

Seasonal changes in above- and belowground carbohydrate concentrations of ponderosa pine along a pollution gradient

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Summary Seasonal patterns of carbohydrate concentration in coarse and fine roots, stem or bole, and foliage of ponderosa pine (*Pinus ponderosa* Laws) were described across five tree-age classes from seedlings to mature trees at an atmospherically clean site. Relative to all other tree-age classes, seedlings exhibited greater tissue carbohydrate concentration in stems and foliage, and greater shifts in the time at which maximum and minimum carbohydrate concentration occurred. To determine the effect of environmental stressors on tissue carbohydrate concentration, two tree-age classes (40-year-old and mature) were compared at three sites along a well-established, long-term O₃ and N deposition gradient in the San Bernardino Mountains, California. Maximum carbohydrate concentration of 1-year-old needles declined with increasing pollution exposure in both tree-age classes. Maximum fine root monosaccharide concentration was depressed for both 40-year-old and mature trees at the most polluted site. Maximum coarse and fine root starch concentrations were significantly depressed at the most polluted site in mature trees. Maximum bole carbohydrate concentration of 40-year-old trees was greater for the two most polluted sites relative to the cleanest site: the bole appeared to be a storage organ at sites where high O₃ and high N deposition decreased root biomass.

Keywords: carbohydrate allocation, mature trees, multiple stressors, ozone exposure, *Pinus ponderosa*.

Introduction

Ponderosa pine (*Pinus ponderosa* Laws) is one of the most widespread conifers in the western USA, and is a common component of western mid-elevation forests (Oliver and Ryker 1994). It typically occurs on well-drained sites with low nutrient content, where the majority of precipitation occurs during the winter months. This species is also one of the most sensitive of the conifers to oxidant pollution (Miller et al. 1983), and its response has been studied extensively across a pollution gradient from west to east in the San Bernardino Mountains, California (Grulke 1999, Miller and McBride 1999). Along this gradient, hourly O₃ concentration declines linearly from 80 to 60 ppb (24 h basis, April 15 through Octo-

ber 15 (Grulke 1999)) and nitrogen deposition declines exponentially from 20–40 to 6–9 kg ha⁻¹ (Kiefer and Fenn 1997) (Table 1). Combined with seasonal drought in this Mediterranean climate, the pollutant exposure significantly influences forest tree carbon acquisition, retention and allocation.

Although considerable research has been conducted to understand the potential impacts of tropospheric O₃ on ponderosa pine, most of the work has been on seedlings using controlled exposure chambers (US EPA 1996, Grulke 1999). Few studies have examined ontogenetic shifts in species sensitivity to O₃. For example, seedlings of giant sequoia (*Sequoiadendron giganteum* Bucholz) had much greater stomatal conductance (*g_s*), and thus O₃ uptake, than mature trees (Grulke and Miller 1994). Conversely, red oak seedlings (*Quercus rubra* L.) had lower *g_s* and O₃ uptake than mature trees (Samuelson and Edwards 1996). Allocation priorities also shift as trees mature and seedling studies may be of limited value in predicting responses of large trees to O₃ (Gower et al. 1995). A better understanding of pollutant effects at various stages of tree development is important to fully evaluate their impact on natural stands.

Ozone exposure, drought and nitrogen availability have all been shown to have significant effects on resource acquisition and allocation patterns. Ozone exposure and drought are both carbon-limiting stressors. Although both ultimately reduce total plant biomass, they shift allocation within the plant in different ways, at different times, and through different mechanisms. Ozone exposure reduces carbohydrate allocation to roots (ponderosa pine seedlings: Tingey et al. 1976, Andersen and Rygielwicz 1995; Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco): Gorissen et al. 1991). Needle (Norway spruce (*Picea abies* (L.) Karst): Luethy-Krause and Landolt 1990) and stem (loblolly pine (*Pinus taeda* L.) seedlings: Spence et al. 1990) carbohydrate concentrations have been shown to increase in response to short-term exposure to elevated O₃. Drought stress increases monosaccharide concentration in needles, and increases allocation to roots on both a short-term (Koppenall et al. 1991) and seasonal basis (Hermann and Peterson 1969). Amendments of N enhance carbon uptake and shift allocation to aboveground tissues in seedlings (loblolly pine: Kuhns and Gjerstad 1988), and in

Table 1. Summary of physical characteristics at the four study sites. Abbreviation: nd = not determined.

	Crestline	Strawberry Peak	Barton Flats	Lassen
Latitude (N)	34°14'05"	34°14'00"	34°09'42"	40°20'11"
Longitude (W)	117°19'12"	117°08'12'	116°51'00"	121°35'04"
Elevation (m)	1800	2240	1820	1700
Hourly O ₃ (ppb) ¹	79 [80]	69 [76]	62 [64]	38 [42]
Soil N (%) ²	0.20	0.11	0.08	0.01
N deposition (kg ha ⁻¹ y ⁻¹) ³	20–40	nd	6–9	nd
Annual ppt (cm) ⁴	98	96	90	115

¹ Summarized from Grulke (1999); unbracketed values are representative of mesic years and bracketed values are representative of years with < 80% of the average precipitation.
² Summarized from Grulke et al. (1998) for the upper 8 cm of mineral horizon.
³ Summarized from Kiefer and Fenn (1997); values for N deposition are broad due to variations in canopy structure.
⁴ Averaged over 17 years, 1980 through 1997 (San Bernardino County Water Resources, <http://www.co.san-bernardino.ca.us/trnsprtn/pwg/default.htm>) for the southern California sites; and for 1994 only at Lassen (this data set).

plantations (ponderosa pine: Powers and Reynolds 1999; red pine (*Pinus resinosa* Ait.): Haynes and Gower 1995). However, some of the effects reported from chamber exposure studies are short-lived, lasting only months (Luethy-Krause and Landolt 1990) or a year (Kelly et al. 1993). The research presented here describes responses of trees exposed to pollutants for over 40 years.

