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Isozyme markers associated with O_3 tolerance indicate shift in genetic structure of ponderosa and Jeffrey pine in Sequoia National Park, California

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Genetic variation in isozyme markers associated with ozone tolerance differed between parental trees and their progeny in two closely related species of yellow pine.

Abstract

Effects of canopy ozone (O_3) exposure and signatures of genetic structure using isozyme markers associated with O_3 tolerance were analyzed in ∼20-, ∼80-, and >200-yr-old ponderosa (*Pinus ponderosa* Dougl. ex Laws.) and Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.) in Sequoia National Park, California. For both species, the number of alleles and genotypes per loci was higher in parental trees relative to saplings. In ponderosa pine, the heterozygosity value increased, and the fixation index indicated reduction of homozygosity with increasing tree age class. The opposite tendencies were observed for Jeffrey pine. Utilizing canopy attributes known to be responsive to O_3 exposure, ponderosa pine was more symptomatic than Jeffrey pine, and saplings were more symptomatic than old growth trees. We suggest that these trends are related to differing sensitivity of the two species to O_3 exposure, and to higher O_3 exposures and drought stress that younger trees may have experienced during germination and establishment.

Keywords: Genetic structure; Isozymes; *Pinus ponderosa*; *Pinus jeffreyi*; Ozone exposure

1. Introduction

Sequoia National Park (SNP) is the most polluted national park in the U.S.A.: "In the past six years, Sequoia and Kings Canyon has had the most days exceeding the national health standard for ozone of any unit in the National Park System.[1]" The source of the pollution is long distance transport of secondary products of fossil fuel combustion into the park from both San Francisco and the San Joaquin Valley (Bytnerowicz et al., 2002). The 12-h average of ozone (O_3) in Sequoia National Park was 63 ppb over the 5-month growing season (May–September) from 1987 through 2003 (National Park Service Air Resources Division database). Over that time period, air quality in the park exceeded the state standard 32 days per year on average (Fig. 1).

The onset of poor air quality in Sequoia National Park is unknown, but possible levels of O_3 exposure can be inferred from increases in upwind population (by county), assumptions in the relationships between population and fossil fuel consumption, and constructed long term O_3 concentrations from other locations. Fossil fuel consumption was correlated with population increase in California, until more stringent air

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[1] National Parks Conservation Association report, http://www.npca.org/turningpoint/; and National Park Service Air Resources Division air quality database for ozone, http://www2.nature.nps.gov/air/Monitoring/network.cfm#data.html. Subsequent references to this database will be cited as the NPS ARD air quality database.

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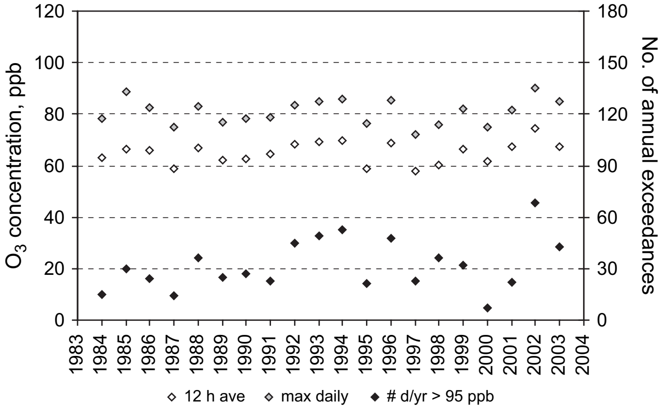

Fig. 1. Daily O_3 concentrations for 12 h (open diamonds), maximum daily O_3 concentrations (gray diamonds), and number of exceedances of California state air quality standards (black diamonds) over the 5-month period. May through September. Data were provided from the National Park Service Air Quality Division.

quality standards were achieved in the mid 1990s (Fig. 2). The longest record of O_3 concentrations were constructed (correcting for older methodologies) for a site in the western San Bernardino Mountains in the Transverse Range (Lee et al., 2003) subject to downwind transport of pollutants from Los Angeles, CA. Lee et al.'s (2003) analysis suggests a steady increase in O_3 concentrations from 1963 to peak values in the late 1970s and early 1980s, followed by a decrease in O_3 concentration by 1999 to 1960 levels. The rate of human population increase in the counties upwind differed between the southern Sierra Nevada and the Transverse Range (Fig. 3). In the Transverse Range, the first foliar symptoms at ambient (elevated) O_3 concentrations were observed in 1953 in the Transverse Range (Asher 1956, as seen in Tingey et al., 2004), with a population of ~5 million people in Los Angeles metropolitan area. This population corresponded to an estimated annual fossil fuel consumption of ~80,000 barrels of petroleum (projected from Fig. 2), with emission transport ~100 km. In Sequoia National Park, the first foliar symptoms at ambient (elevated) O_3 concentrations were observed in 1983 in Sequoia National Park (Miller et al., 1995), with a population of ~8 million people in San Francisco Bay and San Joaquin Valley combined. This population corresponded to an estimated annual fossil fuel consumption of ~140,000 barrels of petroleum, with emission transport ~290 km. We can say with some measure of confidence that the ~20-yr-old saplings in this study germinated and established in a moderately high O_3 concentration environment, the ~80- and >200-yr-old mature trees germinated and established in a low O_3 concentration environment (1914–1921 on average for mature trees), and old growth trees were established prior to industrialization.

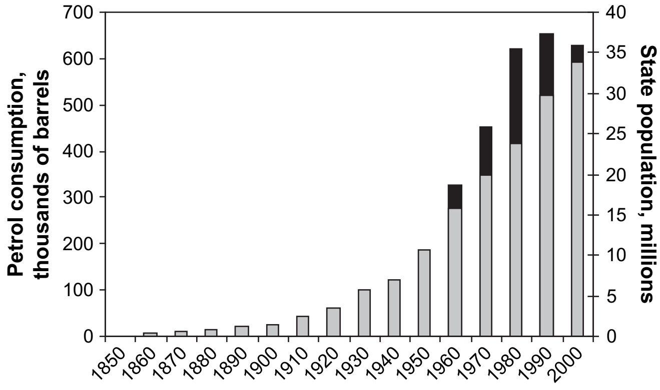

Fig. 2. California state population (2000: http://countingcalifornia.cdlib.org/; 1990: http://censtats.census.gov/; ≤1980: http://factfinder.census.gov/) and annual petroleum consumption (http://www.eia.doe.gov/) in decadal increments. The apparent reduction in petrol consumption (per human) declined in 2000 relative to 1990. Consumption of other fossil fuels (e.g., natural gas and coal) had consistent consumption rates from 1964 through 1988 (2000 billion cubic feet and 2000 short tons, respectively; http://www.eia.doe.gov/). Then, natural gas consumption doubled until the mid 1990s), and so were less related to increases in state population.

