection because they confer adaptation to the environment and enhance individual fitness. In most cases, indirect evidence is used to infer that a trait is under strong natural selection, including (1) physiological data that link the trait to enhanced survival and growth and (2) patterns of genetic variation that suggest the trait has been influenced by natural selection, including levels of population differentiation that exceed those observed for neutral genetic markers, and consistent associations between genotypes and location or environmental variables (Howe et al. 2003). Studies of adaptive traits typically include survival, height growth, fall and spring frost hardiness, drought hardiness, vegetative bud phenology (i.e., the timing of fall bud set or spring bud flush), and the frequency of second flushing.

In natural stands, early height growth is important because Douglas-fir grows poorly in heavy shade (e.g., compared to western hemlock [*Tsuga heterophylla*], or western redcedar [*Thuja plicata*]), often regenerates in dense stands following fire, and is browsed by deer and elk (Hermann and Lavender 1990). At the sapling stage, rapid height growth is needed to become a dominant part of the forest canopy, thereby facilitating survival, as well as early and prolific seed production.

The importance of frost and drought hardiness is obvious, given some of the harsh environments in which Douglas-fir grows. Vegetative bud phenology is important because it is related to annual height growth and the ability to withstand frosts and droughts. Trees that begin elongating too early in the spring are likely to be damaged by late spring frosts, whereas trees that grow too late into the fall are likely to be damaged by summer droughts, early fall frosts, and winter cold (Campbell and Sorensen 1973; Griffin and Ching 1977; White 1987; Howe et al. 2003). A delay in bud set of a single week can increase fall frost damage by 18 to 25% (Campbell and Sorensen 1973; Rehfeldt 1983a). Because early bud set also limits annual shoot elongation, there is an important tradeoff between height growth and fall frost hardiness (Howe et al. 2003).

The timing of bud flush is affected by chilling and flushing requirements (Howe et al. 1999; 2003). High chilling requirements help prevent mid-winter bud flush and cold injury in climates with winter temperatures that fluctuate above and below freezing. In contrast, early bud flush may be advantageous in cold environments that have very short growing seasons (i.e., to maximize seasonal growth), whereas late bud flush may be advantageous in mild climates that have particularly variable spring temperatures and high risks of late spring frosts (Campbell and Sugano 1979).

Second flushing occurs when a tree stops elongating and sets a bud, then flushes a second time in the same growing season. Young trees may second flush if temperature and moisture conditions are favorable in late summer. Second flushing promotes annual height growth, but also predisposes trees to damage from fall frosts (Rehfeldt 1979a; Anekonda et al. 1998).

In summary, a large component of climatic adaptation involves the synchronization of a tree's annual growth cycle to the prevailing frost-free and drought-free growing seasons. Poor synchronization may lead to poor height growth, inadequate lignification, needle and twig damage, mortality caused by freezing of buds, and increased susceptibility to disease (Campbell 1986). Therefore, the lengths of the frost-free and drought-free growing seasons influence much of the geographically based genetic variation we see in forest trees (Morgenstern 1996). Douglas-fir exhibits high levels of genetic variation for each of these adaptive traits—within and among varieties, provenances, and populations (Silen 1978; Sorensen 1979; Campbell 1986; Rehfeldt 1989). Population differences in other adaptive traits, such as resistances to diseases, insects, and mammals are also present (e.g., McDonald 1979; Hoff 1987), but not well characterized.

2. Long-Term Field Tests vs. Short-Term Seedling Tests. Patterns of genetic variation in adaptive traits have been studied using long-term field tests and short-term experiments in outdoor nurseries, greenhouses, or growth chambers (hereafter referred to as seedling tests). The long-term field tests consist of everything from wide-ranging provenance tests to progeny tests of parent trees from limited geographic areas (i.e., single breeding zones). The seedling tests often include imposed environmental treatments (such as cold or drought) and detailed physiological analyses.

Long-term field tests and seedling tests have a few key differences that must be considered when their results are interpreted (White and Ching 1985). First, the long-term field tests provide *direct* information, whereas the seedling tests provide *indirect* information for designing seed transfer guidelines and breeding zones (Adams and Campbell 1981). Second, short-term seedling experiments focus on the juvenile stage of tree development, which is substantially different from that of older, more mature trees (Ritchie and Keeley 1994). Compared to mature trees, Douglas-fir seedlings set bud much later in the growing season, are more prone to second flush, and are less cold hardy (Rehfeldt 1983b). Therefore, patterns and amounts of genetic variation change as trees mature (White and Ching 1985). Third, seedlings in short-term tests are frequently subjected to cold and drought treatments to specifically measure their resistances to these environmental stresses. In contrast, trees in long-term field tests may never experience severe frosts or droughts, or may be exposed for only a small fraction of their life span (e.g., single growing season). Fourth, seedling tests are often more precise (i.e., have higher heritabilities) than long-term field tests, often include more genotypes, and are generally used to measure many more traits (e.g., Campbell 1986). Although many of the same traits could be measured in the field, it would be enormously more difficult and expensive, although the results may be more relevant. Compared to the large number of mostly adaptive traits measured in seedling experiments, survival and growth (e.g., height, diameter, or volume) are often the only traits reported from long-term field tests (e.g., Silen and Mandel 1983). Therefore, it is usually easier to uncover subtle genetic differences using seedling experiments. Finally, non-genetic differences in seed weight, environmental preconditioning (Johnsen 1988), or other maternal effects that are influenced by the environment in which the seed was produced play a larger role in seedling tests when they are present. These factors may lead to inflated estimates of the associations between parental genotypes and their environment. Overall, seedling experiments typically involve a detailed assessment of adaptive traits in the first one to three years, whereas long-term field tests measure overall tree growth over a much longer period of time. In the following sections, we describe climatic, geographic, and topographic patterns of genetic variation in Douglas-fir based on results from long-term field tests and seedling experiments.

3. Varietal Differences. Compared to the coastal variety, the Rocky Mountain variety of Douglas-fir experiences a colder, drier climate with shorter growing seasons. These environmental differences, however, are not abrupt, and west to east clines in genetic variation have developed along corresponding temperature and moisture gradients. Based on long-term provenance tests, there is a corresponding decrease in height growth from west to east. Pacific coast populations grow fastest, populations from interior British Columbia and the northern and southern Rocky Mountains are intermediate, whereas populations from the intermountain region and east slope of the Rocky Mountains grow the slowest (Silen 1978). Compared to coastal populations, interior populations set bud earlier and grow slower in a wide range of environments (Irgens-Moller 1967a; Wright et al. 1971; Rehfeldt 1977; Silen 1978; Sorensen 1979). At the seedling stage, inland populations are also better able to tolerate fall frosts, winter cold, and droughts (Ferrell and Woodward 1966; Pharis and Ferrell 1966; Wright et al. 1971; Rehfeldt 1977). Furthermore, the poor survival of coastal sources planted in the Rocky Mountains, Michigan, and Eastern Europe has been attributed to poor winter hardiness (Wright et al. 1971; Silen 1978). Because interior seedlings are more sensitive to daylength (Irgens-Moller 1962), some of these differences may be tied to differences in short-day-induced bud set and cold acclimation. Compared to interior provenances, coastal provenances are more tolerant of both *Rhabdocline* and Swiss needle cast diseases (Ferre 1955; Rohmeder 1956; Stephan 1973).

4. Within-Variety Patterns of Genetic Variation. Detailed genecological studies have mostly focused on specific regions, breeding zones, or watersheds of either coastal Douglas-fir or Rocky Mountain Douglas-fir. In the largest study, wind-pollinated families from about 1,300 parents in Oregon and Washington are being studied in seedling nursery tests (J. B. St.Clair, pers. comm.). Overall, genecological studies confirm the overriding importance of temperature and moisture regimes in shaping genetic variation throughout the species range. Compared to species such as western white pine (*Pinus monticola*) and western redcedar, Douglas-fir is particularly responsive to these selective forces, and is considered an adaptive specialist. Mean genetic differences in adaptive traits can be detected among populations that are separated by as little as 100 to 200 m in elevation (Rehfeldt 1979a,b). Our discussion of adaptive genetic variation will focus on these two selective forces separately.

5. Genecological Models and Maps. Many of the relationships described below were uncovered using genecological modeling—a set of multivariate statistical approaches commonly used to study the relationships between adaptive traits and either climatic or location variables (Campbell 1986). The genotypic data for genecological modeling comes from common garden studies of wind-pollinated seedlots collected from wild parents. The variation among populations is usually partitioned using principal components analysis (PCA). PCA is a statistical method for finding linear combinations of adaptive traits that can be grouped together and described by a single composite variable, or principal component. PCA is useful for reducing a large set of interrelated traits into a smaller number of principal components (often 2–3) that explain most of the variation. The first principal component (PC1), for example, may largely reflect variation in height growth, second flushing, and the timing of bud set, whereas the second (PC2) may largely reflect variation in the timing of bud flush and spring frost damage. Population means for the composite traits (i.e., factor scores) are calculated, and multiple regression is used to determine which location variables are significantly associated with the population means. The resulting genecological models can then be used to: (1) quantify the degree of genetic change that is associated with various environmental, geographic, or topographic gradients and (2) produce genecological maps, which are visual representations of the relationships between adaptive trait variation (i.e., factor scores) and location or climatic variables (e.g., O'Neill and Aitkin 2004). These genecological maps (which are analogous to topographic maps) can be constructed using geographic information systems (GIS).

6. Temperature-Related Patterns of Variation. Colder environments are associated with a number of highly correlated climatic variables, such as shorter frost-free growing seasons, colder temperatures during the winter months, and colder average annual temperatures. Populations from colder locations typically set bud earlier in the fall, have fewer growth flushes, less annual height growth, and are more tolerant of fall frosts and winter cold (Sweet 1965; Griffin 1974; Griffin and Ching 1977; Aitken et al. 1996; Balduman et al. 1999; J. B. St.Clair, pers. comm.). Therefore, the suite of traits described above is characteristic of populations from higher elevations, more northern latitudes, and (at least for coastal Douglas-fir) greater distances from the Pacific Ocean (Sweet 1965; Kung and Wright 1972; Griffin and Ching 1977; Larsen 1981; Sorensen 1983; Balduman et al. 1999).

Patterns of variation have also been described for the timing of bud flush and spring frost hardiness, but these patterns are neither as strong nor consistent as for the traits described above (Sweet 1965; Sorensen 1967; Griffin and Ching 1977; Howe et al. 2003). In general, it seems that trees from colder locations, higher elevations, and greater distances from the ocean flush slightly earlier and are, therefore, somewhat more susceptible to cold damage in the spring (Campbell and Sugano 1979; Balduman et al. 1999). Contradictory or inconclusive results from other studies probably result from insufficient sampling (Munger and Morris 1936; Sweet 1965).

7. Moisture-Related Patterns of Variation. Patterns of genetic variation are also associated with the availability of water, which is mainly a function of rainfall and temperature, but also humidity, solar radiation, and wind. Southern populations of both varieties are exposed to hot, dry climates with early summer droughts (Silen 1978). In Douglas-fir seedlings, there is a corresponding trend toward increasing drought hardiness from north to south—particularly from the northern, mesic areas along the coast to the hot, dry region of southwestern Oregon and California (coastal variety), and from the intermountain region to the southern Rockies (Rocky Mountain variety) (Pharis and Ferrell 1966; Heiner and Lavender 1972; Kung and Wright 1972; Larsen 1981). In coastal Douglas-fir, populations from southern Oregon flush earlier, set bud earlier, and grow slower than more northern populations (Lavender et al. 1968; Lavender and Overton 1972; Heiner and Lavender 1972). Genetic clines are also associated with the pronounced west to east decrease in precipitation and humidity—eastern populations set bud earlier, have less annual height growth, and are more drought hardy (Pharis and Ferrell 1966; Sorensen 1979).

Much of our detailed knowledge about genetic variation in drought hardiness traits comes from studies that focused on genetic variation in southwestern Oregon and northern California. Because of the harsh environments and difficulty with plantation establishment, southwestern Oregon received extra attention through the Forest Intensive Research (FIR) program (e.g., Campbell 1986, 1991). In a genecological study of southwestern Oregon populations, trees from higher elevations, farther south, and farther east were shorter, set bud earlier, had less winter damage, and were more drought hardy at the seedling stage (Campbell 1986; White 1987). Furthermore, clines in drought hardiness followed gradients in summer precipitation (White 1987). In other studies of trees from southwestern Oregon, inland populations from drier environments had slower rates of shoot elongation, earlier bud set, and higher root:shoot ratios compared to coastal populations (Campbell 1986; Joly et al. 1989). Interestingly, however, genetic variation in drought hardiness was more closely related to temperature regimes than to rainfall patterns in this region (White 1987). Because early bud set is strongly correlated with drought hardiness, this relationship may result from an adaptation to the colder temperatures and short growing seasons at high elevations, rather than to drought per se (Sorensen 1983; White 1987). High-elevation populations are also better able to survive winter drought (Larsen 1981).

8. Disease- and Insect-Related Patterns of Variation. Resistances to a few fungal pathogens and insect pests also show geographic patterns of genetic variation. Douglas-fir populations from northern California are more resistant to the Cooley spruce gall adelgid (*Gilletteella cooleyi*) than are populations from Oregon, Washington, and British Columbia (Stephan 1987). As discussed above, the coastal variety of Douglas-fir is more tolerant of *Rhabdocline* and Swiss needle cast diseases than is the Rocky Mountain variety (Ferre 1955; Rohmeder 1956; Stephan 1973). Furthermore, within the coastal variety, tolerance to Swiss needle cast increases near the coast, at lower elevations, and as one moves northward from California (reviewed in Johnson 2002).

9. Summary and Implications. A large body of information demonstrates that patterns of genetic variation in adaptive traits are associated with temperature and moisture regimes. In general, the best growth potential occurs in populations from the best growing environments (i.e., mild climates with plenty of moisture) (Silen and Mandel 1983; Monserud and Rehfeldt 1990; Hernandez et al. 1993). Although climate models are now being used to study adaptive traits in relation to predicted climatic variables, these climatic variables are only slightly better than location variables for explaining patterns of genetic variation in Douglas-fir (Balduman et al. 1999; J. B. St.Clair, pers. comm.). The numerous reports of associations between adaptive traits and location variables support the conclusion that temperature and moisture are the major selective forces influencing patterns of adaptive variation. Differences in elevation are particularly important because genetic differences have been reported within small geographic areas (Balduman et al. 1999; Rehfeldt 1989). Within both varieties, west to east clines are substantial (Kung and Wright 1972; Rehfeldt 1978; Griffin and Ching 1977; Campbell and Sorensen 1978; Sorensen 1983; J. B. St.Clair, pers. comm.). Differences in latitude are subtler, and genetic differentiation is less pronounced. The latitudinal clines that do exist seem to be stronger in the Rocky Mountains than in the coastal region, presumably because of the moderating effects of the Pacific Ocean (Hernandez et al. 1993). Moisture regimes seem to be particularly important drivers of genetic differentiation in the southern parts of the species range where the climates are hot and dry, and the growing seasons are limited by summer drought. Each of these patterns of genetic variation must be carefully considered when breeding and deployment strategies are designed. Although genecological modeling consistently detects statistically significant relationships between population means and either climatic or location variables, the proportion of population variation that remains unexplained is often large. In the long-term studies of coastal Douglas-fir that were reported by Balduman et al. (1999), the amount of unexplained variation in cold hardiness and growth phenology ranged from 65 to 83%. In seedling studies of Rocky Mountain Douglas-fir, however, the amount of unexplained variation was much lower—only about 21% (Rehfeldt 1989). Although better results might be achieved if climatic variability and the frequency of extreme climatic events were taken into account (Balduman et al. 1999), the nature of this unexplained variation remains an important area of research.

What are the implications of these patterns of genetic variation? First, large elevational transfers of seed (which are possible within relatively small geographic areas) can have adverse effects. Based on field tests of 6- to 7-year-old trees, for example, Balduman et al. (1999) predicted that (on average) seed transferred to a site 600 m higher in elevation would experience a 15% increase in fall cold damage. Rehfeldt (1989) studied seedlings from northern Idaho and western Montana and detected genetic differences between populations separated by only 240 m in elevation—which corresponded to a difference of 20 frost-free days. Based on data from two coastal breeding zones, Silen and Mandel (1983) predicted that a transfer of seed 305 m (1,000 feet) upward would result in a 5 % increase in height at ages 10 to 12. A similar (but weak) trend has been seen in other breeding zones as well (K. Jayawickrama, unpubl. data). Balduman et al. (1999) also predicted that damage from spring frosts would increase if genotypes were moved toward the coast, whereas damage from fall frosts would increase if genotypes were moved away from the coast. North-south transfers within a given elevation are likely to be less risky, especially along the coast.

These patterns, however, reflect differences among population means, yet large amounts of genetic variation also exist within populations. In fact, within-population variation for adaptive traits often equals or exceeds the variation found among populations (Wheeler et al. 1990; Rehfeldt 1979a; Howe et al. 2003). In contrast, White (1987) reported that differences in drought tolerance among populations were much larger than differences among families. Although local patterns of adaptive variation can be used for designing seed transfer guidelines and breeding zones (Campbell 1986; Silen and Mandel 1983), they are not useful for making selections within breeding populations. For example, Balduman et al. (1999) used long-term field tests to study the relationship between parent tree environment and cold hardiness traits within two breeding zones, and concluded that these associations were too weak to be an effective tool for tree improvement.

E. Long-Term Provenance Tests

In addition to providing an understanding of genecology, provenance tests can shed light on two important questions: what is the best provenance to plant in a particular region, and what is the extent of provenance by environment interaction. In this section, we describe what is known about the long-term performance of seed sources in those regions that have large Douglas-fir breeding programs—i.e., western North America, Europe, and New Zealand. In contrast to the results from seedling genecological studies, which have been used to justify relatively small breeding zones in the Pacific Northwest (Silen 1978), results from long-term provenance tests have generally been viewed as evidence that larger breeding zones are appropriate (Woods 1993; Stonecypher et al. 1996).

1. Pacific Northwest. Two provenance tests have been established in western Washington and western Oregon. The first test was established in 1912 by the U.S. Forest Service. Wind-pollinated progeny from 120 parents from 13 locations were planted at five test sites in western Oregon and Washington (Munger and Morris 1936). Unfortunately, this test has produced few robust analyses and conclusions because of problems with its initial design (Adams and Campbell 1981). Nonetheless, early measurements (e.g., 17 to 18 years old from seed) indicated that provenance by plantation interactions were small and that seed transfer among mild sites in the region would be successful (Munger and Morris 1936; Ching and Hinz 1978). For example, several sources grew well at all five test plantations that spanned an elevational range of 1,000 m and a latitudinal range of about 3° (Munger and Morris 1936; reviewed in Stonecypher et al. 1996). In contrast, interactions seemed to be present when the performance of high and low elevation sources were compared at high and low elevation sites, a result that is not surprising given that elevation is consistently identified as an important factor in genecological studies. Based on later measurements, Silen suggested that these elevational interactions increased over time, and that increased mortality and reduced volume growth of some of the better seed sources had resulted in changes in rank that became apparent around age 30, and were large by age 50 (Silen 1965, 1966a, 1978). The significance of these reports, however, has been questioned. Stonecypher et al. (1996), for example, pointed out that Silen did not support these conclusions with "complete and detailed analyses."

The only widely distributed, long-term provenance test in the Pacific Northwest was planted in 1959 in Oregon, Washington, British Columbia, and northern California (Ching 1965). Unfortunately, only 16 seed sources were tested, the experiment has low statistical precision, and results from many of the 17 plantations were never published (White and Ching 1985). Because results from plantations damaged by animals, fire, frost, and drought were not reported (Ching and Hinz 1978), provenance by environment interactions may be underestimated. Nonetheless, measurements over 25 years indicate that growth rates vary significantly among seed sources, and interactions between source and planting site are mostly unimportant (White and Ching 1985). Furthermore, Ching and Hinz (1978) found no evidence to suggest that a provenance is best adapted to the location from which it came. Three of four seed sources from Vancouver Island were always among the tallest, whereas two others (i.e., the southernmost source and one high elevation source) were consistently among the shortest (White and Ching 1985). However, this test also provides evidence that seed source rankings may change over time. One provenance that performed poorly on five test sites at age nine was average or superior by age 25 (White and Ching 1985). This test also suggests that plantations and sources from southwestern Oregon should be considered distinct, which is consistent with results from genecological studies (White and Ching 1985). In an archive plantation in Oregon, the local provenance had one of the largest mean diameters, but sources from as far away as Vancouver Island and northwest Washington were not significantly different from the local source (Gamble et al. 1996).

Because of the limitations of the provenance tests in Washington and Oregon, it is difficult to draw definitive conclusions about the extent of genotype by environment interactions. This, and other factors, has resulted in two very different conclusions about the appropriate size of breeding zones in this region (discussed below). Despite their limitations, these tests are widely viewed as providing little evidence for strong provenance by environment interaction, unless seed transfer involves large elevational distances (Adams and Campbell 1981; White and Ching 1985; Stonecypher et al. 1996; Johnson 1997; but see Silen 1978).

In British Columbia, provenance tests tell the same general story. Ching and Hinz (1978) summarized 20-year results from five provenance test plantations in British Columbia, whereas White and Ching (1985) reported on two low-elevation coastal plantations at age 25. At age 25, the five fastest-growing provenances were from British Columbia, Washington, and Oregon, but not from California (White and Ching 1985). Illingworth (1978) analyzed data from four series of provenance tests in coastal British Columbia and concluded that there is no clear relationship between growth and latitude or elevation for sources collected from an extensive region along the coast. In general, the sources from the areas with optimal growing conditions (i.e., warm and moist) grew the best, the performance of local sources was generally inferior, and sources from the north coast and transition zones sources were shorter overall.

Another provenance test of 102 sources was planted in 1971 at a single site in coastal British Columbia and measured in 1986 (Sziklai 1990). In this test, four of the five fastest-growing provenances came from Washington, and the fifth came from British Columbia. Sziklai (1990) also noted that selection at age seven might be risky, but this was based on a consistently declining performance of a single source from the coast of southwestern Oregon. Overall, the results from coastal British Columbia provenance tests were used to conclude that (1) genetic differences are not strong among coastal British Columbia populations, (2) maladaptation does not seem to be a problem unless interior sources are planted along the coast, and (3) only a few breeding zones are needed in this region (Woods 1993).

A provenance test of 64 interior provenances was established on a single site in the southern interior of British Columbia (Jaquish 1990). Provenances from a large area within British Columbia and Washington grew well, and the growth of the northern sources was closely associated with the elevation from which they were collected.

2. Europe. Extensive information is available from the IUFRO (International Union of Forest Research Organizations) provenance tests that were established in 33 countries during the 1960s, 1970s, and 1980s (Breidenstein et al. 1990). Interior provenances perform poorly in Europe, whereas provenances from a large area in Washington and Oregon (excluding southwest Oregon) combine good growth, good form, late flushing, and low susceptibility to fall frosts (Breidenstein et al. 1990; Anon. 1998; EURFORGEN 2003).

One hundred and eight test sites in Europe and British Columbia were classified into four groups based on climatic conditions: (1) northeastern Europe, (2) northwestern Europe and southwestern British Columbia, (3) northwestern British Columbia, and (4) southern Europe. Analyses of height and survival indicated that (1) provenances from Washington grew the fastest in all site groups, (2) additional provenances from northern Oregon grew fast in areas with mild climates (i.e., groups 2 and 4), (3) some specific provenances grow fast and are stable across all groups of sites, and (4) height growth decreases as the elevation of origin increases. In Spain, most of the fastest-growing provenances came from a wide area covering 27 contiguous seed zones, including 4 zones in southwestern British Columbia, 13 zones in western Washington, and 10 zones in northwestern Oregon (Hernandez et al. 1993). In Europe (as in British Columbia), high-elevation provenances and provenances from latitudinal extremes grow slower (Illingworth 1978; Breidenstein et al. 1990; Hernandez et al. 1993).

3. New Zealand. Provenance trials in New Zealand indicate that coastal provenances from southern Oregon and northern California are the most productive (R. L. Knowles, pers. comm.). Based on these results, new seed collections were made in the best provenances in 1993, and new provenance-progeny trials were established in 1996.

4. Summary and Implications. Based on provenance tests from throughout the world, there is a consistent tendency for the best seed sources to come from large areas that are climatically similar to the planting site. Except for plantations on harsh sites, the amount of seed source by environment interaction is often low for most provenances, suggesting that breeding zones can encompass large areas that have sufficient climatic homogeneity. Provenances that are likely to interact with planting site are likely to come from high elevations, latitudinal extremes, and southwestern Oregon (at least for coastal Douglas-fir). Transfers among mild environments can span large geographic distances, presumably because the environments in which a particular seed source does well recur at many locations across the landscape (Rehfeldt 1990). In short, the environmental distance is far more important than the geographic distance for evaluating seed transfer (Adams and Campbell 1981). This is important because most breeding programs concentrate on the lower-elevation, milder sites—and most of the high-elevation areas have been dropped from the second-generation breeding programs (discussed below).

In many tree species, there is a consistent increase in growth when seed sources are transferred from milder to harsher climates (Namkoong 1969; Silen 1978). However, unless long-term field performance has been measured, this approach is risky because evidence of maladaptation may not show up for many years, and perhaps only after exposure to extreme climatic events (Silen 1978; Adams and Campbell 1981). Therefore, all of the major North American Douglas-fir breeding programs used local provenances as their first-generation breeding population, although the geographic extent of the parents varied dramatically. The lack of high-quality, region-wide provenance tests in Oregon and Washington has been a major impediment to answering fundamental questions about seed movement and breeding zones in this region. In retrospect, it is unfortunate that the comprehensive provenance tests that were established in Europe and British Columbia were never planted in Washington and Oregon, the region from which most of the provenances were collected.

F. Quantitative Genetics and Inheritance

An understanding of genetic and environmental variation is important for designing breeding strategies, picking suitable mating designs, designing field tests, and predicting genetic gains. Key pieces of information include relative amounts of additive vs. non-additive genetic variance, genetic and environmental variances, heritabilities, and genetic correlations (Namkoong and Kang 1990). Most information on quantitative genetic parameters is derived from analyses of wind-pollinated families collected from natural populations (as compared to advanced-generation breeding populations). Furthermore, genetic parameters differ depending on whether they are calculated among or within natural populations (e.g., Howe et al. 2003). The results presented below are based on within-population (or within-breeding-zone) analyses because these are most relevant for improving breeding populations.

1. Additive vs. Non-Additive Genetic Variance. Additive genetic variance, which is the variance of breeding values, is the main reason that progeny resemble their parents and the main determinant of a population's response to selection (Falconer and Mackay 1996). Non-additive genetic variance consists of all other types of genetic variation, including dominance, interaction, and disequilibrium variance (Falconer and Mackay 1996). Non-additive genetic variance, which does not contribute to population improvement via recurrent selection, is the variation that causes the progeny of specific crosses to perform differently from what is predicted by the breeding values of their parents. For Douglas-fir growth traits, the ratio of non-additive to additive genetic variation is usually less than half (i.e., 50%). This is best illustrated by two large experiments in which the ratio of SCA (specific combining ability) variance to GCA (general combining ability) variance was estimated from a large number of 6-parent diallels planted across many sites in the Pacific Northwest. In one series of experiments planted in Oregon and Washington (65 6-parent disconnected diallels), the ratio of SCA to GCA variance averaged 46% for height growth at ages 6 or 8 (Stonecypher et al. 1996). In another series of experiments planted in British Columbia (36 half-diallels), the average ratio was 36% for three growth traits (age-7 height = 34%, age-12 height = 32%, and age-12 volume = 43%; Yanchuk 1996). Despite these values, Yanchuk (1996) concluded that the additional gain from using SCA (i.e., by making crosses between specific parents) would only be about 3% for volume and 1% for height (i.e., as compared to producing seed from the best parents based on GCA).

Douglas-fir breeders are mainly interested in the additive genetic variance because most breeding strategies rely on improving populations via recurrent selection, and because most improved materials are produced via wind-pollinated seed orchards, which do not capture the non-additive component. Non-additive genetic variation, however, can be captured if breeding programs deploy clones or full-sib families. Clones capture all of the non-additive variation, whereas full-sib families capture only a quarter or less of the various sources of non-additive variation.

