Allozyme variation in interior Douglas-fir: association with growth and resistance to western spruce budworm herbivory

Zhong Chen, Thomas E. Kolb, Karen M. Clancy, Valerie D. Hipkins, and Laura E. DeWald

Abstract: We used starch gel electrophoresis to investigate levels of genetic variation between trees of interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca*) that were phenotypically resistant versus susceptible to defoliation by the western spruce budworm (*Choristoneura occidentalis* Freeman). We also investigated the association between allozyme variation and tree growth traits. Overall, the phenotypically resistant trees had a lower allelic heterozygosity (p = 0.020) compared with susceptible trees. However, this difference between resistant and susceptible trees primarily occurred at the Buena Vista, Colorado, site rather than the Deckers, Colorado, and Jacob Lake, Arizona, sites. Among 25 loci we examined, the resistant trees also had a higher frequency of the most common alleles (p = 0.057) and a higher proportion of homozygous genotypes, especially at loci *FEST-1* (p = 0.004), *ACO-1* (p = 0.080), and *6PGD-1* (p = 0.084). The higher allelic heterozygosity in susceptible trees was mainly due to their higher proportion of uncommon and (or) rare alleles. Compared with susceptible trees, resistant trees had higher mean radial growth rates (p = 0.047) and less temporal variability in growth rate over 25 years (p = 0.037). Mean radial growth rate and average tree heterozygosity were not related at any site (p = 0.316). Relationships between temporal variability in growth rate and tree heterozygosity were inconsistent among sites. Our results suggest that phenotypic differences in resistance of interior Douglas-fir to western spruce budworm defoliation are partly caused by genetic differences among trees.

Résumé : Les auteurs ont utilisé l'électrophorèse sur gel d'amidon afin de comparer l'ampleur de la variabilité génétique entre des phénotypes de sapin de Douglas de l'intérieur (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca*) résistants ou susceptibles à la défoliation par la tordeuse des bourgeons de l'épinette de l'Ouest (*Choristoneura occidentalis* Freeman). Les auteurs ont également étudié la relation entre la variabilité d'alloenzymes et les caractères de croissance des arbres. Globalement, l'hétérozygotie allélique des arbres résistants était plus faible (p = 0,020) que celle des arbres susceptibles. Cependant, cette différence entre arbres résistants et susceptibles a été principalement observée pour le site de Buena Vista au Colorado plutôt que pour les sites de Deckers au Colorado et Jacob Lake en Arizona. Parmi les 25 loci étudiés, les arbres résistants affichaient également une fréquence plus élevée d'allèles les plus communs (p = 0,057) et une plus grande proportion de génotypes homozygotes, spécialement aux loci *FEST-1* (p = 0,004), *ACO-1* (p = 0,080) et *6PGD-1* (p = 0,084). L'hétérozygotie allélique plus élevée remarquée chez les arbres susceptibles découlait principalement de leur plus grande proportion d'allèles rares ou peu communs. Comparativement aux arbres susceptibles, les arbres résistants affichaient des taux de croissance radiale plus élevés (p = 0,047) et une variabilité temporelle des taux de croissance moindre (p = 0,037) sur une période de 25 ans. Le taux moyen de croissance radiale et l'hétérozygotie moyenne des arbres ne démontraient pas d'association significative pour aucun site (p = 0,316). Les relations entre la variabilité temporelle du taux de croissance et l'hétérozygotie des arbres n'étaient pas congruentes d'un site à l'autre. Les résultats suggèrent que les différences phénotypiques de résistance du sapin de Douglas de l'intérieur à la tordeuse des bourgeons de l'épinette de l'Ouest sont dues en partie à des différences génétiques entre les arbres.

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Introduction

Knowledge of relationships among allelic heterozygosity of trees, their growth rates, and their resistance to environmental and biotic (e.g., insect herbivores) stresses is relevant to understanding evolutionary change as well as providing information that might be used in tree-improvement programs (Bergmann et al. 1989; Brown 1989; Weeden and Wendel 1989; Raja et al. 1997). Numerous studies have shown a variety of relationships between allelic heterozygosity of trees and quantitative and (or) qualitative traits. For example, lodgepole pine (*Pinus contorta* Dougl. ex Loud.) trees with high heterozygosity had lower variability in radial

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growth rate than trees with low heterozygosity (Knowles and Mitton 1980). However, El-Kassaby (1982) reported little association between allozyme variation and quantitative traits in coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) trees. Furthermore, Ledig et al. (1983) reported that the relationship between allele heterozygosity and growth traits in pitch pine (*Pinus rigida* Mill.) trees could be positive, negative, or neutral depending upon the site. Recently, Schmidtling and Hipkins (1998) reported no relationship between allozyme variation and height or volume growth in longleaf pine (*Pinus palustris* Mill.).

There is evidence for selection of trees with high heterozygosity on sites with high stress or large variability in environmental conditions. For example, ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) trees growing on south-facing sites with highly variable temperatures were more heterozygous than trees growing on north-facing slopes (Mitton 1995). Allelic heterozygosity of trees can also differ between soil types. Pinyon pine (*Pinus edulis* Engelm.) trees growing in nutrient-poor, extremely dry soils derived from cinders had higher allelic heterozygosity for the enzyme glycerate dehydrogenase (GLY) than those growing in sandy-loam soils (Mopper et al. 1991). However, heterozygosity at three other loci (isocitrate dehydrogenase (*IDH*), peroxidase (*PER*), and glucose phosphate isomerase (*PGI*)) did not differ between soil types in this study (Mopper et al. 1991). Likewise, several studies have shown a positive association between allelic heterozygosity and tolerance to air pollution in forest trees (Müller-Starck 1985; Bergmann and Scholz 1987). The results of these studies are consistent with the hypothesis that tree populations in highly heterogeneous environments will tend to have greater genetic variation than populations in less variable environments, because a higher number of heterozygous loci may increase fitness in variable environments by coding for a variety of proteins that help maintain physiological processes (McDonald and Ayala 1974). High heterozygosity at single or multiple gene loci not only may confer fitness advantages upon individuals but also constitutes the basis for evolutionary flexibility and response to changing environments (Strauss 1987; Meffe and Carroll 1997). However, trees with lower heterozygosity may be favored in less heterogeneous environments or environments with one dominate stress factor (Gregorius et al. 1985). The association between heterozygosity and fitness could vary over species, environments, and measurement scales (Strauss 1987).

Few studies have addressed the association between variation in tree allozymes and resistance to insect herbivores. Mopper et al. (1991) reported that pinyon pine trees that were resistant to attack by the shoot moth (*Dioryctria albovitella* Hulst) had greater heterozygosity at the *PER* and *IDH* loci than susceptible trees, and they suggested that higher heterozygosity might facilitate tree resistance to insect herbivory. However, Krabel and Petercord (2000) found that higher heterozygosity at the *IDH* locus was associated with more beech scale (*Cryptococcus fagisuga*) infestation in European beech (*Fagus sylvatica* L.) trees. These results indicate no consistent relationship between heterozygosity at the *IDH* locus and tree resistance to insect herbviory.

