THE IMPACT OF OZONE ON SEQUOIA SEEDLING STEM STRUCTURE: IMPLICATIONS FOR SEEDLING SURVIVAL

Running Heading:

OZONE AND SEEDLING STEM STRUCTURE

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ABSTRACT

Giant sequoia seedlings (Sequoiadendron giganteum [Lindl.] Buchh.) were exposed during the 4th to 7th months after germination to three different atmospheric concentrations of ozone: charcoal-filtered air, ambient air and a 50% increase in ozone over ambient in open top chambers. The study was conducted in the Giant Forest Grove, Sequoia National Park, California, U.S.A. An increase in ozone by 50% over ambient significantly reduced stem diameter. The reduction in total stem diameter is manifest in reduced thicknesses of cortex and xylem cylinder. No reductions in the phloem thickness were observed. The reduction in the diameter of the xylem cylinder is, in part, due to a reduction in the number of secondary tracheids per radial file. Last formed tracheids were significantly larger in radial diameter with thicker cell walls. The number of phloem fibers was also reduced.

The reduction in the xylem cylinder size, number of tracheids per radial file and phloem fibers in conjunction with larger xylem elements and no reduction in the thickness of phloem conductive tissues suggests physiological functions of water and photosynthate transport are conserved at the expense of mechanical elements in stem tissue. The mechanical requirements of a small seedling may not be as critical for seedling establishment and survival as is transport between roots and the canopy.

KEY WORDS: Sequoiadendron, ozone, stem architecture, anatomy

Introduction

The majority of ecophysiological studies on the impact of the atmospheric pollutant ozone on tree seedlings have focused on the measure of photosynthesis and respiration (Grulke et al. 1989; Kozlowski & Constantinidou 1986; Reich 1987; Sasek & Richardson 1989), morphometric data (Grulke et al 1989; Shafer, Heagle & Camberato 1987) and foliar damage (Duriscoe & Stolte 1989; Kozlowski & Constantinidou 1986).

Ozone is known to damage leaves and induce premature leaf loss, resulting in a reduction in the photosynthetic capacity of the tree (Kozlowski & Constantinidou 1986; Miller et al 1963). Associated with the damage to photosynthetic tissue is an increase in tissue respiration (Grulke et al. 1989; Reich 1987). Decreased photosynthesis and increased respiratory demands of the damage leaf tissues results in a reduction in the amount of photosynthate available for growing tissues in the stem (Reich 1987). Ozone induced reductions in stem growth have been reported in seedlings (Grulke et al. 1989; Kozlowski & Constantinidou 1986; Shafer, Heagle & Camberato 1987) and implicated in mature trees (Peterson & Arbaugh 1988; Peterson, Arbaugh & Robinson 1989; Peterson et al. 1987; Williams & Williams 1986). The direct impact of the observed growth reduction on the physiological function of transport and the biomechanical function of support in the stem are not understood. Any change in either function may directly effect the

survivorship of both seedlings and mature trees.

The object of this study is to observe changes in the stem anatomy of sequoia (Sequoiadendron giganteum [Lindl.] Buchh.) seedlings exposed to different concentrations of ozone. These data are interpreted with regard to the structural and functional relationships the different tissues have within the seedling stem and the possible implication these changes will have on seedling survival.

Methods and Materials

All plant material was obtained from an ozone fumigation/filtration study conducted in the Giant Forest Grove, Sequoia National Park, CA, U.S.A., (Grulke et al. 1989) during the summer of 1988. Seedlings of giant sequoia were germinated in leach tubes (145 cc) containing soil from the sequoia grove at the Pacific Southwest Forest and Range Experiment Station, Riverside, CA, U.S.A. and in Giant Forest Grove (1,920 m elevation). Seedlings were transferred to open top chambers in early June. The air in one group of chambers was filtered using charcoal. A second group had the atmospheric concentration of ozone increased 50% over the ambient conditions. The last group of chambers had ambient air. The exact daily concentrations of ozone concentrations are reported in Grulke et al. (1989). In the fall of 1988 five Riverside germinated and five Giant Forest germinated seedlings were removed from each of the three chambers

per treatment group and prepared for histological analysis. Each treatment group consisted of a total of 15 seedlings.

Stems were fixed in a 10% (vol:vol) aqueous acrolein solution in ice. The samples were then dehydrated through an ethanol series and were paraplast infiltrated (Berlyn & Miksche 1976). The stems were microtome sectioned at 15 um directly below the whorl of cotyledons (first needles) in the hypocotyl. This standardized the location of all samples for the anatomical analysis to reduce any possibility of variability induced by sampling at different locations in the stem. The sections were then stained using a safranin and fast green (Berlyn & Miksche 1976).