2. Heritabilities and Amounts of Genetic Variation. Estimates of genetic gain are important for judging alternative breeding strategies and for financial analyses of breeding programs. Additive genetic gains are a function of the selection intensity (i), additive genetic variance ($\sigma^2_{\text{additive}}$), and the narrow-sense heritability (h^2), or ratio of $\sigma^2_{\text{additive}}$ to total phenotypic variance ($\sigma^2_{\text{phenotypic}}$) (i.e., gain = $ih\sigma_{\text{additive}}$). One of the most important indicators of potential breeding success is heritability, the proportion of observed variation (i.e., phenotypic variation) that is controlled by genetics. Broad-sense heritability (H^2) is the ratio of total genetic variation (i.e., additive plus non-additive variation) to total phenotypic variation [$H^2 = (\sigma^2_{\text{additive}} + \sigma^2_{\text{non-additive}}) / (\sigma^2_{\text{additive}} + \sigma^2_{\text{non-additive}} + \sigma^2_{\text{environment}})$]. Narrow-sense heritability (simply called heritability, or h^2) is the ratio of additive genetic variation to total phenotypic variation [$h^2 = \sigma^2_{\text{additive}} / (\sigma^2_{\text{additive}} + \sigma^2_{\text{non-additive}} + \sigma^2_{\text{environment}})$]. Trait heritabilities have received a great deal of attention because they integrate information on genetic and environmental variation, and because they can be altered to increase genetic gains, primarily by reducing environmental variability in genetic tests and by increasing family size to increase family heritabilities.

Because heritability is the ratio of genetic to phenotypic variation, the amount of environmental variation has an important influence on breeding progress. In forestry, environmental variation is particularly large for growth traits, except when tests are conducted in carefully controlled nursery or greenhouse environments. Most progeny tests are planted on sites typical of plantation forestry, which are usually more variable than agricultural sites because of less site preparation, less site maintenance, and larger tests that encompass more land. Furthermore, heritabilities can vary considerably among progeny test sites for the same population. Johnson et al. (1997), for example, examined age-15 height in six breeding programs in which 90 to 150 families were planted across 6 to 12 sites in each program. Within each program, the heritabilities ranged enormously among sites (i.e., 0.00–0.33, 0.09–0.29, 0.05–0.39, 0.19–0.41, 0.09–0.41, and 0.07–0.18).

In forest trees, heritabilities are low for most traits, typically averaging less than 0.30 (Table 6.1; Cornelius 1994). Nonetheless, traits vary in their degree of genetic control (h^2) and the relative amount of genetic variation (i.e., additive genetic coefficient of variation = σ_{additive}/mean). Some traits, such as wood density, have high heritabilities but low genetic variation, whereas other traits such as volume growth, stem defects, and some branching traits have low heritabilities but high levels of variation (Cornelius 1994).

Table 6.1. Median and mean values of individual-tree narrow-sense heritability and additive genetic coefficient of variation (AGCV) for seven traits or trait types compiled from 67 published studies (from Cornelius 1994).

	Heritability		AGCV	
Trait or trait type	Median	Mean	Median	Mean
Height	0.25	0.28	8.50	11.10
Diameter	0.19	0.23	8.60	9.10
Volume	0.18	0.21	20.30	23.10
Straightness	0.26	0.28	11.65	16.25
Morphological and structural	0.23	0.23	8.80	14.73
Specific gravity (wood density)	0.48	0.50	5.10	5.34
Branching traits	0.24	0.26	8.40	16.30

Douglas-fir heritabilities follow the same patterns as seen in other forest trees: low to moderate for growth traits, fall cold hardiness, drought hardiness, stem defects, and branch size—and moderate to high for wood density, bud flush, bud set, spring cold hardiness, and branch angle (Table 6.2). Douglas-fir breeding programs emphasize productivity, which is measured as height, diameter, or stem volume (estimated from height and diameter measurements). Because these are the most frequently measured traits, we know more about their heritabilities than we do for other traits. Heritabilities for height, diameter, and stem volume typically range from 0.10 to 0.30. Three studies examined the heritability of Douglas-fir height growth in detail. The heritability was 0.13 in a large series of 6-parent, half-diallels planted across 11 sites in British Columbia (Yeh and Heaman 1987). In a similar experiment of 6-parent diallels in Oregon and Washington, the heritability averaged 0.13 (0.00–0.40) at individual test sites at ages 6 to 8, and 0.16 (0.08–0.28) in other half-sib and full-sib tests at ages 8 to 12 (Stonecypher et al. 1996). Johnson et al. (1997) reported an average heritability of 0.16 (0.00–0.38) for 51 test plantations in six breeding programs. Compared to the first experiment (Yeh and Heaman 1987), the heritabilities from the latter two experiments are biased upward because they are single-site estimates in which the variation due to the genotype by environment interaction is confounded with the family variation (Stonecypher et al. 1996; Johnson et al. 1997).

For growth traits, the genotype by environment interaction is typically about one-quarter to one-third as large as the additive genetic variance (Yeh and Heaman 1987; Stonecypher et al. 1996; Johnson 1997), and the

Table 6.2. Mean heritabilities for common traits measured in Douglas-fir progeny tests under field conditions. Only experiments in which the trees were at least 4 years old are included.

Trait^z															
HT	DIA	WD	BF	BS	2nd FLUSH	CH (spring)	CH (fall)	FK/ RB	SIN/ CROOK	BR ANGLE	BR DIA	TAPER	SNC	Age (sites)^y	Citation
0.21	—	—	1.00	0.90	0.25	0.98	0.25	—	—	—	—	—	—	5–7 (m)	Aitken and Adams 1995^{x,w}
0.32	—	—	0.78	0.42	0.66	0.59	0.17	—	—	—	—	—	—	5–7 (s)	Aitken and Adams 1995^{v,w}
—	—	—	—	—	—	—	0.16	—	—	—	—	—	—	9 (m)	Aitken et al. 1996
~0.22	~0.33	~0.74	~0.88	—	—	—	—	—	—	—	—	—	—	9–16 (s)	Bastien et al. 1985^u
0.13^t	—	—	—	—	—	—	—	—	—	—	—	—	—	3–4 (m)	Campbell 1972
0.30	0.31	—	0.92	—	—	—	—	—	0.39	0.49	—	—	—	6–12 (s)	Christophe and Birot 1979; Birot and Christophe 1983
0.27	0.23	—	—	—	—	—	—	0.20	0.16	—	—	—	—	11 (m)	Howe and Jayawickrama 2002
0.18	0.21	—	—	—	—	—	—	—	—	—	—	—	—	10–15 (s)	Johnson et al. 1997
0.29	0.30	—	0.79	—	—	—	—	—	—	—	—	—	0.18	10–13 (s)	Johnson et al. 2002
0.14	0.16	—	—	—	—	—	—	—	—	0.73	0.26	0.10	—	12 (m)	King et al. 1988a; 1992
—	0.23	0.90	—	—	—	—	—	—	—	—	—	—	—	12 (s)	King et al. 1988b
—	—	—	0.90	0.81	—	—	—	—	—	—	—	—	—	14 (s)	Li and Adams 1993
—	—	—	0.74	—	—	—	—	—	—	—	—	—	—	13–16 (m)	Li and Adams 1993

—	0.19	—	0.87	—	—	—	—	—	—	—	—	—	—	15 (s)	Li and Adams 1994
~0.10[s]	—	—	—	—	—	—	—	—	—	—	—	—	—	12 (s)	Magnussen and Yanchuk 1994
—	—	—	0.85	0.42	—	0.77	0.21	—	—	—	—	—	—	7 (m)	O'Neill et al. 2000[w]
—	—	—	0.52	0.25	—	—	—	—	—	—	—	—	—	4 (s)	Rehfeldt 1983b[r]
0.17	0.27	0.52	—	—	—	—	—	—	—	—	—	0.24	—	18 (s)	St.Clair 1994
0.15	—	—	—	—	—	—	—	—	—	—	—	—	—	6–12 (s)	Stonecypher et al. 1996
—	0.08	—	—	—	—	—	—	0.08	0.13	—	—	—	—	12–13 (m)	Temel and Adams 2000
0.10	0.12	—	—	—	—	—	—	—	—	—	—	—	—	6 (m)	Yeh and Heaman 1982
0.13	—	—	—	—	—	—	—	—	—	—	—	—	—	7 (m)	Yeh and Heaman 1987

---

[z] Traits are total height (HT) (except as noted); stem diameter (DIA); wood density (WD); time of spring bud flush (BF); time of fall bud set (BS); second flushing (2nd FLUSH); cold hardiness (CH); forking and/or ramicorn branches (FK/RB); sinuosity or crookedness (SIN/CROOK); branch angle (BR ANGLE); branch diameter (BR DIA); stem taper (TAPER); and foliage health in the presence of the Swiss needle cast pathogen (SNC). "Sinuosity" has been used to describe two physiologically distinct traits, stem waviness in the first few interwhorls at the top of the tree (SIN, sensu Campbell 1965) and overall crookedness throughout the bole (CROOK).

[y] Age at the time of measurement and indication of whether the heritabilities were calculated across multiple sites (m), or are applicable to single sites (s), in which case they would be inflated because the variance due to genotype by environment interaction is confounded with the genetic variance.

[x] Two test sites in the coastal breeding zone.

[w] Alternative analyses of some of these data are presented in Aitken and Adams (1995) and O'Neill et al. (2000).

[v] One test site in the Cascade breeding zone.

[u] These heritabilities were estimated from the figure on page 17 (ANNEX 8) of Bastien et al. (1985).

[t] Total height increment during the third and fourth growing seasons.

[s] Estimated from Figure 4 in Magnussen and Yanchuk (1994).

[r] Nursery study.

genetic correlations among sites within breeding zones are about 0.6 to 0.8 (Johnson 1997). Because growth traits have low heritabilities, most breeding programs rely heavily on among-family selection to obtain genetic gain. Heritabilities (i.e., repeatabilities) of family means tend to be much higher (0.60–0.90) than individual tree heritabilities because families are typically planted on four or more sites and are usually represented by more than 60 progeny. As a result, advanced-generation selections are heavily weighted by the family performance compared to the within-family performance. The added benefit of replicating families over multiple sites is that it is possible to reduce the impact of genotype by environment interaction by finding families that perform well and are stable across a breeding zone (Stonecypher et al. 1996).

Heritabilities must also be examined in the context of stand age. In Douglas-fir breeding programs, selections are often made between the ages of 10 and 20, well before the harvest age of 40 or more. Heritabilities for growth traits slowly increase with age (Johnson et al. 1997). Although some of this increase may be associated with intrinsic changes in the relative proportion of genetic to environmental variation, most of the increase that occurs after crown closure is probably artifactual. As stands age and inter-tree competition increases, the fundamental tenet of good experimental design (i.e., no correlation between genotype and environment) no longer holds. If the trees that grow larger during the early life of the stand do so because of their genetic makeup, then genotype is confounded with an environmental effect (i.e., degree of suppression), thereby upwardly biasing estimates of genetic variation, heritability, and gain (Yanchuk 1996). Because tree roots also spread with time, the subsurface environment may exert a similar influence. Because of competition, there is a window of opportunity during which it is possible to obtain unbiased estimates of genetic variance and heritability (i.e., before competition becomes a factor). Unless the tests are systematically thinned, estimates of genetic variance and heritabilities are likely to be inflated after crown closure occurs around age 8 to 15, depending on the site.

Studies examining heritabilities for other traits are fewer, and often include fewer families and sites. Except for survival and growth, cold adaptation traits are the best-studied adaptive traits in Douglas-fir. These traits include vegetative bud phenology (i.e., bud set and bud flush), fall cold hardiness, and spring cold hardiness. In Douglas-fir and other conifers, the heritability of fall cold hardiness tends to be low to moderate, often about the same as for growth traits (O'Neill et al. 2000; Howe et al. 2003). The heritabilities of spring cold hardiness, bud set, and bud flush tend to be higher (i.e., moderate to high), with bud flush having the highest heritability of any measured trait in some studies (O'Neill et al. 2000; Howe et al. 2003). Heritabilities of stem defects (i.e., ramicorn branches, forks, and sinuosity) are low, about the same as for growth. Nonetheless, because these traits have high genetic variability, potential gains are large (Table 6.1). As for other tree species, the heritability of wood density is high but genetic variation is low. Therefore, proportional gains in wood density may be comparable to genetic gains in growth traits. The heritability of branch size (i.e., relative to stem diameter) is low, whereas the heritability for branch angle is high (Table 6.2). Although genetic gains are possible, these traits are not considered important enough to be treated as primary or secondary selection criteria.

3. Genetic Correlations. Genetic correlations are important because breeders may cause undesirable changes in some traits by selecting for other correlated traits, and because some traits can be used as indirect selection criteria for other traits that are harder or more expensive to measure. Selection is mostly based on growth, and adverse genetic correlations have been repeatedly demonstrated between growth and cold hardiness, bud set, ramicorn branches, and wood density (discussed below). Therefore, it might be wise for breeders to monitor these traits in their improved populations to ensure that undesirable changes are not occurring.

In Douglas-fir and other species, increased growth is associated with increased second flushing, later bud set, and increased cold injury in the fall, and these adverse relationships are stronger among, as compared to within, populations (Howe et al. 2003). In contrast, there is no consistent correlation between growth and either spring cold injury or bud flush (Howe et al. 2003). Wood density consistently shows an adverse genetic correlation with growth, and this association is stronger for diameter growth than for height growth. In two studies in which the adverse genetic correlation was strong (< -0.50), wood density decreased by 3 to 6.5% by selecting for increased growth using a 10% selection intensity (King et al. 1988b; Vargas-Hernandez and Adams 1991). One reason for this modest loss in wood density is that density has a high heritability, but a small coefficient of additive genetic variation ($\sigma_{additive}$/mean) (Table 6.1; Bastien et al. 1985; King et al. 1988b; St.Clair 1994). Increased growth is also positively associated with ramicorn branching, an important stem defect (Howe and Jayawickrama 2002). This relationship seems to exist, in part, because second flushing leads to greater seasonal height growth, but aberrant second flushing is also a major cause of ramicorn branches. Increased growth is associated to a lesser extent with a more sinuous stem and more forking.

Genetic correlations are valuable when they allow a breeder to use indirect selection. Although stem volume is the primary trait of interest, selection is usually practiced on height and diameter measurements. Not surprisingly, these three traits are highly correlated, with genetic correlations usually 0.80 or higher (Birot and Christophe 1983; Yeh and Heaman 1982; Johnson et al. 1997). Nonetheless, much lower (but positive) genetic correlations between height and diameter are sometimes found (e.g., 0.45; King et al. 1988a).

Other important relationships exist between vegetative bud phenology and frost hardiness. The timing of bud set in first-year seedlings (which have seasonally indeterminate growth) has a negative genetic correlation with fall frost hardiness—trees that set bud earlier have greater frost hardiness. In saplings (which exhibit seasonally determinate growth), bud set occurs much earlier in the summer and this relationship is weak (Li and Adams 1993; O'Neill et al. 2000). The positive correlation between spring bud flush and spring frost hardiness is stronger and less influenced by tree age. Trees that flush later in the spring are more frost hardy. Because of these relationships, it is possible to use bud phenology (which is easy to measure) as an indirect measure of fall and spring cold hardiness. Nonetheless, the use of bud set as an indirect selection criterion is limited because the correlation between bud set and fall cold hardiness is not very strong in older trees (O'Neill et al. 2000). Correlations between cold hardiness in the spring and fall seem to vary by population. In Douglas-fir seedlings and saplings, they were either uncorrelated (Cascade population), or had a weak to moderate negative correlation (coast population) (O'Neill et al. 2000, 2001).

4. Estimates of Genetic Gain. How much genetic gain is possible in Douglas-fir? Predicted gains in age-12 height and volume were reported for the British Columbia Ministry of Forests (BCMoF) program based on the assumption that the top 10% of parents (n = 22) are selected from all parents that were tested in four series of half-diallel tests (Yanchuk 1996). Gains in height and volume were predicted to be 8.8 and 22.4%, respectively, if the selected parents are placed in a seed orchard where mating is random and there is no pollen contamination or inbreeding. Furthermore, these gains would only increase slightly (i.e., up to 9.4 and 25.2%, respectively) if specific crosses were made to capture SCA (Yanchuk 1996). Genetic gains are expected to be even larger in the Northwest Tree Improvement Cooperative (NWTIC) program because selection intensities could be much higher (i.e., because of the large numbers of parents tested; K. J. Jayawickrama, unpubl. data). Realized gains may be lower than predicted gains, however, mostly because of pollen contamination (if controlled-pollination is not used), the effects of genotype by environment interactions that are not fully measured in the test plantations, and violations of other assumptions used to predict genetic gain.

Results from a block-plot, realized gain trial established at five sites in the northern Oregon Cascades were recently published by St.Clair et al. (2004). Five years after planting, realized gains for selected groups of families were similar to the gains predicted from their performance in progeny tests using single-tree plots. Realized genetic gains were about 6% for height, 8% for diameter, and 28% for stem volume, compared to predicted genetic gains of about 8% for height, 7% for diameter, and 25% for stem volume. Age-7 results were reported for a trial established on five sites in British Columbia (Woods et al. 1995a; Stoehr and Bird 2003). Again, realized genetic gains were similar to predicted gains, with mid-gain (i.e., moderately selected) families showing a 10% gain in height over unimproved seedlots, and top-crosses (i.e., crosses among elite parents) showing a gain of nearly 17%.

G. Sociological and Political Factors

The public believes it has an important responsibility to maintain the long-term health of our forests. The public certainly feels this way about public lands in the Pacific Northwest—and sometimes extends these views to large industrial forests as well. Tree breeders must, therefore, operate within the social and political climates, as well as the physical and economic climates in which they operate (Howe et al. 2005). Despite these constraints, very large breeding programs exist for a handful of the world's most important forest tree species, including Douglas-fir.

VI. BREEDING GOALS AND OBJECTIVES

The main goals of Douglas-fir breeding programs are to improve the economic value of tree crops and maintain adaptability (Table 6.3). At the same time, most breeders also acknowledge the need to maintain flexibility in case markets (and breeding objectives) change, and to ensure that improved populations have sufficient genetic variation for continued gains in the future. Although the value of tree crops is determined on a per-hectare basis, the traits used for selection (i.e., specific breeding objectives) are typically measured on individual trees. This is because it is difficult and expensive to measure the performance of many seedlots (e.g., families) on a per-hectare basis. Nonetheless, breeders must be

Table 6.3. Typical breeding goals, objectives, and traits of interest for Douglas-fir breeding programs in North America.

Breeding goal	Breeding objective (i.e., primary traits of interest)	Relative importance^z		Selection criterion^x
		Designing breeding zones (and populations)^y	Selecting within breeding populations	
Increase crop value	Large stem volume	2	1	Stem height (y) Stem diameter (y) Estimated stem volume (y) Growth model parameters (n)
	High stem quality	3	2	Ramicorn branches (y) Forks (y) Stem sinuosity (y) Second flushing (p)
	Wood quality (i.e., stiff and strong wood)	3	2	Wood density (y) Microfibril angle (n) Modulus of elasticity (MOE) (n) Modulus of rupture (MOR) (n)
	Small knots	3	3	Branch size (p) Branch angle (p)

Maintain adaptability	Spring frost hardiness	1	2–3	Spring artificial freeze test (p) Spring bud flush (y)
	Fall frost hardiness	1	2	Fall artificial freeze test (p) Fall bud set or growth cessation (y)
	Drought hardiness	1	3	Summer artificial drought test (p)
Disease resistance	Swiss needle cast resistance	3	2	Foliage health or diameter growth in the presence of Swiss needle cast (y)
	Rhabdocline needle cast resistance	3	2	Visual assessment in the field (y)
	Armillaria root rot resistance	3	2	Visual assessment in the field (y) Artificial inoculation (p)
	Phellinus root rot resistance	3	2	Visual assessment in the field (y) Artificial inoculation (p)

---

[z] 1 = Always or almost always considered; 2 = sometimes considered, or used as a secondary criterion in two-stage selection; 3 = never or almost never considered.

[y] First-generation breeding populations generally consist of genotypes collected from the breeding zone in which they will be used.

[x] y = yes, trait is always or sometimes used; p = potential for use (trait is not used, but information exists to allow its use); n = no, trait has never been used and there is little information to guide its use.

mindful of the assumptions involved in defining breeding objectives on a per-tree basis when the real goal is to increase the value of the entire crop.

A. Primary Breeding Objectives

The two main breeding goals—increasing crop value and maintaining adaptability—are generally met in different ways. "Adaptability," which usually refers to the ability to tolerate both frosts and droughts, is typically maintained through the use of appropriate breeding zones (Table 6.3). Most of the parents in first-generation breeding populations were selected from the breeding zones in which they will be used, so maladaptation should be not be a problem (i.e., damage from cold and drought should be comparable to that seen in natural populations). In contrast to adaptability, tree value is generally improved by selecting and breeding the most valuable genotypes within these well-adapted populations.

Tree value is primarily determined by stem volume, and secondarily by stem quality and wood properties such as wood density. Therefore, the primary breeding objective for Douglas-fir, and most other forest trees, is to increase volume growth (Campbell 1964; Wheat and Silen 1977; Silen and Wheat 1979; Lester 1986). Greater growth would result in either greater yields at harvest, or permit the use of shorter rotations. The key traits used as predictors of rotation-age volume are diameter at breast height (DBH) and total height—typically measured anywhere from ages five to 20. Although there is interest in exploring measures of growth per se (e.g., growth model parameters) (Cherry and Howe 2004), the advantages of these approaches are not yet known for Douglas-fir.

B. Secondary Breeding Objectives

Improvements in wood quality (i.e., wood properties) and stem quality are secondary in importance because their impact on tree value is neither as great (nor as quantifiable) as it is for stem volume (Table 6.3). The single most important wood property is wood density (e.g., wood specific gravity) because dense wood is associated with stiffer and stronger wood, as well as increased pulp yields. Important stem defects include forks and ramicorn branches. Forked stems are often formed if the terminal leader is damaged or killed, and two branches subsequently assume equal dominance. Ramicorn branches are excessively large, upright branches. Forks and ramicorn branches either reduce stem volume and log length (by making a portion of the stem unmerchantable), or result in low-grade products because of the large knots that are formed. Stem sinuosity is stem waviness in the top few interwhorls of the tree (Campbell 1965). Although stem sinuosity is sometimes used as a selection criterion (Table 6.3), it has little impact on tree value because it only affects a small cylinder of wood in the center of the tree (Spicer et al. 2000).

These secondary traits (i.e., wood density, ramicorn branching, forking, and sinuosity) are often considered in breeding programs, but receive much less weight than stem volume. Genotypes with many stem defects, for example, are unlikely to be included in future breeding populations (regardless of their growth rate), and selections for increased wood density are often made only among the fastest-growing genotypes (i.e., two-stage selection).

Other undesirable traits include large branch diameters and upright branches (i.e., small branch angles), both of which cause large knots. These "tertiary" traits, however, are usually ignored when selection decisions are made (Table 6.3). Branch size and branch angle are clearly associated with knot size, but small differences in knot size have little impact on product value. The relatively small impact that the secondary and tertiary traits have on tree value is exacerbated by the dearth of quantitative data that could be used to derive economic weights for multi-trait selection indices. We have excellent information on the value of gains in stem volume, but little information on the value of improvements in stem quality and wood properties. Although milling studies could be used to derive accurate economic weights for breeding programs, these studies are expensive, and are unlikely to reduce the prime focus on stem volume. Because genetic gains in any single trait decrease as more traits are included in a breeding program, there is little rationale for including these tertiary traits in current breeding programs.

Despite the current focus on stem volume, stem quality and wood properties should be monitored because breeding objectives can change. As new products and manufacturing processes arise—and if machine grading becomes more common—the importance of stem quality and wood properties is likely to increase. Furthermore, rotations are becoming shorter and more of the wood is being harvested from the juvenile core of the tree. Compared to mature wood produced outside of the juvenile core, juvenile wood is inferior for most solid wood products. Therefore, it may become necessary to focus more heavily on wood properties simply to maintain the overall quality of the wood that is harvested. Furthermore, we know little about the genetics of the "real" traits of interest—traits such as wood stiffness and strength. Wood density is used as an indirect measure of these mechanical properties, but other fundamental wood properties, such as microfibril angle, might be useful as well (MeGraw 2002; Table 6.3). Because breeding progress is slow, breeding objectives must be viewed within the context of the products we intend to make in 50 to 100 years, not solely on the products we are making today.

In addition to increasing tree value, most programs seek to maintain sufficient physiological adaptability and genetic variation. In fact, the existence of breeding programs specifically designed to maintain adaptability and variability is a hallmark of Douglas-fir breeding. The most important adaptive traits are cold and drought hardiness. Genotypes that can tolerate the normal, year-to-year frosts and droughts are likely to be included in advanced generations if genotypes are selected based on their superior growth in field tests at age 10 to 15. In addition, trees must be able to survive and prosper after the unusually severe frosts and droughts that sometimes occur (e.g., Duffield 1956).

Unlike stem volume, adaptive traits are infrequently considered as specific traits when genotypes are selected for the next generation (Table 6.3). Instead, this breeding objective is typically pursued through the design of appropriate breeding zones. The reasons for this are three-fold. First, because severe frosts and droughts are rare, it is difficult to measure frost and drought hardiness under normal progeny test conditions. Second, it is difficult to predict how these rare events will affect production plantations and, thus, the value of improving cold and drought hardiness. Third, inexpensive, artificial tests can be used to measure cold and drought hardiness in seedling nursery tests and in the field (at least for cold hardiness), but they add complexity to testing programs. Although costs can be lessened by measuring other traits that are correlated with cold and drought hardiness (e.g., bud phenology; Stonecypher et al. 1996), this approach would be considerably less effective than selecting for cold and drought hardiness directly, except for the timing of spring bud flush, which has a strong positive genetic correlation with spring frost hardiness (O'Neill et al. 2000). Other traits that have low to moderate genetic correlations with cold and drought hardiness include the timing of fall bud set and the propensity for second flushing (Howe et al. 2003). Despite the potential for within population selection, adaptability is typically maintained through the design of appropriate breeding zones. Although frost and drought hardiness are rarely measured directly, it might be wise for breeding programs to monitor these traits because selection for increased growth (i.e., without considering adaptive traits) can reduce adaptability (see *Genetic correlations*) and because the rarity of severe frosts and droughts has the potential to instill a false sense of adaptive security.

Few insect and disease problems have risen to the level where they form key components of Douglas-fir breeding programs. Nonetheless, tolerance to Swiss needle cast has become a breeding objective in some coastal areas where this disease is impacting Douglas-fir plantations.

Recent studies suggest that Swiss needle cast tolerance can be improved via selection and breeding (Johnson 2002; Johnson et al. 2002). Root rots caused by *Armillaria* and *Phellinus* also cause problems, and studies are underway to determine whether there is genetic variation in resistance to these fungal pathogens (B. C. Jaquish, pers. comm.; R. Sturrock, pers. comm.).

A major challenge to breeders is that the two primary breeding goals—improving value and maintaining adaptability—often conflict with one another. Increased growth, for example, is associated with increased cold damage, a greater number of stem defects, and lower wood density, particularly at the population and provenance levels (Howe et al. 2003; see *Genetic correlations*). Furthermore, genetic variation itself may be adaptive—and genetic gain can only be achieved by reducing genetic variation in the trait of interest (Fig. 6.3). Breeding strategies for Douglas-fir seek to balance these conflicting objectives.