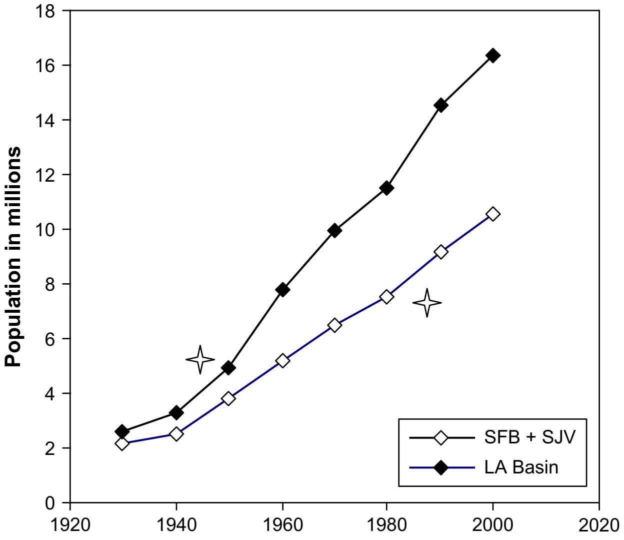

Fig. 3. Comparison of population increase of Los Angeles (LA Basin) and the San Francisco Bay and San Joaquin Valley (SFB + SJV; see website sources provided in Fig. 2). Stars indicate the year of the first foliar O_3 symptoms in the San Bernardino Mountains downwind from LA air basin (in 1961; Miller and McBride, 1975), and in 1983 in Giant Forest, Sequoia National Park downwind from SFB + SJV (in 1983; Miller et al., 1995).

In the southern Sierra Nevada, reduced basal area growth was observed in mature ponderosa ("PP," *Pinus ponderosa* Dougl. ex Laws.) and Jeffrey pine ("JP," *Pinus jeffreyi* Grev. & Balf.), both species of yellow pine, and was associated with moderately high O_3 exposures over the previous several decades (Peterson et al., 1987, 1991). Foliar O_3 injury of mature trees of PP, JP (Duriscoe, 1990), lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), sugar pine (*Pinus lambertiana* Dougl.), and seedlings of giant sequoia (*Sequoiadendron giganteum* Bucholz.) (Miller et al., 1995) occurs under current ambient O_3 concentrations in Sequoia National Park. In southern California, basal area growth (Arbaugh et al., 1999) and simulated whole tree biomass (Tingey et al., 2004) of PP increased when air quality improved due to more stringent air quality standards.

Ponderosa pine has been previously identified as the most sensitive western conifer to elevated O_3 exposure (Miller et al., 1983). Although closely related, PP and JP differ in sensitivity to oxidant pollution. Stomatal conductance of JP is generally 10–25% lower than that of PP, implying lower O_3 uptake (N. Grulke, unpublished data). Jeffrey pine also has greater needle concentrations of antioxidants, so that the O_3 that does enter the needle is more efficiently detoxified (M. Tausz, unpublished data).

Greater foliar O_3 injury of both PP and JP is associated with lower net plant carbon balance. The net carbon balance of both PP (Grulke et al., 2002; Coyne and Bingham, 1982) and JP (Patterson and Rundel, 1989) is significantly reduced by O_3 exposure. Ozone exposure can also significantly alter within-plant carbon allocation (Grulke et al., 2001; Grulke and Balduman, 1999), rendering younger tree age classes more susceptible to other environmental stressors such as drought and pathogens (Miller et al., 1982).

Environmental stress can affect the genetic structure of populations in different ways, e.g., through changes in the frequency of alleles and genotypes due to viability and/or fertility selection (Müller-Starck, 1985; Bergmann and Scholz, 1987; Ruetz and Bergmann, 1989), or via changes in genetic variation (heterozygosity, allele and genotype polymorphism) at the individual and population level (Müller-Starck, 1985, 1989; Bergmann and Scholz, 1989; Geburek et al., 1987; Oleksyn et al., 1994). Previous research on PP and other conifers documented significant shifts in genetic structure in response to either O_3 sensitivity (Staszak et al., 2004) or to elevated O_3 exposure (Bergmann and Scholz, 1989).

Because extant saplings germinated and established in an elevated oxidant pollution environment in Sequoia National Park, and previous generations did not, we hypothesized that the genetic structure of progeny might differ from that of parental trees in isozyme markers associated with O_3 tolerance. We tested for differences in genetic structure of seven enzyme systems and expression of canopy O_3 injury among ~20-, ~80-, and >200-yr-old PP and JP trees. This paper contributes to an understanding of the process of genetic change in populations exposed to O_3.

2. Materials and methods

2.1. Research site

The study site was located on the western slope of the south-central Sierra Nevada Mountains, on the southern edge of Giant Forest, in Sequoia National Park, California (36.6°N, 118.73°W; 1905 m). The mid-elevation forest (Sierra Nevada mixed conifer zone, *sensu* Barbour, 1988) was dominated by both PP and JP trees in a mixed age stand with white fir (*Abies concolor* (Gord. & Glend.) Lindl.), incense cedar (*Libocedrus decurrens* Torr.), California black oak (*Quercus kelloggii* Newb.), and sugar pine (*P. lambertiana* Dougl.) in the area studied. Trees were sampled within a 5 km long transect, 500 m wide, following the 1900 m topographic line (±40 m) from Sunset Rocks to just south of the road access to Crescent Meadow. The long term air quality monitoring station is located within the belt transect (Fig. 1).

2.2. Collection of plant material

Thirty-five trees in each of three age classes for both species were sampled, targeting selection of saplings (S, ~20-yr-old); mature (M, ~80-yr-old); and old growth (O, >200-yr-old), for a total of ~210 trees. Dormant lateral buds (3–5 per tree) were collected from each tree on the southwestern aspect in November 2000 for isozyme analysis. Buds were placed in cryovials and transported and stored in liquid nitrogen (without buffer) until processed. For each tree, basal diameter (for saplings) or diameter at 1.5 m (for mature and old trees) was measured, and approximate age was determined from cambium tissue to the center of the bole at 20× magnification from an increment core. Assessment of foliar injury was made in August 2001 and 2003, a typical time for canopy health assessment. The canopy assessment consisted of counting the number of live needle age classes retained on the branch, estimating the level of chlorotic mottling on 2- and 4-yr-old needles, and measuring within-whorl needle retention, branchlet diameter, and needle length of the 2-yr-old whorl on five mid-canopy secondary branches, 3–4 branches back on mid-canopy, primary branches. On each tree, branches for foliar symptoms were sampled on all aspects. These attributes were effective in assessing the level of O_3 injury in PP (Grulke and Lee, 1997) and JP (Grulke et al., 2003). A Forest Pest Management (FPM) score, an additional O_3 injury scoring system, was assigned to each tree on the basis of the first needle age class that exhibited >15% chlorotic mottle over 30% of the needles in that whorl (Pronos and Vogler, 1981). This assessment was concurrent with the multi-attribute analysis above.

2.3. Isozyme analyses

Bud tissue of each tree was placed in a mortar and homogenized with the extraction buffer in liquid nitrogen and stored at −80 °C until analyzed. The extraction buffer was composed of: 0.1 M Tris–HCl pH 8.0 (100 ml), PVP-40 (10 g), sucrose (10 g), EDTA-2Na (0.17), dithiothreitol DTT (0.15 g), ascorbic acid (0.02 g), bovine albumin 0.10 g), NAD (0.05 g), NADP (0.035 g), and pyridoxal-5-phosphate (0.005 g). Although over 30 enzymes were assayed, little to no variation was observed in 23 of the enzymes between the two species. In the remaining seven enzyme systems, eight loci in JP and eight loci in PP varied sufficiently: fluorescent esterase (FEST, 3.1.1.1), glutamate–oxaloacetate transaminase (GOT, 2.6.1.1), malate dehydrogenase – two loci (MDH, 1.1.1.37), menadione reductase (MNR, 1.6.99.2), phosphoglucomutase (PGM, 2.7.5.1), shikimate dehydrogenase (SHDH, 1.1.1.25), and triose phosphate isomerase (TPI, 5.3.1.1), using the techniques of starch gel electrophoresis (Conkle et al., 1982).