The first objective of this research was to determine whether tissue carbohydrate concentration or its seasonal pattern differed between five tree-age classes ranging from seedlings to mature ponderosa pine trees. This objective was carried out in an atmospherically clean site near Lassen Volcanic National Park, California. The second objective was to describe how environmental stressors modified tissue carbohydrate concentration and the date of maximum and minimum tissue concentration. Because mature trees have much larger storage volumes and larger pool sizes, 40-year-old and mature trees were compared at three sites varying in the level of environmental stressors.

Research sites

Forest plots were all representative of the Sierran mixed conifer zone (*sensu* Barbour 1988). The atmospherically cleanest site (38 to 40 ppb hourly average) was located near the southeastern corner of Lassen Volcanic National Park (LS) in the southern Cascade Mountains of California near the northern limit of the forest type. Three sites were chosen along a pollution gradient east of Los Angeles, CA: a high pollution site at the western-most edge of the San Bernardino Mountains, Crestline (CR, near Camp Paivika); a site with moderate pollution near the eastern edge of the mountain range, Barton Flats (BF, near Camp Osceola); and between these two sites, an area with moderately high pollution, Strawberry Peak (SP, close to Twin Peaks). A summary of site location and physical characteristics is given in Table 1.

This study was initiated in a drought year (1994) with 20% less than average precipitation (115-year average: 93 ± 4 cm; Big Bear Dam, San Bernardino County Water District, monthly precipitation data). The onset of drought stress

ranged from mid-July for seedlings (N.E. Grulke, unpublished results) to early August for older tree-age classes (Grulke et al. 1998). By early September, trees at the most northerly site experienced the highest predawn xylem potential (–1.1 MPa), and the three southern California sites had similar, low predawn xylem potentials for 40-year-old trees (CR: –1.5 MPa, SP: –1.6 MPa, BF: –1.7 MPa; Grulke et al. 1998).

Methods

At each site, a regionally typical stand was chosen, a plot was established in a multi-age stand, and the three to six most representative trees in each tree-age class were selected for intensive measurements (Grulke et al. 1998, Grulke 1999). Representative trees were selected that had statistically average values, relative to the stand population of that tree-age class, for the following characteristics: bole or stem diameter, total height, percent chlorotic mottle of 1-year-old needles, number of needle age classes retained, and the distance to the nearest intra- and interspecific tree as a measure of inferred competition (summarized for Lassen by N.E. Grulke, unpublished results; and for the southern Californian sites in Grulke et al. 1998). There were too few mature trees with accessible branches within the plots, so additional trees near the plots were included for intensive measurements. Mature trees were dominant forest trees. The mature trees averaged 252 ± 43 years at LS, 193 ± 13 years at BF, 85 ± 3 years at SP, and 158 ± 20 years at CR, all determined at 1.4 m height.

The trees selected were widely separated and had no canopy or root interaction; individual trees were used as within-site replications. The distribution of ponderosa pine at LS and BF was characterized by clumps of trees, separated by open areas, typical of droughty environments (Grulke 1999). Ponderosa pine was more evenly distributed at SP and CR. For example, 85% of the population of the 40-year-old tree-age class was less than 3.5 m from another ponderosa pine; only 3% of the population was within 5.0 m of another ponderosa pine at all sites.

For the atmospherically clean site, four tree-age classes

were compared: 3- to 9-year-old ($n = 6$), 21- to 40-year-old ($n = 3$) and 41- to 60-year-old ($n = 3$) tree-age classes, and mature trees ($n = 3$). Foliage only was sampled from an additional tree-age class at LS (11- to 20-year-old, $n = 3$). For comparison with the southern California sites, the 21- to 40-year-old and 41- to 60-year-old tree-age classes were combined because tissue concentrations or their seasonal patterns did not differ significantly. At the southern California sites, only the 40-year-old ($n = 6$) and mature trees ($n = 3$) were compared.

Tissue for carbohydrate analysis was sampled on a monthly basis from early June to mid-September. At LS, seedlings were destructively harvested during each sampling period to obtain sufficient material for analysis. Seedlings did not have coarse roots. All current-year (available after mid-July) and 1-year-old needles were sampled from seedlings, excluding the terminus and the top and bottom branchlet on each seedling. For all older tree-age classes, two to three fascicles each of current-year and 1-year-old needles were sampled from three primary, lateral branches and collated within a tree. Primary branches sampled were on the southern aspect of the canopy, and received a minimum of 2–3 h of direct solar irradiance per day. Foliage from the 11- to 20-year-old trees was sampled from the third, fourth and fifth lateral branch from the terminus. For older tree-age classes, foliage was sampled in the upper part of the lower third of the canopy, at approximately 2 m height for the 21- to 40-year-old and 41- to 60-year-old trees and at 15–18 m height for the mature trees. Bole and coarse roots (> 1 cm in diameter) were sampled using a 0.635-cm-diameter increment borer to obtain three cores of approximately 1.5-cm diameter each. Bark was removed before processing. Coarse and fine roots were sampled 1.5 to 2 m from the base of the tree. The bark on bole cores and the outer reddish brown sheath on roots was removed before processing.

Sampled tissue was placed in cryovials in the field, and immediately placed in flasks of liquid nitrogen for transport to the laboratory. Tissue was stored at -80°C until lyophilization. Dried tissue was ground, then stored in vials in bags of drierite until analysis. Carbohydrate fractions (glucose, fructose, starch) were determined on solution extracts using an LC gradient pump module with a PAD2 detector (Dionex Corp., Sunnyvale, CA) (Wilson et al. 1995). Data were analyzed with AI450 software (Dionex Corp.). Blanks were run every 20 samples, duplicates every 10 samples, and internal laboratory starch solutions were run at the beginning and end of each batch of 50 samples.