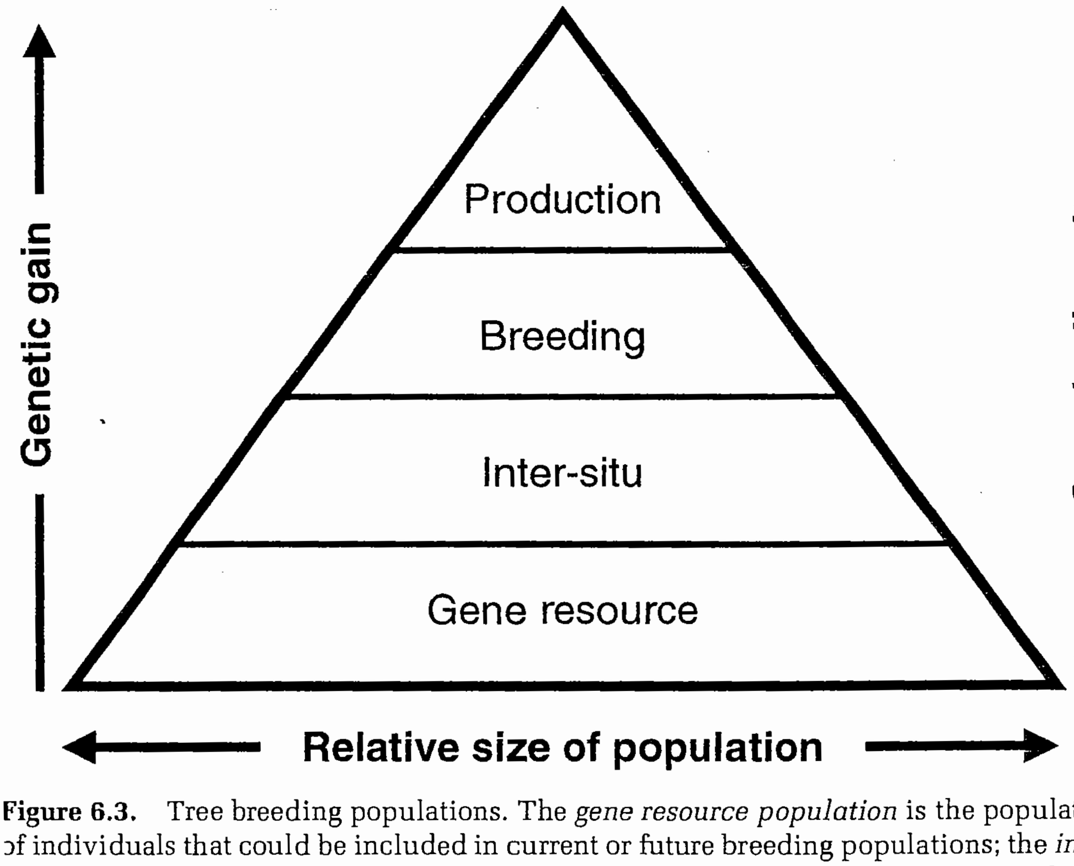

Figure 6.3. Tree breeding populations. The *gene resource population* is the population of individuals that could be included in current or future breeding populations; the *inter-situ population* is the population of individuals in progeny tests, archives, and clone banks that are not included in the breeding or production populations; the *breeding population* is the group of parents that will be used to produce offspring for the next cycle of selection; and the *production population* is the subset of individuals from the breeding popuation that is used to produce propagules for reforestation (see text for details). This figure shows the inverse relationship between genetic diversity (and population size) vs. genetic gain (modified from R. D. Burdon, pers. comm.; cited in Johnson et al. 2001).

VII. OVERVIEW OF TREE BREEDING METHODS

In this section, we describe the key elements of a typical conifer breeding program. In many respects, these programs resemble agronomic and horticultural crop breeding. Nonetheless, they contain elements that are often unfamiliar to breeders of maize, tomato, apple, and even landscape trees—particularly because of their long-term perspective, concentration on population improvement, and focus on maintaining genetic diversity. In fact, tree breeding is more similar to animal breeding than to many other types of plant breeding. In the absence of a breeding program, tree improvement typically involves the planting of source identified wild seed according to seed transfer guidelines or seed zone recommendations. Because we are focusing on breeding per se, we will not discuss seed zones and seed transfer guidelines in detail. Readers interested in these topics should consult reviews by Adams and Campbell (1981), Campbell (1986), Morgenstern (1996), and Randall (1996).

A typical tree breeding program has four main steps, which may occur simultaneously (Wright 1976; Zobel and Talbert 1984; Morgenstern 1996). The first step is to delineate breeding zones. A breeding zone is a set of environments within which the genotypes from a particular breeding population can be safely planted—i.e., resulting in well-adapted plantations that will meet forest management objectives. The second step is to develop one or more breeding populations for each breeding zone. In the first generation, breeding populations may be selected from wild stands within each breeding zone; from superior, non-local populations that have been identified based on provenance tests; or from landraces. The first approach is common for Douglas-fir within its native range, whereas the second approach has been used for Douglas-fir in Europe and the southern hemisphere. The third step is to field test the progeny of the selected parents and pursue advanced-generation breeding within each breeding population. The fourth step is to produce genetically improved materials for outplanting. In most Douglas-fir programs, this involves establishing the best genotypes from each breeding population in separate wind-pollinated seed orchards.

A. Breeding Zones

Tree crops are typically planted across large, environmentally diverse landscapes, managed extensively, and harvested decades later. Compared to the loss of an annual crop, the loss of a forest crop could wipe out an investment that has lasted for decades. Therefore, biological and economic risks are generally greater for tree crops than for annual, agronomic crops—and maintenance of long-term adaptability is critical. The first defense against maladaptation is the design of appropriate breeding zones. A key decision in any breeding program is the appropriate number, size, and locations (or environmental characteristics) of the breeding zones. The greater the number of breeding zones, the easier it is to ensure long-term adaptability and manage genotype by environment interactions. However, breeding, testing, and selection programs must be replicated for each zone. Furthermore, seed orchards will be needed for each breeding zone, at least for programs that rely on wind-pollinated seed orchards. Therefore, one of the most important decisions in any breeding program is the appropriate size and number of breeding zones. This decision involves a balance between decreasing breeding program costs (i.e., by using fewer breeding zones) and decreasing the biological and financial risks associated with maladaptation (i.e., by using more breeding zones).

B. Population Improvement and Genetic Diversity

For species that are planted within their native range (like Douglas-fir), gene conservation and diversity issues are critically important. Therefore, most breeding programs rely on population improvement, rather than cultivar development. Unlike many agronomic and horticultural crops where a single genotype (or genetically homogeneous cultivar) may be planted over a vast number of hectares, most organizations seek to maintain substantial genetic variation within their breeding and planting programs. Therefore, tree breeders deal almost exclusively with populations, and rarely pursue the kinds of individual-focused, pedigree breeding that is commonly practiced in agronomic and horticultural crops. Although exceptions may exist for intensively managed, short-rotation species such as poplars (*Populus*) and willows (*Salix*), this is the rule for species such as Douglas-fir that are mostly propagated by seed and extensively managed on long rotations. Even when clonal deployment is considered, a mixture of clones is typically recommended—either as true mixtures, or as clonal blocks. This constrains the operational use of clones and genetically engineered trees.

C. Early Selection

Because of the long rotations in forest trees, selections must be made well before harvest age. In Douglas-fir, final selections are commonly made when the trees are about 10 to 15 years old, compared to harvest ages of 40 to 70 years. Based on age-age correlations from more than 51 progeny test sites in Oregon, Johnson et al. (1997) concluded that per-year gains are maximized when selections are made for height at age 10, and for diameter at age 13. Magnussen and Yanchuk (1993) used stochastic simulation of Douglas-fir age-age correlations to evaluate the risks associated with different selection ages. If field tests are used to select the tallest families before age 15, they concluded that family sizes of at least 20 trees per family are needed, whereas family sizes should be above 40 if selections are made before age 10. For within-family selection, they concluded that the "safe" age for making selections is age 17 or older.

Various scenarios have been investigated to further reduce the selection age (i.e., below what is optimal using standard progeny tests). These methods of "early selection" include measuring young seedlings (≤ 2 years old) in controlled environments (Lambeth et al. 1982), bareroot nurseries (Riitters and Perry 1987; Adams et al. 2001; Vargas-Hernandez et al. 2003), or container nurseries (Adams et al. 2001; Vargas-Hernandez et al. 2003). In these experiments, low to moderate correlations (e.g., 0.3–0.5) were found between family means for some seedling traits (e.g., height, dry-weight, bud set) and the height or volume of the same families in field tests at ages 6 to 15. Although these correlations are not large enough to permit final selections to be made at very young ages, they are large enough to practice "early culling" using two-stage selection. Adams et al. (2001), for example, concluded that the costs of standard progeny tests could be reduced by 18% (i.e., from $371,000 to $306,000) by culling the poorest 25% of families based on first-year height growth, then planting only the remaining 75% of families in long-term field tests. Other experiments suggest that early culling would also be effective for some branch traits (Vargas-Hernandez et al. 2003). Furthermore, to improve the tolerance of Douglas-fir to Swiss needle cast disease, early selection in the field at age two was 25 to 100% as efficient as waiting until age 10 or 12 (Temel and Johnson 2001).

Another option is to practice early selection using older trees (up to age 7) in "farm-field" tests. Farm-field tests are progeny tests established using intensive site preparation, close spacing, and nearly complete weed control (Woods et al. 1995b). From ages 3 to 7, there was a high correlation (≥ 0.71) between family height in a farm-field test and both the height and volume of the same families in standard progeny tests. Unlike the very early tests described above, the farm-field tests would not be valuable for early culling. This is because the trees could not be transplanted to standard progeny tests after the test is complete (as is done with early culling). On the other hand, farm-field tests might provide greater gains per year by allowing final selections to be made earlier, but (presumably) this would entail using them as replacements for standard progeny tests, which seems unlikely.

Because it takes so long for the financial benefits of tree improvement to be realized, discount rates have a major impact on the economics of tree breeding. The financial benefits of alternative breeding programs are particularly sensitive to discount rates—and modest up-front costs must be balanced by large returns when the stands are harvested. These factors tend to favor low-cost approaches to genetic improvement and exclusion of secondary traits that have ill-defined impacts on tree value.

VIII. BREEDING PROGRAMS

A. North America

In North America, most Douglas-fir improvement is carried out by the Northwest Tree Improvement Cooperative (NWTIC), Inland Empire Tree Improvement Cooperative (IETIC), British Columbia Ministry of Forests (BCMoF), and Weyerhaeuser Company (Table 6.4). These four organizations are responsible for developing improved materials planted by private companies, tribal governments, and public agencies in the U.S. and Canada. The federal agencies include the USDA-Forest Service (USFS) and USDI-Bureau of Land Management (BLM). The state agencies include the Idaho Department of Lands, Montana Division of Forestry, Oregon Department of Forestry (ODF), and Washington Department of Natural Resources (WDNR). Because the approach to Douglas-fir improvement differs among these four organizations, it is important to understand their organizational differences, tree improvement histories, and core philosophies. In subsequent sections, we will expand on their alternative approaches to selection, breeding, testing, seed orchards, vegetative propagation, and deployment of improved genotypes. Earlier summaries of the North American Douglas-fir breeding programs were given by Woods (1993) and Lipow et al. (2003).

1. Northwest Tree Improvement Cooperative (NWTIC). The NWTIC evolved from the Industrial Forestry Association (IFA) breeding program and the subsequent IFA-PNW Progressive Tree Improvement Program. In the 1950s, a small group of government agencies, forestry companies, and forestry associations began independent tree improvement programs (Hagenstein 1986). Booth-Kelly Lumber Company, Crown Zellerbach Corporation, the IFA, Port Blakely Mill Company, Simpson Timber

Table 6.4. Major Douglas-fir genetic improvement programs in North America (updated from Woods 1993).

	Region and program				
	U.S. Pacific Northwest		British Columbia		U.S. Intermountain
Characteristic	NWTIC[z]	Weyerhaeuser[y]	BCMoF coastal[x]	BCMoF interior[x]	IETIC[w]
First generation					
No. of breeding zones	109	6 (low elevation)[v]	2	8	13
No. of parents tested	26,000[u]	3,500	660	1,661	2,503
Mating designs	OP, polymix, single-pair full-sibs	OP, diallel, polymix, single-pair full-sibs	OP, half-diallel, factorial	OP	OP
No. of field tests	835	400	130	32	43
Second generation					
No. of breeding zones	8	3 (low elevation)	2	5	Not planned
No. of parents to be tested	2,000	1,043	300	Not determined	Not planned
No. of families to be tested	2,600[t]	4,886	930	Not determined	Not planned
Mating designs	Unstructured full-sibs	Tester, positive assortative matings, correctively-mated full-sibs[s]	Polycross, half-diallel, double-pair mating	Factorial	Not planned
No. of field tests	95	101	36	Not determined	Not planned
Breeding objectives					
Primary (selection criteria)[r]	Adaptability (BZ) Volume (HT, DIA)	Adaptability (BZ) Volume (HT, DIA) Stem quality (RB, FK, SIN)	Adaptability (BZ) Volume (HT, DIA) Wood quality (WD)	Adaptability (BZ) Volume (HT, DIA)	Adaptability (BZ) Volume (HT, DIA) Fall frost hardiness (BS)

Secondary (selection criteria)[q]	Stem quality (RB, FK, SIN) Wood quality (WD)	Wood quality (WD) Frost hardiness (BF) Disease resistance (NC)	Stem quality (RB, FK, SIN) Frost hardiness (FD)	Wood quality (WD) Stem quality (RB, FK, SIN)	Disease resistance (RR, NC)

---

[z] NWTIC is Northwest Tree Improvement Cooperative. Data are from K. J. Jayawickrama.

[y] Weyerhaeuser data (U.S. breeding program up to 2003) are from C. A. Dean.

[x] BCMoF is British Columbia Ministry of Forests. Coastal data are from M. U. Stoehr and interior data are from B. C. Jaquish.

[w] IETIC is Inland Empire Tree Improvement Cooperative. Data are from M. L. Rust.

[v] Although the Weyerhaeuser program originally had 6 low elevation and 6 high elevation breeding zones (Stonecypher et al. 1996), data for only the 6 low elevation zones were reported by Woods (1993).

[u] Sum of the number of parents tested in each breeding zone. The actual number of parents tested is slightly less because some parents were tested in more than one breeding zone.

[t] Including families growing in nurseries in 2004.

[s] The number of crosses per parent was based on each parent's relative performance and genetic diversity targets.

[r] Primary breeding objectives are those that are almost always considered when making selection decisions. Adaptability refers to the goal of maintaining or enhancing both cold and drought hardiness (i.e., multiple breeding objectives). Selection criteria (in parentheses) refer to the traits on which selection is based. BZ indicates that the breeding objectives are mostly achieved through the design of appropriate breeding zones (and breeding populations) based on a complex suite of traits (e.g., multiple traits measured in field and nursery tests), as well as other geographic, physiographic, and climatological information (see Table 6.3 and text). Selection criteria that are typically used to select within breeding populations include stem height (HT), stem diameter (DIA), ramicorn branching (RB), forking (FK), stem sinuosity (SIN), wood density (WD), damage from natural frosts in the spring or fall (FD), timing of spring bud flush (BF), timing of fall bud set or growth cessation (BS), tolerance to *Armillaria* and *Phellinus* root rots (RR), and tolerance to *Rhabdocline* and Swiss needle cast diseases (NC). "Sinuosity" has been used to describe two physiologically distinct traits, stem waviness in the first few interwhorls at the top of the tree (Campbell 1965) and overall crookedness throughout the bole. See Table 6.3 for more information on breeding goals, breeding objectives, and selection criteria (i.e., traits of interest).

[q] Secondary breeding objectives are those that are sometimes considered, or those used as secondary criteria in two-stage selection. Abbreviations are as described above.

Company, Timber Service Company, USFS, and Weyerhaeuser Company were among the first to select coastal Douglas-fir trees and establish grafted (i.e., clonal) seed orchards. The IFA played a key role in this process by hiring a forest geneticist, John Duffield, to guide their tree improvement efforts (Hagenstein 1986).

During the 1960s, the USFS Pacific Northwest Forest and Range Experiment Station (PNW Station) played an important role in Douglas-fir improvement. In 1966, the PNW Station joined forces with the IFA to form the IFA-PNW Progressive Tree Improvement Program, which was described as a simple, low-cost program that would appeal to medium- and smaller-sized forest landowners in western Oregon and western Washington (Silen 1966b). The Progressive Program was first described by Roy Silen in 1966, and then summarized in subsequent publications (Silen 1966b; Wheat and Silen 1977; Silen and Wheat 1979). The IFA-Progressive Program eventually evolved into the Northwest Tree Improvement Cooperative (NWTIC) in 1986. Although the USFS, BLM, Georgia-Pacific, Simpson, and WDNR operated independent programs for many years, these programs are now part of the NWTIC. As of 2004, the NWTIC is housed at Oregon State University and consists of 27 member organizations (i.e., forest industries, tribal governments, one federal agency, and state agencies within the U.S. and Canada).

Distinctive features of the Progressive Program included the assumption that local seed sources are best (i.e., rather than choosing seed sources based on provenance tests), low-intensity selection of first-generation parents (rather than intensive phenotypic, or "plus-tree," selection), use of many small breeding zones (mostly less than 60,703 ha; 150,000 acres), and the use of very large breeding populations (Silen 1966b; Silen and Wheat 1979). This conservative approach was intended to ensure adaptability of the first-generation breeding populations, which consisted of parents selected from natural stands within the breeding zone. Although breeding zones have been consolidated and breeding populations are being reduced (Table 6.4), the NWTIC program is [illegible] one of the largest tree breeding programs in the world.

The Progressive Program was implemented by forming local, geographically based cooperatives to share the costs and benefits of tree improvement. The members of a particular cooperative consist of organizations that manage lands within the same geographic area and have common tree improvement needs. In the first generation, these individual cooperatives covered one to 20 breeding zones (Silen and Wheat 1979). When the establishment of first-generation tests was completed in 1993, 21 first-generation cooperatives had been formed, and the Douglas-fir zone west of the Cascades was blanketed with 109 breeding zones ranging from the Canadian border to northwest California. More than 26,000 first-generation parents have been evaluated based on more than 3 million progeny test trees (Lipow et al. 2003). This large number of breeding zones was recently reduced to eight second-generation zones, and the number of parents used in advanced-generation breeding is expected to be about 2,000 (Table 6.4).

Today, the NWTIC serves as an umbrella organization that coordinates the breeding and testing activities for seven independent cooperatives that have breeding programs for eight second-generation breeding zones. The NWTIC is essentially a regional confederacy of autonomous, local cooperatives. NWTIC staff provides technical direction; program coordination; administrative support; and data management, analysis, and interpretation to the local cooperatives. In contrast, the costs of breeding and testing are shared among the members of these local, second-generation cooperatives. Seed orchards are managed by individual organizations or by seed orchard cooperatives that are mostly independent of the NWTIC. The main advantage of cooperatives is that expensive breeding programs are not duplicated among many organizations. The main drawbacks are the loss of competitive advantage (i.e., because improved genotypes are shared among cooperators) and the challenge of balancing different priorities, organizational cultures, and levels of funding (Prudham 2003).

2. British Columbia Ministry of Forests (BCMoF). Because 95% of the forestland in British Columbia is publicly owned (i.e., Crown land), private companies operate through various license agreements with strong oversight from the BCMoF. Historically, tree improvement programs were coordinated by cooperative tree improvement councils consisting of the BCMoF, forest companies, Canadian Forest Service (CFS), and universities. A Plus Tree Board was formed in the 1960s, followed by the Coastal Tree Improvement Council in 1979, and the Interior Tree Improvement Council in 1981. The Coastal and Interior Tree Improvement Councils were subsequently merged into the Forest Genetics Council of British Columbia (FGC) in 1998. The FGC is a multi-stakeholder group that coordinates and directs the operational tree improvement programs, management of seed orchards, gene conservation, and forest genetics extension activities. Within this framework, the responsibility for Douglas-fir breeding falls to the BCMoF, whereas the responsibility for producing improved materials for reforestation is shared between the forest industry and the BCMoF.

First-generation selection and testing began around 1960 for coastal Douglas-fir and 1980 for interior Douglas-fir. The early improvement efforts of Alan Orr-Ewing focused on making intensive plus-tree selections, developing inbred lines (for subsequent outcrossing), creating interracial hybrids, and testing provenances within coastal British Columbia (Orr-Ewing 1954; Orr-Ewing et al. 1972; Heaman 1977). After Gene Namkoong was hired as a consultant in the early 1970s, the focus changed to using structured mating designs (e.g., half-diallels) to create a pedigreed breeding population, estimate quantitative genetic parameters, determine the size and importance of genotype by environmental interactions, and provide family information for roguing seed orchards (Heaman 1977). In the coastal program, half-diallel, factorial, and open-pollinated mating designs have been used to test about 660 parents in 130 field tests (Table 6.4). In the interior program, an open-pollinated mating design was used to test about 1,661 parents in 32 field tests (Table 6.4).

3. Inland Empire Tree Improvement Cooperative (IETIC). The IETIC was formed in 1968 to develop improved ponderosa pine (*Pinus Ponderosa*) for the Inland Empire (i.e., eastern Washington, eastern Oregon, northern Idaho, and western Montana). The IETIC is now pursuing genetic improvement of ponderosa pine, western larch (*Larix occidentalis*), western white pine (*Pinus monticola*), lodgepole pine (*P. contorta*), and Douglas-fir for planting in eastern Washington, northern Idaho, and western Montana. The IETIC is housed at the University of Idaho and consists of 19 organizations, including private industries, federal agencies, state agencies, tribal governments, and universities. The USFS is the largest landowner in the region.

The Douglas-fir species group was formed in 1974, and cooperators began selecting first-generation parent trees shortly thereafter. In most of the 13 breeding zones, 200 to 300 trees were selected, and more than 2,500 first-generation parents have been field tested to date (Table 6.4). IETIC members have established four Douglas-fir seed orchards in the region. Compared to the Pacific Northwest, Douglas-fir is relatively less important in the Inland Empire and vies for attention with a number of other commercially important conifers. The number of hectares planted to Douglas-fir has declined in recent years because many sites have abundant natural reproduction and serious root diseases can be a problem in plantations. For these reasons, the Douglas-fir breeding program in the Inland Empire is much less intensive than it is in the Pacific Northwest, and no advanced-generation breeding is planned.

4. Weyerhaeuser. Weyerhaeuser Company has managed a large and sustained tree breeding program in Douglas-fir since 1963. One of their key assumptions was that rigorous phenotypic selection of superior trees in natural stands (i.e., accounting for competitive and environmental influences) would produce genetic gain in growth (Stonecypher et al. 1996). Therefore, the foundation of their program was an intensive plus-tree selection program in natural stands aged 25 to 80 years (Stonecypher et al. 1996). Some 3,500 parents were selected in six breeding zones (i.e., with high and low elevation splits) covering Weyerhaeuser lands in western Washington and western Oregon (Table 6.4). The primary objective of the first-generation program was to improve growth and stem quality (Woods 1993). Selection, breeding, and testing of a large second-generation population are almost complete, and the third generation of improvement is underway.

Based on extensive field tests of adaptability and stability (i.e., low genotype by environment interaction), Weyerhaeuser significantly increased the size of their breeding zones in the U.S. There are now three low-elevation zones, one in Washington and two in Oregon. Although Weyerhaeuser typically produces improved reforestation stock from wind-pollinated orchard seed, some elite material was produced from rooted cuttings during the 1990s (Ritchie 1993). Nonetheless, the current long-term goal is to produce elite material via somatic embryogenesis and wind-pollinated seed orchards.

B. Europe

European interest in Douglas-fir tree improvement has grown in conjunction with its importance in European forestry. Many European countries and organizations are involved in provenance research on Douglas-fir through a comprehensive set of provenance trials established by the International Union of Forest Research Organizations (IUFRO) in the 1960s (Fletcher 2002). Seeds from 176 provenances collected throughout the range of Douglas-fir were distributed to 55 research organizations in 33 countries throughout the world (Breidenstein et al. 1990). These tests were used to identify the best provenances for Europe, and a second collection was made in the 1980s that concentrated on Washington provenances.

The EUDIREC (European Douglas-fir Improvement Research Cooperative) project is a recent collaboration among research organizations in Belgium, France, Germany, Italy, Spain, and the United Kingdom (Héois 2000). Their objectives are to develop a database for European gene resources, test Douglas-fir provenances and families in the field and in controlled environments, build a common breeding population for Douglas-fir, and improve methods of vegetative propagation and seed production.

C. New Zealand

Douglas-fir is second only to radiata pine in importance as a plantation species in New Zealand. Large provenance trials were established in 1957 and 1959 by I. J. Thulin and results were summarized in the mid-1960s and in 1974 (Sweet 1965; R. L. Knowles, pers. comm.). A Douglas-fir breeding program was begun in 1970 by M. D. Wilcox of the New Zealand Forest Research Institute, and updated in 1987 by C. J. A. Shelbourne based on the most current provenance test results (R. L. Knowles, pers. comm.).

In 1993, the Douglas-fir Cooperative was formed—a research-industry cooperative that is now managed by Forest Research, a Crown Research Institute (Douglas-fir Cooperative 2004). The cooperative has 12 regular members and 14 associate members, including seed producers, forest growers, manufacturers, and consultants. One objective of the cooperative is to advance technology in plantation-grown Douglas-fir by developing and deploying improved genetic materials. Although their traits of interest include growth, stem form, and needle cast resistance (Douglas-fir Cooperative 2004), the breeding program will focus on improving wood stiffness (R. L. Knowles, pers. comm.). Improved seed will be produced in clonal seed orchards, and they may use vegetative multiplication to produce operational quantities of elite material from control-pollinated seed.

IX. BREEDING AND TESTING METHODS

A. Overall Breeding Strategies

Each of the North American programs is using recurrent selection to improve breeding populations for specific breeding zones. Recurrent selection aims to progressively improve breeding values by increasing the frequency of desirable alleles. This approach is particularly compatible with the production of improved seed in wind-pollinated seed orchards, which is the main way that improved planting stock is produced. In the 1960s and 1970s, the BCMoF investigated other breeding methods, but these were eventually abandoned. These included a wide-crossing project between "local" and "non-local" coastal parents and the development of inbred lines for outcrossing (Heaman 1977). Recurrent selection is also the foundation of the breeding programs in New Zealand and Europe.

B. Breeding Zones—Theoretical Considerations

Breeding zones and seed zones are used to manage the deployment of trees from breeding programs and wild seed collections, respectively. A breeding zone is a group of sites across which a breeding population can be planted and expected to perform well. Because long-term survival and growth are crucial for achieving all breeding objectives, breeding zones are generally delineated with these and other adaptive traits in mind. A seed zone is a group of sites from which wild seed can be collected and expected to perform well when deployed anywhere within the same seed zone. Breeding zones, not seed zones, are the main concern for organizations that have breeding programs. Some of the most important decisions in a breeding program are the appropriate number, sizes, and locations of the breeding zones. Although adaptability and genetic gain can be increased by decreasing the size and increasing the number of breeding zones, this is expensive because separate breeding programs are needed for each zone.

Breeding zones and seed zones are delineated by building models that predict (1) the group of genotypes that are genetically suited to the environment at a specific planting site and (2) the group of sites that are environmentally suitable for a particular group of genotypes (Rehfeldt 1990). In seed zone delineation, the genotypes are derived from seed collected from indigenous trees that have specific locations. In breeding zone delineation, the genotypes are members of a breeding population, which do not have a specific location of origin after crossing begins in advanced generations. Within the optimal breeding zone, the genotype by environment interaction would be zero. That is, the genotypes would have the same relative performance everywhere. In the real world, however, breeding zones are delineated by balancing the size of the genotype by environment interaction against financial and other practical considerations.

Discrete zones are usually circumscribed geographic areas (i.e., "circles on a map"), but could consist of a collection of non-contiguous sites that have similar environmental characteristics (Rehfeldt 1990; O'Neill and Aitken 2004). Seed transfer guidelines are alternatives to discrete seed zones that specify how far a seedlot can be safely moved from its native location to alternative planting sites, essentially defining a separate seed zone for each wild stand (i.e., indigenous breeding population; Campbell 1974a, 1974b, 1986). Furthermore, seed transfer guidelines are continuous because a certain level of risk can be associated with whatever transfer distance is contemplated. In contrast to seed zones (which are almost unlimited in number), the number of advanced-generation breeding populations will always be modest. Therefore, discrete breeding zones are the rule. Nonetheless, the discrete nature of breeding zones is still a biological simplification because materials from multiple breeding populations may do equally well on some sites, performance and risk vary continuously both within and among breeding zones, and the relative performance of genotypes varies among sites within a zone (i.e., genotype by environment interaction is not zero).

An objective way to delineate breeding zones is to (1) estimate the genotype by environment interaction among all sites within the region of interest, (2) decide on the maximum interaction to be allowed within a breeding zone (or the desired number of zones), and then (3) group the sites according to this criterion. Two vastly different approaches have been used to delineate breeding zones: *direct* approaches based on long-term field tests of breeding materials, and *indirect* approaches based on seedling tests of natural populations (i.e., genecological tests).