For each tree age class of each species, gene and genotype frequency, mean number of alleles (*A*/*L*) and genotypes (*G*/*L*) per locus, observed heterozygosity (Ho), expected heterozygosity (He), genotype polymorphism index (Pg), and fixation index (*F*) (Jain and Workman, 1967; Nei and Roychoudhury, 1974; Kahler et al., 1980) were calculated using the GEN computer program (Yeh et al., 1999). Allele and genotype frequencies for the compared groups of trees were evaluated using a homogeneity chi-square test, assuming equal frequency in both groups (Elandt-Johnson, 1971). Statistical significance is reported here at the $p \leq 0.05$ level.

3. Results

3.1. Allele and genotype frequencies

For both PP and JP, the mean number of alleles and genotypes increased with increasing tree age (Table 1). The mean number of alleles per locus for PP was 2.125, 2.500, and 2.500 for saplings, mature, and old growth trees, respectively. The mean number of genotypes per locus for PP was 3.000, 3.250, and 3.125 for

Table 1
Mean number of alleles and genoptypes per locus for the three tree age classes of ponderosa and Jeffrey pine (O, old growth; M, mature; S, saplings)

Species	Alleles			Genotypes		
	S	M	O	S	M	O
Ponderosa pine	2.125	2.500	2.500	3.000	3.250	3.125
Jeffrey pine	2.000	2.250	2.375	2.500	2.875	3.000

saplings, mature, and old growth trees, respectively. Similar tendencies were noted for JP: 2.000, 2.250 and 2.375 alleles per locus, and 2.500, 2.875 and 3.000 genotypes per locus for saplings, mature, and old growth trees, respectively.

In saplings, mature, and old growth trees of PP, there were 17, 20, and 20 alleles, and 24, 26 and 25 genotypes, respectively. The same tendency was found for JP: in saplings, mature, and old growth trees, there were 16, 18, and 19 alleles and 20, 23 and 24 genotypes, respectively.

Differences in allele frequency between the age classes of PP trees were significant for allele *SHDH-1*, *SHDH-2* and in the frequency of genotypes for genotypes *SHDH-11*, *SHDH-22* and *MDH3–12* (Table 2). For JP, significant differences in the allele frequency among the tree age classes were found for alleles *FEST-2*, *SHDH-2*, *SHDH-3*, *TPI-1* and *TPI-2* and in genotype frequency for genotype *TPI-11* (Table 3).

3.2. Heterozygosity

The observed (Ho) and expected heterozygosity (He) for the three tree age classes of PP and JP are shown in Tables 4 and 5. For PP, the average observed heterozygosity for all studied loci was higher in the older trees by 24%. The reverse tendency was noted for JP with higher observed heterozygosity in younger trees compared to old growth by 34%. The most distinct differences in observed heterozygosity for the three age classes of PP were found for loci *TPI* and *MDH5*, and for JP, loci *FEST*, *MDH3*, *TPI* and *SKDH*.

3.3. Genotype polymorphism index

The genotype polymorphism index (Pg) for the three age classes of trees of PP and JP is shown in Tables 4 and 5. There was no significant difference in Pg among tree age classes of PP. In JP, Pg was higher in younger trees than in old growth trees.

3.4. Fixation index

The mean value of the fixation index (F) describes whether populations are in Hardy–Weinberg equilibrium (Tables 4 and 5). For PP and JP, an excess of homozygotes was found in the studied groups of trees. The average fixation indices (F) were similar for PP and JP. However, there were differences in the F index among tree age classes for each species. For PP,

Table 2
Statistically significant differences in frequency of alleles and genotypes (frequencies in parentheses) among the three tree age classes of ponderosa pine (O, old growth; M, mature; S, sapling)

Alleles

Locus	Allele	Age class		Chi-square
SKDH1	1	O (0.86)	M (0.46)	7.38
SKDH1	2	O (0.00)	M (0.42)	25.00
SKDH1	2	O (0.00)	S (0.43)	25.65

Genotypes

Locus	Genotype	Age class		Chi-square
MDH3	12	O (0.22)	M (0.57)	4.41
SKDH1	11	O (0.78)	M (0.38)	4.22
SKDH1	22	O (0.00)	S (0.35)	8.36

Table 3
Statistically significant differences in frequency of alleles and genotypes among the three tree age classes of Jeffrey pine (O, old growth; M, mature; S, sapling)

Alleles

Locus	Allele	Age class		Chi-square
FEST	2	O (0.03)	S (0.15)	3.96
SKDH1	2	O (0.00)	S (0.17)	8.65
SKDH1	3	O (0.17)	M (0.00)	8.55
SKDH1	3	O (0.17)	S (0.04)	3.95
TPI-1	1	O (0.74)	M (0.40)	6.11
TPI-1	1	O (0.74)	S (0.42)	5.35
TPI-1	2	O (0.26)	M (0.60)	8.22
TPI-1	2	O (0.26)	S (0.54)	6.07

Genotypes

Locus	Genotype	Age class		Chi-square
TPI-1	11	O (0.63)	M (0.23)	5.62
TPI-1	11	O (0.63)	S (0.27)	4.39

Table 4
Observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F) and genotypic polymorphism index (Pg) for the three age classes of ponderosa pine (O, old growth; M, mature; S, sapling)

Locus	Population	He	Ho	F	Pg
FEST	O	0.0000	0.0000	–	0.0000
	M	0.0997	0.0000	1.0000	0.0997
	S	0.1588	0.0870	0.4524	0.2344
GOT1	O	0.1653	0.1818	−0.1000	0.2975
	M	0.0000	0.0000	–	0.0000
	S	0.0000	0.0000	–	0.0000
MDH3	O	0.3512	0.2727	0.2235	0.5124
	M	0.4321	0.5263	−0.2179	0.5429
	S	0.3147	0.2174	0.3093	0.4612
MDH5	O	0.1684	0.1818	−0.0798	0.3099
	M	0.2368	0.2632	−0.1111	0.4211
	S	0.1238	0.1304	−0.0534	0.2344
MNR	O	0.5539	0.4762	0.1845	0.7392
	M	0.6080	0.4211	0.3075	0.7590
	S	0.5992	0.4545	0.2414	0.7727
PGM	O	0.0444	0.0455	−0.0233	0.0868
	M	0.0512	0.0526	−0.0270	0.0997
	S	0.0000	0.0000	–	0.0000
SKDH	O	0.3605	0.2727	0.2436	0.5331
	M	0.6496	0.1579	0.7569	0.7147
	S	0.4915	0.1739	0.6462	0.6200
TPI-1	O	0.3853	0.4091	−0.0617	0.5661
	M	0.4806	0.4211	0.1239	0.6704
	S	0.3488	0.3478	0.0027	0.5331
Mean value	O	0.2574	0.2300	0.1065	0.3806
	M	0.3198	0.2303	0.2799	0.4134
	S	0.2546	0.1764	0.3072	0.3570
Mean	O + M + S	0.2772	0.2122	0.2312	0.3837

Table 5
Observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F) and genotypic polymorphism index (Pg) for the three age classes of Jeffrey pine (O, old growth; M, mature; S, sapling)