Results

Tree-age class differences in carbohydrate concentration at the clean site

In general, starch concentration was at a maximum early in the growing season, and declined exponentially through the growing season (Figure 1). Starch concentrations in all tissues declined when branch and needle growth were concurrent (N.E. Grulke, unpublished results). Monosaccharide concentration

increased in fine roots throughout the growing season, but was more constant over the growing season for coarse roots, stems or boles, and needles. Because fructose and glucose individually had the same phenology as total monosaccharide concentration, and each accounted for half of the monosaccharide concentration, the data are not presented or discussed.

Seedlings had the greatest stem and needle monosaccharide concentration relative to bole tissue in all other tree-age classes throughout the growing season. Carbohydrate concentrations were greater in seedling tissue by 2- to 7-fold for all comparisons where statistical significance was indicated ($P \leq 0.05$; Figure 1). Fine-root monosaccharide concentration of seedlings was significantly greater in mid-August than that of all other tree-age classes. Monosaccharide concentration of current-year needles of seedlings was 1.5- to 2-fold greater relative to all older tree-age classes for mid-August and mid-September.

Tissue carbohydrate concentrations were similar among the older tree-age classes, but there were exceptions. Both seedlings and mature trees completed transfer of belowground carbohydrate for aboveground growth earlier than the other two tree-age classes, as inferred from changes in tissue carbohydrate concentration. Fine-root starch concentrations of seedlings and mature trees were similar at the mid-July sampling date, and were significantly lower than those of the 21- to 40-year-old and 41- to 60-year-old trees (at $P = 0.05$). Fine-root monosaccharides of mature trees were lower than in all other tree-age classes at the end of September. Bole starch in mature trees was one third that in all other tree-age classes by mid-August.

Tissue carbohydrate concentrations in relation to pollutant exposure

The conventional way to compare sites is to average the carbohydrate concentration of all trees within a tree-age class on a single sampling date, as was done to compare tree-age classes at LS. However, within-site asynchrony in phenology increased with intensity of environmental stress (drought and air pollution exposure). Therefore, the comparison of sites was based on individual tree performance within a site, e.g., magnitude and date of occurrence of maximum and minimum starch and monosaccharide concentration, as determined from individual tree phenology (Tables 1 and 2; Figures 2 and 3). Root, bole and foliar carbohydrate concentrations are presented for the 40-year-old and mature trees. Because seasonal patterns of carbohydrate concentration differed between LS and the southern California sites, statistical comparisons were made only among the southern California sites. For example, in northern California, monosaccharide concentration of fine roots generally increased through the growing season. In southern California, fine-root monosaccharide concentration was characterized by a parabolic seasonal response, with peak values in early August at the most polluted site (CR), early September for the central site (SP), and a broad maximum peak between early July and late August at the least polluted site (BF).

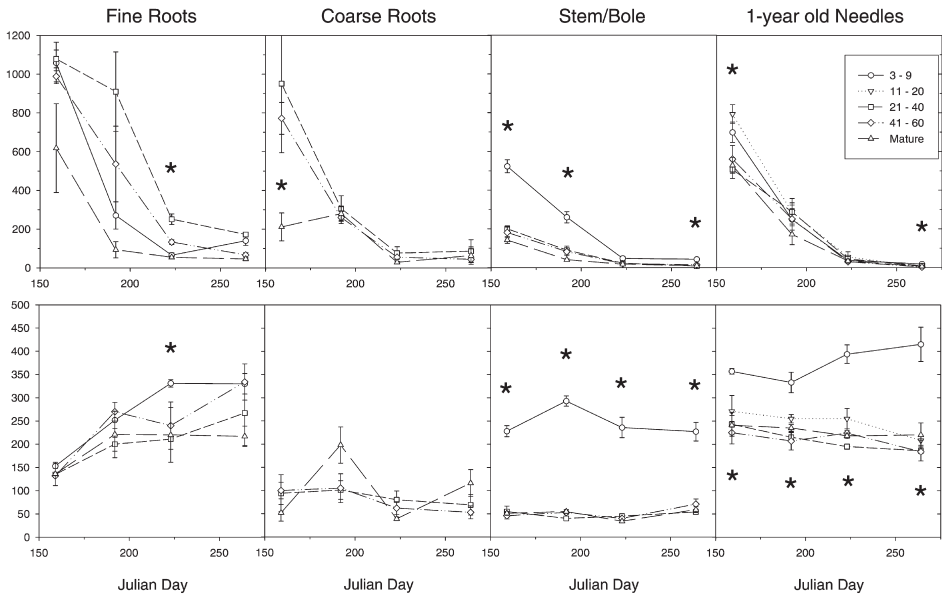


Figure 1. Seasonal course of carbohydrate concentration for fine and coarse roots, stem, and 1-year-old needles of five tree-age classes of ponderosa pine. In all cases except the seedlings (where $n = 6$), the symbol represents the mean $\pm$ 1 SE of three individuals. Sampling dates where carbohydrate concentration was significantly different at $P \leq 0.05$ are indicated with an asterisk.

Maximum root starch concentrations were lowest at the most polluted site (CR) for both tree-age classes, but the difference between sites was statistically significant only for fine and coarse roots of mature trees (Figure 2). The maximum root

monosaccharide concentration was lower at the more polluted sites (SP, CR) for both tree-age classes (Figure 3). The minimum fine- and coarse-root starch concentrations did not differ significantly among the southern California sites, except for

Table 2. Maximum and minimum starch concentrations ($\mu\text{mol g}^{-1}$) and date of occurrence (Julian day). Differing lower case letters indicate statistical differences between paired southern California sites (t -test, $P = 0.05$).