The following parameters were measured using an ocular micrometer: Stem width, thickness of the cortex tissue, thickness of the xylem central cylinder, thickness of the phloem, last formed tracheid cell radial diameter and lumen diameter, radial epidermal cell width, radial epidermal cell wall thickness and cuticle thickness. The number of primary and secondary xylem cells (tracheids) and phloem cells in a radial file, and the number of fibers in the phloem were counted in each representative transverse section.

An analysis of variance (ANOVA) was performed on individual data sets for anatomical characteristics using a computer program by SAS (SAS Institute 1985). When a group of means were found to differ significantly, a multiple comparison of means was performed using the Duncan's multiple-range test to determine

which means differed significantly from other means in each individual data set (SAS Institute 1985).

Results

Since the seedlings germinated at Riverside were older, the stems were consistently larger than those germinated at Giant Forest. For this reason, the two groups were treated separately in all statistical analyses. There is a significant reduction in the stem diameter of Riverside germinated seedlings exposed to a 50% increase in ozone. A similar trend of stem diameter reduction exists in the Giant Forest seedlings, however it is not significant. These results are similar for cortex thickness, and xylem cylinder diameter (Tables 1 and 2).

No difference were observed in phloem thickness in either Riverside or Giant Forest seedlings (Table 2). Significantly fewer xylem cells per radial file were produced in response to a 50% increase in ozone in both the Giant Forest and Riverside seedlings (Table 3). Seedlings of Giant Forest origin grown in either elevated ozone or filtered air produced significantly fewer phloem cells per radial file than conspecific seedlings growing in ambient air (Table 3). This effect was only observed in the Giant Forest seedlings for phloem cells per radial file (Table 3). There was a significant reduction in the number of phloem fibers in response to increased ozone exposure in both germination groups (Table 3).

Last formed tracheids were significantly larger in radial diameter in Giant Forest germinated seedlings grown under ambient and increased ozone conditions. A similar trend without any significant difference can be observed in the Riverside germination group (Table 2). A trend to increased radial tracheid wall thickness with increase exposure to ozone can be inferred from the data presented in Table 2 (tracheid diameter - lumen diameter).

Stem epidermal cells from the elevated ozone Riverside group were significantly smaller in radial diameter, but no differences were observed in the Giant Forest seedlings (Table 4). Radial wall thickness and cuticle thickness was significantly reduced with an increase in ozone exposure in the Giant Forest group. No differences were observed in these two parameters in the Riverside group (Table 4).

Discussion

Grulke et al. (1989) reported decreased photosynthate available in ozone damaged giant sequoia seedlings and an increased respiration demand of tissues to facilitate repair. These studies were conducted using seedlings from the experiment outlined above. The decrease in photosynthate impacted the growth and development of all tissues within the plant resulting in reductions in root weight and height growth and adversely affected the root/shoot ratio (Grulke et al. 1989). Usually,

tissues which are critical to the survival of the plant receive priority in the allocation of carbon for growth (Kramer & Kozlowski 1979). The data derived from the chamber studies do indicate that a reduction in growth occurs in response to ozone fumigation. This can be seen as a reduction in stem diameter, cortical tissue thickness and the diameter of the xylem central cylinder and xylem cells per radial file (Tables 1, 2 and 3). The thickness of the phloem does not appear to be significantly affected (Table 2) despite the significant decrease in the number of phloem cells per radial file in the Giant Grove germinated seedlings grown in filtered air and elevated ozone (Table 3). What is interesting is the significant decrease in the number of phloem fibers in both Giant Forest and Riverside germinated seedlings in response to a 50% increase of ozone.

These data may suggest a re-partitioning of resources to different tissues, favoring the physiological function of the stem over the mechanical requirements. In such a small seedling the mechanical requirements should not be large and could be reduced with a minimum of compromise to the integrity of the biomechanical structure. Resources appears to be moved away from supporting tissues, such as the xylem and the phloem fibers. Phloem fibers function as storage and mechanical support cells and are not necessary to facilitate transport (Esau 1977). The conducting cells of the phloem may be a stronger sink for photosynthate during development since most of the reductions observed in the phloem are not significant between treatments.

Phloem is a living tissue while actively functioning in the transport of photosynthate, where as the xylem is dead when it is physiologically active. Therefore, the phloem has a higher metabolic requirement for photosynthate after differentiation, in addition to the developmental requirement.