Locus	Population	He	Ho	F	Pg
FEST	O	0.0260	0.0263	−0.0133	0.0512
	M	0.1189	0.1250	−0.0511	0.2257
	S	0.2604	0.2308	0.1136	0.4112
GOT1	O	0.0512	0.0526	−0.0270	0.0997
	M	0.0408	0.0417	−0.0213	0.0799
	S	0.0000	0.0000	–	0.0000
MDH3	O	0.1908	0.1053	0.4483	0.2812
	M	0.2491	0.2083	0.1638	0.3924
	S	0.1420	0.1538	−0.0833	0.2604
MDH5	O	0.1243	0.1316	−0.0585	0.2341
	M	0.0799	0.0000	1.0000	0.0799
	S	0.0000	0.0000	–	0.0000
MNR	O	0.5313	0.3889	0.2680	0.7037
	M	0.5877	0.6250	−0.0635	0.7257
	S	0.5355	0.6154	−0.1492	0.6746
PGM	O	0.0512	0.0526	−0.0270	0.0997
	M	0.0000	0.0000	–	0.0000
	S	0.0000	0.0000	–	0.0000
SKDH	O	0.2836	0.0789	0.7216	0.3532
	M	0.3377	0.1250	0.6298	0.4132
	S	0.3469	0.2692	0.2239	0.5237
TPI-1	O	0.3324	0.2105	0.3667	0.4765
	M	0.4575	0.3750	0.1803	0.6215
	S	0.5296	0.3077	0.4190	0.6893
Mean value	O	0.1989	0.1308	0.3420	0.2874
	M	0.2339	0.1875	0.1985	0.3173
	S	0.2268	0.1971	0.1309	0.3199
Mean	(O + M + S)	0.2199	0.1718	0.2238	0.3082

homozygosity level decreased from younger trees to old growth. JP had the opposite response.

3.5. Genetic distances

Comparison of genetic distance indices based on the frequency of alleles (SN) and genotypes (SH) indicated that average genetic distances were two times higher between the tree age classes in PP than in JP (Table 6). Genetic distances between parental and young trees of PP and JP were distinctly higher than the genetic distances between the two species. Comparison of genetic similarities' indices based on SN and SH indicated that in both species, trees in sapling and mature age classes were the most similar genetically. Comparative SN values ranged between 0.01 and 0.06.

Table 6
Genetic similarity coefficients among age classes in both pine species

	Jeffrey pine			Ponderosa pine		
	SN O	SN M	SN S	SN O	SN M	SN S
SH O		0.963	0.973		0.983	0.974
SH M	0.944		0.994	0.945		0.985
SH S	0.945	0.985		0.962	0.956	

SN indicates frequency of alleles and SH indicates frequency of genotypes.

3.6. Level of O_3 injury in the two species and across tree age classes

Individual tree health as determined by canopy health attributes previously shown to be responsive to O_3 exposure exhibited significant differences in the number of live whorls retained (old growth, 2003), chlorotic mottle of 2-yr-old needles (saplings in both 2001 and 2003; mature trees in 2003), and branch diameter (saplings and mature trees in 2003) between PP and JP (Table 7). Individual canopy health attributes differed significantly among tree age classes for percent branch foliated (both species, both years), needle elongation growth (both species in 2001; JP in 2003), and branch elongation and diameter growth (both species, both years). A commonly used index of O_3 injury in yellow pine throughout the western United States did not significantly differ among tree age classes for either species or year (Table 7). However, the higher score in 2003 for both species suggests an overall improved canopy health in that year, better canopy health for parental vs. progeny, and better canopy health for JP vs. PP.

To integrate the response of the individual morphological attributes within a species and tree age class, a detrended coordinate analysis (DCA) was applied to both the 2001 and 2003 data (PC-ORD, 1999). The coefficient of the ordinations was >0.95, using three axes. The tree age classes had different morphological responses in the 2 yr of the study, but it is clear that canopy health attributes of old growth PP and JP differed from that of younger tree age classes (Fig. 4). Old growth PP differed significantly from old growth JP only in eigenvector 3 in 2001. In general, eigenvalues of sapling and mature trees of both species did not significantly differ. The exceptions were only for JP: eigenvector 3 in 2001, and eigenvectors 1 and 2 in 2003. In 2001, the 2nd eigenvector significantly differentiated the two species in both mature and old growth tree age classes, and the 3rd eigenvector significantly differentiated the two species in both old growth and saplings. In 2003, the two species were differentiated significantly only for eigenvector 1 for saplings.

4. Discussion

Adaptation to environmental stress contributes to altered genetic structure of forest tree species (Bergmann and Scholz, 1989). Environmental stress may be sufficiently severe that sensitive individuals are lost in a population and specific genes change in frequency, resulting in changes in the genetic structure of the population (Bergmann and Scholz, 1989). In general, less homozygotic individuals are better adapted to environmentally stressful conditions (Hedrick, 1976, 1978; Lewontin, 1974; Zouros and Foltz, 1987). However, under extreme environmental conditions, a decreased level of heterozygosity may also be found because only special genotypes can survive in such environments (Prus-Glowacki and Godzik, 1991; Prus-Glowacki and Nowak-Bzowy, 1992; Scholz,

Table 7
Morphological attributes of three tree age classes of ponderosa and Jeffrey pine

	Ponderosa pine			Jeffrey pine		
	Saplings	Mature	Old growth	Saplings	Mature	Old growth
Tree age, yr	17 ± 1	86 ± 8	>200	18 ± 1	79 ± 6	>200
Bole diameter, cm	6 ± 1	48 ± 2	106 ± 4	5 ± 0	42 ± 2	99 ± 3
FPM score						
2001	3.3 ± 0.3	3.4 ± 0.2	3.6 ± 0.2	3.6 ± 0.1	3.7 ± 0.2	4.1 ± 0.2
2003	3.8 ± 0.5	4.4 ± 0.4	4.3 ± 0.1	4.0 ± 0.2	4.0 ± 0.2	4.2 ± 0.2
No. live whorls						
2001	4.3 ± 0.3	4.2 ± 0.2	4.5 ± 0.2	4.2 ± 0.2	3.9 ± 0.2	4.4 ± 0.2
2003	4.9 ± 0.3	5.0 ± 0.2	5.4 ± 0.2*	4.8 ± 0.2	4.6 ± 0.2	4.7 ± 0.2*
% Branch foliated						
2001	63 ± 4a	69 ± 3a	56 ± 2b	62 ± 3(1)	68 ± 2(1)	54 ± 2(2)
2003	67 ± 5a	72 ± 2a	57 ± 3b	73 ± 3(1)	72 ± 2(1)	52 ± 2(2)
Chlorotic mottle, %, 2-yr-old needles						
2001	10 ± 2*	7 ± 1	6 ± 1	7 ± 1*	6 ± 1	5 ± 1
2003	11 ± 6a*	3 ± 0b§	3 ± 0b	4 ± 0*	5 ± 1§	3 ± 0
Chlorotic mottle, %, 4-yr-old needles						
2001	36 ± 9	40 ± 9	33 ± 6	36 ± 5	27 ± 6	28 ± 7
2003	21 ± 4	16 ± 4	21 ± 2	16 ± 2	26 ± 5	30 ± 6
Needle length, mm						
2001	180 ± 8a	190 ± 4a	151 ± 8b	193 ± 7(1)	179 ± 6(2)	151 ± 6(3)
2003	181 ± 2	176 ± 8	152 ± 8	202 ± 6(1)	177 ± 5(2)	150 ± 5(3)
Branch length, mm						
2001	9 ± 3a	32 ± 2b	94 ± 9c	30 ± 16(1)	29 ± 2(1)	75 ± 7(2)
2003	25 ± 2a	24 ± 2a	52 ± 8b	27 ± 1(1)	23 ± 2(2)	52 ± 7(3)
Branch diameter, mm						
2001	9.2 ± 9.6a	10.0 ± 0.5a	7.4 ± 0.5b	10.7 ± 0.4(1)	10.1 ± 0.5(1)	7.4 ± 0.5(2)
2003	9.6 ± 0.3a§	8.4 ± 0.4b*	7.4 ± 0.3c	11.0 ± 0.3(1)§	10.3 ± 0.3(1)*	7.6 ± 0.3(2)

Except for chlorotic mottle, mean ± 1 S.E. were reported for 2-yr-old tissue. Within a year, within a species, differing letters (ponderosa pine) or numbers (Jeffrey pine) denote statistical significance. Comparisons between years were not made due to differences in sample size from 2001 to 2003 (not all individuals relocated in 2003). Statistical significance is reported at the $P = 0.05$ level using paired t-tests (SAS, 2003). § and * denote significant differences between values within the row of data in Table 7.