	Maximum starch concentration				Minimum starch concentration			
	40-year-old	Date	Mature	Date	40-year-old	Date	Mature	Date
<i>Fine roots</i>								
Lassen	1022 $\pm$ 32	168 $\pm$ 6	618 $\pm$ 229	159 $\pm$ 0	119 $\pm$ 24	264 $\pm$ 0	46 $\pm$ 1	264 $\pm$ 0
Barton Flats	867 $\pm$ 217 a	160 $\pm$ 8 a	797 $\pm$ 111 a	149 $\pm$ 2 a	181 $\pm$ 41 a	248 $\pm$ 12 a	48 $\pm$ 17 a	246 $\pm$ 12 a
Strawberry Peak	1050 $\pm$ 208 a	134 $\pm$ 6 b	981 $\pm$ 53 ab	189 $\pm$ 10 a	116 $\pm$ 36 a	265 $\pm$ 6 a	89 $\pm$ 31 a	238 $\pm$ 18 a
Crestline	718 $\pm$ 157 a	168 $\pm$ 6 a	552 $\pm$ 72 ac	179 $\pm$ 19 a	137 $\pm$ 50 a	230 $\pm$ 19 a	54 $\pm$ 31 a	257 $\pm$ 12 a
<i>Coarse roots</i>								
Lassen	668 $\pm$ 202	172 $\pm$ 8	317 $\pm$ 16	185 $\pm$ 13	36 $\pm$ 5	250 $\pm$ 9	21 $\pm$ 5	237 $\pm$ 14
Barton Flats	879 $\pm$ 262 a	177 $\pm$ 6 a	891 $\pm$ 81 a	181 $\pm$ 0 a	223 $\pm$ 90 a	233 $\pm$ 24 a	56 $\pm$ 10 a	270 $\pm$ 0 a
Strawberry Peak	734 $\pm$ 126 a	166 $\pm$ 6 a	668 $\pm$ 161 a	179 $\pm$ 0 a	145 $\pm$ 51 a	253 $\pm$ 8 a	186 $\pm$ 19 b	247 $\pm$ 12 a
Crestline	531 $\pm$ 130 a	177 $\pm$ 11 a	294 $\pm$ 37 b	188 $\pm$ 22 a	183 $\pm$ 53 a	213 $\pm$ 11 a	87 $\pm$ 40 a	246 $\pm$ 11 a
<i>Bole</i>								
Lassen	180 $\pm$ 19	164 $\pm$ 5	142 $\pm$ 17	159 $\pm$ 0	9 $\pm$ 1	257 $\pm$ 7	10 $\pm$ 3	264 $\pm$ 0
Barton Flats	96 $\pm$ 35 a	175 $\pm$ 15 a	99 $\pm$ 18 a	181 $\pm$ 0 a	13 $\pm$ 5 a	256 $\pm$ 9 a	7 $\pm$ 0 a	270 $\pm$ 0 a
Strawberry Peak	225 $\pm$ 31 b	144 $\pm$ 6 bc	150 $\pm$ 31 a	179 $\pm$ 0 a	9 $\pm$ 2 a	253 $\pm$ 8 a	48 $\pm$ 9 b	271 $\pm$ 0 a
Crestline	255 $\pm$ 8 b	154 $\pm$ 0 ac	112 $\pm$ 5 a	191 $\pm$ 18 a	10 $\pm$ 2 a	244 $\pm$ 12 a	36 $\pm$ 10 b	227 $\pm$ 9 b
<i>One-year-old needles</i>								
Lassen	536 $\pm$ 46	164 $\pm$ 5	528 $\pm$ 41	159 $\pm$ 0	4 $\pm$ 3	257 $\pm$ 7	5 $\pm$ 3	264 $\pm$ 0
Barton Flats	522 $\pm$ 28 a	151 $\pm$ 0 a	564 $\pm$ 44 a	151 $\pm$ 0 a	19 $\pm$ 3 a	246 $\pm$ 8 a	20 $\pm$ 2 a	258 $\pm$ 12 a
Strawberry Peak	359 $\pm$ 23 b	134 $\pm$ 6 ab	365 $\pm$ 19 b	153 $\pm$ 0 a	15 $\pm$ 1 a	271 $\pm$ 0 a	22 $\pm$ 5 a	271 $\pm$ 0 a
Crestline	90 $\pm$ 18 c	173 $\pm$ 19 ac	187 $\pm$ 81 b	208 $\pm$ 36 a	22 $\pm$ 3 a	209 $\pm$ 13 b	47 $\pm$ 4 b	198 $\pm$ 18 b
<i>Current-year needles</i>								
Lassen	73 $\pm$ 12	223 $\pm$ 0	76 $\pm$ 38	223 $\pm$ 0	20 $\pm$ 5	264 $\pm$ 0	21 $\pm$ 7	264 $\pm$ 0
Barton Flats	79 $\pm$ 14 a	239 $\pm$ 10 a	125 $\pm$ 22 a	234 $\pm$ 0 a	38 $\pm$ 11 a	252 $\pm$ 8 a	47 $\pm$ 10 a	251 $\pm$ 19 a
Strawberry Peak	35 $\pm$ 7 b	228 $\pm$ 4 ac	40 $\pm$ 1 a	216 $\pm$ 8 b	16 $\pm$ 1 bc	271 $\pm$ 0 ab	17 $\pm$ 6 bc	271 $\pm$ 0 a
Crestline	28 $\pm$ 1 b	214 $\pm$ 5 bc	103 $\pm$ 38 a	235 $\pm$ 1 a	20 $\pm$ 2 ac	248 $\pm$ 10 ac	32 $\pm$ 5 ac	229 $\pm$ 20 a