The most direct way to delineate breeding zones is to plant long-term field tests over a large number of diverse sites, and then use this information to identify groups of genotypes and sites that have an acceptably low genotype by environment interaction. Using this approach, breeding zones and breeding populations are determined simultaneously, and there are no a priori assumptions about which genotypes constitute an appropriate breeding population, or which sites constitute an appropriate breeding zone. Two things are needed: (1) a measure of genotype by environment interaction to judge the suitability of the breeding zones, and (2) a method for grouping the sites. Direct measures of genotype by environment interaction commonly include interaction variances from analyses of variance (Stonecypher et al. 1996) and among-site genetic correlations (Johnson 1997). Sites with a low interaction variance or high among-site genetic correlation would be preferentially included within the same breeding zone. Methods used to group the sites include subjective approaches, value maximization, and various clustering techniques (Roberds and Namkoong 1989; Roberds et al. 1990; O'Neill and Aitken 2004).

This direct approach is empirical and involves few assumptions. The main disadvantages are the high cost of field tests, difficulty in allocating untested sites to breeding zones, and the time it takes to obtain the long-term data needed to delineate zones with sufficient confidence. Furthermore, because the trees only experience a small sample of yearly environments, the results describe past growth, and may not predict future performance. The best information for delineating breeding zones in Washington and Oregon comes from a set of widely distributed genetic tests planted by Weyerhaeuser (Stonecypher et al. 1996). Data from the first-generation Progressive Program have also been used to address breeding zone questions (Silen and Mandel 1983; Johnson 1997; Balduman et al. 1999), but these analyses only included families that were planted within their first-generation breeding zones, so conclusions are limited.

In the indirect approach, seedling genecological studies are used to delineate breeding zones (or seed zones). Open-pollinated families are collected from wild trees, tested in common garden studies, and the magnitude of genetic differences among populations is used to delineate breeding zones (e.g., O'Neill and Aitken 2004). In essence, the "genetic distance" between two populations in a seedling test is used as a surrogate for the genotype by environment interaction measured in long-term field tests. If two populations perform very differently in a common garden test (i.e., if they have a large genetic distance), then we assume that there would be a large genotype by environment interaction between genetic tests planted at the locations from which the seeds were collected. Differences in multivariate population means for quantitative traits (O'Neill and Aitken 2004) and allozyme markers (Westfall and Conkle 1992) have been used in indirect tests to delineate breeding zones.

The indirect approach is based on some important assumptions. The main assumptions are that the (1) adaptability and productivity of local populations are nearly optimal, (2) genetic variation in the traits that are measured results from natural selection, (3) observed patterns of genetic variation reflect underlying differences in the environment, and (4) traits measured are relevant to breeding programs (Campbell 1986; Rehfeldt 1990; Morgenstern 1996). If each of these is true, then one might expect that the genetic differences measured in genecological tests would be highly correlated with the level of genotype by environment interaction that would be measured in field tests (the key criterion for delineating breeding zones). This final relationship also requires that trees respond to plantation environments and natural environments in the same way, levels of genetic resolution in seedling common-garden trials are relevant to field conditions, and select breeding populations have the same patterns of genotype by environment interaction as do natural populations. This last assumption is unlikely because stability probably increases when genotypes are chosen based on their performance across many sites (Stonecypher et al. 1996). Furthermore, breeding for stability has been proposed as a good way to increase breeding zone size (Rehfeldt 1990).

Using the indirect approach, the biggest challenge is how to determine the appropriate sizes of the breeding zones (or seed zones). Campbell developed a measure called "relative risk" that can be used to estimate the relative (but not absolute) risk of seed transfer and delineate seed zones. Relative risk is the degree of non-overlap between the frequency distributions of additive genotypes for two populations (Campbell 1986). Rehfeldt (1990) used least-significant-differences (LSDs) among population means, which he suggested should be less than 0.20 (i.e., an 80% chance of detecting a genetic difference). O'Neill and Aitken (2004) used cluster analysis to minimize the sum of squared differences among population means, but breeding zone size was ultimately determined by deciding how many zones to use. Other approaches used to delineate seed zones could also be used (e.g., differential systematics coefficient; DSC) (Parker 2000).

Despite the many assumptions involved, the indirect approach has four main advantages. First, the data needed to delineate breeding zones can be acquired quickly. Second, the resulting breeding zones are likely to be conservative. Third, the number of sites (i.e., parent tree locations) that can be evaluated is enormous. A recent genecological study of Douglas-fir in Oregon and Washington, for example, included more than 1,300 families (J. B. St.Clair, pers. comm.). Fourth, the long-term adaptability of local populations is presumably guaranteed, although optimal productivity may not be.

The biggest disadvantage of the indirect approach is that the relationship between the genetic distance measured in indirect tests and the genotype by environment interactions measured in long-term field tests is unknown. With field data, we can directly explore how genetic gains and financial returns change under different breeding zone scenarios, but this is not possible using indirect approaches. Instead, breeding zone size will be determined using strictly theoretical or arbitrary criteria. Other disadvantages include the fact that mature phenotypes are not measured and trees are never tested in the environments where they will be planted, so traits such as disease resistance may be ignored.

Questions surrounding the appropriate size of breeding zones persist. Although some information is available from widely planted tests (Stonecypher et al. 1996), the data needed to construct optimal breeding zones via direct methods does not exist for most programs. Therefore, breeders must balance information from imperfect field tests with indirect tests that sample many parts of the region intensively. Genecological approaches are excellent for identifying the most important environmental gradients to consider when breeding zones are defined. Elevational and east-west distances, for example, should usually be given greater consideration then north-south distances. Genecological approaches are also valuable for identifying which sites are more or less similar to one another. Similar environments recur across the landscape and can be identified using genecological modeling (Rehfeldt 1990). Therefore, it is now feasible to develop fine-scaled, non-contiguous breeding zones using genecological analyses, geographic information systems (GIS), and advanced climate models (O'Neill and Aitken 2004). Optimally, sets of similar environments would be grouped into breeding zones based on data from long-term field tests. Genecological approaches are poor for predicting how genotypes will perform in long-term field tests and for deciding how large breeding zones should be. Indirect approaches would be more valuable if we knew the relationship between genetic distances measured in genecological tests and genotype by environment interactions measured in long-term field tests. Finally, no matter how breeding zones are delineated, they should be validated and refined by planting genotypes well beyond current breeding zone boundaries. There are no alternatives to long-term tests that intensively sample environmental heterogeneity (Rehfeldt 1990). Without this, breeding zones are likely to be overly conservative and breeding programs are likely to be much more costly than they need to be.

C. Tree Breeding Populations

Different types of populations are used in tree breeding, including gene resource, inter-situ, breeding, and production populations (Fig. 6.3; Burdon 1988; Johnson 1998a; Yanchuk 2001a). The size and makeup of these populations are important elements of all Douglas-fir breeding programs.

The *gene resource population*, which is the population of individuals that could be included in current or future breeding populations, includes trees in the production, breeding, and inter-situ populations, as well as trees in the wild (i.e., in situ populations; Fig. 6.3). Because the entire species (and even closely related species) can be considered the gene resource population, this population is shared among all breeding populations. To maintain adaptability, however, most of the alleles for a particular breeding program will come from a limited geographical or ecological region. Furthermore, the use of trees from wild stands will be rare in advanced generations, and most infusions of genetic material will come from the inter-situ populations described below. Because the gene resource population is the foundation of all genetic improvement, its conservation is critical (Yanchuk 2001a; Lipow et al. 2004). The gene resource population should be large enough to maintain low-frequency alleles that could be valuable for meeting current and future breeding objectives. Because breeding programs are large, and because Douglas-fir is a major component of western forests, Douglas-fir has a vast gene resource population that contains large stores of genetic variation.

The *inter-situ population* is the population of individuals in progeny tests, archives, and clone banks that are not included in the breeding or production populations (Fig. 6.3; Yanchuk 2001a). For the most part, each breeding population can be considered to have its own inter-situ population, which is usually the population from which the breeding population was derived. The trees in the inter-situ population provide a link between the gene resource and breeding populations—one or both parents are known and the trees will often have an intermediate level of genetic improvement. Because of the large first-generation selection and testing programs, most inter-situ populations have tens of thousands of individuals.

The *breeding population* is the group of parents that will be used to produce offspring for the next cycle of selection (Fig. 6.3). The breeding population is associated with a particular breeding zone, which is a geographical or ecological area in which the individuals of the breeding population are expected to perform well for the traits of interest. The breeding population is usually evaluated by establishing field tests within its corresponding breeding zone. The size of the breeding population is an important factor to consider in long-term breeding. In long-term, recurrent selection programs, breeders often choose a population size that they intend to maintain over many generations. In Douglas-fir breeding, these stable population sizes have not yet been met in most programs (Table 6.4). High selection intensities are being used on very large first-generation populations to dramatically reduce the size of the first-generation breeding populations. In advanced generations, the sizes of the breeding populations are likely to be even smaller than they are in the second generation. If the breeding population is too small, genetic variation will be lost to genetic drift, and inbreeding will become a problem, but if it is too large, the breeding and testing program will be prohibitively expensive. Based on these considerations, Yanchuk (2001a) concluded that an effective population size (N_e) of only 20 to 80 is sufficient to maintain genetic variation, avoid inbreeding, and allow for multiple-trait breeding. White (1992) and Johnson et al. (2001) reviewed the literature and concluded that an N_e of 20 to 50 can sustain several generations of breeding. These are effective population sizes, however, not the actual number of individuals. For Douglas-fir, Johnson (1998a) recommended that the census number of individuals (N) should be 150–200 to maintain genetic diversity and gain for 10 generations (if inbreeding is managed effectively). More individuals may be needed if new traits become important in the future, especially if those traits are rare (Johnson et al. 2001). In contrast, Yanchuk (2001a) suggested that a breeding population of about 80 individuals would contain adequate amounts of quantitative genetic variation. Over many generations, the N_e of the breeding population should be monitored and unrelated genotypes should be infused as necessary (Yanchuk 2001a). Despite these theoretical considerations, operational programs tend to have breeding populations of 300–400 individuals (White 1992; Johnson et al. 2001). Within the NWTIC program, for example, most second-generation breeding populations have about 300 individuals to minimize inbreeding in sublines and seed orchards, and to allow the option of reorganizing them into multiple breeding populations in the future.

Breeding populations may be divided into subpopulations to manage inbreeding, conserve genetic variation, and increase genetic gains by focusing breeding efforts on the very best selections. The advantages and disadvantages of various subpopulation approaches were reviewed by Johnson (1998a). *Multiple breeding populations* with different breeding objectives may be used to maintain genetic variation for a diversity of traits, thereby maintaining the flexibility to change breeding objectives in the future (Namkoong 1976). Viewed as a whole, Douglas-fir has multiple breeding populations that are being bred for optimal performance in different environments (i.e., breeding zones). In *stratified breeding populations*, or *nucleus breeding*, the breeding population is stratified into an elite population and a main population (Cotterill et al. 1988; Cotterill 1989). Most of the breeding effort is focused on the elite population (nucleus) to maximize genetic gain, and less effort is devoted to the main population, which mainly serves as an inter-situ source of genetic variation. *Sublines* are unrelated subsets of the breeding population that have a common breeding objective (i.e., unlike multiple populations). Sublines are used to manage inbreeding—breeding occurs within, but not between sublines, and the trees that are deployed are derived from crossing between sublines. Although inbreeding will develop in the sublines, it does not occur in the trees that are deployed. Sublines with only a small number of individuals are problematic because inbreeding will develop quickly and it is difficult to use very high selection intensities to achieve high genetic gains. If improved seed were produced in wind-pollinated seed orchards, the number of sublines should be as large as the number of orchard clones (e.g., at least 20 to 25). This will add substantial complexity to the breeding program. In contrast, if trees are deployed via control-pollinated seed orchards or vegetative propagation, then only two sublines would be needed to avoid inbreeding. The alternative approach for avoiding inbreeding is to carefully monitor and restrict the relatedness of crosses made in the breeding population.

The *production population* is a subset of the best individuals from the breeding population that is used to produce propagules for reforestation (Fig. 6.3). In Douglas-fir, the production population usually consists of individuals that have been grafted into seed orchards and managed to produce high quantities of open-pollinated or control-crossed seed. In other cases, the production population consists of young seedlings from elite crosses that are used to produce rooted cuttings for outplanting. In the future, the production population may consist of tissue cultures that are used to produce somatic embryos for clonal deployment. Propagules from the production population may be outplanted to the entire breeding zone, or to restricted deployment zones. Although most orchard seed is currently planted throughout its breeding zone, controlled crosses are sometimes made between the very best parents, and the resulting trees may be outplanted on only the very best sites (e.g., "high-site" deployment zone). In most cases, however, the breeding and deployment zones are the same. The size of the production population is an important consideration. Wind-pollinated seed orchards should probably contain at least 20 to 25 clones to ensure that the effective population size of the seed crop is 10 or greater (Johnson 1998a).

D. Mating Designs—Theoretical Considerations

Mating designs have two main functions. The first function is to provide *information* (e.g., breeding values, genotypic values, genetic and environmental variances) on which to select the best parents or families, and to predict genetic gains. The second function is to *create new genotypes* for the next round of testing and selection. Stonecypher et al. (1996) referred to the first function as the "progeny test function," and the second as the "recurrent selection function." Half-sib mating designs, which include open-pollinated and polycross (polymix) designs, are generally good for fulfilling the progeny test function, whereas full-sib designs, including single-pair, diallel, and factorial designs, are generally better for meeting the recurrent selection function. Because no mating design is optimal for both functions, half-sib and full-sib designs are often used together in a complementary fashion (Burdon and Shelbourne 1971).

Five main types of mating designs have been used in Douglas-fir breeding: (1) open-pollinated families, (2) polycross families, (3) unstructured full-sibs (e.g., single-pair or double-pair matings), (4) structured full-sibs (e.g., diallels and factorials), and (5) complementary designs.

1. Open-Pollinated Mating Design. Open-pollinated, or wind-pollinated, families are often used to estimate breeding values of trees in wild stands or seed orchards. Although we refer to open-pollinated families as "half-sibs," they actually consist of a mixture of half-sibs, full-sibs, and a small percentage of selfs. The main advantage of using open-pollinated families is that they do not require controlled crosses, which are particularly difficult and expensive to do on large, widely distributed trees in the wild. Open-pollinated families are good for estimating breeding values, although differences in the makeup of the pollen pool results in some bias. Furthermore, because trees vary greatly in the proportion of selfed seed they produce, differences in selfing can affect family performance in open-pollinated genetic tests (Sorensen 1973; Sorensen and White 1988). Open-pollinated families are not good for generating new genotypes for recurrent selection because the male parent is unknown.

2. Polycross Mating Design. Polycross families are usually created by crossing a seed (assigned female) parent with a mix of pollen from a number of pollen (assigned male) "testers." Although a single male can be crossed with a number of female testers, this approach is more complex and time consuming. Polycross families are better than open-pollinated families for estimating breeding values because each female is crossed with the same pollen pool. They are similar to open-pollinated families in that the male parents of the resulting progeny are usually unknown. For this reason, they are rarely used to generate new genotypes for recurrent selection. This situation may change, however, now that highly variable genetic markers are available for Douglas-fir and other tree species (Slavov et al. 2004). Using SSR (simple sequence repeat) markers, for example, it may be possible to test polycross families in the field, make provisional forward selections based on the combined performance of the maternal family and individual tree, determine the male parent of the provisional selections using genetic markers, then make final selections by considering the performance of both the male and female parent (Lambeth et al. 2001). Using this approach, the number of parental combinations that are tested in the field is much larger than it is using full-sib crosses. Therefore, one should have a greater chance of finding offspring that result from crosses between the very best parents. Nonetheless, this approach remains untested and may not work well for subline breeding in advanced generations (i.e., once the genotypes in the subline become related). In subline breeding, the testers should come from outside of the subline so that the parents are evaluated using outcrossed progeny, rather than closely related testers. If the testers are not in the subline, however, the polycross progeny will not be suitable for recurrent selection because the selections will not conform to the subline structure. Furthermore, relatedness among the trees in the subline may eventually hinder one's ability to determine the paternal parent using genetic markers (Lambeth et al. 2001).

In addition to providing good estimates of parental breeding values, polycross designs can be used to select which full-sib families to use for making forward selections (i.e., by calculating mid-parent values). Even when dominance is present, polycrosses are nearly as effective as full-sib families for estimating the performance of full-sib crosses (Johnson 1998a).

3. Unstructured Full-Sib Mating Designs. Unstructured full-sib families result from crosses among parents that are not organized according to a structured mating design such as a factorial or diallel (discussed below). In forest trees, most unstructured full-sib designs include each parent in only one or a few crosses (e.g., single-pair or double-pair matings in which each parent is used in one or two crosses). Single-pair or double-pair mating designs are often used because they generate new, pedigreed genotypes for recurrent selection, and are both easy and flexible. The choice of male and female parents is often opportunistic (e.g., based on which trees are flowering), rather than being pre-planned. Their main advantage is that crossing programs can be completed quickly, thereby maximizing genetic gain per year. In particular, the limited availability of male or female flowers is much less of a constraint than it is for structured full-sib designs. The parents can be mated randomly, or based on either assortative or nucleus mating, in which the better parents are used in more crosses (Johnson 1998a). The main drawback to single-pair and double-pair matings is that they are not as good for estimating the general combining ability (GCA) of the parents or the specific combining ability (SCA) of the cross (because each parent is used in only a few crosses). Nonetheless, Johnson (1998b) used simulations to conclude that gains from backwards selection for GCA increase very little when more than two or three crosses are used per parent. Even with substantial non-additive genetic variation (e.g., simulations in which the dominance and additive variation were assumed to be equal), three crosses per parent were enough to estimate breeding values and select parents reasonably well (Johnson 1998b).

4. Structured Full-Sib Mating Designs. Structured full-sib designs, such as diallels or factorials, can also be used to produce new pedigreed genotypes for selection, but are better than the unstructured designs for estimating GCA and SCA. Instead of complete designs, partial disconnected diallels and disconnected factorials are typically used in forest trees because large, complete designs are usually not practical. Using these designs, breeding values can be estimated that are mostly free of non-additive genetic effects and new genotypes can be selected that have known male and female parents (Stonecypher et al. 1996). Although estimates of GCA and SCA may be biased by epistasis, dominance, and linkage disequilibrium, these effects should be small and not a serious problem in applied breeding programs (Stonecypher et al. 1996; Yanchuk 1996). The main disadvantages of full-sib designs are their complexity and the time they take to complete. Insufficient flowering, for example, usually makes it impossible to complete all crosses in a single year. Furthermore, for designs with many crosses per parent, many full-sib families will be produced, but relatively few unrelated progeny.

5. Complementary Mating Designs. Because single mating designs are not optimal for meeting all breeding objectives, a combination of complementary designs may be desirable (Burdon and Shelbourne 1971). The most common complementary approach is to combine open-pollinated or polycross families with unstructured full-sib families (Cotterill and Jackson 1989). The main advantage of this approach is that multiple objectives can be met without using the time-consuming and complex factorial or diallel designs. Complementary approaches combine the advantages of each mating design—the ability to estimate breeding values using open-pollinated or polycross families, and the production of new pedigreed genotypes using unstructured full-sibs. The main disadvantage (i.e., compared to using unstructured full-sib families alone) is the added cost of creating and testing the open-pollinated or polycross families. Assuming that open-pollinated seed orchards are used, Cotterill and Jackson (1989) predicted that gains would be greatest using half-diallels, a little less using a complementary combination of open-pollinated families and single-pair matings, and lowest using single-pair matings alone. They emphasized that the complementary design would probably produce the greatest gains per year. Furthermore, the choice of design depends on financial considerations and economic assumptions, as well as how the mating design affects field testing (discussed in Johnson 1998a).

E. Field Test Designs—Theoretical Considerations

Field tests are used to meet many objectives. Most importantly, they are used to estimate the genetic worth of individual trees and families. Depending on the method of deployment, field tests should focus on accurately estimating breeding values, genotypic values, or the mean performance of specific crosses. When field tests are used to identify the best families (e.g., to select the best parents based on progeny tests), they should be designed to accurately assess family performance across a range of sites. In contrast, when field tests are used for within-family selection, they should be designed to facilitate the direct comparison of trees from the same family. This can be done using large, unreplicated family blocks, or by clonally replicating each genotype and testing them in a replicated field design. The mating design used may influence the choice of field design, particularly in advanced generations. When a complementary design is used, for example, it may be unnecessary to test the full-sib families in replicated field tests unless the goal is to capitalize on SCA. By planting the families in unreplicated family blocks, within-family selection may be easier and more effective than when members of a family are scattered among plantations and blocks. A detailed discussion of the interactions between mating designs and field tests was given by Johnson (1998a). Field tests are also used to estimate genetic and environmental variances, genetic correlations, heritabilities, and genetic gains. These objectives are usually secondary, however, because this information already exists from many past studies. Finally, field tests are used to characterize genotype by environment interactions and provide information that can be used to refine breeding zone boundaries.

Field designs for forest trees must address three main challenges: (1) substantial environmental heterogeneity within and among planting sites, (2) large numbers of genotypes to be tested, and (3) mortality. These challenges are usually met by establishing tests across a range of sites, keeping the blocks (replications) small, and planting enough trees per genotype that reasonable amounts of mortality can be tolerated.

Field tests must be established on multiple sites that represent the range of environments in the deployment zone. This is important for evaluating the extent of genotype by environment interactions and the stability of specific genotypes. It is also helpful to establish tests beyond the expected deployment zones so that breeding zone boundaries can be refined. By comparing predicted gains under different test scenarios, Johnson (1998a) concluded that four sites should be adequate for evaluating the height growth of families in an existing breeding zone. If only three sites provide usable data (i.e., if one site is lost), the expected gain is still 85% of what is expected from using six sites. The number of sites needed would increase when new breeding zone boundaries are being evaluated. Based on empirical data and theoretical considerations, Cotterill and James (1984) concluded that 10 to 20 trees should be tested per family, depending on the heritability of the trait. This number is a balance between providing enough trees to estimate family means, and enough families to practice among-family selection. In programs that seek to characterize genotype by environment interactions and evaluate breeding zone boundaries, each site must be able to stand alone. Therefore, 10 to 20 trees are needed per family on each site. Because of mortality, at least 20 trees should be planted per site to ensure that 10 to 20 healthy trees are available when the tests are measured in later years.

F. First-Generation Strategies

1. First-Generation Breeding Zones. Because western North America is mountainous and environmentally heterogeneous, the first-generation breeding zones were small. In the southeastern U.S., for example, breeding zones are much larger because of greater environmental homogeneity and less pronounced local adaptation. Most breeding zones in Oregon and Washington were established as part of the Progressive Program based on the assumption that local populations are best, and genotypes are adapted to a narrow range of environmental conditions. Compared to other programs, the number of first-generation breeding zones (109) was huge. Because information on patterns of genetic variation was "fragmentary," breeding zones were based on geographical, ecological, climatic, and land ownership patterns, rather than on genetic data (Silen 1966b). Although small breeding zones ensured adaptability, they also ensured that regional Douglas-fir breeding would be very expensive. First-generation zones were defined as ecologically similar units having a size less than 60,700 ha (150,000 acres) and an elevational range less than 304 m (1,000 ft), breeding populations were selected from wild stands within these zones, and the breeding populations were tested within these same zones (Silen and Wheat 1979). Because the breeding zones and populations were chosen a priori, different breeding populations were not systematically tested in the same experiment or outside their zone of origin, although over 1,400 families were included in tests in other (mostly adjacent) breeding zones.

The Weyerhaeuser program covers a much smaller and less heterogeneous land base than the other programs—about 0.9 million ha (2.2

million ac.) in western Washington and Oregon in 1996 (Stonecypher et al. 1996). Compared to the Progressive Program, Weyerhaeuser used larger breeding zones, resulting in only 12 first-generation breeding zones—six geographical areas with two elevational zones in each area. Their advanced-generation breeding program concentrates on the six low-elevation zones (≤ 610 m) (Stonecypher et al. 1996; Table 6.4). In contrast to the Progressive Program, Weyerhaeuser undertook a wide testing program to test their first-generation breeding zones.

Breeding zones for Rocky Mountain Douglas-fir were delineated based on genecological studies (Rehfeldt 1979a, 1982, 1983a). The IETIC established 13 first-generation breeding zones consisting of seven large regional zones in eastern Washington, northern Idaho, and western Montana, with elevational bands within each regional zone. The BCMoF interior program used results from Rehfeldt's genecological studies and biogeoclimatic classifications to establish eight first-generation breeding zones, with separate first-generation testing programs within each zone (Woods 1993). The original breeding zones for the coastal BCMoF program were based on existing seed zones, but were later merged into two large first-generation zones—a low-elevation maritime zone and a zone covering the transition area between the coast and interior (Woods 1993). The decision to expand the breeding zones was based on results from provenance tests indicating that genetic differences among coastal provenances were not strong and maladaptation was not a problem (Woods 1993).

2. Selection of First-Generation Parents. Once first-generation breeding zones were delineated, parents were selected for the first-generation breeding populations. Each North American program decided to focus on selecting trees from wild stands within their respective breeding zones. That is, no program relied solely on exotic seed sources, although parents from western Washington were tested in the coastal BCMoF program. The methods used to select the parents ranged from non-intensive, mostly random selection of trees, to intensive methods of phenotypic selection in the field. In all cases, the selected parents were widely separated to avoid selecting related trees, and efforts were made to sample the entire breeding zone (i.e., all potential reforestation environments).

The Progressive Program (now NWTIC) emphasized low-intensity, "roadside" selections and immediately began testing the parents using open-pollinated seed collected from the trees in the wild. This approach was taken because intensive phenotypic (mass) selection was judged to be ineffective and perhaps risky (Silen 1966b), and rapid progress was important. This approach was designed to be a quick, low-cost way of initiating tree improvement on a large scale. Rather than relying on phenotypic selection in the field, the main objective was to quickly select about 3 trees per 405 ha (1,000 acres) of forestland, then test the parents using open-pollinated progeny tests. Phenotypic selection was not used because the trees were selected from natural stands that had a great deal of variability in microsite and stand density. The only selection criteria were that dominant, well-formed trees with cones were chosen. A novel aspect of the Progressive Program was that crossing was done on field selections to produce full-sib families for seedling seed orchards.

In contrast to the Progressive approach, other North American programs used intensive phenotypic selection in the field. Selections were typically chosen based on the growth rate of the tree relative to its neighbors, and scions (stem cuttings) were grafted into seed orchards or clone banks where full-sib families were produced for progeny testing. The latter approach delayed progeny testing, but was designed to achieve quick gains from mass selection in the field. Based on the assumption that heritabilities would be relatively high in even-aged stands of Douglas-fir, the BCMoF coastal program (Alan Orr-Ewing) began making intensive phenotypic selections in 1957 (Heaman 1977; Yeh and Heaman 1987). These plus-trees were grafted into clonal seed orchards and breeding arboreta, and became available for crossing in the early 1970s (J. C. Heaman, pers. comm.).

The IETIC used a comparison-tree approach based on relative diameter growth and seed availability to make their field selections (Fins 1983). Weyerhaeuser used a similar comparison-tree approach that emphasized diameter growth per unit of growing space, but also considered stem form and crown quality (Stonecypher et al. 1996). The selected plus-trees were grafted into seed orchards and clone banks in the 1960s, and a backup orchard was established in 1973. Stonecypher et al. (1996) reported that plus-tree selection by Weyerhaeuser resulted in a 4.5% improvement in juvenile height compared to the unselected controls, which could translate into a 9% gain in height for seedlings derived from a clonal seed orchard of the selected parents (i.e., in the progeny of superior × superior parents).

The first phase of the European and New Zealand programs was to find the best provenance via provenance testing. These programs are now in their second phase—selecting the best parents from the best provenances. In New Zealand, analyses of 13-year-old provenance tests demonstrated that coastal seed sources from southern Oregon and northern California grow the fastest (R. L. Knowles, pers. comm.). Earlier breeding efforts that focused on Washington seed sources were de-emphasized, and trees from the fog-belt of southern Oregon and northern California were chosen to form most of their breeding population. Focusing on these provenances, about 400 trees were recently selected from (1) commercial plantations of known origin, (2) older genetic tests, (3) clonal archives, and (4) recent open-pollinated progeny tests derived from new seed collections in North America (R. L. Knowles, pers. comm.). As in New Zealand, forward selections from provenance-progeny tests will play a major role in Douglas-fir breeding in Europe. The large network of IUFRO provenance-progeny trials in Europe will provide a diverse genetic base for advanced-generation breeding.