1990). Examples of both adaptation strategies have been reported for conifers in response to heavy metals or excessive sulphur dioxide exposure (Mejnartowicz, 1983; Scholz and Bergmann, 1984; Geburek et al., 1987; Prus-Glowacki and Nowak-Bzowy, 1989, 1992; Prus-Glowacki and Godzik, 1991; Prus-Glowacki et al., 2006). Decreased genetic variation from the elimination of sensitive individuals constitutes an erosion of the gene pool, and can reduce the adaptability of the population to local conditions as well as future changes in the environment.

Although not generally believed to be strong enough to induce changes in population genetic structure, the genetic basis of O_3 tolerance was first established in Bel W-3 tobacco (Taylor, 1968). Subsequently, the genetic basis of O_3 tolerance has been reported for a number of species, including: potato (*Solanum tuberosum* L., DeVos et al., 1982), bladder campion (*Silene cucubalus*, Ernst et al., 1985), and onion (*Allium cepa* L., Engel and Gabelman, 1989). Perhaps the best example of the genetic basis of O_3 tolerance in a tree species was established for aspen (*Populus tremuloides* Michx., Karnosky, 1977), with subsequent field verification (Berrang et al., 1989). Most recently, O_3 resistant mature ponderosa pine were found to be more heterozygous than O_3 sensitive individuals in a forest exposed consistently to long term, high O_3 exposure (Staszak et al., 2004).

In our study, the mean number of alleles, as well as the mean number of genotypes per locus, exhibited the same tendency in both species: the number of alleles and genotypes increased with increasing age of trees. Wright's index (F) indicated an excess of homozygotes in both species but in PP, homozygosity was higher in the two younger tree age classes relative to old growth trees, and the observed heterozygosity was higher in old growth trees by 24%. In JP, homozygosity was higher in the old growth tree age class relative to saplings, and the observed heterozygosity was higher in saplings by 34%. These trends were reflected in the fixation index for both species. Considering that PP is more sensitive to oxidant pollution than JP (Miller et al., 1983), it is reasonable that the two species exhibit different adaptive responses to oxidative stress. More heterozygotic saplings relative to parental trees would be expected if significant environmental stress were imposed on younger tree age classes, selecting for survival of more heterozygotic seedlings, through greater success of progeny resulting from outbreeding, not from local parental crosses (e.g., JP). The average genetic distance indices (SN and SH) between tree age classes in PP and JP were similar

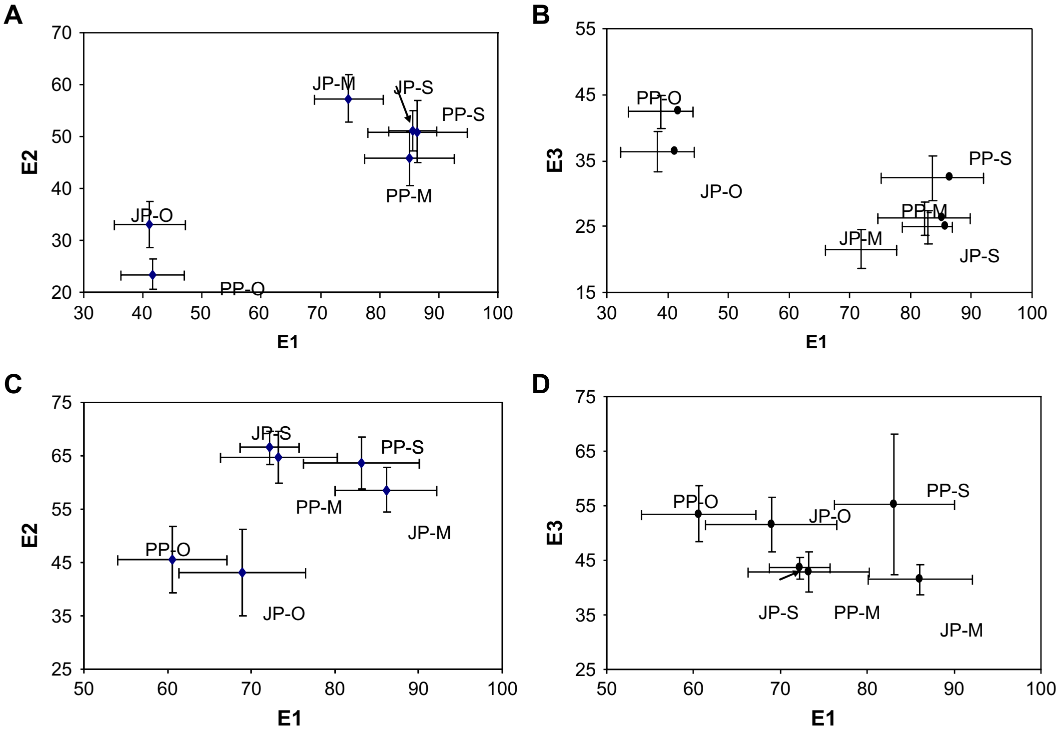

Fig. 4. Detrended correspondence analysis (DCA) for 2001 (A, eigenvector 1 vs. 2; B, eigenvector 1 vs. 3) and 2003 (C, 1 vs. 2; D, 1 vs. 3). The points and error bars (mean ± 1 S.E. for eigenvalues) are identified by species (PP, ponderosa pine; or JP, Jeffrey pine) and by tree age class (O, old growth; M, mature; S, sapling).

suggesting similar adaptations. In an earlier publication we demonstrated that O_3-tolerant individuals of PP exhibited higher heterozygosity than O_3-sensitive individuals (Staszak et al., 2004). Judging from genetic similarity indices, the most pronounced changes in genetic structure occurred between younger (sapling and mature) tree age classes and the old growth trees. Assuming that O_3 exposure did not increase significantly until after 1945 (see Fig. 3), these data suggest that mature trees also experienced sufficient environmental stress (from age 30 to 80 yr) to influence genetic structure.

Differences in frequency of alleles and genotypes, mean number of alleles and genotypes per locus, level of heterozygosity (Ho) and genotypic polymorphism (Pg) between young and older tree age classes suggest that changes in the genetic structure of both PP and JP have occurred. The greatest differences in heterozygosity were found for loci *SKDH*, *FEST*, *MNR*, *MDH-C*, and *TPI*. Most of these enzyme loci except for *TPI* have been previously identified as suitable markers for studying changes in the genetic structure caused by pollution (Bergmann and Scholz, 1985, 1987, 1989; Geburek et al., 1986; Prus-Glowacki and Godzik, 1995; Prus-Glowacki et al., 1999).