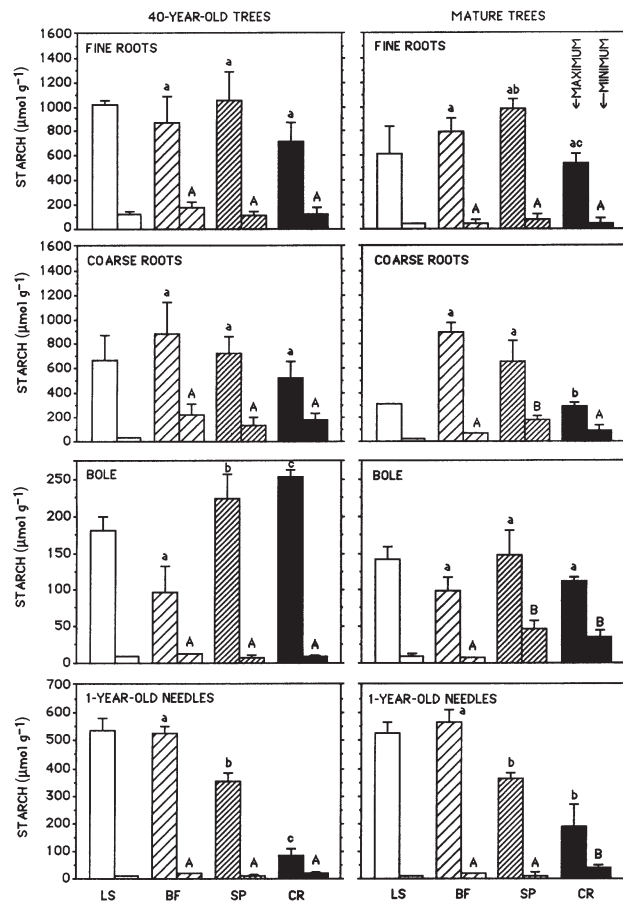


Figure 2. Seasonal maximum starch concentration of tissues for six 40-year-old and three mature ponderosa pine trees at each site. Sites are arranged in order of increasing O_3 exposure: LS = Lassen; BF = Barton Flats; SP = Strawberry Peak; CR = Crestline. Because seasonal carbohydrate patterns differed between northern and southern California, statistical comparisons were made only among the southern California sites. Values for the northern California samples were included for comparison only. Bar height represents the mean maximum tissue concentration; the error bar indicates ± 1 SE of the mean. Differing letters indicate statistically significant differences.

coarse-root starch of mature trees at the moderately high pollution site (SP), which was significantly higher (Table 2). The minimum fine- and coarse-root monosaccharide concentrations, and their timing, did not differ significantly among the southern California sites for either tree-age class (Table 3).

The 40-year-old trees at the more polluted sites (SP, CR) had 2.5-fold greater bole starch concentration (Figure 2) than trees at the moderately polluted site (BF). Maximum bole monosaccharide concentration was greater at the most polluted site (CR) relative to SP and BF. Maximum bole starch and monosaccharide concentrations of mature trees were not significantly different between any of the sites. Minimum bole starch of 40-year-old trees did not differ significantly among the southern Californian sites, but was elevated among the more polluted sites (SP, CR) for mature trees. Minimum bole monosaccharides were elevated for the two more polluted

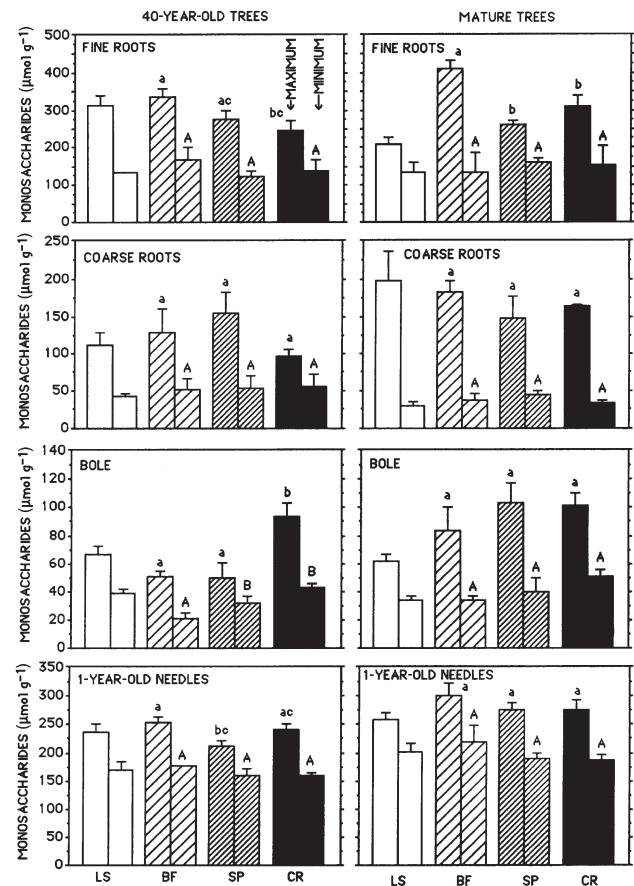


Figure 3. Seasonal maximum monosaccharide concentration of ponderosa pine tissues for six 40-year-old and three mature ponderosa pine trees at each site. Sites are arranged in order of increasing O_3 exposure: LS = Lassen; BF = Barton Flats; SP = Strawberry Peak; CR = Crestline. Because seasonal carbohydrate patterns differed between northern and southern California, statistical comparisons were made only among the southern California sites. Values for the northern California samples were included for comparison only. Bar height represents the mean maximum tissue concentration; the error bar indicates ± 1 SE of the mean. Differing letters indicate statistically significant differences.

sites for 40-year-old trees, but did not differ for mature trees among southern California sites. When belowground starch concentration differed significantly, the maximum for 40-year-old trees and minimum for mature trees occurred earlier in the growing season (Tables 1 and 2). The timing of maximum or minimum bole monosaccharide concentration did not differ significantly among the southern California sites.