Douglas-fir is one of the most intensively studied and important commercial timber trees in western North America (Lassoie 1982; Hermann and Lavender 1990; Harlow et al. 1996) and includes coastal (var. *menziesii*) and interior (var. *glauca*) varieties. The interior variety is more frequently defoliated by the western spruce budworm (*Choristoneura occidentalis* Freeman). Repeated severe defoliation can reduce growth of Douglas-fir trees and cause top-kill or death of the entire tree, hence decreasing individual tree fitness and stand productivity (Brookes et al. 1986). Nevertheless, some trees show no obvious signs of defoliation when others within the same stand are heavily defoliated (Clancy et al. 1993). This apparent difference in resistance to budworm defoliation could result from various factors including uneven distribution of budworms, spatial variation in budworm mortality agents, or differences among trees in resistance mechanisms caused by genetic or environmental influences. However, no studies have addressed relationships among allozyme variation, tree growth rates, and tree resistance to the western spruce budworm in interior Douglas-fir.

The overall objective of our study was to compare genetic variation between interior Douglas-fir trees that are phenotypically resistant versus susceptible to budworm defoliation and to examine the relationship between allozyme variation and tree growth rates. We hypothesized that (*i*) the phenotypically resistant trees are genetically different from the susceptible trees, (*ii*) the phenotypically resistant trees have a greater allelic heterozygosity, and (*iii*) allele heterozygosity is positively related to tree growth rate and temporal stability in growth rate (i.e., resistant trees have greater heterozygosity and growth rate than susceptible trees).

Materials and methods

Tree characteristics

After population outbreaks of the western spruce budworm in Colorado and northern Arizona in the mid-1980s, twenty-four pairs of phenotypically resistant and susceptible trees were selected in 1988 and 1989 from two sites: near Deckers, Colo., in the Pike National Forest, and near Jacob Lake, Ariz., in the Kaibab National Forest. In 1994, an additional 16 pairs of resistant and susceptible trees were selected from a third site near Buena Vista, Colo., in the San Isabel National Forest. A pair of resistant and susceptible trees was defined as two trees that grew within 30 m of each other in the same stand, and with similar height, DBH (diameter at breast height, 1.3 m), aspect, slope, soil type, and microclimate (Clancy et al. 1993). The phenotypically resistant trees had healthy crowns and had sustained only light defoliation, whereas the susceptible trees had been heavily defoliated for several years (Clancy et al. 1993). Height and DBH were measured for all the selected trees. Yearly radial growth increment was measured on one increment core sample per tree removed at the breast height in the fall of 1990 (Deckers and Jacob Lake sites) or in the summer of 1995 (Buena Vista site).

Open-pollinated seeds were collected from the selected trees at the Deckers site in 1993 and 1994 and from trees at the Jacob Lake and Buena Vista sites in 1994. However, because it was not possible to collect enough seeds from each of the 40 pairs of trees for allozyme analysis, we used only those trees from which a sufficient number of seeds were collected. This included seven resistant and five susceptible trees from the Buena Vista site, seven resistant and seven susceptible trees from the Deckers site, and six resistant and four susceptible trees from the Jacob Lake site. There was no difference between the age of resistant (75.7 ± 6.2 years; mean ± SE) and susceptible trees (81.3 ± 6.7 years) at the Deckers and Jacob Lake sites (one-way ANOVA, $p = 0.549$). The mean age of susceptible trees (98.8 ± 5.6 years) was approximately 15 years older

than the age of resistant trees (84.0 ± 4.8 years) at the Buena Vista site (one-way ANOVA, $p = 0.072$). The seeds were sealed in plastic bags and stored in a freezer.

Enzyme electrophoresis

The seeds were soaked in 1% hydrogen peroxide for 48 h at room temperature and then germinated for 3–5 days (in December 1999) in Petri dishes lined with filter paper soaked in 1% hydrogen peroxide. After germination, embryo tissues were removed and discarded, and haploid megagametophytes were ground in three drops of 0.2 M phosphate extraction buffer (pH = 7.5). Ten megagametophyte samples per tree were prepared. With this sample size for each tree, the probability of inferring the genotype of an individual maternal tree correctly was $1 - (0.5)^{10-1} = 0.998$, assuming 1:1 segregation of alleles (Na'iem et al. 1991; Lamy et al. 1999). Each megagametophyte sample was absorbed onto six Whitman filter-paper wicks (2.0 × 14.0 mm). All samples were frozen at –70°C and partially thawed at room temperature prior to the electrophoresis.

The thawed wicks were inserted into 11% potato starch gels (Sigma Chemical Co.). Each gel set accommodated 6 groups of samples, and each group contained 10 samples. Two wicks soaked with red pine (*Pinus resinosa* Ait.) megagametophytes were inserted at the beginning and end of the samples within each group; these served as controls to assist in enzyme interpretation. A common Douglas-fir megagametophyte control wick (9019) was inserted between the first and second sample groups. Methods of sample preparation and electrophoresis followed the general methodology of Conkle et al. (1982), except that most enzyme stains were somewhat modified as outlined in NFGEL (1995).

Twenty-five loci coded in 16 enzyme systems were analyzed using three buffer systems. Leucine aminopeptidase (LAP-1 and LAP-2, EC 3.4.11.1), phosphoglucose isomerase (PGI-2, EC 5.3.1.9), phosphoglucomutase (PGM-1 and PGM-2, EC 5.4.2.2), aconitase (ACO-1 and ACO-2, EC 4.2.1.3), and fluorescent esterase (FEST-1 and FEST-2, EC 3.1.1.1) were run with a lithium borate buffer (LB, pH 8.3). Glycerate-2-dehydrogenase (GLYDH, EC 1.1.1.29), uridine diphosphoglucose pyrophorylase (UGPP-1, UGPP-2, and UGPP-3, EC 1.2.1.8), glucose-6-dehydrogenase (G6PD, EC 1.1.1.49), glutamic oxaloacetate transminase (GOT-1, GOT-2, and GOT-3, EC 2.6.1.1), superoxide dimutase (SOD, EC 1.15.1.1), glutamic dehydrogenase (GDH, EC 1.4.1.2), and catalase (CAT, EC 1.11.1.6) were run with a sodium borate buffer (SB, pH 8.8). Diaphorase (DIA-1, EC 1.6.99), malate dehydrogenase (MDH-1 and MDH-4, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6PGD-1, EC 1.1.1.44), and isocitrate dehydrogenase (IDH-1, EC 1.1.1.42) were run with a morpholine citrate buffer (MC6, pH 6.1). The laboratory work was completed in January of 2000 at the National Forest Genetics Electrophoresis Laboratory (NFGEL, Camina, Calif.).