Although we have inferred changes in O_3 concentrations over the lifetimes of the three tree age classes, other environmental stressors may have had an effect on the genetic structure of yellow pine populations in Sequoia National Park. These stressors include periodic severe drought stress, pathogen and insect infestations, fire disturbance, land use changes, excess nitrogen deposition, and long term increases in temperature. Based on a 70-yr precipitation record in Sequoia National Park, there were two periods which would have induced extreme drought stress in ponderosa pine, inferred from physiological measurements made in southern California on PP (Grulke et al., in press): 1959–1961 (averaging 54% of the long term average annual precipitation record), and 1987–1990 (averaging 62% of the long term average; Fig. 5). These multiple-year droughts resulted in elevated mortality in yellow pine in the park (Warner, 1987). It is likely that the saplings in this study established during years of largely above-average precipitation 1978–1986, just prior to the 4-yr, late 1980s drought. The saplings would likely have experienced the greatest drought stress because they were ~10-yr-old (vs. ~60-yr-old for mature trees) at that time.

There was an historic bark beetle outbreak (prior to precipitation records for the region) in 1913–1918 affecting yellow pine (Miller 1940, as seen Warner, 1987) (reported epidemic bark beetle infestations are indicated with arrows on Fig. 5). Although the saplings were cored at the base of the stem, the mature trees were cored at 1.4 m, and so were at least that height at the time of the bark beetle infestation in the mid 1900s. It is likely that the mature trees in this study established during years of largely above-average precipitation 1900–1910. The current mature trees thus also likely experienced drought stress early in their establishment.

Fire records in the park have been tracked since 1921 (Caprio and Graber, 2000). Prescribed fire affected one-third of the mature and old growth trees in this study in 1972 (www.nps.gov/archive/seki/fire/fire_map.htm), prior to the establishment of one-third of the saplings in this study. Forest stands are not managed except in the immediate vicinity of

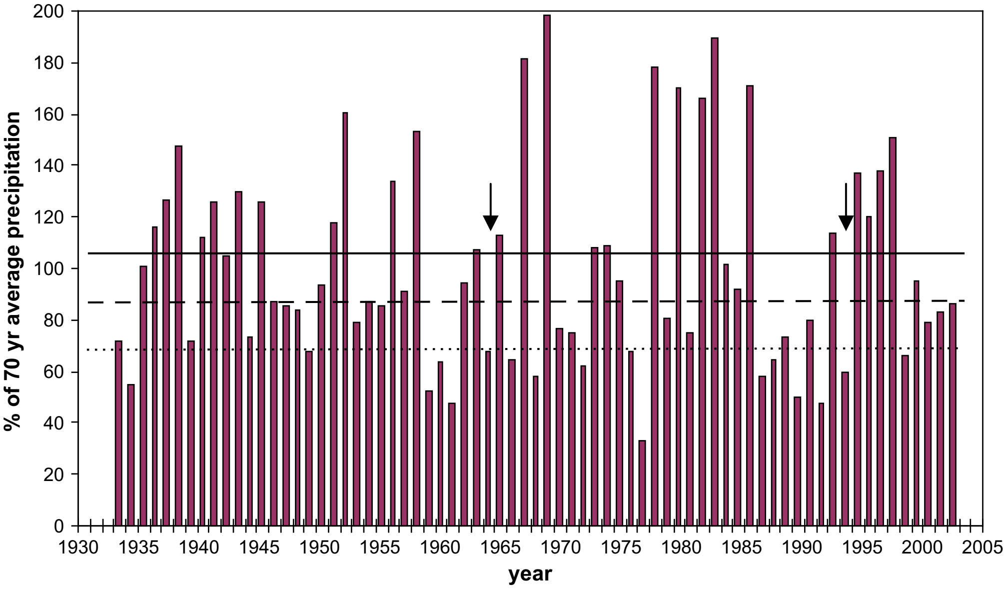

Fig. 5. Precipitation record for Giant Forest (until 1968) and Lodgepole (after 1968) in Sequoia National Park. Dashed line indicates physiologically defined moderate drought stress and dotted line indicates extreme drought stress for ponderosa pine (Grulke, 1999 for moderate drought stress and Grulke, unpublished data for extreme drought stress). Arrows indicate periods of expected high bark beetle attacks based on level of physiological drought stress (Grulke et al., in press).

developed areas (Warner, 1987). Near Sunset Rocks, a campground was abandoned in the early 1970s. Approximately one-quarter of the trees (in all age classes) established outside of the campground. Otherwise, land use has changed little since the park was established in 1890. Although nitrogen deposition accompanies O_3 transport into the park, total annual deposition rates are low (~6 kg ha^{-1} yr^{-1}; Fenn et al., 2003).

Temperature records exist from 1931 to present, although the location of the station was changed from Giant Forest to Lodgepole (6 km north) in November 1968, a site of greater cold air drainage. Compared to the 70-yr record, daily minimum and maximum air temperatures were 3.7 and 0.5 °C greater than the average for the period prior to and including 1968. After 1968, daily minimum and maximum air temperatures were 6.5 and 2.7 °C lower than that of the 70-yr record. Because of the shift in station location, we cannot rule out the potential role of temperature in our observations. Higher temperature, increased drought stress, and increased O_3 exposure can all contribute to increased oxidative stress in plants (Elstner and Osswald, 1994).

The level of drought experienced modifies the expression of foliar O_3 symptoms (Grulke, 2003). In 2001 and 2003 when canopy O_3 injury was assessed, the total annual precipitation was similar (80% of the 70-yr record, Fig. 5), but 2001 was preceded by an average precipitation year (95%, 2000) and a below-average precipitation year (65%, 1999). The canopy assessment in 2003 was preceded by two below-average precipitation years of 80%. The FPM score suggests that canopy health was improved in 2003 relative to 2001, that old growth trees had greater canopy health than saplings, and that canopy health of PP was greater than JP in 2003 but poorer than JP in 2001 (Table 5). In this study, the FPM score was not sensitive enough to elucidate statistically significant differences among tree age classes or between the two species. Using a more quantitative, multivariate assessment of the attributes of canopy health, an ordination demonstrated significant differences among the tree age classes (Fig. 4). The strongest differences were between the old growth trees and the younger tree age classes.

Foliar retention in yellow pine is highly responsive to O_3 exposure, drought stress, and nitrogen deposition (Grulke and Balduman, 1999). Old growth trees had significantly less within-whorl needle retention (as indicated by lower % branch foliated; Table 5) in both species, in both years. Older needles within a whorl, and more O_3-symptomatic needles are sloughed prematurely (see % branch foliated), which contributes to an apparent decrease in chlorotic mottle in old growth trees (Grulke, 2003), especially when combined with drought stress. Chlorotic mottle (definitive response to O_3 exposure, Miller et al., 1996) of 2-yr-old needles was significantly greater in PP saplings than in JP saplings in both years (Table 5), and in mature PP in 2003. The level of chlorotic mottle can decrease within a needle age class (the whorl) when the more O_3-injured needles are lost (e.g., with O_3 and/or drought). A reduction in within-whorl needle retention would explain the differences observed because significantly more needles were retained within-whorl in saplings and mature trees. Chlorotic mottle of 4-yr-old needles mirrored that of the FPM score: PP had greater chlorotic mottle than JP in 2001 but less chlorotic mottle than JP in 2003.

Differences in frequency of alleles and genotypes, mean number of alleles and genotypes per locus, level of heterozygosity (Ho) and genotypic polymorphism (Pg) between saplings and old growth trees suggest that changes in the genetic structure of both PP and JP populations have occurred. We selected isozyme markers associated with O_3 tolerance. Because both drought and O_3 induce oxidative stress, both stressors could have contributed to the observed shifts in genetic structure. Our data suggest that an increase in pollution over the last ∼50 yr, and episodic drought stress may have affected the genetic structure of two species of yellow pine in Sequoia National Park but to a greater degree in PP, a species more sensitive to oxidant pollution.