The maximum starch concentration of 1-year-old needles was significantly lower for the more polluted sites (SP, CR) than for the least polluted site (BF) for both tree-age classes (Figure 2, Table 2). Minimum needle starch content was significantly elevated only in the most polluted site (CR) for mature trees. One-year-old needle monosaccharide content was significantly lower at SP in 40-year-old trees, but it did not differ significantly among the southern California sites for mature trees. In general, when foliar carbohydrate concentrations

Table 3. Maximum and minimum monosaccharide concentrations ($\mu\text{mol g}^{-1}$), and date of occurrence (Julian day). Significance at $P = 0.05$ for the t -test performed between sites is indicated by differing letters.

	Maximum monosaccharide concentration				Minimum monosaccharide concentration			
	40-year-old	Date	Mature	Date	40-year-old	Date	Mature	Date
<i>Fine roots</i>								
Lassen	313 $\pm$ 25	258 $\pm$ 6	210 $\pm$ 17	250 $\pm$ 14	134 $\pm$ 3	170 $\pm$ 11	136 $\pm$ 25	159 $\pm$ 0
Barton flats	336 $\pm$ 22 a	201 $\pm$ 11 a	409 $\pm$ 24 a	218 $\pm$ 16 a	166 $\pm$ 34 a	210 $\pm$ 27 a	133 $\pm$ 51 a	230 $\pm$ 40 a
Strawberry Peak	280 $\pm$ 20 ac	223 $\pm$ 13 a	263 $\pm$ 7 b	217 $\pm$ 9 a	126 $\pm$ 12 a	168 $\pm$ 22 a	164 $\pm$ 8 a	207 $\pm$ 16 a
Crestline	246 $\pm$ 25 bc	221 $\pm$ 13 a	311 $\pm$ 31 b	238 $\pm$ 17 a	137 $\pm$ 29 a	172 $\pm$ 9 a	154 $\pm$ 54 a	240 $\pm$ 29 a
<i>Coarse roots</i>								
Lassen	112 $\pm$ 17	183 $\pm$ 11	198 $\pm$ 40	198 $\pm$ 0	42 $\pm$ 4	264 $\pm$ 0	29 $\pm$ 6	202 $\pm$ 21
Barton Flats	129 $\pm$ 32 a	208 $\pm$ 16 a	182 $\pm$ 15 a	181 $\pm$ 0 a	54 $\pm$ 13 a	216 $\pm$ 24 a	38 $\pm$ 9 a	248 $\pm$ 22 a
Strawberry Peak	156 $\pm$ 26 a	212 $\pm$ 9 a	150 $\pm$ 28 a	198 $\pm$ 10 ac	56 $\pm$ 14 a	234 $\pm$ 18 a	46 $\pm$ 4 a	216 $\pm$ 19 a
Crestline	97 $\pm$ 10 a	172 $\pm$ 9 b	164 $\pm$ 2 a	210 $\pm$ 0 bc	56 $\pm$ 16 a	233 $\pm$ 14 a	34 $\pm$ 3 a	269 $\pm$ 0 a
<i>Bole</i>								
Lassen	67 $\pm$ 6	229 $\pm$ 22	62 $\pm$ 5	242 $\pm$ 22	39 $\pm$ 3	200 $\pm$ 11	34 $\pm$ 2	223 $\pm$ 0
Barton Flats	51 $\pm$ 4 a	196 $\pm$ 18 a	83 $\pm$ 17 a	193 $\pm$ 12 a	21 $\pm$ 4 a	204 $\pm$ 23 a	34 $\pm$ 5 a	234 $\pm$ 0 a
Strawberry Peaks	51 $\pm$ 10 a	180 $\pm$ 14 a	104 $\pm$ 13 a	189 $\pm$ 10 a	33 $\pm$ 4 b	225 $\pm$ 25 a	42 $\pm$ 8 a	235 $\pm$ 0 a
Crestline	93 $\pm$ 10 b	201 $\pm$ 18 a	101 $\pm$ 9 a	204 $\pm$ 5 a	43 $\pm$ 3 b	228 $\pm$ 16 a	51 $\pm$ 5 a	235 $\pm$ 1 a
<i>One-year-old needles</i>								
Lassen	237 $\pm$ 16	189 $\pm$ 12	259 $\pm$ 12	194 $\pm$ 35	169 $\pm$ 15	257 $\pm$ 7	202 $\pm$ 15	229 $\pm$ 35
Barton Flats	253 $\pm$ 9 a	171 $\pm$ 20 a	301 $\pm$ 22 a	191 $\pm$ 40 a	176 $\pm$ 8 a	203 $\pm$ 7 a	219 $\pm$ 30 a	192 $\pm$ 11 a
Strawberry Peak	217 $\pm$ 5 bc	227 $\pm$ 28 ac	278 $\pm$ 9 a	232 $\pm$ 39 a	161 $\pm$ 9 a	179 $\pm$ 0 b	191 $\pm$ 8 a	189 $\pm$ 10 a
Crestline	242 $\pm$ 10 ac	254 $\pm$ 10 bc	277 $\pm$ 17 a	217 $\pm$ 37 a	158 $\pm$ 5 a	185 $\pm$ 5 b	187 $\pm$ 9 a	190 $\pm$ 10 a
<i>Current-year needles</i>								
Lassen	226 $\pm$ 23	229 $\pm$ 7	257 $\pm$ 38	264 $\pm$ 0	178 $\pm$ 17	257 $\pm$ 7	234 $\pm$ 31	223 $\pm$ 0
Barton Flats	236 $\pm$ 21 a	243 $\pm$ 9 a	286 $\pm$ 19 a	270 $\pm$ 0 a	173 $\pm$ 14 a	215 $\pm$ 20 a	210 $\pm$ 13 a	214 $\pm$ 0 a
Strawberry Peak	231 $\pm$ 11 a	264 $\pm$ 6 ab	282 $\pm$ 9 a	271 $\pm$ 0 a	167 $\pm$ 13 a	208 $\pm$ 0 a	194 $\pm$ 8 ab	208 $\pm$ 0 ab
Crestline	226 $\pm$ 9 a	219 $\pm$ 10 ac	331 $\pm$ 33 a	235 $\pm$ 1 b	183 $\pm$ 14 a	242 $\pm$ 6 a	243 $\pm$ 15 ac	249 $\pm$ 20 ac