Data analysis

Resistant and susceptible trees from the same site constituted one population. Within each population, the groups of resistant and susceptible trees were regarded as two subpopulations. The combined groups of resistant and susceptible trees from all three sites constituted two pooled populations. Differences in allelic and genotypic structure among these populations were compared with the following measures: mean number of alleles per locus (A), mean number of alleles per polymorphic locus (A_p), percentage of polymorphic loci (P), and observed (H_o) and expected (H_e) allele heterozygosity. The calculations of these genetic parameters were based on equations in Hamrick et al. (1992), Rothe (1994), and Berg and Hamrick (1997). Agreement for observed and expected genotype frequencies in Hardy–Weinberg equilibrium was examined with a χ^2 test. Most calculations were completed with the tools for population genetics analysis software (TFPGA) program (Miller 1997). The differences in A, H_e, and H_o between the subpopulations of resistant and susceptible trees at each site and between the pooled population of resistant and susceptible trees, were determined over 25 loci with paired t tests. However, the differences in A_p between subpopulations and pooled populations of resistant and susceptible trees were determined only at polymorphic loci.

The F (Wright 1965) and G_{ST} statistics (Nei 1977) were used to differentiate populations. Statistic G_{ST} (Nei 1977) over all polymorphic loci was used to measure differentiation among populations; it is an extension of Wright's F_{ST}, which was originally formulated for loci with two alleles (Berg and Hamrick 1997). The G_{ST} is equivalent to F_{ST} with two alleles per locus (Berg and Hamrick 1997): $G_{ST} = D_{ST}/H_T = (H_T - H_S)/H_T$, where D_{ST} is the proportion of genetic diversity among populations, H_S is the mean allelic heterozygosity over populations, and H_T is the total allelic heterozygosity at one locus ($H_T = 1 - \Sigma p_i^2$, where p_i is the mean frequency of the ith allele) (Hamrick et al. 1992; Rothe 1994). Jackknifing and bootstrapping procedures (repeated 1000 times) were performed with the TFPGA program (Miller 1997) to estimate sample standard deviations and 95% confidence intervals over all loci.

Average radial growth was calculated at 5-year intervals for each of the 36 trees used in the electrophoretic analysis over the 25 years prior to sampling (1966–1990 for the Deckers and Jacob Lakes sites; 1970–1994 for the Buena Vista site). Because trees from Deckers and Jacob Lake were different from those from Buena Vista in tree age and the years included in the radial growth measurement, we separated them for data analysis. For trees at the Deckers and Jacob Lake sites, we assessed the effects of trait (resistant vs. susceptible), site (Deckers vs. Jacob Lake), and their interaction on mean 5-year radial growth rate using a multivariate analysis of variance (MANOVA) with tree age as a covariant variable. For trees at the Buena Vista site, we determined the influence of trait on the mean 5-year radial growth rate using MANOVA with tree age as a covariate. The difference in mean 5-year radial growth rate between resistant and susceptible trees pooled over all sites was compared using a one-way analysis of variance (ANOVA).

The frequency of alleles at each locus was used for principal component analysis (PCA). This technique condenses the variance–covariance structure to a linear combination of the original variables that accounts for as much of the original total allozyme variation as possible (Dillon and Goldstein 1984), to partition variation among the populations by using fewer allozyme principal components. Correspondence analysis (Dillon and Goldstein 1984) was used to display the distribution pattern of allozymes and to show spatial relationships among populations. A simple regression model was used to describe the association between the average tree heterozygosity and mean 5-year radial growth and its coefficient of variation (CV) over 25 years. All statistical analyses were completed with SAS (SAS Institute Inc. 1990) and SAS JMP (SAS Institute Inc. 1995).

Results

Allelic structure

Overall, the phenotypically resistant trees had a different allele structure than the susceptible trees, but this difference varied among sites (Table 1). At the Buena Vista site, the susceptible trees had higher H_o and H_e than the resistant trees ($p \leq 0.009$). At the Jacob Lake site, the resistant trees had higher A and A_p than the susceptible trees ($p \leq 0.043$). However, at the Deckers site, there were no significant differences between the resistant and susceptible trees in any of the genetic parameters we measured ($p \geq 0.465$). Pooled

Table 1. Comparison of genetic parameters (mean ± 1 SE) over 25 loci for interior Douglas-fir trees that were phenotypically resistant versus susceptible to western spruce budworm defoliation at three sites.

Site	Trait	N^a	A^b	$A_p{}^c$	$H_e{}^d$	$H_o{}^e$	P (%)^f
Buena Vista,	Resistant	7	1.600±0.153	1.882±0.189	0.167±0.046	0.137±0.045	44
Colorado	Susceptible	5	1.800±0.153	2.716±0.154	0.266±0.049	0.228±0.054	60
	p		0.096	0.096	0.004*	0.009*	na^g
Deckers,	Resistant	7	1.600±0.141	1.882±0.169	0.181±0.047	0.147±0.037	48
Colorado	Susceptible	7	1.640±0.151	1.941±0.181	0.195±0.050	0.171±0.056	48
	p		0.664	0.668	0.574	0.465	na
Jacob Lake,	Resistant	6	1.640±0.140	1.941±0.160	0.189±0.044	0.127±0.032	52
Arizona	Susceptible	4	1.480±0.131	1.706±0.166	0.174±0.049	0.155±0.046	40
	p		0.043*	0.041*	0.558	0.517	na
Over all sites	Resistant	20	2.000±0.173	2.470±0.151	0.177±0.042	0.138±0.033	52
	Susceptible	16	1.960±0.187	2.600±0.163	0.214±0.046	0.185±0.050	56
	p		0.714	0.718	0.020*	0.071	na

Note: Asterisks indicate a significant difference at $\alpha = 0.05$; paired t test over 25 loci between the subpopulations and pooled populations of resistant and susceptible trees.

[a]Number of trees examined.
[b]Number of alleles per locus.
[c]Number of alleles per polymorphic locus.
[d]Expected allelic heterozygosity.
[e]Observed allelic heterozygosity.
[f]Percentage of polymorphic loci (95% criteria).
[g]Not applicable.

over all sites, the susceptible trees had a higher H_e (p = 0.020) and P than the resistant trees, which indicated higher genetic variation for the susceptible trees (Table 1).