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References

Arbaugh, M.J., Peterson, D.L., Miller, P.R., 1999. Air pollution effects on growth. In: Miller, P.R., McBride, J.R. (Eds.), Oxidant Air Pollution Impacts in the Montane Forests of Southern California: A Case Study of the San Bernardino Mountains. Ecological Studies, vol. 134. Springer-Verlag, New York, pp. 179–207.

Barbour, M.G., 1988. Californian upland forests and woodlands. In: Barbour, M.G., Billings, W.D. (Eds.), North American Terrestrial Vegetation. Cambridge University Press, New York, pp. 131–164.

Bergmann, F., Scholz, F., 1985. Effects of selection pressure by SO_2 pollution on genetic structure of Norway spruce (*Picea abies*). In: Gregorius, H.R. (Ed.), Population Genetics in Forestry. Lecture Notes in Biomathematics, vol. 60. Springer-Verlag, New York, pp. 267–275.

Bergmann, F., Scholz, F., 1987. The impact of air pollution on the genetic structure of Norway spruce. Silvae Genetica 36, 80–83.

Bergmann, F., Scholz, F., 1989. Selection effects of air pollution in Norway spruce (*Picea abies* L.) populations. In: Scholz, F., Gregorius, H.R., Rudin, D. (Eds.), Genetic Effects of Air Pollutants in Forest Tree Populations. Springer-Verlag, New York, pp. 143–160.

Berrang, P., Karnosky, D.F., Bennett, J.P., 1989. Natural selection for ozone resistance in *Populus tremuloides*: field verification. Canadian Journal Forest Research 19, 519–522.

Bytnerowicz, A., Tausz, M., Alonso, R., Jones, D., Johnson, R., Grulke, N., 2002. Summer-time distribution of air pollutants in Sequoia National Park, California. Environmental Pollution 188, 187–203.

Caprio, A.C., Graber, D.M., 2000. Returning Fire to the Mountains: Can We Successfully Restore the Ecological Role of Pre-EuroAmerican Fire Regimes to the Sierra Nevada? USDA Forest Service, Rocky Mountain Research Station Publication 15, vol. 5, pp. 233–245.

Conkle, M.T., Hodgkiss, P.D., Nunnally, L.B., Hunter, S.C., 1982. Starch Gel Electrophoresis of Pine Seed: A Laboratory Manual. USDA Forest Service. PSW-General Technical Report 64.

Coyne, P.I., Bingham, G.E., 1982. Variation in photosynthesis and stomatal conductance in an ozone-stressed ponderosa pine stand: light response. Forest Science 28, 257–273.

DeVos, N.E., Hill Jr., R.R., Pell, E.J., Cole, R.H., 1982. Quantitative inheritance of ozone resistance in potato. Crop Science 22, 992–995.

Duriscoe, D.M., 1990. Cruise Survey of Oxidant Air Pollution Injury to *Pinus ponderosa* and *Pinus jeffreyi* in Saguaro National Monument, Yosemite National Park, and Sequoia and Kings Canyon National Parks. NPS/AQD-90/003. Air Quality Division, USDI National Park Service, Denver, CO, 68 p.

Elandt-Johnson, R.C., 1971. Probability Models and Statistical Methods in Genetics. Wiley and Sons, Inc., New York, pp. 345–390.

Elstner, E.F., Osswald, W., 1994. Mechanisms of oxygen activation during plant stress. Proceedings of the Royal Society of Edinburgh 102B, 131–154.

Engel, R.L., Gabelman, W.H., 1989. Inheritance and mechanism for resistance to ozone damage in onion, *Allium cepa* L. Journal of the American Society for Horticultural Science 5, 423–430.

Ernst, W.H.O., Tonneijck, A.E.C., Pasman, F.J.M., 1985. Ecotypic response of *Silene cucubalus* to air pollutants (SO_2, O_3). Journal of Plant Physiology 118, 439–450.

Fenn, M.E., Haeuber, R., Tonnesen, G.S., Baron, J.S., Grossman-Clarke, S., Hope, D., Jaffe, D.A., Copeland, S., Geiser, L., Rueth, H.M., Sickman, J.O., 2003. Nitrogen emissions, deposition, and monitoring in the western United States. BioScience 53 (4), 391–403.

Geburek, T.H., Scholz, F., Bergmann, F., 1986. Variation in aluminum-sensitivity among *Picea abies* L. Karst. seedlings and genetic differences between their mother trees as studied by isozyme-gene-markers. Angewandte Botanik 60, 451–460.

Geburek, T.H., Scholz, F., Knabe, W., Vorneweg, A., 1987. Genetic studies by isozyme gene loci on resistance in sensitivity in an air polluted *Pinus silvestris* field trial. Silvae Genetica 36, 49–53.

Grulke, N.E., 1999. Physiological responses of ponderosa pine to gradients of environmental stressors. In: Miller, P.R., McBride, J. (Eds.), Oxidant Air Pollution Impacts in the Montane Forests of Southern California: The San Bernardino Mountain Case Study. Ecological Studies, vol. 134. Springer-Verlag, New York, pp. 126–163.

Grulke, N.E., 2003. Physiological basis of ozone injury assessment in Sierra Nevadan conifers. In: Bytnerowicz, A., Arbaugh, M., Alonso, R. (Eds.), Assessment of Ozone Distribution and its Effects on Sierra Nevada Ecosystems. Developments in Environmental Science, vol. 2. Elsevier Publishers, The Hague, Netherlands, pp. 55–81.

Grulke, N.E., Andersen, C.P., Hogsett, W.E., 2001. Seasonal changes in above- and belowground carbohydrate concentrations of ponderosa pine along a pollution gradient. Tree Physiology 21, 173–181.

Grulke, N.E., Balduman, L., 1999. Deciduous conifers: high nitrogen deposition and ozone exposure effects on ponderosa pine. Water, Air and Soil Pollution 166, 235–248.

Grulke, N.E., Johnson, R., Jones, D., Monschein, S., Nikolova, P., Tausz, M., 2003. Variation in morphological and biochemical O_3 injury attributes of Jeffrey pine within canopies and between microsites. Tree Physiology 213, 923–929.

Grulke, N.E., Lee, E.H., 1997. Assessing ozone-induced foliar injury in ponderosa pine. Canadian Journal Forest Research 27, 1658–1668.

Grulke, N.E., Paine, T., Minnich, R., Riggan, P. A link between air pollution and forest susceptibility to wildfire: a case study for the San Bernardino Mountains. In: Proceedings of the Assessment of Threats to Forest Health, July 11–13, 2006, Boulder, CO, PNW-General Technical Report, 10 pp. Available from: <http://www.srs.fs.usda.gov/threats/>, in press.

Grulke, N.E., Preisler, H., Rose, C., Kirsch, J., Balduman, L., 2002. Evaluating the role of drought stress on ozone uptake in ponderosa pine. New Phytologist 154, 621–632.

Hedrick, P.W., 1976. Genetic variation in a heterozygous environment. II. Temporal heterogeneity and directional selection. Genetics 84, 145–157.

Hedrick, P.W., 1978. Genetic variation in a heterozygous environment. V. Spatial heterogeneity in finite populations. Genetics 89, 398–401.

Jain, S.K., Workman, P.L., 1967. Generalized *F*-statistics and the theory of inbreeding and selection. Nature 214, 674–678.

Kahler, A.L., Allard, R.W., Krzakowa, M., Wherhahn, C.F., Nevo, E., 1980. Associations between phenotypes and environment in the slender wild oat (*Avena barbata*) in Israel. Theoretical and Applied Genetics 56, 31–47.