3. Mating Designs in the First Generation. Each of the North American programs included some open-pollinated families in their testing program (Table 6.4). The less intensive IETIC and interior B.C. programs relied solely on open-pollinated tests, but the larger breeding programs (i.e., NWTIC, BCMoF coastal program, and Weyerhaeuser) used additional mating designs to meet some of their objectives.

In addition to open-pollinated families, the Progressive Program used single-pair matings to generate full-sib families to plant in seedling seed orchards. Their plan was to quickly make roadside selections, test the parents using open-pollinated progeny tests, cross the selected parents using single-pair matings, establish full-sib seedling seed orchards, then periodically rogue the orchards as increasingly reliable information became available from the open-pollinated tests (Silen and Wheat 1979; Silen and Wanek 1986). Seedling seed orchards were used to circumvent the problem of graft incompatibility, which was a serious problem in the clonal orchards that were about eight years old at the time.

In the early 1970s, the BCMoF began making crosses in their breeding arboreta. Although they began using a factorial (NC II) design and single-pair matings, they quickly switched to half-diallels because this design can be used to meet both primary objectives (J. C. Heaman, pers. comm.). The current foundation of the BCMoF coastal program is a large and ambitious series of half-diallels that were planted over many years and planting sites. Their basic design is a 6-tree half-diallel that consists of 15 crosses (i.e., no selfs and no reciprocal crosses; Heaman 1977; Woods 1993). From 1976 to 1986, 62 disconnected, half-diallels were planted on 88 test sites. The diallels were grouped into eight series, and each series was tested on 11 sites (Heaman 1977; Yanchuk 1996). Some diallels were included in more than one series to facilitate among-series comparisons. This core program was augmented by other designs. Open-pollinated families and polycrosses were used to test other parents from western Washington and British Columbia, factorial designs were used to evaluate wide crosses, and selfing was used in an attempt to develop highly inbred lines for outcrossing (Heaman 1977; Woods 1993). In contrast to the coastal program, the less intensive interior program relied solely on open-pollinated families to estimate breeding values.

Weyerhaeuser began making crosses in their grafted seed orchards in the late 1960s (C. A. Dean, pers. comm.). They began testing open-pollinated families, polycrosses, and single-pair matings, but switched to 6-parent diallels in 1980 (Woods 1993; Stonecypher et al. 1996). Their first-generation field tests were planted from 1969 to 1985 (C. A. Dean, pers. comm.; Stonecypher et al. 1996).

The New Zealand program began using a polycross design to estimate breeding values and full-sib crosses to produce new genotypes for the next round of selection. This approach, however, was unsuccessful because not enough seed could be produced in their clonal archives. Therefore, a revised breeding plan was developed that uses open-pollinated seed to meet both of these objectives (R. L. Knowles, pers. comm.).

4. Field Designs in the First Generation. Virtually all first-generation tests were established as randomized, complete block designs that were planted across multiple sites. To reduce block size in the NWTIC tests, large numbers of families were grouped into smaller "sets" of about 30 families each, and the sets were planted in balanced, complete block designs. The families were assigned to these sets randomly, or by grouping them according to geography (i.e., parent tree location). The most common design in the NWTIC program was the "reps-in-sets" design in which the blocks (or replications) belonging to each set were grouped together. Because the blocks were planted adjacent to one another on a site, each set was essentially a separate experiment. In the less common "sets-in-reps" design, the sets were randomized within blocks, which makes it easier to compare families in different sets. For both designs, a typical NWTIC test might consist of 150 to 300 families planted in 3 to 4 blocks of 4-tree noncontiguous plots at each of 8 to 10 sites. On average, 12 to 16 trees per family were planted at each site, and a total of 96 to 120 trees per family were tested in each experiment.

The BCMoF coastal program tested a large number of half-diallels by allocating them to eight series that were planted over a 10-year period. In most cases, each series had about 150 families planted on 11 sites using 4 blocks of 4-tree row-plots, and the families were fully randomized within each block (i.e., the families within a diallel were not planted adjacent to one another; Fu et al. 1999). In some of the early tests, 2 blocks of 9-tree row plots were used (Yeh and Heaman 1987).

The IETIC planted their first progeny tests in 1982, usually using a "sets-in-reps" design. Families were randomly assigned to sets, and three test sites were established per elevation band in most zones. At each site, each family was planted in 4 blocks using a randomized, 9-tree, non-contiguous plot design. In some tests, the fourth block was planted using 9-tree family block-plots, rather than in non-contiguous plots.

The basic Weyerhaeuser field design consisted of a randomized complete block design with 8 blocks of 4-tree, non-contiguous plots. The main exception was that 8-tree plots were used for a common set of non-selected "control" seedlots that were planted at each site within a region. In 1977, they switched to using an interlocking block design that allows trees to be thinned while still maintaining an equal representation of trees per family. Other field designs that included as many as 151 families and 20 test sites were used to study genotype by environment interactions (Stonecypher et al. 1996).

The intensity of site preparation for field tests varies from complete stump removal and intensive weed control to less intensive management. Fencing is essential to protect seedlings from the deer, elk, rabbits, hares, porcupines, and mountain beaver. Fencing costs are a major financial burden for field testing programs. Measurements in long-term field tests are typically made every three to five years, and often include stem height and diameter, ramicorn branches, forks, and stem sinuosity. The presence of root rot and needle cast disease has been measured in some tests where these diseases are prevalent.

Short-term, "farm-field" tests and adaptability tests have been used to a limited extent. The IETIC established short-term trials on mild agricultural sites. These tests were designed for a final measurement three to four years after planting. In some tests, height was measured periodically during the growing season to determine the timing of height growth cessation (i.e., date of bud set). These data were used as an indirect measure of fall frost hardiness.

G. Second-Generation Strategies

Methods of advanced-generation breeding and testing are usually different from those used in the first generation. Breeding zone boundaries have been changed, open-pollinated mating designs are rarely used, selection at the family level is lower, within-family selection becomes more important, and field test designs evolve as data and experience accumulate.

1. Second-Generation Breeding Zones. Most of the second-generation programs are increasing the size of their breeding zones because information now suggests that the first-generation zones were unnecessarily small. Changes include everything from a dramatic reduction (NWTIC) to no decrease in the number of breeding zones from the first to second generation (coastal program, BCMoF).

Based on a review of the literature, Randall (1996) and Randall and Berrang (2002) concluded that seed zones could be expanded in a north-south direction, which suggested that the sizes of the breeding zones could be increased as well. At the same time, Weyerhaeuser demonstrated that a substantial number of their families perform well on mild, low-elevation sites across large areas (Stonecypher et al. 1996). Based on tests designed to measure genotype by environment interactions, Stonecypher et al. (1996) concluded that it is doubtful whether separate breeding zones are needed for Weyerhaeuser lands within Oregon and Washington. Instead, they suggested that improved families could be deployed on their low-elevation lands based on parental performance and stability, rather than on their first-generation breeding zones.

NWTIC field tests were also used to examine first-generation breeding zones. Johnson (1997) analyzed genetic correlations among field tests within six breeding zones, and concluded that the first-generation zones were not too large. Balduman et al. (1999) analyzed the relationship between parent tree location and cold hardiness traits in two breeding zones and came to the same conclusion. Because different first-generation breeding populations were not systematically tested in the same experiment or outside their zones of origin, these studies are mostly suitable for determining how much smaller the first-generation zones should be, rather than how much larger. Nonetheless, because some families were planted outside their zone of origin, there is some potential for examining the consequences of larger breeding zones in the future. In contrast to the results discussed above, Campbell (1992) detected significant genotype by environment interactions among field tests within several breeding zones in Oregon. Stonecypher et al. (1996) also found significant genotype by environment interactions within Weyerhaeuser breeding zones, but less than 20% of the families contributed appreciably to the interaction, and there was no relationship between family height rank and stability.

Although most results from these direct field tests suggest that larger breeding zones are appropriate, these larger zones are provisional and should be validated because none of these studies used rotation-aged trees. In contrast to the results from direct field tests, much of the genecological research that was completed from the mid-1970s until the early 1990s emphasized the importance of local adaptation. Although these indirect studies were sometimes used to justify small breeding zones, the results from long-term field tests that suggested otherwise could not be ignored.

The NWTIC merged about half of their first-generation zones (109) into eight new zones for the second generation (Table 6.4), and will focus on identifying broadly adapted genotypes in future generations. The remaining, mostly high-elevation zones and zones comprising federal lands in southwestern Oregon, were omitted because no second-generation breeding is planned. The lands now covered by the second-generation programs do not extend above 900 m (about 3,000 feet). Because environmental and genetic gradients are less pronounced latitudinally, the new breeding zones are much longer in a north-south direction. Furthermore, because they tend to follow bands of similar elevation, there are no elevational divisions within each zone. Today, there are seven "metacooperatives" that manage breeding programs for these eight zones (a metacooperative is an amalgamation of multiple first-generation cooperatives). Selections from multiple first-generation breeding programs have been combined to form the second-generation breeding population for each new zone (about 300 selections per second-generation breeding population). Furthermore, the field tests for these selections are designed to test the validity of these larger second-generation zones.

Based on their first-generation wide-testing program, Weyerhaeuser reduced their number of low-elevation breeding zones from six to three (one in Washington and two in Oregon; Table 6.4). In Washington, four first-generation breeding zones were consolidated into a single second-generation zone, and the original first-generation breeding populations were organized into four sublines. In Oregon, the two low-elevation breeding zones were retained. The BCMoF is retaining two breeding zones for their coastal program, and the interior program has reduced their breeding zones from eight to five. Because the IETIC has no plans for advanced-generation breeding in Douglas-fir, they will continue to use their first-generation breeding zones, which now stand at thirteen (Table 6.4).

2. Mating Designs in the Second Generation. In the NWTIC program, the parents of the second-generation breeding population include first-generation parents (i.e., "backward selections") and "forward selections" from wind-pollinated progeny tests and full-sib seedling seed orchards. At the parent or family level, a 10% selection intensity is being used, mostly based on age-15 height. Wood density and stem quality are also being considered when this information is available.

Each second-generation breeding population of about 300 parents is subdivided into about 10 sublines of 20 to 40 individuals to manage inbreeding and conserve genetic variation. These sublines are designed to contain individuals that are genetically similar with respect to adaptive traits (e.g., from the same first-generation breeding zone). Other "elite" sublines are also being created that contain the top 10 to 20% of all parents in the second-generation breeding population, but without regard to geographic origin. Within each subline, an unstructured, full-sib family mating design is being used for both the progeny test and recurrent selection functions. Each parent is being crossed with two or three other parents to estimate parental GCA and to permit selection at the family level. If information is needed to rogue a wind-pollinated seed orchard, then three crosses are being used per parent because no complementary GCA tests are planned. Across all second-generation programs, about 2,600 crosses and 95 test plantations are planned. A complementary mating design is not being used because breeding values can be estimated from a small number of full-sib crosses when the parents are unrelated (Johnson 1998a). When inbreeding becomes a problem in advanced generations, a complementary mating design of full-sib crosses and polycrosses may be used. After this strategy was developed, Lambeth et al. (2001) suggested that polycross breeding with paternity analysis would be a good alternative to a complementary mating design. A pilot study of this approach would be valuable for Douglas-fir.

Weyerhaeuser used a somewhat similar approach in their second-generation program. Forward selections from their first-generation progeny tests (i.e., full-sib and half-sib tests) were allocated to sublines within each of three breeding zones, then crossed using a complementary mating design involving test crosses, positive assortative matings, and correctively-mated full-sib families. These mating designs were used to (1) estimate genetic parameters and develop efficient breeding strategies; (2) estimate the breeding values of the parents, full-sib families, and individual progeny; (3) produce new genotypes for the next cycle of selection; and (4) predict genetic gain. The number of crosses for each parent was determined by its relative performance and by genetic diversity targets. The controlled crosses began in 1991, the first field tests were planted in 1994, and the last mainline tests were established in 2003 (C. A. Dean, pers. comm.).

The BCMoF coastal program is using a complementary mating design in their second generation. Forward selections were made in their 6-parent diallel tests and the resulting breeding population was divided into sublines. Crossing began in the late 1990s using about 15 full-sib crosses per subline. In addition, the breeding values of the forward selections are being estimated using polycrosses (10 parents per pollen mix). Field tests for 16 sublines have already been established in the field.

3. Field Designs in the Second Generation. The NWTIC is using two types of field designs in their second-generation breeding program—a design for ranking families and making selections, and another for testing long-term stability. The *family-ranking and selection* tests are designed to give precise estimates of family means and genotype by environment interactions. Most of these tests use an "alpha" design, which is a type of incomplete block design (Patterson and Williams 1976). A smaller number of these tests were established using a sets-in-reps design. The boundaries of both the first-generation and second-generation breeding zones were considered when the test sites were chosen. Overall, five to six test plantations are being established in each new, second-generation breeding zone, with 20 trees per family per site planted in single-tree plots. For the tests that are already in the ground, the number of full-sib crosses ranges from 143 to 283. The trees will probably be measured twice—about seven and 12 years from seed, or when the trees are about 15 and 30 feet tall. Measurements for all NWTIC tests will include height, diameter, stem form, and (perhaps) cold hardiness and the timing of bud flush.

The NWTIC *long-term stability* tests are designed to test adaptability and stability of families over half a rotation (i.e., > 25 years). These tests are being established using a sets-in-reps design (with sets consisting of families from the same subline, or first-generation breeding zone), and interlocking blocks that will leave a balanced number of trees in each family after thinning. In addition to testing long-term stability, these tests will be used for ranking families and making selections.

The field tests used in Weyerhaeuser's second-generation program were designed to test a total of 96 individuals from each parent or full-sib cross across three to four field sites. All sites were intensively prepared, fenced, and maintained to obtain high-quality genetic data. The BCMoF is using two field designs, one for each component of their complementary mating design. The full-sib families are being planted in 25-tree blocks on each of two, intensively prepared sites (i.e., including removal of stumps). The polycrosses are being established on standard forest sites. The full-sib family blocks and GCA tests are being established at the same time.

X. PRODUCTION OF IMPROVED MATERIALS FOR REFORESTATION

A. Introduction

Breeding strategies must mesh with the methods used to produce genetically improved trees for reforestation. Virtually all Douglas-fir breeding programs focus on recurrent selection for general combining ability. This approach is largely dictated by the fact that improved Douglas-firs are almost exclusively produced via seed from wind-pollinated seed orchards. Breeding strategies are likely to change if other production approaches gain favor. Production via rooted cuttings is the preferred method in species that are easy to root, such as *Populus* or redwood (*Sequoia sempervirens*), but rooting is inefficient and costly in Douglas-fir. Other production approaches are biologically possible and used in a few species (e.g., loblolly pine, *Pinus taeda*), but are uncommon in Douglas-fir and most other species. These include controlled mass pollination (CMP), supplemental mass pollination (SMP), and somatic embryogenesis.

B. Seed Orchards

1. Conventional Seed Orchards. Improved seedlings of Douglas-fir are typically grown from seed produced in conventional, wind-pollinated seed orchards. Conventional orchards consist of large (≥ 15 m tall), widely spaced trees that are intensively managed to produce large amounts of high-quality seed. These orchards can remain productive for decades, and yields of 28 kg of seed per hectare (25 pounds/acre) are not uncommon (Cress and Daniels 1990). In 1990, seed orchards of coastal Douglas-fir covered nearly 1,012 hectares (2,500 acres) in the Pacific Northwest, with a predicted cumulative reforestation potential of 4.45 million hectares (11 million acres) by the year 2000 (Cress and Daniels 1990). Seed orchards are also common in other regions of the world. At least 13 clonal orchards covering 120 hectares have been established in France, and several have been established in Belgium, Denmark, and Germany (Chollet 1986; Héois et al. 1995). Although other production approaches may gain favor in the future (e.g., miniaturized seed orchards, rooted cuttings, and somatic embryogenesis), conventional seed orchards will remain the dominant approach for many years to come.

In the Pacific Northwest, production of orchard seed began in the Dennie Ahl orchard in 1962 (Wheat 1966), and by 1986, about 50% of the seed requirements for the Progressive Program and 30% of the requirements for British Columbia were met with genetically improved seed (Cafferata 1986; Arnott 1986). Seed production has increased over time, with thousands of pounds of orchard seed currently produced each year. Although data are unavailable for Oregon and Washington, the production of orchard seed in British Columbia was 344 kg (for coastal Douglas-fir) and 6 kg (for the interior variety) in 2003 (Anon. 2004). Weyerhaeuser has been deploying genetically improved orchard seed since 1980, and all of their seedlings have been grown from improved seed since 1986. Today, the same is true for many other organizations as well. On productive orchard sites, wind-pollinated seed can be produced for about 0.7 cents each (S. Lipow, pers. comm.), which translates to only 1–2% of the cost of planting Douglas-fir, including the costs of site preparation, seedling production, and planting. Thus, wind-pollinated orchards are a highly cost-effective way to boost the productivity of Douglas-fir plantations.

Seed orchards can be classified as either *clonal* or *seedling* seed orchards, and may contain either *tested* or *untested* genotypes. Most first-generation orchards of Douglas-fir were established as *clonal* orchards by grafting scions from field selections onto seedling rootstock that were planted at wide spacings (e.g., 6 × 6 m). In clonal orchards, multiple ramets (copies) of each clone are separated from one another to maximize outcrossing. Orchard layouts vary widely in the number and arrangement of clones and ramets. In Douglas-fir, these layouts include random designs, randomized complete blocks, systematic designs, and arrangements based on the geographic origin of the parents (Cress and Daniels 1990). Detailed design considerations for wind-pollinated orchards have been discussed by Giertych (1975) and Hodge and White (1993).

John Duffield pioneered the use of Douglas-fir clonal orchards in the 1950s, but these early orchards ran into problems with graft incompatibility, a type of graft failure caused by the tree's defense response (Silen and Copes 1972; Copes 1989). To avoid graft incompatibility, the Progressive Program made crosses among their better field selections and established full-sib seedling seed orchards (Silen and Wanek 1986), but this approach was abandoned when graft-compatible rootstock became available. Donald Copes began studying graft incompatibility in 1964. He found that graft rejection is highly heritable (Copes 1974), and by the mid-1970s, seeds from graft-compatible families were widely available. Today, seedling seed orchards are rare, and virtually all scions are grafted onto graft-compatible rootstock (Copes 1999). Other cultural treatments, such as bark scoring, are also valuable for alleviating graft incompatibility problems in established orchards (Copes 1989).

Tested orchards contain parents that have been chosen based on progeny test results, whereas *untested* orchards (which may be established when a breeding program is initiated) contain trees that have been chosen based on their phenotype alone. Tested orchards may be established using tested parents, or by roguing (i.e., removing) undesirable clones in untested orchards based on progeny tests results. In either case, orchards that contain only the best first-generation selections are typically referred to as 1.5 generation orchards. In the first generation, the IFA, BCMoF, and Weyerhaeuser programs grafted scions from field selections directly into clonal seed orchards. As information from progeny tests became available, these orchards were rogued based on progeny performance. This approach was desirable because it minimized the time until the orchards began producing seed, but also led to uneven spacing and gaps in the orchards because of the large number of clones that had to be removed. Most second-generation orchards are established as tested orchards. Compared to rogued orchards, these orchards will have more ramets per clone when they are established, and a better distribution of trees when they are in full production.

Now that graft incompatibility is no longer a problem, virtually all Douglas-fir orchards are established by grafting scions from select trees onto young seedlings, either directly in the field, or onto trees temporarily growing in pots. Because it takes eight or more years for the grafts to develop large crowns that can carry large cone crops, some orchard managers have begun to accelerate cone production by grafting multiple scions onto a single rootstock, or by substantially increasing the density of the trees (see *Miniaturized Seed Orchards*).

The number of clones in an orchard is a balance between the need to keep the number of clones small (i.e., to increase selection intensity and genetic gain) and the need to have enough clones to avoid inbreeding and maintain genetic diversity. If the clones are unrelated, inbreeding is rarely a concern because selfing (i.e., individual or clonal selfing) is low at the mature seed stage, and selfs are unlikely to survive culling during seedling production. Therefore, the main effect of selfing is to lower seed production. The inclusion of related clones in an orchard is more of a concern because inbred seedlings derived from matings between close relatives may not be culled in the nursery and could adversely affect the performance of plantations. Therefore, related individuals are usually excluded from the same orchard and the number of clones in the orchard is usually determined by balancing genetic gain with genetic diversity. From a diversity standpoint, the effective population size (N_e) of orchard seed is more relevant than the census number of clones (N). The province of British Columbia, for example, stipulates that all orchard crops must have an N_e of 10 or more (British Columbia Ministry of Forests 2004). Clonal differences in male or female fertility and non-random mating tend to reduce N_e, whereas pollen contamination tends to increase N_e (i.e., compared to N). Furthermore, non-random mating, fertility differences, and pollen contamination are all common in Douglas-fir orchards (discussed below). Based on data from a wide variety of conifer seed orchards, 0.5 is a conservative estimate for the ratio of N_e to N (Johnson 1998a; Kang et al. 2001). Based on this estimate, Johnson (1998a) used genetic theory to compare genetic gains in relation to genetic diversity, and concluded that a final orchard population of 20 to 25 clones would be reasonable for Douglas-fir (see Fig. 1 in Johnson 1998a). Johnson and Lipow (2002) came to the same conclusion after reviewing studies that either examined genetic diversity in seed orchards (based on allozymes) or risks associated with clonal deployment. In contrast, many of the operational Douglas-fir orchards have had 50 to 150 or more clones. This dramatically limits genetic gains and is well beyond what is needed to maintain adequate genetic diversity. In contrast, if the number of clones were at the lower end of the spectrum, it would be wise for seed orchard managers to closely monitor the N_e of their orchard seed using molecular genetic markers. Unfortunately, the amount of genetic diversity that should be retained is mostly based on theoretical considerations because empirical data on the relationship between genetic diversity and long-term performance are unavailable.

Roguing is an important part of orchard management. Many first-generation orchards were established with a large number of untested clones, or clones for which only sibling (i.e., not progeny) information is available. Roguing then takes place after progeny test results become available. When orchards are established using tested clones, some managers skew the clonal makeup of the orchard towards the best parents (e.g., 75% of the ramets may come from the top 50% of the clones). In either case, the final number of clones in the orchard must be low enough to achieve adequate genetic gain, but high enough to maintain an adequate N_e.

Orchard managers seek to maintain orchard vigor, promote early seed production, and produce large quantities of genetically improved seed at regular intervals over the life of the orchard. This is done via grafting, flower stimulation, roguing, pollen management, weed control, irrigation, fertilization, frost protection, and pest control. Because these activities are costly, a rapid return on investment is important.

One of the key management activities in Douglas-fir orchards is flower stimulation. Flower stimulation generally refers to the enhanced production of male and female strobili on trees that are already competent to flower. Flowering can be increased using stem injections of gibberellic acid (i.e., a mixture of GA_4 and GA_7), wounding the trees with stem girdles or root pruning, and/or fertilizing with calcium nitrate (Ebell 1972; Ross et al. 1985; Wheeler et al. 1985; Woods 1989). These treatments are applied in the spring, and mature seeds are available two growing seasons later. Therefore, flower-stimulating treatments are usually applied every other year. Flowering can be enhanced as early as two years after grafting using $GA_{4/7}$ and wounding (Cherry et al., unpubl. data), whereas calcium nitrate is generally used on older trees. Using these techniques, either alone or in combination, cone and pollen production can be increased more than 10-fold, but work is still needed to reliably promote flowering on juvenile seedlings and recalcitrant genotypes. The mechanisms by which these treatments stimulate flowering have been the source of much speculation, but little focused research. The availability of advanced approaches in molecular biology and genomics should now make this a more tractable research question. A great deal of operational flowering research has been done in North America, and cooperative research is also underway in France, Belgium, Spain, and Italy (Héois 2000; Philippe et al. 2004).

Pollen management is a particularly important aspect of seed orchard management that affects both genetic gain and genetic diversity. Douglas-fir pollen management was reviewed in detail by Webber (1995) and Webber and Painter (1996). Pollen represents half the potential genetic gain in an orchard, but is difficult to control. In conventional orchards, the optimal situation is to have all seeds fathered by other orchard trees, and all parents contributing equally to the seed crop. Unfortunately, neither goal is achieved in wind-pollinated orchards.

Pollen contamination (which is measured as the proportion of orchard seeds that are fathered by non-orchard trees) is typically high because most Douglas-fir orchards are located near other Douglas-fir trees—either in native stands, or in other orchards containing trees from other breeding zones. Pollen contamination in conventional orchards often exceeds 40% (Smith and Adams 1983; Wheeler and Jech 1986a; Adams et al. 1997; Slavov 2004), which could reduce genetic gains by 20% or more (depending on the genetics of the surrounding stands), and potentially increase maladaptation, if the contamination comes from trees from other breeding zones (Webber and Painter 1996). As genetic gains increase in advanced generations, the adverse impact of pollen contamination will increase as well.

Several techniques are used to reduce pollen contamination. One way is to increase the amount of orchard pollen reaching the trees. Flower stimulation can be used to create large quantities of pollen in the orchard, and orchard blowers (e.g., helicopters or large fans pulled behind tractors) can be used to release large clouds of pollen, hopefully before peak pollen shed occurs in the surrounding stands (Sorensen and Webber 1997). In addition, some orchard managers graft new, second-generation selections into rogued, first-generation orchards that contain mature trees that are already producing large amounts of pollen. The first-generation trees are then removed after the second-generation trees mature and begin producing enough pollen of their own. Finally, methods have been developed for the collection, storage, and delivery of pollen, so that orchard pollen can be applied to unprotected female strobili via SMP (Copes 1985; Copes et al. 1991, 1995). SMP success is measured as the percentage of seed that are fathered by the pollen that is applied via SMP. Although the success of SMP can be greater than 50% (Wheeler and Jech 1986b, 1988, 1992), its success depends on many factors (Webber and Painter 1996). In operational settings, Webber and Painter (1996) suggest that a long-term success rate of only 20 to 30% is likely to be achieved. In addition to reducing pollen contamination, SMP can be used to increase seed yields when pollen is limiting, balance paternal contributions, and create elite full-sib crosses (Webber 1995). Controlled mass pollination can also be used to exclude foreign pollen, but this is more expensive.

A second way to reduce pollen contamination is to reduce the amount of background pollen reaching the trees. Overhead irrigation can be used to cool the orchard and delay flowering relative to non-orchard trees, potentially reducing the amount of outside pollen that is present during peak orchard receptivity (Silen and Kean 1969). Cooling also shortens the duration of female receptivity and pollen shed, thereby increasing the number of clones with the potential to mate with one another (Fashler and El-Kassaby 1987). Webber and Painter (1996) conclude that bloom delay is the most consistently effective way to reduce contamination and improve parental balance.

Condensing the time of female receptivity and pollen flight also alleviates some of the problems associated with unequal mating within the orchard. In wind-pollinated orchards, male and female parents contribute very unequally to the seed crop (El-Kassaby and Askew 1991; Stoehr et al. 1998; Slavov 2004). Furthermore, mating is not random.

Trees with early pollen-shed preferentially mate with trees that are receptive early, and trees with late pollen-shed mate more often with trees that are receptive late (Slavov 2004).

In addition to the reduction in N_e caused by non-random mating, these early- and late-receptive clones are more prone to pollen contamination (El-Kassaby and Ritland 1986; Slavov 2004). Therefore, it may be possible to reduce pollen contamination by harvesting seed selectively, but this will reduce pollen contamination only slightly because only a small proportion of the clones will fall into unusually early or late phenology classes (Slavov 2004).

Although each of these approaches can reduce pollen contamination, their effectiveness has not been fully evaluated. Now that highly variable SSR genetic markers have been developed for Douglas-fir (Slavov et al. 2004), it is possible to precisely measure pollen contamination and test the effectiveness of these treatments. SSR markers are also valuable for measuring clonal selfing, determining the genetic contributions of each clone to the orchard crop, characterizing deviations from random mating, and uncovering seed contamination and mislabeled ramets (Slavov 2004).