Among the 25 loci we examined, *FEST-2*, *GOT-1*, *SOD*, *GDH*, *CAT*, *DIA-1*, *MDH-4*, and *IDH* were monomorphic (i.e., no genetic variation occurred at these loci), and they were excluded from further analyses. The other 17 loci were polymorphic and had 2 to 5 alleles per locus, and 8 of the 17 polymorphic loci had 3 alleles per locus (Table 2). At each locus, the allele that had the highest frequency (most often >0.5) was defined as the most common allele; otherwise, it was defined as an uncommon allele. On average, the frequency of most common alleles in resistant trees (0.818 ± 0.044) was marginally significantly higher than that in susceptible trees (0.778 ± 0.047) over 17 polymorphic loci (paired t test, p = 0.057). We defined uncommon alleles with a frequency ≤0.05 as rare alleles. Further, unique alleles were defined as those that did not occur in both resistant and susceptible trees. That is, unique alleles only occurred in one tree phenotype (i.e., either resistant or susceptible) but not in both phenotypes. Consequently, most common, uncommon, and rare alleles were identified by their frequency, whereas unique alleles were identified by their exclusive occurrence in either resistant or susceptible tree phenotypes. Resistant trees had six unique alleles (e.g., allele 3 of *LAP-2*, allele 2 of *PGI-2*, allele 2 of *PGM-1*, allele 3 of *ACO-2*, allele 4 of *GOT-3*, and allele 3 of *MDH-1*), whereas the susceptible trees had five unique alleles (e.g., allele 6 of *LAP*-2, alleles 4 and 5 of *PGI-2*, allele 3 of *UGPP-1*, and allele 4 of *UGPP-3*). Moreover, several of these unique alleles were rare (e.g., alleles 3 and 6 of locus *LAP-2*, alleles 2 and 4 of *PGI-2*, allele 2 of *PGM-1*, allele 3 of *ACO-2*, allele 3 of *UGPP-1*, allele 4 of *GOT-3*, and allele 3 of *MDH-1*) (Table 2).

Genetic structure and diversity

The values of H_o for loci *ACO-2* and *PGI-2* in the subpopulation of susceptible trees and for *UGPP-1* in the subpopulation of resistant trees at the Buena Vista site were significantly different from the H_e based on the Nei's (1978) unbiased calculation (χ^2 test, $p \le 0.025$). Differences between H_o and H_e were also significantly different for loci *ACO-2*, *6PGD-1*, and *UGPP-1* in the subpopulation of susceptible trees at Deckers site and for locus *FEST-1* in the subpopulation of resistant trees at Jacob Lake site (χ^2 test, $p \le 0.014$). Thus, most loci did not deviate significantly from the Hardy–Weinberg equilibrium. Moreover, compared with the resistant trees, the genotypes of susceptible trees had a higher proportion of heterozygotes but a lower proportion of homozygotes, particularly at loci *FEST-1*, *ACO-1*, and *6PGD-1* (G test, $p \le 0.084$) (Fig. 1). This result was also consistent with our conclusion that the susceptible trees overall had a significantly higher H_e than the resistant trees pooled over three sites (Table 1).

The H_T values averaged 0.2823 over pooled resistant and susceptible populations and ranged from 0.0247 at *MDH-1* to 0.6296 at *6PGD-1* (Table 3). The H_T averaged 0.2789 over the three site populations and ranged from 0.0274 at *MDH-1* to 0.6321 at *6PGD-1* (Table 3). Because H_S was much greater than D_{ST}, most genetic diversity occurred within these populations. Some loci such as *PGM-2*, *ACO-1*, *FEST-1*, *GLYDH*, *UGPP-1*, *UGPP-2*, and *6PGD-1* had a higher genetic diversity than others, indicating that the genetic variation varied over different loci. However, loci with a high total genetic diversity did not necessarily have a higher genetic differentiation between populations (e.g., *GLYDH* and *6PGD-1*) (Table 3).

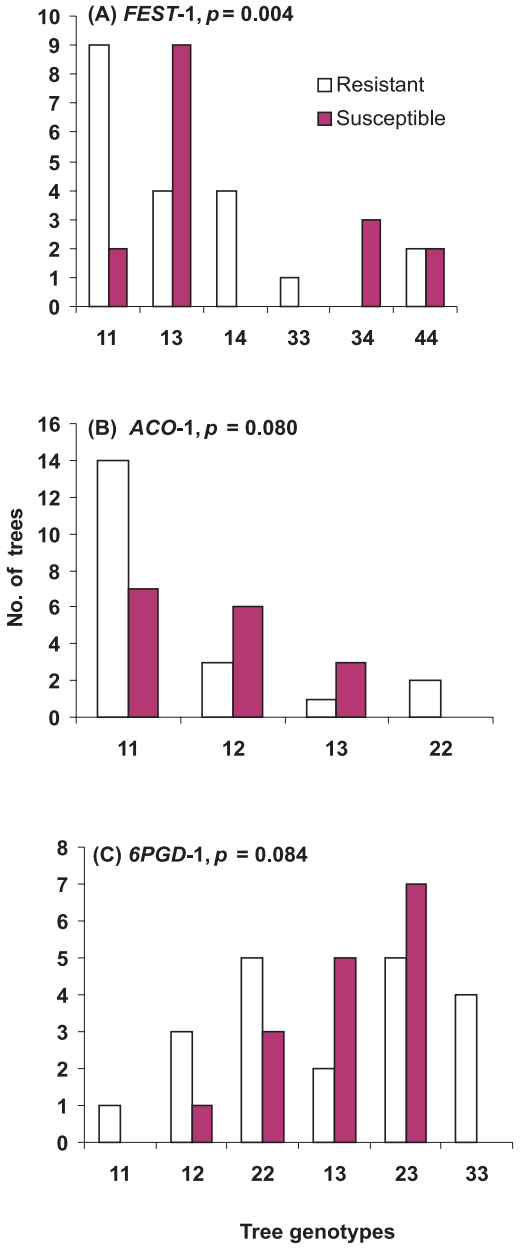
The mean F_{IS}, a measure of the deviation of the genotypic proportion from Hardy–Weinberg equilibrium, was 0.1435 over all loci (95% C I from 0.025 to 0.332) for the pooled resistant and susceptible trees (Table 3). Eleven of the 17 polymorphic loci had a positive F_{IS} value (Table 3), implying an excess of homozygosity; 6 of 17 loci (*LAP-1*, *LAP-2*, *UGPP-2*, *G6PD*, *GOT-2*, and *GOT-3*) had a negative F_{IS} value (Table 3), indicating an excess of heterozygosity.

Table 2. Comparison of allele frequencies at 17 polymorphic loci for pooled populations of interior Douglas-fir trees from southern Colorado and northern Arizona that were phenotypically resistant and susceptible to western spruce budworm defoliation.