The reduction in the number of xylem cells per radial file in response to ozone exposure may be compensated for during development by the production of larger tracheids (Tables 2 and 3). Fewer xylem cells would reduce the efficiency of water transport in the stem. However, an increase in cell lumen diameter would effectively increase the volume of water that could be transported in the stem by effectively reducing resistance to water movement.

The data derived from the epidermal layer is not very clear. There was a significant decrease in the radial epidermal cell diameter in the Riverside seedling group, however, this was not observed in the Giant Forest group (Table 4). Significant decreases were observed in the radial wall thickness and cuticle thickness in the Giant Forest group, but not in the Riverside group. Reductions in the epidermis and cuticle in response to ozone exposure may indicate an increased susceptibility to desiccation and pathogens.

The effectiveness of the physiological and biomechanical function of the seedling stem is critical to survival and establishment within the first year of growth. The stem facilitates the movement of water, minerals and photosynthate

between the roots and canopy. All of these tissues require a share of the reduced available photosynthate as is evidenced by the reduced growth observed in the roots, stem and canopy. Reduced stem vigor in either the physiological or the biomechanical function could contribute to a reduction in the ability of the seedling to establish and survive in the environment.

Unfortunately, seedling mortality data were not collected during the course of this study. However, in consideration of the observed increase in the concentration of ozone in the giant sequoia habitat (Pedersen & Cahill 1989) in combination with increased visible damage to mature trees (Duriscoe & Stolte 1989; Peterson, Arbaugh & Robinson 1989), the effect of ozone on seedling growth and survivorship should not be ignored. Reduced seedling vigor and survivorship in response to air pollution is as important as mature tree mortality in the study of forest decline.

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Table 1. Diameters of stems and central xylem cylinders measured directly below the cotyledon whorl of giant sequoia seedlings exposed to ozone. A mean (n=15) followed by a different letter is significantly different from other means within a column at $p>0.05$.

Treatment	Stem Diameter (mm)		Xylem Cylinder Diameter (mm)	
	Giant Forest	Riverside	Giant Forest	Riverside
Filtered	0.87 A	1.37 A	0.29 A	0.52 AB
Ambient	0.86 A	1.40 A	0.32 A	0.59 A
Ambient + 50%	0.83 A	1.22 B	0.27 A	0.45 B

Table 2. Thickness of cortical and phloem tissues and radial diameters of tracheid cells and lumens measured directly below the cotyledon whorl of giant sequoia seedlings exposed to ozone. A mean followed by a different letter is significantly different from other means within a column at $p>0.05$.

Treatment	Cortex (mm)		Phloem (um)		Tracheid Diameter (um)		Lumen Diameter (um)	
	Giant Forest	Riverside	Giant Forest	Riverside	Giant Forest	Riverside	Giant Forest	Riverside
Filtered	0.27 A	0.43 A	53.7 A	88.1 A	7.2 B	7.9 A	3.0 A	3.2 A
Ambient	0.27 A	0.40 AB	59.0 A	85.8 A	8.8 A	9.4 A	4.1 A	4.2 A
Ambient + 50%	0.27 A	0.36 B	50.2 A	92.2 A	9.3 A	8.9 A	4.0 A	3.9 A

Table 3. Number of cells per radial file in the xylem and phloem and the number of phloem fibers measured directly below the cotyledon whorl of giant sequoia seedlings exposed to ozone. A mean followed by a different letter is significantly different from other means within a column at $p>0.05$.

Treatment	# of Xylem Cells		# of Phloem Cells		# of Phloem Fibers	
	Giant Forest	Riverside	Giant Forest	Riverside	Giant Forest	Riverside
Filtered	8.5 AB	16.0 AB	8.5 B	14.6 A	23.9 AB	141.4 AB
Ambient	9.5 A	18.1 A	10.5 A	14.6 A	41.8 A	178.4 A
Ambient + 50%	7.6 B	14.7 B	7.8 B	14.2 A	15.3 B	101.4 B

Table 4. Epidermal cell radial diameter, radial cell wall thickness and cutical thickness measured directly below the cotyledon whorl of giant sequoia seedlings exposed to ozone. A mean followed by a different letter is significantly different from other means within a column at $p>0.05$.

Treatment	Epidermal Cell Size (um)		Radial Cell Wall Thickness (um)		Cutical Thickness (um)	
	Giant Forest	Riverside	Giant Forest	Riverside	Giant Forest	Riverside
Filtered	17.0 A	23.1 A	4.7 A	4.2 A	2.6 B	3.9 A
Ambient	19.2 A	24.1 A	4.0 AB	4.4 A	3.4 A	3.9 A
Ambient + 50%	17.7 A	16.6 B	3.6 B	4.4 A	2.5 B	3.9 A