During the early life of the orchard, it is important to promote rapid vegetative growth and large crowns, but later, it may be more important to limit vegetative growth to promote flowering. Most orchard managers monitor the nutritional status of the soil and tree foliage to guide fertilizer treatments. It is also necessary to control seed and cone insects with pesticides. Therefore, pesticide testing and registration is an important ongoing effort. Because late spring frosts cause serious damage during peak receptivity of the strobili, frost protection is frequently needed. Some frost protection is possible by mixing the air with fans and helicopters, or by warming the air with smudge pots, gas-fired blowers, or under-crown watering, but these methods are often partially effective at best.

The main advantage of conventional orchards is that large amounts of seed can be produced on relatively few trees with minimal crown management. Despite their success, conventional, wind-pollinated orchards have a few key limitations (Sweet 1995). First, because they are wind-pollinated, pollen contamination is a problem and intra-orchard mating is uneven. Second, because the trees are large, it is difficult and costly to harvest cones, apply pesticides, prevent frost damage, use overhead irrigation for bloom delay, and make elite crosses using CMP or SMP. Finally, because the trees are widely spaced, they require a great deal of land and per-hectare seed yields are low during the early life of the orchard. Because of these limitations, various miniaturized seed orchard designs are being used or considered in Douglas-fir and other species (Sweet 1995; Webber and Painter 1996; Héois 2000).

2. Miniaturized Seed Orchards. Miniaturized seed orchards (MSOs) are promising alternatives to conventional seed orchards. We use MSOs to denote outdoor orchards in which the trees are planted at close spacings, and then maintained at a height of only 2 to 4m. Using this approach, seeds are produced close to the ground on many small trees, rather than on a few, much larger trees. Other terms have been used to describe particular types of MSOs, including HAPSOs (hedged artificially-pollinated seed orchards), monoclonal orchards, and micro-orchards (Sweet 1995).

The goal of most MSOs is to facilitate controlled pollination via CMP or SMP. This is accomplished by keeping the trees small and planting them in clonal rows so that controlled pollination and other management techniques can be easily applied from the ground to individual clones (Sweet and Krugman 1977). Control-pollinated orchards allow breeders to eliminate pollen contamination and produce both elite families and specialty breeds (e.g., cold hardy families for deploying on particularly harsh microsites). Furthermore, a wind-pollinated orchard with 25 clones would require a long-term breeding program with 25 sublines to be able to establish 25 unrelated clones in the orchard. A control-pollinated orchard, in contrast, would only require two sublines because controlled crosses can be restricted to parents in different sublines. This would dramatically reduce the complexity of long-term breeding.

Although the driving force behind many MSOs is the ability to use controlled-pollination, they have other benefits as well. In some cases, seed orchard managers are moving toward miniaturized seed orchards because land is scarce for conventional orchards, but it is not yet clear whether per-hectare seed yields will be higher for MSOs or not. Second, once they are established, the costs of MSO management should be lower because the crowns are closer to the ground, thereby facilitating seed collection, pest management, frost protection, and bloom delay. Third, because there are more trees per hectare, a given quantity of seed can be produced earlier in the life of the orchard (i.e., assuming that orchard acreage is held constant). In conventional orchards, genetic gains are delayed (and financial returns are reduced) because of the long time lag between seed orchard establishment and the production of commercial quantities of genetically improved seed. Many first-generation orchards took 10 to 15 years to produce useful amounts of seed (Cress and Daniels 1990), whereas seed production in MSOs is possible as early as three years after grafting because of their high densities (Cherry et al., unpubl.).

MSOs are becoming increasingly popular for the production of horticultural tree crops and forest tree seed (Jackson 1989). In New Zealand, for example, radiata pine orchards were changed from mostly open-pollinated orchards to control-pollinated MSOs in 1984 (Sweet 1995). At least five Douglas-fir MSOs have been established in recent years in the Pacific Northwest. Although seed is now being produced from the oldest of these orchards, much remains to be learned about the management, costs, and potential yields of Douglas-fir MSOs.

Despite their potential advantages, MSOs remain unproven in Douglas-fir. MSOs will cost more to establish than conventional orchards because more trees are needed to produce equal amounts of seed. This should be offset, however, by earlier production of large seed crops. Some management costs will increase, such as the costs of extra labor needed to keep the trees small. Finally, there are many biological questions that need to be answered before they can be considered fully operational.

C. Vegetative Propagation

1. Advantages of Vegetative Propagation. Compared to sexual propagation via seed, vegetative propagation has four main advantages. First, the problem of pollen contamination, which is inherent to wind-pollinated seed orchards, is eliminated. Second, by avoiding sexual reproduction, non-additive genetic variation can be captured to obtain greater genetic gains. Third, because vegetative propagation is possible from young seedlings and even immature embryos, improved materials can be tested almost immediately via clonal tests, rather than having to wait until the trees flower to conduct progeny tests. This means that deployment of improved, tested genotypes can occur earlier using vegetative propagation. Fourth, the genetic uniformity of clones should facilitate reforestation, stand management, harvesting, and processing, and permit smaller tests to be used for research and breeding. Vegetative propagation can also be used in conjunction with seed-based strategies to multiply or "bulk up" small numbers of seedlings produced by crossing elite parents. Although current methods of vegetative propagation are too expensive to be used operationally, vegetative propagation is an active area of research and costs should decrease. Douglas-fir can be vegetatively propagated via rooted stem cuttings, somatic embryogenesis, organogenesis, and grafting, but only rooted cuttings and somatic embryogenesis have the potential to produce large numbers of trees for outplanting, and only rooted cuttings have been deployed operationally.

2. Methods of Vegetative Propagation. Rooted cuttings are widely used to propagate forest tree species, including angiosperms and conifers. Compared to other methods of vegetative propagation, rooted cuttings have three main advantages—the technology is low-tech, does not require much labor, and once the cuttings have formed roots, they can be handled like seedlings throughout the rest of the reforestation process. The main disadvantages are that only modest numbers of cuttings can be produced from a single tree (i.e., without serial propagation), and once they are established in the field, they may have problems with plagiotropic (i.e., non-upright) growth. Propagation of Douglas-fir from rooted cuttings has been studied for more than 50 years (Griffith 1940), and has been actively pursued since the 1970s (Copes 1977; Ritchie 1993). In general, stem cuttings from young trees are treated with plant growth regulators (e.g., IBA, NAA), then cultured under mist, often with bottom heat and aggressive treatment with fungicides (Copes and Mandel 2000). Rooting success is often high, but varies by genotype and declines rapidly with the age of the ortet (Copes and Mandel 2000). Furthermore, the trees are expensive and often show plagiotropic growth. During the 1990s, Weyerhaeuser produced and deployed millions of Douglas-fir rooted cuttings (see *Genotype multiplication* below). They produced their rooted cuttings from rapidly growing, 1-year-old seedlings by rooting them in the greenhouse, and then transferring them to containers or bareroot nurseries for another year of growth before being outplanted.

Somatic embryogenesis is the process whereby embryos are formed from vegetative (somatic) tissues without sexual reproduction. In Douglas-fir, embryos from immature seed can be induced to form embryogenic tissue cultures that can be repeatedly subcultured and multiplied to produce millions of clonal replicates. Although the starting embryos are usually derived from full-sib crosses between elite parents, the full genetic potential of the embryo is unknown when the cultures are initiated. Therefore, it is important that embryogenic cultures can be cryogenically stored, and then used to produce trees for outplanting once the embryogenic lines have been tested in the field. Because of the vast numbers of trees that can be produced, somatic embryogenesis is the most promising approach to large-scale clonal forestry (Farnum et al. 1983). At least two companies (Weyerhaeuser and CellFor) have large research programs aimed at developing commercially viable somatic embryogenesis for Douglas-fir (Sutton 2002; Gupta et al. 2003). Although large field tests with hundreds of Douglas-fir clones have already been established, large-scale operational plantations are still many years away.

Unfortunately, somatic embryogenesis is labor intensive, difficult to mechanize, and biologically challenging. Labor may be as much as 60% of the total production costs, and efficient methods for storing and converting the embryos into somatic seedlings are still being developed (Sutton 2002; Gupta et al. 2003). Current areas of research, including the development of production systems based on liquid media and deployment via manufactured seed, would allow somatic embryos to be handled just like orchard seed is handled today (Sutton 2002; Gupta et al. 2003).

Organogenesis is the de novo production of plant organs. The induction of roots on stem cuttings is one example of ex vitro organogenesis. Whole trees can also be recovered using in vitro organogenesis—i.e., by inducing shoot meristems on tissues cultured in vitro, and then inducing these shoots to form roots. Organogenesis can be used to produce Douglas-fir trees from seedling cotyledons and other tissues (Goldfarb et al. 1991a), but this system will never be able to compete with rooted cuttings or somatic embryogenesis because it is too costly and slow (i.e., shoot and root induction are separate processes). Furthermore, because only juvenile tissues can be used, the clones are untested and the number of ramets that can be produced is limited by the amount of cotyledonary tissue available (i.e., unless the primary ramets are subsequently propagated by rooted cuttings or some other method). Although grafting is an extremely valuable technique for establishing clonal seed orchards, it cannot be used to establish production plantations because it is too costly.

3. Genotype Multiplication. The production of elite full-sib families can be enhanced by using vegetative propagation to "bulk-up" a small number of full-sib seedlings. Throughout much of the 1990s, Weyerhaeuser used this approach to deploy large numbers of rooted cuttings (Ritchie 1993). Elite trees were crossed to generate full-sib families, and the progeny were used as motherstock (ortets) from which the cuttings were taken. Forty or more cuttings were rooted from each seedling, and these were combined to create large half-sib or full-sib families (i.e., containing mixtures of siblings and clones). These families were subsequently mixed, or deployed in pure family blocks. Although the trees performed well, this program is being discontinued because the costs are too high. Instead, Weyerhaeuser's long-term goal is to capture genetic gain by deploying trees ("somatic seedlings") derived from somatic embryos.

4. Clonal Forestry. Clonal forestry is the production, testing, and deployment of clones. It is distinguished from other types of forestry that use vegetative propagation (such as genotype multiplication) by its use of clonal testing. Clonal forestry is widely used in species that are easy to propagate from stem cuttings (e.g., *Populus, Eucalyptus, Sequoia, Cryptomeria*), but is rarely practiced in most conifers. One advantage of true clonal forestry is the ability to capture non-additive genetic variation. In Douglas-fir, the ratio of non-additive to additive genetic variation seems to be about 0.5 or less for most commercially important traits (see *Quantitative genetics*). Therefore, it should be possible to achieve some additional gains by testing and deploying clones, rather than full-sib families or wind-pollinated seedlots. A second advantage is the ability to begin field tests immediately after the clones are available, rather than having to wait until seed production begins, which is the case for normal progeny tests designed to measure GCA or SCA.

The major hurdle to clonal forestry is its cost. Producing reforestation stock via rooted cuttings or somatic embryos can be orders of magnitude more expensive than seedlings. Another concern is whether a sufficient number of clones can be developed given the economic and biological constraints of the production technologies. Although virtually all trees can be efficiently propagated via seed, many genotypes do not perform well in rooted cutting or somatic embryogenic systems. The challenge for large-scale clonal forestry systems in Douglas-fir will be to produce a sufficient number of genotypes for each of the breeding zones that exist in the region.

5. Genetic Testing. Vegetative propagation can also be used to efficiently test genotypes in the field. By estimating breeding values or genotypic values from clonal replicates, rather than from siblings, field testing is more precise and effective. This approach has been used to map quantitative trait loci in Douglas-fir (Wheeler et al. 2005) and to improve genetic gains in tree breeding programs using clonal replication. In particular, the effectiveness of within-family selection is greatly improved by clonally replicating and testing multiple genotypes within each full-sib family (Isik et al. 2004).

D. Deployment

There is a close link between the production of improved materials and their deployment. On the one hand, options for deployment are constrained by the methods used to produce the improved materials, but deployment goals can also drive changes in production technology. Because improved seed can be efficiently produced in wind-pollinated seed orchards, Douglas-fir is mostly deployed as heterogeneous collections of seedlings from a single orchard. Nonetheless, there has been a trend toward planting seedlots of greater uniformity and genetic gain. Because many of the Pacific Northwest orchards are now producing surplus seed, some companies are collecting and deploying seed from only the very best parents. The seedlings being deployed may come from a bulk collection of seed from the best parents in the orchard, or may consist of half-sibs from a single orchard clone. Other organizations are producing high-gain, control-pollinated seed to be deployed as seedlings (i.e., without genotype multiplication). In mature, highly productive orchards, this can be a surprisingly cost-effective approach. In addition, Weyerhaeuser used rooted cuttings to multiply seedlings from elite crosses, and was able to deploy full-sib family blocks on a modest scale (Ritchie 1993). The long-term goal of many forestry organizations is to practice true clonal forestry, at least on their best sites (Farnum et al. 1983). This deployment goal has the potential to drive major changes in seedling production strategies—from seed orchards to somatic embryogenesis, and perhaps manufactured seed. This deployment goal may also force changes in breeding strategy—from strategies that focus exclusively on additive genetic variation, to ones that focus more heavily on both additive and non-additive variation. Because of the high costs of clonal forestry, a shift in this direction will provide pressures to reduce the genetic diversity of the trees being outplanted and keep the number of breeding zones small (Sutton 2002), although this is not a necessary outcome. Clones could be deployed as small blocks of single clones or as clonal mixtures. Nonetheless, it will still be many years before a large number of clonal test plantations are established across the region. Therefore, there is little to suggest that that clonal deployment will compete with seed-based deployment of Douglas-fir within the next few decades.

XI. BIOTECHNOLOGY

In the context of Douglas-fir breeding, biotechnology is the application of science and engineering technology to the genetic improvement and deployment of Douglas-fir trees. Four areas of biotechnology have current or potential application to forest tree breeding and deployment: vegetative propagation, transgenics, molecular genetic markers, and genomics (Yanchuk 2001b).

A. Vegetative Propagation

Uses of rooted cutting technology, somatic embryogenesis, and manufactured seed for vegetative propagation have already been discussed. By facilitating clonal forestry, these biotechnologies have the potential to dramatically change tree breeding and deployment, but are not yet economically viable, at least for Douglas-fir. Furthermore, somatic embryogenesis is the most promising mode for producing transgenic trees (van Frankenhuyzen and Beardmore 2004), although operational deployment of transgenic Douglas-fir is highly unlikely in the foreseeable future (discussed below).

B. Transgenics

Plant transformation is the process whereby genes are introduced, become stably integrated, and expressed in a plant. We refer to plants that have been transformed as "transgenic," regardless of the origin of the genes. At least 33 species of forest trees have been genetically transformed (van Frankenhuyzen and Beardmore 2004). Although transgenic Douglas-firs have not been documented in the literature, at least one transgenic tree has been produced (D. D. Ellis, pers. comm.). The beta-glucuronidase (GUS) gene was inserted into Douglas-fir via biolistic transformation of somatic embryos. The GUS gene was stably incorporated and expressed in one tree, which was grown to age three. Transformation was verified via the presence of GUS enzyme activity and PCR-based detection of GUS gene fragments in Douglas-fir DNA (D. D. Ellis, pers. comm.). This report is consistent with the fact that other species in the pine family (Pinaceae) have been transformed (van Frankenhuyzen and Beardmore 2004). Douglas-fir trees can be recovered from somatic embryogenic tissue cultures (Gupta et al. 2003), and foreign genes are expressed in Douglas-fir tissues (Goldfarb et al. 1991b). Despite this report, there has been no long-term, concerted effort to develop efficient transformation protocols for Douglas-fir. In some species, transgenic trees have already been tested in the field, but it may be 15 to 20 years before any are released for commercial use (at least in Canada; Hadley et al. 2001), and transgenic Douglas-fir will lag behind, if they are deployed at all.

Why is there so little interest in transgenic Douglas-fir? First, we know of no single-gene or oligogenic traits that are valuable enough to warrant the large investments needed to develop transgenic Douglas-fir. Growth and adaptability are highly polygenic and no single phenotype would do well over a very large area; there is only a modest economic incentive to improve wood quality; no insect or disease pests are important enough to warrant the development of large resistance breeding programs, let alone transgene technology; and traits such as herbicide resistance are not as valuable for long-rotation trees as they are for annual agronomic crops. Furthermore, there is ample genetic variation to make progress via traditional breeding, so only novel traits are likely to attract real interest. The most compelling reason to have transgene technologies on hand would be to counteract the potentially serious problems caused by exotic insect or disease pests. Both gypsy moth (*Lymantria dispar*) and the sudden oak death pathogen (*Phytophthora ramorum*) are potential problems for Douglas-fir in the future. Furthermore, given that most programs seek to maintain substantial genetic diversity (even if clonal deployment becomes a reality), the development and long-term testing of large numbers of transgenic genotypes is an expensive proposition. Finally, public perceptions and regulatory hurdles may be insurmountable. Despite the technical and economic hurdles, environmental concerns have become the main obstacle to public acceptance and regulatory approval of transgenic trees (van Frankenhuyzen and Beardmore 2004). A decision to plant genetically transformed trees among native forests will receive close scrutiny, mainly because of concerns about transgenes introgressing into native populations. To prevent this, robust mechanisms for imparting sterility may be required for these trees to be deployed (Strauss et al. 1995). We must also pay careful attention to the effects of transgenic trees on other organisms and ecosystem processes (van Frankenhuyzen and Beardmore 2004), particularly for traits such as insect resistance. Use of the insect toxin gene from *Bacillus thuringiensis* to confer insect resistance is one of the more realistic applications of genetic transformation in forest trees (van Frankenhuyzen and Beardmore 2004).

C. Molecular Genetic Markers

Molecular genetic markers (mostly allozymes) have been widely used in Douglas-fir tree improvement and gene conservation programs since the early 1980s. DNA-based markers are beginning to supplant allozymes for most traditional uses, and have also made new uses possible, including the construction of genetic maps, analyses of quantitative trait loci (QTL), and association genetics.

Allozymes were used throughout the 1980s and early 1990s to study Douglas-fir pollen contamination, seed orchard management, genotype mislabeling, population structure, genetic diversity, and mating systems (El-Kassaby et al. 1981; Neale and Adams 1985; Merkle and Adams

1987; Li and Adams 1989; Wheeler and Jech 1992; El-Kassaby and Ritland 1996; Adams et al. 1997). Since the mid-1980s, the use of DNA-based markers has steadily increased, including RFLPs (Neale et al. 1986; Jermstad et al. 1994), RAPDs (Krutovskii et al. 1998), microsatellites (Amarasinghe and Carlson 2002; Slavov et al. 2004), and single nucleotide polymorphisms (SNPs; Krutovskii et al. 2004). RFLPs were first used to confirm the paternal inheritance of chloroplast DNA in Douglas-fir (Neale et al. 1986) and to study the evolutionary relationships between Douglas-fir and its relatives (Strauss et al. 1990). RFLPs and RAPDs were subsequently used to construct linkage maps of Douglas-fir and comparative maps between Douglas-fir and pine (Jermstad et al. 1998; Krutovskii et al. 1998; Krutovsky et al. 2004). These linkage maps were the foundation of a long-term project to map QTL for important growth and adaptive traits. Scientists at the U.S. Forest Service Institute of Forest Genetics and Weyerhaeuser collaborated to create two large, clonally replicated full-sib families for pedigree-based QTL mapping and verification. Phenotypic measurements in nursery, greenhouse, and field tests were used to map QTL for height growth, second flushing, fall cold hardiness, spring bud flush, and spring cold hardiness (Jermstad et al. 2001a, 2001b, 2003; Wheeler et al. 2005). Six to 10 QTL regions have been repeatedly detected for these traits. Although the percentages of phenotypic variance explained were usually less than 10%, some QTL explained as much as 50% or more of the genetic variation in some experiments (Jermstad et al. 2001a). These results, and results from other species, support an oligogenic or polygenic model of inheritance for adaptive traits—presumably involving tens to hundreds of loci (Howe et al. 2003). Similar conclusions have been drawn for other traits in forest trees (Sewell and Neale 2000).

Pedigree-based QTL studies reveal much about the genetic architecture of growth and adaptation, and provide a foundation for marker-aided-selection (MAS), which seems to be economically feasible in Douglas-fir (Johnson et al. 2000; Wu et al. 2000). Nonetheless, MAS will be challenging because of the polygenic control of important quantitative traits in forest trees (Howe et al. 2003) and substantial linkage disequilibrium (Strauss et al. 1992). Therefore, markers for MAS will probably be needed within the genes of interest, rather than in distant genomic regions, but these markers are not uncovered using pedigree-based QTL studies because of their low resolution. Therefore, another approach called association genetics may be needed to identify the actual genes responsible for variation in quantitative traits (discussed below).

A newly developed set of SSR markers has been used to measure pollen contamination, selfing, relative paternal contributions, and positive assortative mating with respect to flowering phenology in a Douglas-fir seed orchard (Slavov 2004; Slavov et al. 2004). These SSRs have also been used to fingerprint Douglas-fir trees to uncover mislabeled parental selections in the field and mislabeled ramets in a seed orchard (Slavov 2004). This same approach would be valuable for confirming genotypes that are deployed in clonal forestry programs. Given their high variability, these markers should also be perfect for combining polycross breeding with paternity analysis (Lambeth et al. 2001).

D. Genomics

Genomics is the integrated study of the structure, function, and interactions of all the genes in an organism. It typically involves the use of high-throughput techniques to identify genes, determine their DNA sequences, map their locations on chromosomes, and understand their functions. Because the genome is the basis of all biology, genomics research will facilitate the integration of tree physiology, genecology, gene conservation, and applied tree breeding. Advanced genomics programs exist in a small number of forest trees, including loblolly pine, radiata pine, *Eucalyptus*, and *Populus*. We and others recently organized the Douglas-fir Genome Project to facilitate the development of genomics resources in Douglas-fir (http://dendrome.ucdavis.edu/dfgp/). Ongoing activities include the development of expressed sequence tag (EST) libraries, DNA microarrays, SNP markers, and association genetics. The main objective of this work is to identify the genes responsible for genetic variation in adaptive traits and wood quality.

Association genetics is an emerging population-based approach for identifying genes that are responsible for genetic variation in natural populations (Neale and Savolainen 2004). Genetic association studies involve searching for statistical associations between phenotypes and marker alleles in populations of unrelated individuals (Howe et al. 2003; Neale and Savolainen 2004). Unlike pedigree analyses (used in typical QTL experiments), association studies should be useful for finding markers in the genes of interest, which are more likely to be useful for MAS in many different families. Therefore, the markers developed from association studies might be useful for MAS in typical breeding programs and for studying adaptation and genetic structure in natural populations. Nonetheless, this will only happen after we can reliably explain a reasonable proportion of the phenotypic variation among individuals and populations—perhaps 20% or more. Otherwise, traditional breeding will probably be more efficient. Because we expect that individual markers will only reliably explain a few percent of the phenotypic variation in natural and breeding populations, our long-term goal is to develop robust, multilocus approaches for explaining substantial proportions of genetic variation (Howe et al. 2003). To conduct association studies in forest trees, we will need to (1) identify candidate genes, (2) find useful polymorphisms in candidate genes, (3) develop study populations, (4) phenotype and genotype individuals, and (5) verify associations using independent populations (Howe et al. 2003).

Because complete genome scans using SNP markers are not yet possible in Douglas-fir, it is necessary to focus our efforts on candidate genes—genes believed to have important functional roles in the trait of interest based on indirect or circumstantial evidence. Candidate genes can be identified based on their similarity to genes of known function in other species, co-location with QTL on genetic maps, and patterns of gene expression. Once candidate genes are identified, molecular markers are developed in, or very near, these genes to use in association studies. The markers of choice for association studies are SNPs. Nearly 50 candidate genes have already been placed on a Douglas-fir genetic map, and nucleotide diversity and linkage disequilibrium have been measured (Krutovskii et al. 2004). Using these candidate genes, association studies will begin within the next year (D. B. Neale, pers. comm.).

Although genomic analyses may involve determining the complete DNA sequence of an organism, the cost of doing this is prohibitive, and this is likely to be done for only a handful of model organisms in the near future. The main alternative is to sequence only those genes that are expressed in an individual at any given time. These DNA sequences are called expressed sequence tags, or ESTs. Because the expressed parts of the genome are most easily tied to organism function, an EST sequencing project is a critically important building block of any genomics program. We are developing large EST libraries in Douglas-fir to facilitate candidate gene discovery. Candidates will be identified using all three methods described above: EST sequence similarity to genes in other species, co-location with known QTL on genetic maps, and patterns of gene expression measured using DNA microarrays. Because they are transferable across species, EST markers also facilitate comparative genome analysis (Krutovsky et al. 2004). Microarrays can be used to monitor collective changes in gene expression in response to multiple factors, such as drought, temperature extremes, nutrition, mycorrhizae, insects, disease, and intrinsic developmental programs such as flowering.

XII. GENE CONSERVATION

The impacts of tree breeding and other silvicultural practices on genetic diversity must be carefully considered for ecological, economic, social, and ethical reasons. Except for small, isolated populations, population extirpation is not a serious threat in Douglas-fir. Therefore, tree breeders are mostly concerned with maintaining sufficient genetic diversity for populations to evolve and adapt to changing environmental conditions such as those imposed by exotic pests, air pollution, and climate change. In general, different types and amounts of genetic diversity will be maintained in the gene resource, inter-situ, breeding, and production populations (Fig. 6.3) to (1) permit continued gain in future generations, (2) maintain adaptability, and (3) conserve rare alleles that may be important in the future (Lipow et al. 2003).

In situ and ex situ methods are used to conserve genetic diversity in Douglas-fir. Gene resource populations conserve genetic resources in their native habitats (in situ), whereas other genetic resources are conserved ex situ—in seed banks, clone banks, progeny tests, provenance tests, and seed orchards. Not only does coastal Douglas-fir have extensive in situ genetic resources, but also the largest and most complete collection of ex situ resources of any forest tree (Lipow et al. 2003). More than 4 million progeny from nearly 34,000 parents have been planted on nearly 1,000 sites in western Oregon, western Washington, British Columbia, and northern California (Lipow et al. 2003). Furthermore, seed stores contain seedlots from more than 20,000 parents, and additional materials are planted in Europe, New Zealand, and Chile (Lipow et al. 2004). Despite these huge numbers, the ability of tree breeding populations to meet gene conservation goals has been carefully considered (Yanchuk and Lester 1996; Yanchuk 2001a; Lipow et al. 2003, 2004).

Tree breeders seek to conserve *quantitative genetic variation* because it is associated with adaptability and the ability to obtain genetic gains in economic traits. Breeders may also seek to conserve *rare alleles* based on the hypothesis that they will be important in the future. Although most low-frequency alleles are probably rare because they are deleterious (at least under current conditions), they might confer important phenotypes (such as disease resistance) if conditions change. Alternatively, they might confer novel traits that are not subject to strong positive selection in natural populations. A rare, mutated allele for a gene that normally encodes cinnamyl alcohol dehydrogenase (CAD), for example, may confer desirable wood characteristics in loblolly pine (Gill et al. 2003).

For long-term conservation of quantitative genetic variation, a population of 5,000 trees (i.e., $N_e \geq 1{,}000$) should be more than adequate (Yanchuk 2001a). Based on this target, Lipow et al. (2004) performed a "gap analysis" of Douglas-fir gene resources in western Oregon and western Washington. In situ resources were considered adequate if at least 5,000 mature trees were estimated to occur in protected areas within each genetically distinct area (i.e., ecoregion, breeding zone, or seed zone). Based on these analyses, Lipow et al. (2004) concluded that Douglas-fir genetic resources are well protected in situ, except for a possible gap in the southern Puget lowlands, which has few protected areas. However, genetic resources from this area are well conserved in ex situ reserves. Not only is Douglas-fir well conserved in situ, but the ex situ populations that are associated with breeding programs (e.g., progeny test "inter-situ" populations) are large enough for most gene conservation purposes (Lipow et al. 2003). Douglas-fir genetic resources are also well conserved in British Columbia (Yanchuk and Lester 1996).