Locus	Allele	Resistant (N = 20)[a]	Susceptible (N = 16)
LAP-1	5	0.875[b]	0.906[b]
	6	0.125	0.094
LAP-2	1	0.925[b]	0.875[b]
	2	0.025[c]	0.063
	3	0.025[c,d]	0.000
	4	0.025[c]	0.031[c]
	6	0.000	0.031[c,d]
PGI-2	1	0.975[b]	0.906[b]
	2	0.025[c,d]	0.000
	4	0.000	0.031[c,d]
	5	0.000	0.063[d]
PGM-1	1	0.050[c]	0.125
	2	0.025[c,d]	0.000
	4	0.925[b]	0.875[b]
PGM-2	1	0.075	0.156
	2	0.775[b]	0.719[b]
	3	0.125	0.125
ACO-1	1	0.800[b]	0.688[b]
	2	0.175	0.156
	3	0.025[c]	0.063
ACO-2	1	0.950[b]	0.844[b]
	2	0.025[c]	0.156
	3	0.025[c,d]	0.000
FEST-1	1	0.650[b]	0.406[b]
	3	0.150	0.375
	4	0.200	0.219
GLYDH	1	0.553[b]	0.594[b]
	2	0.395	0.375
UGPP-1	1	0.325	0.406
	2	0.675[b]	0.563[b]
	3	0.000	0.031[c,d]
UGPP-2	1	0.525[b]	0.625[b]
	2	0.475	0.375
UGPP-3	1	0.950[b]	0.875[b]
	2	0.050[c]	0.063
	4	0.000	0.063[d]
G6PD	1	0.975[b]	0.938[b]
	3	0.025[c]	0.063
GOT-2	1	0.025[c]	0.031[c]
	2	0.975[b]	0.969[b]
GOT-3	1	0.950[b]	1.000[b]
	4	0.050[c,d]	0.000
MDH-1	1	0.975[b]	1.000[b]
	3	0.025[c,d]	0.000
6PGD-1	1	0.175	0.188
	2	0.450[b]	0.438[b]
	3	0.375	0.375

[a]Number of trees.
[b]Most common allele with the highest frequency (most often ≥ 0.50) at each locus.
[c]Rare alleles with a frequency ≤ 0.05.
[d]Unique alleles that did not occur in both resistant and susceptible trees.

Fig. 1. Comparison of the distribution pattern of tree genotypes at loci *FEST-1* (A), *ACO-1* (B), and *6PGD-1* (C) between the pooled interior Douglas-fir trees that showed resistance and susceptibility to western spruce budworm defoliation. The *p* value indicates whether the distribution differed between resistant and susceptible trees at each locus with a *G* test.



The value of G_{ST}, a measure of genetic differentiation among populations, ranged from 0 (*GOT-2*) to 0.0387 (*ACO-2*) with a mean of 0.0130 for the pooled population of resistant and susceptible trees (Table 3), suggesting that most genetic diversity (98.7%) occurred within the pooled population and only 1.30% (i.e., G_{ST} = 0.013) occurred between the populations of resistant and susceptible trees. This mean G_{ST} value differed significantly from zero (*t* test, $P < 0.001$), showing the occurrence of genetic differentiation between populations of resistant and susceptible trees. However, because the value of *Nm*, the number of migrants per generation (*Nm* =

Table 3. Genetic diversity and structure between pooled populations of interior Douglas-fir trees that were resistant or susceptible to western spruce budworm defoliation and among three populations of interior Douglas-fir trees from Buena Vista, Colorado; Deckers, Colorado; and Jacob Lake, Arizona.

Locus (alleles)	Genetic diversity			Genetic structure	
	H_S	H_T	D_{ST}	F_{IS}	G_{ST}
Genetic differentiation between populations of resistant and susceptible trees pooled over three sites					
LAP-1 (2)	0.1944	0.1948	0.0005	–0.0995	0.0023
LAP-2 (5)	0.1855	0.1869	0.0014	–0.0474	0.0075
PGI-2 (4)	0.1113	0.1138	0.0026	0.4896	0.0224
PGM-1 (3)	0.1800	0.1822	0.0022	0.2366	0.0121
PGM-2 (3)	0.4108	0.4131	0.0024	0.2151	0.0057
ACO-1 (3)	0.4139	0.4175	0.0036	0.0713	0.0087
ACO-2 (3)	0.1800	0.1872	0.0073	0.5325	0.0387
FEST-1 (3)	0.5808	0.6038	0.0231	0.0597	0.0382
GLYDH (2)	0.5228	0.5233	0.0005	0.3436	0.0010
UGPP-1 (3)	0.4782	0.4832	0.0051	0.7209	0.0105
UGPP-2 (2)	0.4838	0.4888	0.0050	–0.1741	0.0102
UGPP-3 (3)	0.1608	0.1632	0.0024	0.1233	0.0147
G6PD (2)	0.0830	0.0837	0.0008	–0.0241	0.0090
GOT-2 (2)	0.0546	0.0546	0.0000	–0.0004	0.0000
GOT-3 (2)	0.0475	0.0488	0.0013	–0.0241	0.0266
MDH-1 (2)	0.0244	0.0247	0.0004	0.0029	0.0142
6PGD-1 (3)	0.6296	0.6296	0.0000	0.0131	0.0001
Mean	0.2789	0.2823	0.0034	0.1435	0.0130
Genetic differentiation among three populations at different sites pooled over resistant and susceptible trees					
LAP-1 (2)	0.1933	0.1982	0.0049	–0.1105	0.0249
LAP-2 (5)	0.1860	0.1896	0.0036	–0.0460	0.0190
PGI-2 (4)	0.1056	0.1083	0.0027	0.4997	0.0252
PGM-1 (3)	0.1581	0.1678	0.0097	0.2199	0.0580
PGM-2 (3)	0.4006	0.4054	0.0048	0.2247	0.0118
ACO-1 (3)	0.4056	0.4078	0.0022	0.0902	0.0055
ACO-2 (3)	0.1764	0.1775	0.0011	0.5591	0.0064
FEST-1 (3)	0.5789	0.5942	0.0153	0.0924	0.0257
GLYDH (2)	0.4779	0.5176	0.0397	0.3006	0.0768
UGPP-1 (3)	0.4688	0.4749	0.0061	0.7273	0.0129
UGPP-2 (2)	0.4816	0.4891	0.0075	–0.1644	0.0153
UGPP-3 (3)	0.1572	0.1654	0.0082	0.0998	0.0494
G6PD (2)	0.0826	0.0849	0.0023	–0.0345	0.0271
GOT-2 (2)	0.0509	0.0540	0.0031	–0.0476	0.0568
GOT-3 (2)	0.0442	0.0465	0.0023	–0.0336	0.0487
MDH-1 (2)	0.0266	0.0274	0.0008	0.0000	0.0280
6PGD-1 (3)	0.6203	0.6321	0.0118	0.0096	0.0186
Mean	0.2714	0.2789	0.0074	0.1404	0.0300

Note: The mean values of H_S, H_T, D_{ST}, F_{IS}, and G_{ST} are all significantly different from zero ($P < 0.05$, t test). H_S, mean allelic heterozygosity over populations; H_T, total allelic heterozygosity at one locus; D_{ST}, proportion of genetic diversity among populations; F_{IS}, deviation of the genotypic proportion from Hardy–Weinberg equilibrium over all loci; G_{ST}, genetic differentiation among populations ($G_{ST} = D_{ST}/H_T = (H_T - H_S)/H_T$).

$(1 - F_{ST})/4F_{ST}$; Wright 1931), was estimated to be >4 (or G_{ST} value < 0.0588) for all 17 polymorphic loci, this genetic differentiation between the populations of resistant and susceptible trees was not strong. The gene pool diversity (Rothe 1994) in the resistant and susceptible trees was 1.209 and 1.261, respectively. The gene pool distance between resistant and susceptible trees pooled over three sites was 0.0501. Finally, Nei's (1978) genetic identity between the populations of resistant and susceptible trees was 0.994. All these measures indicated that, while genetic differences between the populations of resistant and susceptible trees could be detected, the differences were small in magnitude.