differed significantly among sites, the maximum or minimum value occurred earlier in the growing season. In both tree-age classes, the least polluted site (BF) had a 50% greater starch concentration than the site with moderately high pollution (SP). At the most polluted site (CR), 40-year-old trees were more affected than older trees; 1-year-old needles had 25% (versus 50% for mature trees) of the needle starch concentration at the moderately high (SP) pollution site. Needles of mature trees had significantly greater minimum starch concentration at the most polluted site relative to the intermediate and least polluted sites ($F = 9.72$; $P = 0.013$).

Linkages between below- and aboveground carbohydrate concentrations

Correlation coefficients between tissue types (fine roots, coarse roots, bole, needles) within individual trees were calculated for the maximum starch concentration, and for the date of its occurrence in 40-year-old trees. The maximum coarse- and fine-root starch concentrations were significantly correlated at BF and CR, but not at LS and SP (LS: $r = 0.48$, $P = 0.19$; BF: $r = 0.80$, $P = 0.02$; SP: $r = 0.601$; $P = 0.15$; CR: $r = 0.68$, $P = 0.05$). The timing of the maximum coarse-root starch concentration was highly correlated with that of fine roots only at the most polluted site ($r = 0.88$, $P = 0.002$). Although the lower bole has been used as an analog for coarse-root carbohydrate

concentration, there was no significant correlation between the two tissue types, either in magnitude or phenology. The maximum bole and needle starch concentrations were significantly correlated only at SP ($r = 0.74$, $P = 0.02$), as was its date of occurrence ($r = 0.23$, $P = 0.02$). The maximum starch concentration and its date of occurrence was not significantly correlated between fine roots and needles (LS: $F = 0.117$, $P = 0.41$; BF: $F = 0.197$, $P = 0.67$; SP: $F = 0.268$, $P = 0.62$; CR: $F = 0.204$, $P = 0.66$).

Discussion

Carbohydrate concentrations in tissues of trees differing in age

Ponderosa pine seedlings exhibited significantly greater concentrations of starch and monosaccharides in stems and 1-year-old needles than older tree-age classes (Figure 1). In general, the seasonal trends in starch and monosaccharide concentrations of all tissue types were similar across all tree-age classes. One exception was monosaccharide concentrations in seedling needles, which continued to increase throughout the growing season. This pattern of foliar carbohydrate accumulation has also been observed in loblolly pine seedlings (Edwards et al. 1992). Short-term drought stress in

Norway spruce seedlings increased retention of photosynthate in needles (Gorissen et al. 1994). Although the seasonal pattern of fine-root monosaccharides was similar to that of older tree-age classes, the maximum tissue concentration occurred 1 month earlier. Seedlings experienced low predawn xylem water potential 1 month earlier than all other tree-age classes at Lassen (N.E. Grulke, unpublished results). Greater tissue monosaccharide concentration in seedlings may permit greater resistance to drought.

Carbohydrate concentrations in relation to phenology

It has been suggested that the growth of one plant part is slowed or truncated when growth in another part is initiated as a result of internal competition among sinks for carbon resources (Waring and Schlesinger 1985, Luxmoore et al. 1995). The sequence of growth, and changes in tissue carbohydrate concentration in ponderosa pine, suggests that there are concurrent, strong sinks for carbohydrates over at least half the growing season. Branch elongation growth was initiated in early May and was 90% complete by early July (Grulke et al. 1998; N.E. Grulke, unpublished results). Needle elongation growth was initiated approximately 1 month after branch elongation in early June, and was 90% complete by mid-July in 40-year-old trees. There was a decline in starch in all tissues sampled in the third week of June, corresponding to the peak acceleration of both branch and needle elongation growth.

Growth of fine roots was also concurrent with aboveground elongation growth, and continued through at least mid-July (Grulke et al. 1998). Carbohydrate concentration in belowground tissues reached its seasonal minimum by mid-July. Fine root growth in the summer may be limited both by competing sinks within the plant and low available soil water (Hermann and Peterson 1969). The total pool of resources obtained and retained, its storage location, and the timing of the internal allocation relative to the environmental cue may indicate the degree of stress experienced (Waring 1987). Only a few apparently stressed individuals (one mature tree in the droughty site and two 40-year-old trees in the most polluted site) exhibited greater coarse root carbohydrate concentration in early autumn relative to the total population of trees sampled. However, the spatial and phenological variation of carbohydrate concentration between roots on the same individual is unknown. Coarse roots may have a greater sink strength for carbohydrate. Alternatively, changes in bole carbohydrate were difficult to detect because bole tissue had the lowest concentration but the highest volume of the woody tissues.