What population sizes are needed to conserve rare alleles? Yanchuk (2001a) studied this question based on the assumption that (1) rare alleles must be captured in multiple individuals (e.g., 5 or 20) to be useful in a breeding program, and (2) only alleles that impart phenotypic effects can be captured in a form that is useful (i.e., can be located and purposefully used in breeding). Therefore, it is much easier to "capture" (i.e., conserve and use) dominant alleles that can be identified in heterozygotes than it is to capture recessive alleles that can only be identified in trees that are homozygous for the desired allele. Based on these analyses, Yanchuk (2001a) concluded that a population of 554 trees would have a good (95%) chance of capturing dominant alleles in 20 trees if the allele frequency is 0.025. For recessive alleles, a population of 554 trees will only capture alleles in 20 trees if the allele frequency is almost 10 times higher (i.e., 0.224). In contrast, a population of 55,755 trees would be needed to capture recessive alleles at a frequency of 0.022 (i.e., about the same frequency as for dominant alleles in a population of 554). In short, a population of a few thousand trees (i.e., equivalent to a localized inter-situ or gene resource population) is very effective for capturing rare dominant alleles (i.e., frequencies < 0.005), but hundreds of thousands of trees are needed to capture recessive alleles that are only moderately rare (e.g., < 0.01). Therefore, more geographically extensive inter-situ or gene resource populations are needed to capture these alleles. Nonetheless, given the huge number of trees conserved in all protected areas and ex situ populations, the conservation of these alleles is virtually assured, even if they may not occur in the most desirable genetic backgrounds (Yanchuk 2001a).

XIII. ACKNOWLEDGMENTS

We thank the following for reviewing the manuscript and providing unpublished information: Christine Dean, Barry Jaquish, Leith Knowles, Marc Rust, J. Bradley St.Clair, Michael Stoehr, and Jack Woods. We also thank Frank Sorensen, Charles Tauer, and one anonymous reviewer for providing helpful comments on an earlier draft of the manuscript, and Dana Howe for editorial help.

LITERATURE CITED

Aagaard, J. E., S. S. Vollmer, F. C. Sorensen, and S. H. Strauss. 1995. Mitochondrial DNA products among RAPD profiles are frequent and strongly differentiated between races of Douglas-fir. Mol. Ecol. 4:441–447.

Adams, W. T. 1992. Gene dispersal within forest tree populations. New Forests 6:217–240.

Adams, W. T., and R. K. Campbell. 1981. Genetic adaptation and seed source specificity. p. 78–85. In: S. D. Hobbs, and O. T. Helgerson (eds.), Reforestation of skeletal soils, Proc. Reforestation Workshop, Medford, OR, 17–19 Nov. 1981, Forest Research Laboratory, Oregon State Univ., Corvallis.

Adams, W. T., V. D. Hipkins, J. Burczyk, and W. K. Randall. 1997. Pollen contamination trends in a maturing Douglas-fir seed orchard. Can. J. For. Res. 27:131–134.

Adams, W. T., S. N. Aitken, D. G. Joyce, G. T. Howe, and J. Vargas-Hernandez. 2001. Evaluating efficacy of early testing for stem growth in coastal Douglas-fir. Silvae Genet. 50:167–175.

Aitken, S. N., and W. T. Adams. 1995. Impacts of genetic selection for height growth on annual developmental cycle traits in coastal Douglas-fir. p. AD1: 1–8. In: Evolution of breeding strategies for conifers from the Pacific Northwest. Joint meeting of the IUFRO Working Parties S2.02.05 (Douglas-fir), S2.02.06 (*Pinus contorta*), S2.02.12 (Sitka spruce) and S2.02.14 (*Abies*), Limoges, France, 1–4 Aug. 1995, INRA, Station d'Amélioration des Arbres Forestiers, Ardon, France.

Aitken, S. N., and M. Hannerz. 2001. Genecology and gene resource management strategies for conifer cold hardiness. p. 23–53. In: F. J. Bigras, and S. J. Columbo (eds.), Conifer cold hardiness. Kluwer, Dordrecht, The Netherlands.

Aitken, S. N., W. T. Adams, N. Schermann, and L. H. Fuchigami. 1996. Family variation for fall cold hardiness in two Washington populations of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). For. Ecol. Managem. 80:187–195.

Amarasinghe, V., and J. E. Carlson. 2002. The development of microsatellite markers for genetic analysis in Douglas-fir. Can. J. For. Res. 32:1904–1915.

Anekonda, T. S., W. T. Adams, and S. N. Aitken. 1998. Influence of second flushing on genetic assessment of cold hardiness in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). For. Ecol. Managem. 111:119–126.

Anekonda, T. S., W. T. Adams, and S. N. Aitken. 2000. Cold hardiness testing for Douglas-fir tree improvement programs: Guidelines for a simple, robust and inexpensive screening method. West. J. Appl. For. 15:129–136.

Anonymous. 1998. Best seed origins for Douglas-fir. Making Headway with Forest Research, Coillte Research and Development Infor. Note No. 17, Newtownmountkennedy, County Wicklow, Ireland.

Anonymous. 2003. Lumber production and mill stocks: 2002. U.S. Census Bureau, Current Industrial Reports MA321T(02)-1 (http://www.census.gov/industry/1/ma321t02.pdf).

Anonymous. 2004. Forest Genetics Council of British Columbia 2003/2004 Annu. Rep.

Arnott, J. T. 1986. Douglas-fir stand establishment overview: Coastal British Columbia. p. 219–229. In: C. D. Oliver, D. P. Hanley, and J. A. Johnson (eds.), Douglas-fir: Stand management for the future. College of Forest Resources, Univ. Washington, Seattle.

Balduman, L. M., S. N. Aitken, M. Harmon, and W. T. Adams. 1999. Genetic variation in cold hardiness of Douglas-fir in relation to parent tree environment. Can. J. For. Res. 29:62–72.

Bastien, J.-C., B. Roman-Amat, and G. Vonnet. 1985. Natural variability of some wood quality traits of coastal Douglas-fir in a French progeny test: Implications on breeding strategy. In: Proc. IUFRO Working Party S2.02.05, Vienna, Austria, June 1985.

Bever, D. N. 1954. Evaluation of factors affecting natural reproduction of forest trees in central western Oregon. Oregon State Board of Forestry, Res. Bul. #3.

Birot, Y., and C. Christophe. 1983. Genetic structures and expected genetic gains from multitrait selection in wild populations of Douglas-fir and Sitka spruce. Silvae Genet. 32:141–151.

Breidenstein, J., J.-C. Bastien, and B. Roman-Amat. 1990. Douglas-fir range-wide variation results from the IUFRO data base. p. 2.13–2.26. In: Proc. joint meeting Western Forest Genetics Assoc. and IUFRO Working Parties S2.02-05, 06, 12, and 14 Douglas-fir, contorta pine, Sitka spruce, and *Abies* breeding and genetic resources, Olympia, WA, 20–24 Aug. 1990, Weyerhaeuser Co., Federal Way, WA.

British Columbia Ministry of Forests. 2004. Chief Forester's standards for seed use. British Columbia Ministry of Forests, November 20, 2004, Victoria.

Burdon, R. D. 1988. Recruitment for breeding populations: Objectives, genetics, and implementation. p. 555–572. In: B. S. Weir, E. J. Eisen, M. M. Goodman, and G. Namkoong (eds.), Proc. Second Intl. Conf. Quantitative Genetics, Raleigh, NC, 31 May–5 June 1987, Sinauer, Inc.

Burdon, R. D., and C. J. A. Shelbourne. 1971. Breeding populations for recurrent selection: Conflicts and possible solutions. New Zealand J. For. Sci. 1:174–193.

Cafferata, S. L. 1986. Douglas-fir stand establishment overview: Western Oregon and Washington. p. 211–218. In: C. D. Oliver, D. P. Hanley, and J. A. Johnson (eds.), Douglas-fir: Stand management for the future. College of Forest Resources, Univ. Washington, Seattle.

Campbell, R. K. 1964. Recommended traits to be improved in a breeding program for Douglas-fir. Weyerhaeuser Res. Note. No. 57, Forestry Research Center, Timberland Division, Weyerhaeuser Company, Centralia, WA.

Campbell, R. K. 1965. Phenotypic variation and repeatability of stem sinuosity in Douglas-fir. Northwest Sci. 39:47–59.

Campbell, R. K. 1972. Genetic variability in juvenile height-growth of Douglas-fir. Silvae Genet. 21:126–129.

Campbell, R. K. 1974a. A provenance-transfer model for boreal regions. Rep. Norwegian For. Res. Inst. 31:545–566.

Campbell, R. K. 1974b. Use of phenology for examining provenance transfers in reforestation of Douglas-fir. J. Appl. Ecol. 11:1069–1080.

Campbell, R. K. 1979. Genecology of Douglas-fir in a watershed in the Oregon Cascades. Ecology 60:1036–1050.

Campbell, R. K. 1986. Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. Silvae Genet. 35:85–96.

Campbell, R. K. 1991. Soils, seed-zone maps, and physiography: Guidelines for seed transfer of Douglas-fir in southwestern Oregon. For. Sci. 37:973–986.

Campbell, R. K. 1992. Genotype × environment interaction: A case study for Douglas-fir in western Oregon. USDA For. Serv. Res. Pap. PNW-RP-455, Portland, OR.

Campbell, R. K., and F. C. Sorenson. 1973. Cold acclimation in seedling Douglas-fir related to phenology and provenance. Ecology 54:1148–1151.

Campbell, R. K., and F. C. Sorensen. 1978. Effect of test environment on expression of clines and on delineation of seed zones in Douglas-fir. Theor. Appl. Genet. 51: 223–246.

Campbell, R. K., and A. I. Sugano. 1979. Genecology of bud-burst phenology in Douglas-fir: Response to flushing temperature and chilling. Bot. Gaz. 140:223–231.

Cherry, M. L., and G. T. Howe (eds.). 2004. Proc. Genetics and Growth Modeling Workshop. Vancouver, WA, 4–6 Nov. 2003, Pacific Northwest Research Cooperative Report 21, Oregon State Univ., Corvallis.

Ching, K. K. 1959. Hybridization between Douglas-fir and bigcone Douglas-fir. For. Sci. 5:246–254.

Ching, K. K. 1965. Early growth of Douglas-fir in a reciprocal planting. Forest Research Laboratory Res. Pap. 3, Oregon State Univ., Corvallis.

Ching, K. K., and P. N. Hinz. 1978. Provenance study of Douglas-fir in the Pacific Northwest region. Silvae Genet. 27:229–233.

Chollet, F. 1986. Les vergers à graines forestiers. p. 74–80. In: Amélioration génétique des arbres forestiers. Revue Forestière Française, Numéro Spécial 38.

Christophe, C., and Y. Birot. 1979. Genetic variation within and between populations of Douglas-fir. Silvae Genet. 28:197–206.

Copes, D. L. 1974. Genetics of graft rejection in Douglas-fir. Can. J. For. Res. 4:186–192.

Copes, D. L. 1977. Influence of rooting media on root structure and rooting percentage of Douglas-fir cuttings. Silvae Genet. 26:102–106.

Copes, D. L. 1985. Fertility of Douglas-fir pollen after one year of storage in liquid nitrogen. For. Sci. 31:569–574.

Copes, D. L. 1989. Bark scoring problem grafts in five Douglas-fir seed orchards: A case history. USDA For. Serv. Res. Note PNW-RN-487, Portland, OR.

Copes, D. L. 1999. Breeding graft-compatible Douglas-fir rootstocks (*Pseudotsuga menziesii* (Mirb.) Franco). Silvae Genet. 48:188–193.

Copes, D. L., and N. L. Mandel. 2000. Effects of IBA and NAA treatments on rooting Douglas-fir stem cuttings. New Forests 20:249–257.

Copes, D. L., N. C. Vance, W. K. Randall, A. Jasumback, and R. Hallman. 1991. Vacuum collection of Douglas-fir pollen for supplemental mass pollinations. USDA For. Serv. Res. Note PNW-RN-503, Portland, OR.

Copes, D. L., D. O. O'Rourke, and W. K. Randall. 1995. Field testing a modified duster for supplemental mass pollination in Douglas-fir seed orchards. Tree Planters' Notes 46:118–125.

Cornelius, J. 1994. Heritabilities and additive genetic coefficients of variation in forest trees. Can. J. For. Res. 24:372–379.

Cotterill, P. P. 1989. The nucleus breeding system. p. 36–42. In: Proc. 20th Southern Forest Tree Improvement Conf., Charleston, SC, 26–30 June 1989, Southern Forest Tree Improvement Committee, Springfield, VA.

Cotterill, P. P., and N. Jackson. 1989. Gains expected from clonal orchards under alternative breeding strategies. For. Sci. 35:183–196.

Cotterill, P. P., and J. W. James. 1984. Number of offspring and plot sizes required for progeny testing. Silvae Genet. 33:203–209.

Cotterill, P., C. Dean, J. Cameron, and M. Brindbergs. 1988. Nucleus breeding: A new strategy for rapid improvement under clonal forestry. p. 39–51. In: G. I. Gibson, A. R. Griffin, and A. C. Matheson, Breeding tropical trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Proc. IUFRO Meeting on Breeding Tropical Trees, Pattaya, Thailand, 28 Nov.–2 Dec. 1988, Oxford For. Inst., Oxford, UK.

Cress, D. W., and J. D. Daniels (eds.). 1990. Forest seed orchards of western Oregon, western Washington and northern California. Daniels and Associates Consultants, Centralia, WA.

Doerksen, A. H., and K. K. Ching. 1972. Karyotypes in the genus *Pseudotsuga*. For. Sci. 18:66–69.

Douglas-fir Cooperative 2004. http://www.forestresearch.co.nz/topic.asp?topic=Douglas%20Fir%20Cooperative&title=Douglas%20Fir%20Cooperative

Duffield, J. W. 1956. Damage to western Washington forests from November 1955 cold wave. USDA For. Serv. Pac. Northwest For. Range Exp. Sta. Res. Note 129, Portland, OR.

Ebell, L. F. 1972. Cone-induction response of Douglas-fir to form of nitrogen fertilizer and time of treatment. Can. J. For. Res. 2:317–326.

El-Kassaby, Y. A., and G. R. Askew. 1991. The relation between reproductive phenology and reproductive output in determining the gametic pool profile in a Douglas-fir seed orchard. For. Sci. 37:827–835.

El-Kassaby, Y. A., and K. Ritland. 1986. The relation of outcrossing and contamination to reproductive phenology and supplemental mass pollination in a Douglas-fir seed orchard. Silvae Genet. 35:240–244.

El-Kassaby, Y. A., and K. Ritland. 1996. Impact of selection and breeding on the genetic diversity in Douglas-fir. Biodivers. Conserv. 5:795–813.

El-Kassaby, Y. A., F. C. Yeh, and O. Sziklai. 1981. Estimation of the outcrossing rate of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] using allozyme polymorphisms. Silvae Genet. 30:182–184.

EUFORGEN. 2003. Summary of the meeting, Fourth EUFORGEN Conifers Network Meeting, Pitlochry, Scotland, 18–20 Oct. 2003.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics, 4th ed. Longman Group Ltd., Essex, England.

Farnum, P., R. Timmis, and J. L. Kulp. 1983. Biotechnology of forest yield. Science 219:694–702.

Farrar, J. L. 1995. Trees in Canada. Fitzhenry and Whiteside Ltd., Nat. Res. Canada, Canadian Forest Service, Markham/Ottawa, Ont.

Fashler, A. M. K., and Y. A. El-Kassaby. 1987. The effect of water spray cooling treatment on reproductive phenology in a Douglas-fir seed orchard. Silvae Genet. 36:245–249.

Ferre, Y. de. 1955. Les *Pseudotsuga* del l'Arboretum de Joueou et leur resistance au Phaeocryptus. Trav. Lab. for. Toulouse 6:art 1 [Forestry-Abstracts 1956 017-02982].

Ferrell, W. K., and E. S. Woodard. 1966. Effects of seed origin on drought resistance of Douglas-fir (*Pseudotsuga menziesii*) (Mirb.) Franco. Ecology 47:499–503.

Fins, L. 1983. Douglas-fir tree improvement plan. Inland Empire Tree Improvement Cooperative, Univ. Idaho, Moscow.

Fletcher, A. M. 2002. Introduction of western North American species to Europe and the IUFRO seed collections. In: G. Philippe, and G. Bergevin (eds.), Pacific temperate conifers as native and introduced species: Genetics and sexual reproduction, Proc. IUFRO Working Parties S2.02.5 and S2.01.16, Orleans, France, 27–30 May 2002, Cemagref.

Forest Products Laboratory. 1999. Wood handbook—Wood as an engineering material. USDA For. Serv. Forest Products Laboratory Gen. Tech. Rep. FPL-GTR-113, Madison, WI.

Fu, Y. B., A. D. Yanchuk, and G. Namkoong. 1999. Spatial patterns of tree height variations in a Douglas-fir progeny trial: Implications for genetic testing. Can. J. For. Res. 29:714–723.

Gamble, W. E., W. T. Adams, and J. Tappeiner II. 1996. Survival and growth of Douglas-fir seed sources in the Hospital Tract rangewide source archive plantation. Forest Research Laboratory Res. Contrib. 12, Oregon State Univ., Corvallis.

Gernandt, D. S., and A. Liston. 1999. Internal transcribed spacer region evolution in *Larix* and *Pseudotsuga* (Pinaceae). Am. J. Bot. 86:711–723.

Giertych, M. 1975. Seed orchard designs. p. 25–37. In: R. Faulkner (ed.), Seed orchards. Forestry Comm. Bul. 54, London.

Gill, G. P., G. R. Brown, and D. B. Neale. 2003. A sequence mutation in the cinnamyl alcohol dehydrogenase gene associated with altered lignification in loblolly pine. Plant Biotech. J. 1:253–258.

Goldfarb, B., G. T. Howe, L. M. Bailey, S. H. Strauss, and J. B. Zaerr. 1991a. A liquid cytokinin pulse induces adventitious shoot formation from Douglas-fir cotyledons. Plant Cell Rep. 10:156–160.

Goldfarb, B., S. H. Strauss, G. T. Howe, and J. B. Zaerr. 1991b. Transient gene expression of microprojectile-introduced DNA in Douglas-fir cotyledons. Plant Cell Rep. 10:517–521.

Griffin, A. R. 1974. Geographic variation in juvenile growth characteristics of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) from the coastal ranges of California. Ph.D. Diss., Oregon State Univ., Corvallis.

Griffin, A. R., and K. K. Ching. 1977. Geographic variation in Douglas-fir from the coastal ranges of California. I. Seed, seedling growth and hardiness characteristics. Silvae Genet. 26:149–157.

Griffith, B. G. 1940. Effect of indolebutyric acid, indoleacetic acid, and alpha naphthaleneacetic acid on rooting of cuttings of Douglas-fir and Sitka spruce. J. For. 38:496–501.

Griffin, J. R. 1964. A new Douglas-fir locality in southern California. For. Sci. 10:317–319.

Gupta, P. K., R. Timmis, and W. Carlson. 2003. Somatic embryo development in liquid medium for large-scale propagation of conifer trees. Abstract P-28. In: Proc. Congress on In Vitro Biology, Portland, OR, 31 May–4 June 2003. In Vitro Cell. Dev. Biol. - Plant 39:P-28.

Hadley, M. J., J. S. Tanz, and J. Fraser. 2001. Biotechnology: Potential applications in tree improvement. Forest Genetics Council of British Columbia Extension Note 2.

Hagenstein, W. D. 1986. Douglas-fir forestry from Allen to Mendel. p. 32–38. In: C. D. Oliver, D. P. Hanley, and J. A. Johnson (eds.), Douglas-fir: Stand management for the future. College of Forest Resources, Univ. Washington, Seattle.

Heaman, J. C. 1977. Choosing strategies for a breeding program in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) for coastal British Columbia—A case study. p. 1205–1214. In: A. G. Brown, and C. M. Palmberg (eds.), Proc. FAO/IUFRO Third World Consultation Forest Tree Breeding, Vol. 2, Canberra, Australia, 21–26 March 1977, CSIRO, Canberra, Australia.

Heiner, T. D., and D. P. Lavender. 1972. Early growth and drought avoidance in Douglas-fir seedlings. Forest Research Laboratory Res. Pap.14, Oregon State Univ., Corvallis.

Héois, B. (coordinator). 2000. EUDIREC: Towards a sustainable productive Douglas-fir forest of high quality raw material in Europe. Final report: European Union Project FAIR-CT95-0909.

Héois, B., M. Guibert, G. Philippe, N. Schermann, and J.-C. Bastien. 1995. Les vergers à graines de Douglas français: Une composante majeure du programme d'amélioration vers une organization européenne cooperative. In: Evolution of breeding strategies for conifers from the Pacific Northwest, Proc. IUFRO Working Parties S2.02.05, 06, 12 and 14, Limoges, France, 1–4 Aug. 1995.

Hermann, R. K. 1982. The genus *Pseudotsuga*: Historical records and nomenclature. Forest Research Laboratory, Special publication 2a, Oregon State Univ., Corvallis.

Hermann, R. K., and D. P. Lavender. 1990. *Pseudotsuga menziesii* (Mirb.) Franco. Douglas-fir. p.527–540. In: R. M. Burns, and B. H. Honkala (eds.), Silvics of North America, Vol. 1, Conifers. USDA For. Serv. Agr. Handb. 654.

Hermann, R. K., and D. P. Lavender. 1999. Douglas-fir planted forests. New Forests 17:53–70.

Hernandez, G. T., G. V. Alonso, G. P. Arribas, and J. L. Jenkinson. 1993. Screening Douglas-fir for rapid growth in common-garden tests in Spain. USDA For. Serv. Gen. Tech. Rep. PSW-GTR-146, Albany, CA.

Hodge, G. R., and T. L. White. 1993. Advanced generation wind-pollinated seed orchard design. New Forests 7:213–236.

Hoff, R. J. 1987. Susceptibility of inland Douglas-fir to *Rhabdocline* needle cast. USDA For. Serv. Res. Note INT-375, Ogden, UT.

Howard, J. L. 2001. U.S. timber production, trade consumption, and price statistics 1965 to 1999. USDA For. Serv. Res. Pap. FPL-RP-595, Forest Products Laboratory, Madison, WI.

Howe, G. T., and K. J. Jayawickrama. 2002. Genetics of stem quality in coastal Douglas-fir. p. 67–71. In: K. J. Jayawickrama, and G. Bracher (eds.), Proc. Genetic Improvement of Wood Quality in Coastal Douglas-fir and Western Hemlock. Corvallis, OR, 27 June 2002. Oregon State Univ., Corvallis (http://www.fsl.orst.edu/pnwtirc/publicatons/pnwtirc_pubs_date.htm).

Howe, G. T., J. Davis, Z. Jekni´c, T. H. H. Chen, B. Frewen, H. D. Bradshaw, Jr., and P. Saruul. 1999. Physiological and genetic approaches to studying endodormancy-related traits in *Populus*. HortScience 34:1174–1184.

Howe, G. T., S. N. Aitken, D. B. Neale, K. D. Jermstad, N. C. Wheeler, and T. H. H. Chen. 2003. From genotype to phenotype: Unraveling the complexities of cold adaptation in forest trees. Can. J. Bot. 81:1247–1266.

Howe, G. T., B. Shindler, B. Cashore, E. Hansen, D. Lach, and W. Armstrong. 2005. Public influences on plantation forestry. J. For. (in press).

Illingworth, K. 1978. Douglas-fir provenance trials in coastal British Columbia: Results to six years after planting. p. 411–425. In: Proc. IUFRO joint meeting of Working Parties. Vancouver, B.C., 21 Aug.–9 Sept. 1978.

Irgens-Moller, H. 1962. Genotypic variation in photoperiodic response of Douglas-fir seedlings. For. Sci. 8:360–362.

Irgens-Moller, H. 1967a. Geographical variation in growth patterns of Douglas-fir. Silvae Genet. 17:106–110.

Irgens-Moller, H. 1967b. Patterns of height growth initiation and cessation in Douglas-fir. Silvae Genet. 16:56–58.

Isaac, L. A. 1930. Seed flight in the Douglas-fir region. J. For. 28:492–499.

Isik, F., B. Li, J. Frampton, and B. Goldfarb. 2004. Efficiency of seedlings and rooted cuttings for testing and selection in *Pinus taeda*. For. Sci. 50:44–53.

Jackson, J. E. 1989. Worldwide development of high density planting in research and practice. Acta Hort. 243:17–28.

Jaquish, B. C. 1990. Geographic variation in ten-year height growth of interior Douglas-fir in British Columbia. p. 2.144–2.155. In: Proc. joint meeting Western Forest Genetics Assoc. and IUFRO Working Parties S2.02-05, 06, 12, and 14 Douglas-fir, contorta pine, Sitka spruce, and *Abies* breeding and genetic resources, Olympia, WA, 20–24 Aug. 1990, Weyerhaeuser Co., Federal Way, WA.

Jermstad, K. D., A. M. Reem, J. R. Henifin, N. C. Wheeler, and D. B. Neale. 1994. Inheritance of restriction fragment length polymorphisms and random amplified polymorphic DNAs in coastal Douglas-fir. Theor. Appl. Genet. 89:758–766.

Jermstad, K. D., D. L. Bassoni, N. C. Wheeler, and D. B. Neale. 1998. A sex-averaged genetic linkage map in coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb] Franco var. *menziesii*) based on RFLP and RAPD markers. Theor. Appl. Genet. 97:762–770.

Jermstad, K. D., D. L. Bassoni, K. S. Jech, N. C., Wheeler, and D. B. Neale. 2001a. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir: I. Timing of vegetative bud flush. Theor. Appl. Genet. 102:1142–1151.

Jermstad, K. D., D. L. Bassoni, N. C. Wheeler, T. S. Anekonda, S. N. Aitken, W. T. Adams, and D. B. Neale. 2001b. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir: II. Spring and fall cold-hardiness. Theor. Appl. Genet. 102: 1152–1158.

Jermstad, K. D., D. L. Bassoni, K. S. Jech, G. A. Ritchie, N. C. Wheeler, and D. B. Neale. 2003. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. III. Quantitative trait loci by environment interactions. Genetics 165:1489–1506.

Johnsen, Ø. 1988. After-effects on progenies from orchard clones moved to non-native environments. p. 1–11. In: J. Worrall, J. Loo-Dinkins, and D. P. Lester (eds.), Physiology and genetics of reforestation, Proc. 10th North American Forest Biology Workshop, Vancouver, B.C., 20–22 July 1988, Univ. British Columbia Press, Vancouver.

Johnson, G. R. 1997. Site-to-site genetic correlations and their implications on breeding zone size and optimum number of progeny test sites for coastal Douglas-fir. Silvae Genet. 46:280–285.

Johnson, G. R. 1998a. Breeding design considerations for coastal Douglas-fir. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-411, Portland, OR.

Johnson, G. R. 1998b. Parental GCA testing: How many crosses per parent? Can. J. For. Res. 28:540–545.

Johnson, G. R. 2002. Genetic variation in tolerance of Douglas-fir to Swiss needle cast as assessed by symptom expression. Silvae Genet. 51:80–86.

Johnson, G. R., and S. Lipow. 2002. Compatibility of breeding for increased wood production and long-term sustainability: The genetic variation of seed orchard seed and associated risks. p. 169–179. In: Congruent management of multiple resources, Proc. Wood Compatibility Initiative Workshop, number 18. USDA For. Serv. PNW-GTR-563, Portland, OR.

Johnson, G. R., R. A. Sniezko, and N. L. Mandel. 1997. Age trends in Douglas-fir genetic parameters and implications for optimum selection age. Silvae Genet. 46:349–358.

Johnson, G. R., F. Temel, and K. Jayawickrama. 2002. Genetic studies involving Swiss needle cast. p.38–43. In: G. Filip (ed.), Swiss Needle Cast Cooperative Annual Report, 2002. Forest Research Laboratory, College of Forestry, Oregon State Univ., Corvallis.

Johnson, G. R., N. C. Wheeler, and S. H. Strauss. 2000. Economic feasibility of marker-aided selection in Douglas-fir. Can. J. For. Res. 30:1942–1952.

Johnson, G. R., J. B. St.Clair, and S. Lipow. 2001. Genetic conservation in applied tree breeding programs. p. 215–230. In: B. A. Thielges, S. Sastrapradja, and A. Rimbawanto (eds.), Proc. Intl. Conf. on Ex Situ and In Situ Conservation of Commercial Tropical

Trees, Yogyakarta, Indonesia, 11–13 June 2001, International Tropical Timber Organization (ITTO), Yokohama, Japan.