Genetic diversity and structure also varied among populations from the three different sites. For example, G_{ST} values ranged from 0.0055 (*ACO-1*) to 0.0768 (*GLYDH*) with a mean of 0.030, indicating that about 97.0% of the genetic diversity occurred within populations and only 3.0% occurred among populations (Table 3). However, because of the small population sample size (<15 individuals per population), the G_{ST} value could be inflated (Berg and Hamrick 1997); thus, we advise caution when interpreting these results.

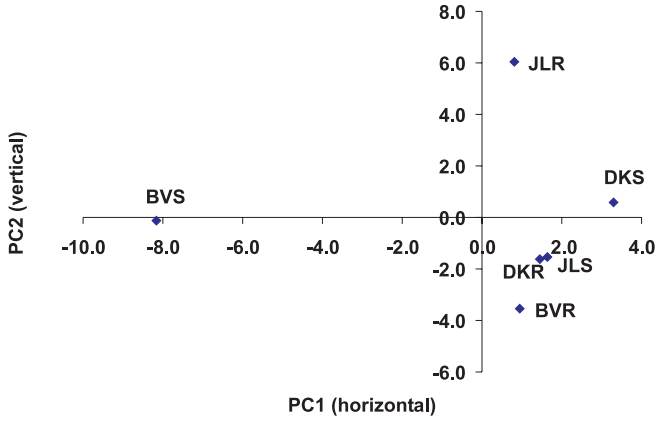
Principal component analysis (PCA) of the allelic heterozygosity showed that the first three principal components accounted for approximately 79% of the total variance in the six subpopulations (i.e., subpopulations of resistant and susceptible trees at each of three sites). The first, second, and third component explained approximately 35.6, 22.8, and 20.5% of the total variance, respectively. The first principal component (PC1) was primarily composed of 11 allozymes that had an absolute loading >0.20, among which PGI-21, PGM-14, UGPP-31, UGPP-34, G6PD-1, and 6PGD-13 were positive, whereas LAP-22, PGI-25, PGM-11, UGPP-13, and G6PD-3 were negative. The second principal component (PC2) was dominated by 10 allozymes that had an absolute loading >0.20, and the third principal component (PC3) contained 13 allozymes that had an absolute loading >0.20. However, some loci such as *UGPP-2*, *GOT-2*, and *MDH-1* accounted for a very small amount of variation based on the first three principal components.

Correspondence analysis based on the first two principal components demonstrated an obvious separation for the subpopulation of susceptible trees at the Buena Vista site (BVS) from the other five subpopulations on PC1 (horizontal axis) (Fig. 2). On the other hand, PC2 (vertical axis) showed separation between the subpopulation of resistant trees at the Buena Vista site (BVR) and resistant trees at the Jacob Lake (JLR) from other subpopulations. This result agrees with a cluster analysis result using the unweighted pair group method with arithmetic means procedure (UPGMA) based on Nei's (1972) original distance (results not shown). The largest genetic distance between the subpopulations of resistant and susceptible trees occurred at the Buena Vista site for PC1 (Fig. 2). Differentiation between the resistant and susceptible trees also occurred at the Jacob Lake site for PC2, whereas the differentiation was weak at the Deckers for both PC1 and PC2 (Fig. 2). Thus, differences in allelic heterozygosity between resistant and susceptible trees at the Buena Vista site made the largest contribution to the difference in allelic heterozygosity between the pooled resistant and susceptible trees over all sites (Table 1).

Relationship between allozyme variation and growth

At the Deckers and Jacob Lake sites, 5-year radial growth rate differed between resistant and susceptible trees (MANOVA, $p = 0.001$). Resistant trees had higher radial growth at every 5-year interval from 1966 to 1990 than susceptible trees (Fig. 3A). The increase in growth of the resis-

Fig. 2. Correspondence analysis of the allelic frequency over six subpopulations of interior Douglas-fir (BVR, resistant trees at Buena Vista, Colo; BVS, susceptible trees at Buena Vista, Colorado; DKR, resistant trees at Deckers, Colorado; DKS, susceptible trees at Deckers, Colorado; JLR, resistant trees at Jacob Lake, Arizona; JLS, susceptible trees at Jacob Lake, Arizona) based on the first two principle components.



tant trees between the 1981–1985 and 1986–1990 measurements, compared with a lack of increase for susceptible trees, may be due to less defoliation of resistant trees or perhaps greater compensatory growth of resistant trees after the western spruce budworm population outbreak ended. Site, the interaction between site and trait, and the tree age (as a covariant) did not influence the 5-year radial growth at the Deckers and Jacob Lake sites (MANOVA, $p \leq 0.159$). That is, there was no difference in the 5-year radial growth of trees between these sites, differences in growth rate between resistant and susceptible trees were similar for both sites, and growth rate was independent of tree age. Finally, the CV for the 5-year radial growth over 25 years did not differ significantly (one-way ANOVA, $p = 0.199$) between resistant (25.67 ± 2.74%) and susceptible (31.03 ± 2.98%) trees at these two sites.

At the Buena Vista site, tree trait had a significant effect on the pattern of temporal variation in 5-year radial growth (MANOVA, $p = 0.003$) (Fig. 3B). The decrease in radial growth rate during the 1975–1979 interval in resistant trees primarily accounted for this difference in pattern (Fig. 3B). There was no significant difference in the mean 5-year radial growth between the resistant and susceptible trees at the Buena Vista site (one-way ANOVA, $p = 0.705$) (Fig. 4). Tree age was a significant covariant on radial growth rate at the Buena Vista site (MANOVA, $p = 0.048$), indicating that radial growth rate also varied with tree age. The CV for the 5-year radial growth over 25 years did not differ significantly (one-way ANOVA, $p = 0.134$) between resistant (30.07 ± 4.75%) and susceptible (42.08 ± 5.63%) trees.

For trees pooled over all three sites, the resistant trees had a significantly greater mean 5-year radial growth rate (ANOVA, $p = 0.047$) but a lower CV in growth over time (ANOVA, $p = 0.037$) than the susceptible trees. The 5-year radial growth rate was 5.83 ± 0.44 and 4.58 ± 0.50 mm in resistant and susceptible trees, respectively (Fig. 4). On the other hand, the CV of the 5-year radial growth rate over 25 years was 27.49 ± 2.44% in resistant trees, and 35.56 ± 2.80% in susceptible trees, which indicated that the susceptible trees had a higher temporal variation in 5-year radial growth rate than the resistant trees.

Fig. 3. Comparison of radial growth over 5-year intervals between interior Douglas-fir trees that showed resistance and susceptibility to western spruce budworm defoliation. The means for radial growth increment at each 5-year interval were least-square means and adjusted to the covariant tree age.