Relationship between carbohydrate concentration and environmental stress

In conifer seedlings, short-term (days to weeks) exposure to elevated O₃ increased retention of recently fixed photosynthate in 1-year-old or current-year needles (Douglas-fir: Gorissen et al. 1994; Norway spruce: Luethy-Krause and Landolt 1990). Medium-term (1–3 years) exposure of conifer seedlings to elevated O₃ also increased retention of photosynthate in 1-year-old needles (loblolly pine: Kupperts and Klumpp 1988, Friend and Tomlinson 1992; shortleaf pine

(*Pinus echinata* Mill.): Paynter et al. 1991). Some of these effects were reversible. For example, Luethy-Krause and Landolt's (1990) short-term response was lost by winter. One-year-old loblolly pine seedlings showed reduced photosynthate allocation to roots in 1-year-old seedlings, but not in the second year of exposure (as reviewed in Kelly et al. 1993).

In response to short-term elevated O₃ exposure, there was no effect on total starch content of 1-year-old or current-year needles of seedlings (Gorissen et al. 1994). In response to medium-term elevated O₃ exposure, total starch content of 1-year-old needles was significantly reduced in seedlings (Kupperts and Klumpp 1988, Kelly et al. 1993, Paynter et al. 1991) and red spruce (*Picea rubens* Sarg.) (Alscher et al. 1989), but current-year foliage was unaffected (Kupperts and Klumpp 1988). In our field study of older trees, needle starch concentration was significantly reduced in both 1-year-old and current-year needles in response to long-term exposure to pollutants. Fertilization did not alter starch content of aboveground tissues of Douglas-fir seedlings (Margolis and Waring 1986), suggesting that oxidant pollution induced the reduction in starch in ponderosa pine.

Conifer seedlings exposed to medium-term elevated O₃ had an increased monosaccharide concentration in needles (Paynter et al. 1991). When nitrogen was augmented, conifer seedlings had significantly increased sugar content in aboveground tissue (Margolis and Waring 1986). In our study of ponderosa pine exposed to a range of long-term O₃ and nitrogen deposition rates, needle monosaccharide concentration was generally similar across sites in the 40-year-old and mature trees. The exception was 40-year-old trees at the site with moderately high O₃ exposure and moderate nitrogen deposition (SP), which had lower foliar monosaccharide concentrations.

The effect of low foliar starch concentration in both current- and 1-year-old needles at the most polluted site is accentuated when low foliar retention is taken into account (Grulke and Balduman 2000). For example, 95% of the foliar biomass of 40-year-old trees at the most polluted site comprised current-year needles, compared to 35% at a site of moderate pollution. Current-year needles have one third to one tenth the starch concentration of 1-year-old needles. The potential consequences of lower needle starch are more severe for 40-year-old trees than for mature trees, which generally have longer-lived needles (N.E. Grulke, unpublished results). Older needles are important sources of photosynthate for newly developing needles and roots (white pine (*Pinus strobus* L.): McLaughlin et al. 1982). Loss of older needles requires greater translocation of photosynthate from current-year needles for root growth.

Elevated O₃ exposure reduced starch concentration or carbohydrate allocation to roots in Norway spruce (Kupperts and Klumpp 1988) and loblolly pine seedlings (Adams et al. 1990). Elevated O₃ exposure and nutrient supply both lowered root biomass and starch concentration in ponderosa pine seedlings (Andersen and Scagel 1997, Andersen et al. 1997). In our study, fine- and coarse-root starch of older trees was significantly reduced at the most polluted site. Fine-root monosaccharide concentration was significantly lower at the most

polluted site for 40-year-old trees and at the two most polluted sites for mature trees. Significantly lower coarse- and fine-root biomass was found at the two most polluted sites for the same 40-year-old trees used in this study (Grulke et al. 1998). Both O₃ and high nitrogen deposition probably contributed to this response.

Bole starch concentration of 40-year-old trees was significantly greater at the two most polluted sites compared to the moderately polluted site. This, combined with reduced root biomass and carbohydrate concentration (Grulke et al. 1998), suggests that the bole is the primary storage location for carbohydrates at sites with moderate to high pollution. In loblolly pine seedlings, high O₃ exposure induced preferential allocation of carbon to stems (Spence et al. 1990). An alternative explanation is that transport of carbohydrates to the roots from the bole changed. McLaughlin et al. (1982) hypothesized that carbohydrate retention in needles after O₃ exposure may result from a change in phloem loading or biochemical pathways. Significantly greater minimum bole starch (mature trees) and monosaccharide (40-year-old trees) concentrations observed in this study suggest that phloem loading is limited at each step of transfer (e.g., going into and out of storage locations). In ponderosa pine seedlings exposed to elevated O₃, the source of carbohydrates for spring root growth was both stems and coarse roots (Andersen et al. 1997). This pattern was not detected in the boles of mature trees, perhaps because their large volumes made changes in carbohydrate concentrations more difficult to detect.

Trees respond differently to the complex interaction of natural and anthropogenic stresses as they mature. As pool size increases with age, trees may have more capacitance with which to supply carbohydrate to actively growing sinks, which greatly affects their ability to tolerate environmental stressors. However, chronic stresses, such as pollutant exposure, that deplete carbohydrate pool sizes may take longer to recharge, leaving older trees more susceptible to other environmental stressors, such as drought (Johnson 1989). Because fewer needle-age classes are retained, decreased needle carbohydrate concentration had a greater impact on the 40-year-old trees than the mature trees. However, despite the large total carbohydrate stores present in mature tree boles, the data presented here suggest that mature ponderosa pine trees are also adversely affected by high O₃ and N exposure, because both fine- and coarse-root starch and fine-root monosaccharide concentrations were depressed relative to mature trees at less polluted sites.

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