Joly, R. J., W. T. Adams, and S. G. Stafford. 1989. Phenological and morphological responses of mesic and dry site sources of coast Douglas-fir to water deficit. For. Sci. 35:987–1005.

Kang, K. S., A. M. Harju, D. Lindgren, T. Nikkanen, C. Almqvist, and G. U. Suh. 2001. Variation in effective number of clones in seed orchards. New Forests 21:17–33.

King, J. N., F. C. Yeh, and J. C. H. Heaman. 1988a. Selection of growth and yield traits in controlled crosses of coastal Douglas fir. Silvae Genet. 37:158–164.

King, J. N., F. C. Yeh, J. C. H. Heaman, and B. P. Dancik. 1988b. Selection of wood density and diameter in controlled crosses of coastal Douglas-fir. Silvae Genet. 37:152–157.

King, J. N., F. C. Yeh, J. C. Heaman, and B. P. Dancik. 1992. Selection of crown form traits in controlled crosses of coastal Douglas-fir. Silvae Genet. 41:362–370.

Krutovskii, K. V., S. S. Vollmer, F. C. Sorensen, W. T. Adams, S. J. Knapp, and S. H. Strauss. 1998. RAPD genome maps of Douglas-fir. J. Hered. 89:197–205.

Krutovskii, K. V., K. D. Jermstad, N. C. Wheeler, G. T. Howe, J. B. St.Clair, and D. B. Neale. 2004. Gene discovery for adaptive traits and candidate gene variation in Douglas-fir. Abstract P622. In: Plant and Animal Genomes XII Conference, San Diego, CA, 10–14 January 2004. http://www.intl-pag.org/12/abstracts/P5i_PAG12_622.html.

Krutovsky, K. V., M. Troggio, G. R. Brown, K. D. Jermstad, and D. B. Neale. 2004. Comparative mapping in the Pinaceae. Genetics 168:447–461.

Kung, F. H., and J. W. Wright. 1972. Parallel and divergent evolution in Rocky Mountain trees. Silvae Genet. 21:77–85.

Lambeth, C. C., R. W. Stonecypher, and B. J. Zobel. 1982. Early testing of Douglas-fir in phytotron environments—The effect of selection trait and genotype-environment interaction. p. 137–148. In: B. A. Thielges (ed.), Proc. 7th North Am. For. Biol. Workshop, Univ. Kentucky, Lexington.

Lambeth, C., B.-C. Lee, D. O'Malley, and N. Wheeler. 2001. Polymix breeding with parental analysis of progeny: An alternative to full-sib breeding and testing. Theor. Appl. Genet. 103:930–943.

Larsen, J. B. 1981. Geographic variation in winter drought resistance of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco). Silvae Genet. 30:109–114.

Lavender, D. P., and W. S. Overton. 1972. Thermoperiods and soil temperatures as they affect growth and dormancy of Douglas-fir seedlings of different geographic origins. Forest Research Laboratory Research Paper 13, Oregon State Univ., Corvallis.

Lavender, D. P., K. K. Ching, and R. K. Hermann. 1968. Effect of environment on the development of dormancy and growth of Douglas-fir seedlings. Bot. Gaz. 129:70–83.

Lester, D. T. 1986. Genetic considerations for Douglas-fir. p. 151–154. In: C. D. Oliver, D. P. Hanley, and J. A. Johnson (eds.), Douglas-fir: Stand management for the future. College of Forest Resources, Univ. Washington, Seattle.

Li, P., and W. T Adams. 1989. Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). Can. J. For. Res. 19:149–161.

Li, P., and W. T. Adams. 1993. Genetic control of bud phenology in pole-size trees and seedlings of coastal Douglas-fir. Can. J. For. Res. 23:1043–1051.

Li, P., and W. T. Adams. 1994. Genetic variation in cambial phenology of coastal Douglas-fir. Can. J. For. Res. 24:1864–1870.

Lipow, S. R., G. R. Johnson, J. B. St.Clair, and K. J. Jayawickrama. 2003. The role of tree improvement programs for ex situ gene conservation of coastal Douglas-fir in the Pacific Northwest. For. Gen. 10:111–120.

Lipow, S. R., K. Vance-Borland, J. B. St.Clair, J. Henderson, and C. McCain. 2004. Gap analysis of conserved genetic resources for forest trees. Conserv. Biol. 18:412–423.

Magnussen, S., and A. D. Yanchuk. 1993. Selection age and risk: Finding the compromise. Silvae Genet. 42:25–40.

Magnussen, S., and A. D. Yanchuk. 1994. Time trends of predicted breeding values in selected crosses of coastal Douglas-fir in British Columbia: A methodological study. For. Sci. 40:663–685.

Marshall, K. A., and D. B. Neale. 1992. Inheritance of mitochondrial DNA in Douglas-fir. Can. J. For. Res. 22:73–75.

McDonald, G. I. 1979. Resistance of Douglas-fir to western spruce budworm: Results of a preliminary progeny test. USDA For. Serv. Res. Note INT-264, Ogden, UT.

MeGraw, R. 2002. Wood quality overview. p. 5–12. In: K. J. Jayawickrama, and G. Bracher (eds.), Proc. Genetic Improvement of Wood Quality in Coastal Douglas-fir and Western Hemlock. Corvallis, OR, 27 June 2002. Oregon State Univ., Corvallis (http://www.fsl.orst.edu/pnwtirc/publicatons/pnwtirc_pubs_date.htm).

Merkle, S. A., and W. T. Adams. 1987. Patterns of allozyme variation within and among Douglas-fir breeding zones in southwest Oregon. Can. J. For. Res. 17:402–407.

Monserud, R. A., and G. E. Rehfeldt. 1990. Genetic and environmental components of variation of site index in inland Douglas-fir. For. Sci. 36:1–9.

Morgenstern, E. K. 1996. Geographic variation in forest trees: Genetic basis and application of knowledge in silviculture. UBC Press, Univ. British Columbia, Vancouver.

Munger, T. T., and W. G. Morris. 1936. Growth of Douglas-fir trees of known seed source. U.S. Dept. Agric. Tech. Bul. 537.

Namkoong, G. 1969. Nonoptimality of local races. p. 149–153. In: Proc. 10th Southern Conf. Forest Tree Improvement, Houston, Texas, 17–19 June 1969, Texas Forest Service and Texas A&M Univ., College Station.

Namkoong, G. 1976. A multiple-index selection strategy. Silvae Genet. 25:199–201.

Namkoong, G., and H. H. Kang. 1990. Quantitative genetics of forest trees. Plant Breed. Rev. 8:139–188.

Neale, D. B., and W. T. Adams. 1985. The mating system in natural and shelterwood stands of Douglas-fir. Theor. Appl. Genet. 71:201–207.

Neale, D. B., and O. Savolainen. 2004. Association genetics of complex traits in conifers. Trends Plant Sci. 9:325–330.

Neale, D. B., N. C. Wheeler, and R. W. Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16:1152–1154.

O'Brien, I. E. W., D. R. Smith, R. C. Gardner, and B. G. Murray. 1996. Flow cytometric determination of genome size in *Pinus*. Plant Sci. 115:91–99.

O'Neill, G. A., and S. N. Aitken. 2004. Area-based breeding zones to minimize maladaptation. Can. J. For. Res. 34:695–704.

O'Neill, G. A., S. N. Aitken, and W. T. Adams. 2000. Genetic selection for cold hardiness in coastal Douglas-fir seedlings and saplings. Can. J. For. Res. 30:1799–1807.

O'Neill, G. A., W. T. Adams, and S. N. Aitken. 2001. Quantitative genetics of spring and fall cold hardiness in seedlings from two Oregon populations of coastal Douglas-fir. For. Ecol. Manage. 149:305–318.

Orr-Ewing, A. L. 1954. Inbreeding experiment with the Douglas-fir. For. Chron. 30:7–16.

Orr-Ewing, A. L., A. R. Fraser, and I. Karlsson. 1972. Interracial crosses with Douglas-fir: Early field results. B.C. For. Serv. Res. Note 55.

Parker, W. H. 2000. Rates of change of adaptive variation in *Picea mariana* visualized by GIS using a differential systematic coefficient. New Forests 20:259–276.

Patterson, H. D., and E. R. Williams. 1976. A new class of resolvable incomplete block designs. Biometrika 63:83–92.

Pharis, R. P., and W. K. Ferrell. 1966. Differences in drought resistance between coastal and inland sources of Douglas-fir. Can. J. Bot. 44:1651–1659.

Philippe, G., S. J. Lee, G. Schüte, and B. Héois. 2004. Flower stimulation is cost-effective in Douglas-fir seed orchards. Forestry 77:279–286.

Prudham, W. S. 2003. Taming trees: Capital, science, and nature in Pacific Slope tree improvement. Ann. Assoc. Am. Geograph. 93:636–656.

Randall, W. K. 1996. Forest tree seed zones for western Oregon. State of Oregon Dept. Forestry, Portland, OR.

Randall, W. K., and P. Berrang. 2002. Washington tree seed transfer zones. Washington Dept. Natural Resources, Olympia.

Rehfeldt, G. E. 1977. Growth and cold hardiness of intervarietal hybrids of Douglas-fir. Theor. Appl. Genet. 50:3–15.

Rehfeldt, G. E. 1978. Genetic differentiation of Douglas-fir populations from the northern Rocky Mountains. Ecology 59:1264–1270.

Rehfeldt, G. E. 1979a. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) populations. I. Idaho and northeast Washington. Heredity 43:383–397.

Rehfeldt, G. E. 1979b. Variation in cold hardiness among populations of *Pseudotsuga menziesii* var. *glauca*. USDA For. Serv. Res. Paper INT-233, Ogden, UT.

Rehfeldt, G. E. 1982. Ecological adaptations in Douglas-fir populations. II. Western Montana. USDA For. Serv. Res. Pap. INT-295, Ogden, UT.

Rehfeldt, G. E. 1983a. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) populations. III. Central Idaho. Can. J. For. Res. 13:626–632.

Rehfeldt, G. E. 1983b. Genetic variability within Douglas-fir populations: Implications for tree improvement. Silvae Genet. 32:9–14.

Rehfeldt, G. E. 1989. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*): A synthesis. For. Ecol. Managem. 28:203–215.

Rehfeldt, G. E. 1990. Adaptability versus zone size: Continuous zones for the Rocky Mountains (USA). p. 2.265–2.275. In: Proc. joint meeting Western Forest Genetics Assoc. and IUFRO Working Parties S2.02-05, 06, 12, and 14 Douglas-fir, contorta pine, Sitka spruce, and *Abies* breeding and genetic resources, Olympia, WA, 20–24 Aug. 1990, Weyerhaeuser Co., Federal Way, WA.

Riitters, K. H., and D. A. Perry. 1987. Early genetic evaluation of open-pollinated Douglas-fir families. For. Sci. 33:577–582.

Ritchie, G. A. 1993. Production of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco rooted cuttings for reforestation by Weyerhaeuser Company. p. 68–72. In: Comb. Proc. Intl. Plant Prop. Soc. 43:68–72.

Ritchie, G. A., and J. W. Keeley. 1994. Maturation in Douglas-fir: I. Changes in stem, branch, and foliage characteristics associated with ontogenetic aging. Tree Physiol. 14:1245–1259.

Roberds, J. H., and G. Namkoong. 1989. Population selection to maximize value in an environmental gradient. Theor. Appl. Genet. 77:128–134.

Roberds, J. H., J. O. Hyun, G. Namkoong, and G. Rink. 1990. Height response functions for white ash provenances grown at different latitudes. Silvae Genet. 39:121–129.

Rohmeder, E. 1956. Professor MUNCHS Anbauversuch mit Douglasien verschiedener Herkunft und anderen Nadelbaumarten im Forstamt Kaiserslautern-Ost 1912 bis 1954. Z. Forstgenet. 5:142–156 [Forestry Abstracts 1957, 018-01483].

Ross, S. D., J. E. Webber, R. P. Pharis, and J. N. Owens. 1985. Interaction between gibberellin A4/7 and root-pruning on the reproductive and vegetative process in Douglas-fir. I. Effects on flowering. Can. J. For. Res. 15:341–347.

Sewell, M. M., and D. B. Neale. 2000. Mapping quantitative traits in forest trees. p. 407–423. In: S. M. Jain, and S. C. Minocha (eds.), Molecular biology of woody plants. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Silen, R. R. 1965. Regeneration aspects of the 50-year-old Douglas-fir heredity study. p. 35–39. In: Proc. 1964 Annual Meet. West. Refor. Coord. Comm., West. For. Conserve. Assoc., Spokane, WA.

Silen, R. R. 1966a. A 50-year racial study of Douglas-fir in western Oregon and Washington. p. 6–7 (Abstract). In: Proc. 1965 Annual. Meet. West. For. Genetics Assoc.

Silen, R. R. 1966b. A simple, progressive, tree improvement program for Douglas-fir. USDA For. Serv. Res. Note PNW-45, Portland, OR.

Silen, R. R. 1978. Genetics of Douglas-fir. USDA For. Serv. Res. Pap. WO-35.

Silen, R. R., and D. L. Copes. 1972. Douglas-fir seed orchard problems—A progress report. J. For. 70:145–147.

Silen, R. R., and G. Kean. 1969. Cooling a Douglas-fir seed orchard to avoid pollen contamination. USDA For. Serv. Res. Note PNW-101, Portland, OR.

Silen, R. R., and N. L. Mandel. 1983. Clinal genetic growth variation within two Douglas-fir breeding zones. J. For. 81:216–220.

Silen, R. R., and J. Wanek. 1986. The J.E. Schroeder forest tree seed orchard in Oregon. J. For. 84:31–37.

Silen, R. R., and J. G. Wheat. 1979. Progressive tree improvement program in Coastal Douglas-fir. J. For. 77:78–83.

Sinclair, T. R. 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Sci. 38:638–643.

Slavov, G. T. 2004. Development and application of SSR markers for measuring gene flow in Douglas-fir. Ph.D. Dissertation, Oregon State Univ., Corvallis.

Slavov, G. T., G. T. Howe, I. Yakovlev, K. J. Edwards, K. V. Krutovskii, G. A. Tuskan, J. E. Carlson, S. H. Strauss, and W. T. Adams. 2004. Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. Theor. Appl. Genet. 108:873–880.

Smith, D. B., and W. T. Adams. 1983. Measuring pollen contamination in clonal seed orchards with the aid of genetic markers. p. 69–77. In: Proc. 17th Southern Forest Tree Improve. Conf., Athens, Georgia, 6–9 June 1983, Univ. Georgia, Athens.

Smith, W. B., J. S. Vissage, D. R. Darr, and R. M. Sheffield. 2001. Forest resources of the United States, 1997. USDA For. Serv. Gen. Tech. Report NC-219, North Central Research Station, St. Paul, MN.

Sorensen, F. C. 1967. Two-year results of a west-east transect-provenance test of Douglas-fir in Oregon. USDA For. Serv. Res. Note PNW-72, Portland, OR.

Sorensen, F. C. 1973. Frequency of seedlings from natural self-fertilization in coastal Douglas-fir. Silvae Genet. 22:20–24.

Sorensen, F. C. 1979. Provenance variation in *Pseudotsuga menziesii* seedlings from the var. *menziesii*—var. *glauca* transition zone in Oregon. Silvae Genet. 28:96–103.

Sorensen, F. C. 1983. Geographic variation in seedling Douglas-fir (*Pseudotsuga menziesii*) from the western Siskiyou Mountains of Oregon. Ecology 64:696–702.

Sorensen, F. C. 1997. Effects of sib mating and wind pollination on nursery seedling size, growth components, and phenology of Douglas-fir seed-orchard progenies. Can. J. For. Res. 27:557–566.

Sorensen, F. C. 1999. Relationship between self-fertility, allocation of growth, and inbreeding depression in three coniferous species. Evolution 53:417–425.

Sorensen, F. C., and J. E. Webber. 1997. On the relationship between pollen capture and seed set in conifers. Can. J. For. Res. 27:63–68.

Sorensen, F. C., and T. L. White. 1988. Effect of natural inbreeding on variance structure in tests of wind-pollination Douglas-fir progenies. For. Sci. 34:102–118.

Spicer, R., B. L. Gartner, and R. L. Darbyshire. 2000. Sinuous stem growth in a Douglas-fir (*Pseudotsuga menziesii)* plantation: Growth patterns and wood-quality effects. Can. J. For. Res. 30:761–768.

St.Clair, J. B. 1994. Genetic variation in tree structure and its relation to size in Douglas-fir. I. Biomass partitioning, foliage efficiency, stem form, and wood density. Can. J. For. Res. 24:1226–1235.

St.Clair, J. B., N. L. Mandel, and K. J. S. Jayawickrama. 2004. Early realized genetic gains for coastal Douglas-fir in the northern Oregon Cascades. West. J. Appl. For. 19: 195–201.

Stein, W. I., and P. W. Owston. 2002. *Pseudotsuga* Carr. (Douglas-fir). p. 1–38. In: F. T. Bonner, and R. G. Nisley (eds.), Woody plant seed manual. USDA For. Serv., Washington, DC. Available at http://ntsl.fs.fed.us/wpsm/Pseudotsuga.pdf (accessed 7/23/04).

Stephan, B. R. 1973. Über anfälligkeit und resistenz von Douglasien-Herkünften gegenüber *Rhabdocline pseudotsugae* (Susceptibility and resistance of Douglas-fir provenances to *Rhabdocline pseudotsugae*). Silvae Genet. 22:149–153.

Stephan, B. R. 1987. Differences in the resistance of Douglas-fir provenances to the wooly aphid *Gilletteella cooleyi*. Silvae Genet. 36:76–79.

Stoehr, M., and K. Bird. 2003. Coastal Douglas-fir. p. 7. In: B. Crown, and R. Painter (eds.), Forest Genetics Council of B.C. Tree Improvement Program Project Report 2002/2003. B.C. Ministry Forests, Victoria.

Stoehr, M. U., B. L. Orvar, T. M. Vo, J. R. Gawley, J. E. Webber, and C. H. Newton. 1998. Application of a chloroplast DNA marker in seed orchard management evaluations of Douglas-fir. Can. J. For. Res. 28:187–195.

Stoehr, M. U., J. E. Webber, C. C. Hollefreund, and R. A. Painter. 2004. Potential pollen contamination effects on progeny from an off-site Douglas-fir seed orchard: 9 year field results. Can. J. For. Res. 34:981–984.

Stonecypher, R. W., R. F. Piesch, G. G. Helland, J. G. Chapman, and H. J. Reno. 1996. Results from genetic tests of selected parents of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) in an applied tree improvement program. For. Sci. 42, Monograph 32:1–35.

Strauss, S. H., A. H. Doerksen, and J. R. Byrne. 1990. Evolutionary relationships of Douglas-fir and its relatives (genus *Pseudotsuga*) from DNA restriction fragment analysis. Can. J. Bot. 68:1502–1510.

Strauss, S. H., R. Lande, and G. Namkoong. 1992. Limitations of molecular-marker-aided selection in forest tree breeding. Can. J. For. Res. 22:1050–1061.

Strauss, S. H., W. H. Rottmann, A. M. Brunner, and L. A. Sheppard. 1995. Genetic engineering of reproductive sterility in forest trees. Mol. Breed. 1:5–26.

Sutton, B. 2002. Commercial delivery of genetic improvement to conifer plantations using somatic embryogenesis. Ann. For. Sci. 59:657–661.

Sweet, G. B. 1965. Provenance differences in Pacific Coast Douglas-fir. 1. Seed and seedling characteristics. Silvae Genet. 14:46–56.

Sweet, G. B. 1995. Seed orchards in development. Tree Physiol. 15:527–530.

Sweet, G. B., and S. L. Krugman. 1977. Flowering and seed production problems, and a new concept of seed orchards. p. 749–759. In: A. G. Brown, and C. M. Palmberg (eds.), Proc. FAO/IUFRO Third World Consultation Forest Tree Breeding, Vol. 2, Canberra, Australia, 21–26 March 1977, CSIRO, Canberra, Australia.

Sziklai, O. 1990. Douglas-fir provenance-progeny test at Haney B.C., Canada. p. 2.313–2.321. In: Proc. joint meeting Western Forest Genetics Assoc. and IUFRO Working Parties S2.02-05, 06, 12, and 14 Douglas-fir, contorta pine, Sitka spruce, and *Abies* breeding and genetic resources, Olympia, WA, 20–24 Aug. 1990, Weyerhaeuser Co., Federal Way, WA.

Temel, F., and W. T. Adams. 2000. Persistence and age-age genetic correlations of stem defects in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). For. Genet. 7:145–153.

Temel, F., and G. R. Johnson. 2001. Early testing of Douglas-fir for Swiss needle cast tolerance. p. 36–40. In. G. Filip (ed.), Swiss Needle Cast Research Cooperative Annual Report, 2001, Forest Research Laboratory, Oregon State Univ., Corvallis.

Timmis, R., J. Flewelling, and C. Talbert. 1994. Frost injury prediction model for Douglas-fir seedlings in the Pacific Northwest. Tree Physiol. 14:855–869.

van Frankenhuyzen, K., and T. Beardmore. 2004. Current status and environmental impact of transgenic forest trees. Can. J. For. Res. 34:1163–1180.

Vargas-Hernandez, J., and W. T. Adams. 1991. Genetic variation of wood density components in young coastal Douglas-fir: Implications for tree breeding. Can. J. For. Res. 21:1801–1807.

Vargas-Hernandez, J. J, W. T. Adams, and D. G. Joyce. 2003. Quantitative genetic structure of stem form and branching traits in Douglas-fir seedlings and implications for early selection. Silvae Genet. 52:36–44.

von Rudloff, E. 1972. Chemosystematics studies in the genus *Pseudotsuga*. I. Leaf oil analysis of the coastal and Rocky Mountain varieties of the Douglas-fir. Can. J. Bot. 50:1025–1040.

Webber, J. E. 1995. Pollen management for intensive seed orchard production. Tree Physiol. 15:507–514.

Webber, J. E., and R. A. Painter. 1996. Douglas-fir pollen management manual, 2nd ed. Research Branch, B.C. Ministry Forests Working Paper 02/1996, Victoria.

Westfall, R. D., and M. T. Conkle. 1992. Allozyme markers in breeding zone designation. New Forests 6:279–309.

Wheat, J. 1966. IFA Tree Improvement Newsletter No. 3, Tree Improvement Laboratory, Rt. 2, Box 3226, Olympia, WA.

Wheat, J., and R. Silen. 1977. Cooperative tree improvement in the Douglas-fir region. p. 1215–1220. In: A. G. Brown, and C. M. Palmberg (eds.), Proc. FAO/IUFRO Third World Consultation Forest Tree Breeding, Vol. 2, Canberra, Australia, 21–26 March 1977, CSIRO, Canberra, Australia.

Wheeler, N. C., and K. S. Jech. 1986a. Pollen contamination in a mature Douglas-fir seed orchard. p. 160–171. In: R. J. Weir (ed.), Proc. IUFRO Conf. on breeding theory, progeny testing and seed orchards, Williamsburg, VA, 13–17 Oct. 1986, North Carolina State Univ., Raleigh.

Wheeler, N. C., and K. S. Jech. 1986b. Estimating supplemental mass pollination (SMP) success electrophoretically. p. 111–120. In: G. E. Caron, A. G. Corriveau, and T. J. B. Boyle (eds.), New ways in forest genetics, Proc. 20th Canadian Tree Improvement Assoc. Meeting, Québec City, Québec, 19–22 Aug. 1985, Canadian Forestry Service, Ottawa.

Wheeler, N. C., and K. S. Jech. 1988. Supplemental mass pollination (SMP) in Douglas-fir seed orchards: Biological efficacy and economic evaluation. Weyerhaeuser Tech. Rep. 050-3210/25.

Wheeler, N. C., and K. S. Jech. 1992. The use of electrophoretic markers in seed orchard research. New Forests 6:311–328.

Wheeler, N. C., C. J. Masters, S. C. Cade, S. D. Ross, J. W. Keeley, and L. Y. Hsin. 1985. Girdling: An effective and practical treatment for enhancing seed yields in Douglas-fir seed orchards. Can. J. For. Res. 15:505–510.

Wheeler, N. C., R. W. Stonecypher, and K. S. Jech. 1990. Physiological characterization of select families to aid in source movement decisions: Supplementing long-term performance trials. p. 2.362–2.377. In: Proc. joint meeting Western Forest Genetics Assoc. and IUFRO Working Parties S2.02-05, 06, 12, and 14, Douglas-fir, contorta pine, Sitka spruce, and *Abies* breeding and genetic resources, Olympia, WA, 20–24 Aug. 1990, Weyerhaeuser Co., Federal Way, WA.

Wheeler, N. C., K. D. Jermstad, K. Krutovsky, S. N. Aitken, G. T. Howe, J. Krakowski, and D. B. Neale. 2005. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. IV. Cold-hardiness QTL verification and candidate gene mapping. Mol. Breed. (in press).

White, T. L. 1987. Drought tolerance of southwestern Oregon Douglas-fir. For. Sci. 33:283–293.

White, T. 1992. Advanced-generation breeding populations: Size and structure. p. 208–222. In: C. C. Lambeth, and W. Dvorak (eds.), Proc. IUFRO Conf. S2.02-08, breeding tropical trees—resolving tropical forest resource concerns through tree improvement, gene conservation, and domestication of new species, Cartagena-Cali, Colombia, 12–16 October 1992, CAMCORE, North Carolina State Univ., Raleigh.

White, T. L., and K. K. Ching. 1985. Provenance study of Douglas-fir in the Pacific Northwest region. IV. Field performance at age 25 years. Silvae Genet. 34:84–90.

Woods, J. H. 1989. Stem girdling to increase seed and pollen production of coast Douglas-fir. B.C. Ministry Forests Res. Note. 103, Victoria.

Woods, J. H. 1993. Breeding programs and strategies for Douglas-fir in North America. p. 1–11. In: Y. S. Park and G. W. Adams (eds.), Breeding strategies of important tree species in Canada. Natural Resources Canada, Fredericton, N.B. Inform. Rep. M-X-186E.

Woods, J. H., and M. Stoehr. 1993. Inbreeding effects on the seed production, nursery production, and early field growth of Douglas-fir. In: Proc. Western Forest Genetics Assoc. Annual Meeting, Oahu, HI, 4–8 October 1993.

Woods, J., J. King, and C. Cartwright. 1995a. Early genetic gains verified in realized-gain trials of coastal Douglas-fir and western hemlock. B.C. Ministry Forests Extension Note 01, Victoria.

Woods, J. H., D. Kolotelo, and A. D. Yanchuk. 1995b. Early selection of coastal Douglas-fir in a farm-field test environment. Silvae Genet. 44:178–186.

Woods, J. H., T. Wang, and S. N. Aitken. 2002. Effects of inbreeding on coastal Douglas-fir: Nursery performance. Silvae Genet. 51:163–170.

Wright, J. W. 1976. Introduction to forest genetics. Academic Press, New York.

Wright, J. W., F. H. Kung, R. A. Read, W. A. Lemmien, and J. N. Bright. 1971. Genetic variation in Rocky Mountain Douglas-fir. Silvae Genet. 20:54–60.

Wu, R. L., Z.-B. Zeng, S. E. McKeand, and D. M. O'Malley. 2000. The case for molecular mapping in forest tree breeding. Plant Breeding Rev. 19:41–68.

Yanchuk, A. D. 1996. General and specific combining ability from disconnected partial diallels of coastal Douglas-fir. Silvae Genet. 45:37–45.

Yanchuk, A. D. 2001a. A quantitative framework for breeding and conservation of forest tree genetic resources in British Columbia. Can. J. For. Res. 31:566–576.

Yanchuk, A. D. 2001b. The role and implications of biotechnological tools in forestry. Unasylva 52:53–61.

Yanchuk, A. D., and D. T. Lester. 1996. Setting priorities for conservation of the conifer genetic resources of British Columbia. For. Chron. 72:406–415.

Yeh, F. C., and C. Heaman. 1982. Heritabilities and genetic and phenotypic correlations for height and diameter in coastal Douglas-fir. Can. J. For. Res. 12:181–185.

Yeh, F. C., and J. C. Heaman. 1987. Estimating genetic parameters of height growth in seven-year-old coastal Douglas-fir from disconnected diallels. For. Sci. 33:946–957.

Zobel, B., and J. Talbert. 1984. Applied forest tree improvement. Wiley, New York.