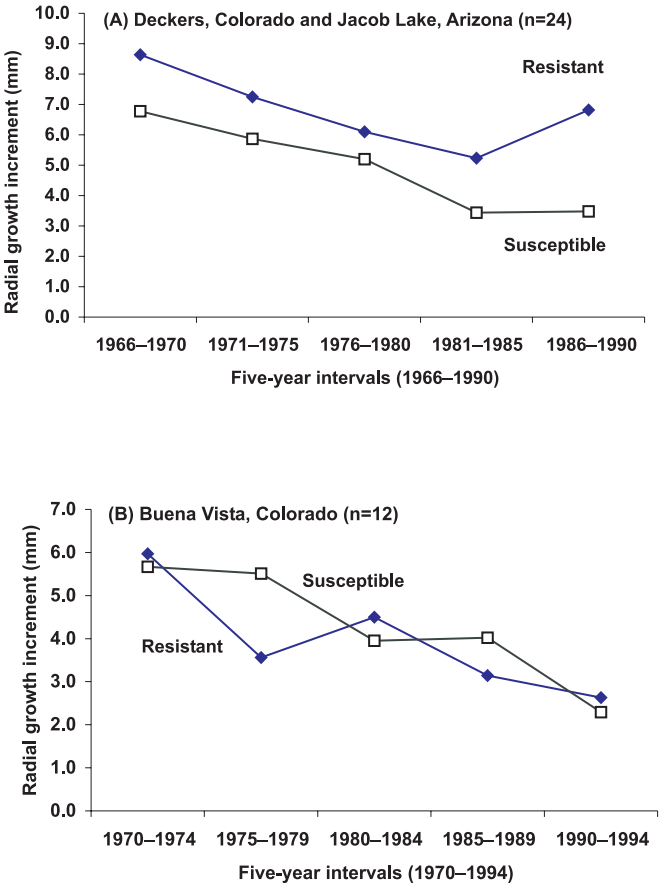
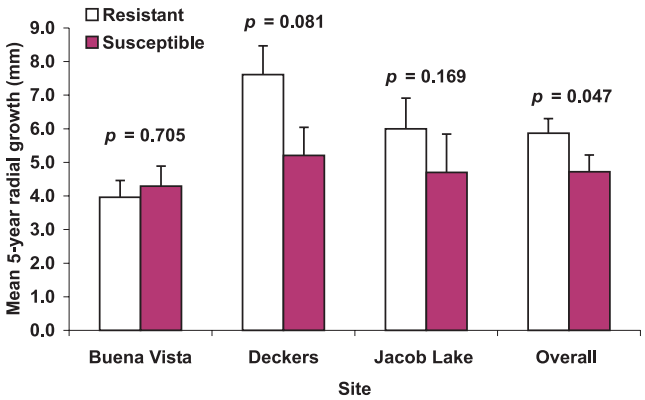
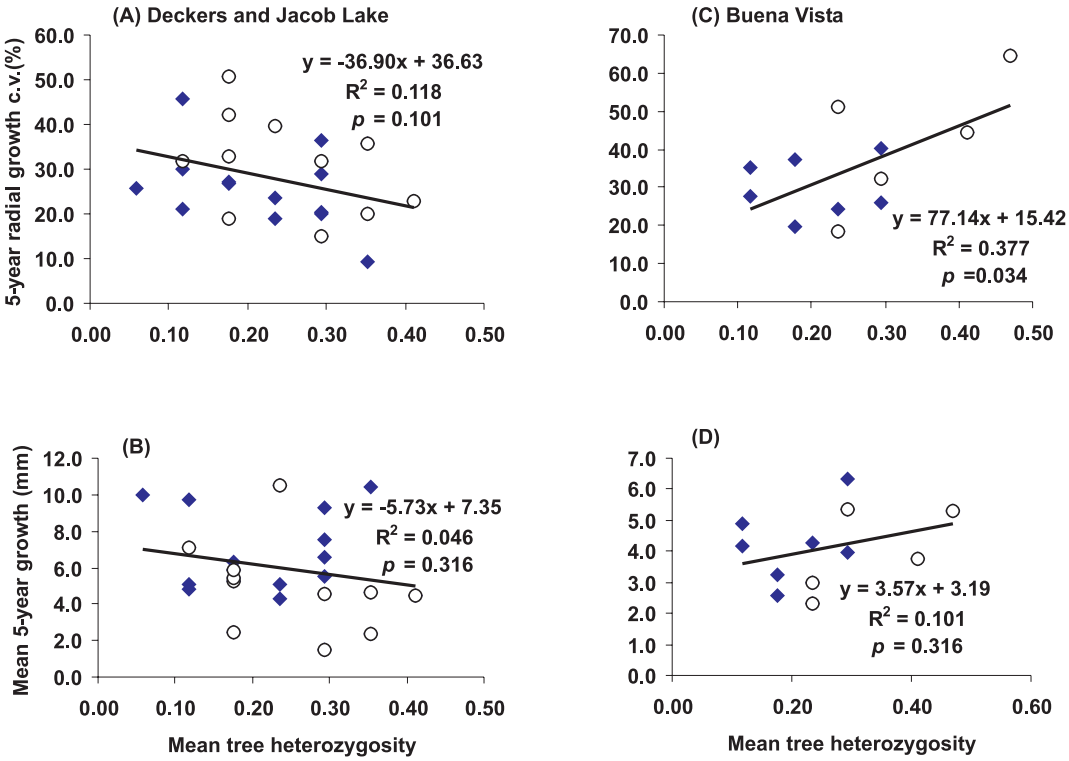


Fig. 4. Mean 5-year radial growth over 25 years (1966–1990 for trees at Deckers and Jacob Lake sites; 1970–1994 for trees at Buena Vista site) between phenotypically resistant versus susceptible trees within each site. The means for radial growth increment at each 5-year interval were least-square means and adjusted to the covariant tree age. Error bars are +1 SE.



A tree's genotype at one particular locus will be either heterozygous (consisting of two different alleles) or homo-

Fig. 5. Mean tree heterozygosity versus the coefficient of variation for 5-year radial growth rate at the Deckers and Jacob Lake sites (A) and Buena Vista site (C) and versus mean 5-year radial growth at the Deckers and Jacob Lake sites (B) and Buena Vista site (D). The solid diamonds and open circles show the phenotypic resistant and susceptible trees, respectively.



zygous (consisting of two same alleles). The average heterozygosity of a tree in this study was calculated as the proportion of heterozygous loci over the 17 polymorphic loci. The CV of the 5-year radial growth over 25 years in trees at Deckers and Jacob Lake sites was negatively associated with mean tree heterozygosity (p = 0.101) (Fig. 5A), but only approximately 12% of the variation in the CV of 5-year radial growth was explained by tree heterozygosity. Furthermore, this pattern was similar in both resistant and susceptible trees at these two sites (Fig. 5A). In contrast, the CV of the 5-year radial growth was positively correlated with mean tree heterozygosity at the Buena Vista site (p = 0.034) (Fig. 5C), where approximately 38% of the variation in the CV of the 5-year radial growth was accounted for by mean tree heterozygosity. However, this positive association occurred primarily because of two susceptible trees (Fig. 5C). Moreover, there was no significant relationship between mean 5-year radial growth and mean tree heterozygosity at any site (p = 0.316) (Figs. 5B and 5D).