Karnosky, D.F., 1977. Evidence for genetic control of response to sulfur dioxide and ozone in *Populus tremuloides*. Canadian Journal of Forest Research 7, 437–440.

Lee, E.H., Tingey, D.T., Hogsett, W.E., Laurence, J.A., 2003. History of tropospheric ozone for the San Bernardino Mountains of southern California, 1963–1999. Atmospheric Environment 37, 2705–2717.

Lewontin, R.C., 1974. The Genetic Basis of Evolutionary Change. Columbia University Press, New York.

Mejnartowicz, L.E., 1983. Changes in genetic structure of Scots pine (*Pinus sylvestris* L.) population affected by industrial emission of fluoride and sulphur dioxide. Genetica Polonica 24, 41–50.

Miller, P.R., McBride, J.P., 1975. Effects of air pollutants on forests. In: Mudd, J.B., Kozlowski, T.T. (Eds.), Responses of Plants to Air Pollution. Academic Press, New York, pp. 195–235.

Miller, P.R., Grulke, N.E., Stolte, K., 1995. Air Pollution Effects on Giant Sequoia Ecosystems. USDA Forest Service, Albany, CA, PSW-General Technical Report 151, pp. 90–99.

Miller, P.R., Longbotham, G.J., Longbotham, C.R., 1983. Sensitivity of selected western conifers to ozone. Plant Disease 67, 1113–1115.

Miller, P.R., Stolte, K.W., Duriscoe, E., Pronos, J., 1996. Monitoring Ozone Air Pollution Effects on Western Pine Forests. USDA Forest Service, Albany, CA. Pacific Southwest Region, General Technical Report 155.

Miller, P.R., Taylor, O.C., Wilhour, R.G., 1982. Oxidant Air Pollution Effects on a Mixed Conifer Forest Ecosystem. U.S. EPA Environmental Research Brief. EPA-600/D-82-278.

Müller-Starck, G., 1985. Genetic differences between tolerant and sensitive beeches (*Fagus sylvatica* L.) in an environmentally stressed forest stand. Silvae Genetica 34, 241–247.

Müller-Starck, G., 1989. Genetic implications of environmental stress in adult forest stands of *Fagus sylvatica* L. In: Scholz, F., Gregorius, H.R., Rudin, D. (Eds.), Genetic Effects of Air Pollutants in Forest Tree Populations. Springer-Verlag, Berlin, pp. 127–142.

National Park Service, Air Resource Division, Sequoia National Park-Lower Kaweah Monitoring Station. Accessible via the NPS web site. Available from: <http://www2.nature.nps.gov/air/Monitoring/network.cfm#data.html>.

Nei, M., Roychoudhury, A.K., 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76, 379–390.

Oleksyn, J., Prus-Glowacki, W., Giertych, M., Reich, P.B., 1994. Relation between genetic diversity and pollution impact in a 1912 experiment with East European *Pinus sylvestris* provenances. Canadian Journal Forest Research 24, 2390–2394.

Patterson, M.T., Rundel, P.W., 1989. Seasonal physiological responses of ozone stressed Jeffrey pine in Sequoia National Park, California. In: Olson, R.K., Lefohn, A.S. (Eds.), Effects of Air Pollution on Western Forests. Air and Waste Management Association, Pittsburgh, PA, pp. 419–428.

PC-ORD, 1999. PC-ORD: Multivariate Analysis of Ecological Data. Version 4. MjM Software Design, Gleneden Beach, OR.

Peterson, D.L., Arbaugh, M.J., Robinson, L.J., 1991. Regional growth changes in ozone-stressed ponderosa pine (*Pinus ponderosa*) in the Sierra Nevada, California, USA. The Holocene 1, 50–61.

Peterson, D.L., Arbaugh, M.J., Wakefield, V.A., Miller, P.R., 1987. Evidence of growth reduction in ozone-stressed Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) in Sequoia and Kings Canyon national Parks. Journal of the Air & Waste Management Association 38, 906–912.

Pronos, J., Vogler, D.R., 1981. Assessment of Ozone Injury to Pines in the Southern Sierra Nevada, 1979/1980. Pacific Southwest Region, USDA Forest Service, Forest Pest Management Report 81-20, 13 p.

Prus-Glowacki, W., Godzik, S., 1991. Changes induced by zinc smelter pollution in the genetic structure of pine (*Pinus sylvestris* L.) seedling populations. Silvae Genetica 40, 184–188.

Prus-Glowacki, W., Godzik, S., 1995. Genetic structure of *Picea abies* trees tolerant and sensitive to industrial pollution. Silvae Genetica 44, 2–3.

Prus-Glowacki, W., Nowak-Bzowy, R., 1989. Demographic processes in *Pinus sylvestris* populations from regions under strong and weak anthropogenous pressure. Silvae Genetica 38, 55–62.

Prus-Glowacki, W., Nowak-Bzowy, R., 1992. Genetic structure of naturally regenerating Scots pine population tolerant for high pollution near a zinc smelter. Water, Air and Soil Pollution 62, 249–259.

Prus-Glowacki, W., Wojnicka-Poltorak, A., Oleksyn, J., Reich, P.B., 1999. Industrial pollutants tend to increase diversity: evidence from field-grown European Scots pine populations. Water, Air and Soil Pollution 116, 395–402.

Prus-Glowacki, W., Chudzinska, E., Wojnicka-Poltorak, A., Kozacki, L., Fagiewicz, K., 2006. Effects of heavy metal pollution on genetic variation and cytological disturbances in the *Pinus sylvestris* L. population. Journal of Applied Genetics 47, 99–108.

Ruetz, W.F., Bergmann, F., 1989. Possibilities of identifying autochthonous high-altitude stands of Norway spruce (*Picea abies* L.) in the Berchtesgaden Alps. Forstwissenschaftliches Centralblatt 108, 164–174.

SAS Institute, Inc., 2003. SAS/STAT User's Guide. SAS Institute, Inc., Cary, NC.

Scholz, F., 1990. Importance of the genetic structure in tree species for forest ecosystems under the influence of air pollutants. In: Ulrich, B. (Ed.), Proceedings of the International Congress on Forest Decline Research: State of Knowledge and Perspectives, pp. 479–497. Friedrichshafen 2–6.10.1989.

Scholz, F., Bergmann, F., 1984. Selection pressure by air pollution as studied by isoenzyme-gene-systems in Norway spruce exposed to sulphur dioxide. Silvae Genetica 33, 238–241.

Staszak, J., Grulke, N.E., Prus-Glowacki, W., 2004. Genetic differences of *Pinus ponderosa* [Dougl. ex Laws.] trees tolerant and sensitive to ozone. Water, Air and Soil Pollution 153, 3–14.

Taylor, G.S., 1968. Ozone injury on Bel W-3 tobacco controlled by at least two genes. Phytopathology 58, 1069.

Tingey, D.T., Hogsett, W.E., Lee, E.H., Laurence, J.A., 2004. Stricter ozone ambient air quality standard has beneficial effect on ponderosa pine in California. Environmental Management 34 (3), 397–405.

Warner, T., 1987. Vegetation Management Plan (for the Development Zone). Sequoia and Kings Canyon National Parks, US Department of the Interior, National Park Service, Western Region, Three Rivers, CA, 140 pp.

Yeh, F.C., Yang, R.-C., Boyle, T.B.J., Ye, Z.-H., Mao, J.X., 1999. PopGene v.1.32, The User-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Zouros, E., Foltz, D.W., 1987. The use of allelic isozyme variation for the study of heterosis. Isozymes: Current Topics in Biological and Medical Research 13, 1–59.