Discussion

The A and H_e values in our study (Table 1) were close to those measured for interior Douglas-fir in the Chuska Mountains in northeastern Arizona (A = 2.19, H_e = 0.248) (Moser 1999). However, the P values in our study (mean 48.7%) were lower than in the Chuska Mountains (76.4%) (Moser 1999). Among the 25 loci we examined, 15 loci (60%) had two or three alleles per locus, two loci (*LAP-2* and *PGI-2*) (8%) had four or five alleles per locus (Table 2), and eight loci (32%) had only one allele per locus. These values are lower than the mean number of alleles per locus for 13 coniferous species (including Douglas-fir) sampled in California, in which about 80% of the loci had three or more alleles (Conkle 1992).

H_e is a function of the percentage of polymorphic loci, the number of alleles per locus, and the evenness of allele frequency within populations (Berg and Hamrick 1997); therefore, it is a composite measure. Compared with the previously reported H_e values for interior Douglas-fir trees, including 0.11 in the Great Basin area (Schnabel et al. 1993), 0.15 for the northern interior variety, and 0.08 for the southern interior variety (Li and Adams 1989), H_e in our study (mean 0.195) was closer to that of the coastal variety. For instance, H_e was 0.16 for the coastal variety from southwestern Oregon (Moran and Adams 1989), 0.18 from 22 breeding zones in southwestern Oregon (Merkle and Adams 1987), and 0.165 from a regionwide study of the coastal variety (Li and Adams 1989). Various factors such as geographic features, demographic history, sampling design, the population size investigated, and the number of gene loci examined may explain these differences.

Analysis of genetic differentiation showed that 98.7% of the variation resided within the pooled populations and the remaining 1.3% (i.e., G_{ST} = 0.013) occurred between the populations of resistant and susceptible trees (Table 3). Regarding our three sites, 97.0% of the variation occurred within the site and the remaining 3.0% (i.e., G_{ST} = 0.030) occurred among sites (Table 3). Our results are similar to Moser's (1999) for Douglas-fir in the Chuska Mountains of northeastern Arizona and are consistent with general patterns of allozyme variation in coniferous trees with wide

geographic ranges: most of the genetic variation occurs within geographic populations (Hamrick et al. 1992).

While numerous studies have addressed the population genetics of Douglas-fir, fewer have compared allozyme variation between trees resistant and susceptible to damaging biotic agents. Moser (1999) found no difference in H_e between Douglas-fir trees infected with dwarf mistletoe (*Arceuthobuim douglasii* Engelm.) and noninfected trees. In contrast, we found differences in H_e (Table 1) and genetic differentiation (i.e., G_{ST} significantly different from zero; Table 3) between trees resistant and susceptible to western spruce budworm defoliation based on field observations of crown condition following an outbreak. However, we note that this genetic differentiation between resistant and susceptible trees primarily occurred at the Buena Vista site rather than at the Deckers and Jacob Lake sites. Without the Buena Vista site, H_e would have been similar between resistant and susceptible trees in our study, similar to Moser's (1999) results for Douglas-fir resistance to dwarf mistletoe. Resistant trees in our study had a greater frequency of most common alleles at 12 loci, particularly at *FEST-1*, *ACO-1*, and *6PGD-1* (Table 2, Fig. 1), than susceptible trees. Similarly, Moser (1999) also found a greater frequency of most common alleles for Douglas-fir trees not infected by dwarf mistletoe compared with infected trees at three loci: *ACO-1*, *IDH-1*, and *G6PD-1*. Thus, higher frequencies of most common alleles might be associated with Douglas-fir resistance to damaging biotic agents.

Our results support our first hypothesis that phenotypically resistant and susceptible trees differ genetically, which implies that resistance of Douglas-fir to budworm defoliation is to a certain extent genetically controlled. However, our results did not support our second hypothesis that phenotypically resistant trees would have a greater H_e. Contrary to our expectation, the susceptible trees had an overall greater H_e than the resistant trees, particularly for trees at the Buena Vista site (Table 1).

Regarding our third hypothesis that H_e would be positively correlated with tree growth rate and temporal stability in growth rate, the CV of the 5-year radial growth over 25 years was associated with mean tree heterozygosity (Figs. 5A and 5C), but the direction and magnitude of this correlation differed among sites. Furthermore, mean 5-year radial growth rate was not related to tree heterozygosity at any site (Figs. 5B and 5D). Thus, our results do not support our third hypothesis that H_e would be positively correlated with tree growth rate. Ledig et al. (1983) reported that the relationship of tree growth to heterozygosity (number of heterozygous loci) in pitch pine was negative, neutral, or positive depending on the stand sampled. Moser (1999) also found no significant linear relationships between growth (e.g., DBH, and height) and tree heterozygosity. Overall, we conclude that there are no general consistent relationships between tree heterozygosity and tree radial growth rate or temporal variation in growth rate in southern interior populations of Douglas-fir, although such relationships may be detected in some individual stands.

Allozyme variation at the *IDH* locus has been investigated in other studies of tree resistance to insects (Mopper et al. 1991; Krabel and Petercord 2000) and in our study also. Mopper et al. (1991) found that pinyon pine trees resistant to shoot moth herbivory were significantly more heterozygous at the *IDH* locus than susceptible trees. In contrast, the association between the IDH enzyme and resistance of host trees to insect herbivory was opposite in European beech: trees with a higher proportion of heterozygous genotypes at the *IDH* gene locus tended to be more infested by beech scales (Krabel and Petercord 2000). Among the 25 enzyme loci investigated in our study, *IDH* was monomorphic and, thus, did not contribute to the genetic variation. Collectively, these findings indicate no general relationship between allozyme variation at the *IDH* locus and tree resistance to insect herbivory.

Conclusions

Allelic heterozygosity and genetic structure and composition were associated with Douglas-fir resistance to western spruce budworm defoliation. Allele frequency differed between resistant and susceptible trees at certain loci, especially *FEST-1*, *ACO-1*, and *6PGD-1*, in which, the resistant trees tended to have higher proportions of homozygous and lower proportions of heterozygous genotypes. Consequently, these three loci might have potential as protein markers to assist in early selection for resistant trees in tree-improvement programs. Furthermore, the resistant trees appeared to have a higher compensatory growth rate after defoliation than the susceptible trees. Overall, trees resistant to defoliation had a significantly higher radial growth rate but a lower allelic heterozygosity than susceptible trees and less temporal variation in 5-year radial growth over 25 years. Relationships between mean tree heterozygosity and the CV of the 5-year radial growth were inconsistent over different sites. Finally, there was no detectable correlation between the mean 5-year radial growth rate and mean tree heterozygosity.

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