

Gas Exchange Rates and Ascorbate and Glutathione Concentrations of *Pinus strobus* Needles Exposed to Ambient Ozone

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대기의 오존에 의한 스트로브 잣나무 잎의 가스교환과 아스코르브산, 글루타치온의 농도 변화

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ABSTRACT

Gas exchange rates and concentrations of ascorbate and glutathione were measured in needles of eastern white pine (*Pinus strobus*) trees differing in foliar sensitivity to ambient oxidant pollution during a ten month period beginning in mid-June, 1988. Current-year needle dry mass and length was 60 to 75% and 45 to 60% less, respectively, in sensitive trees than in a tolerant tree. Net photosynthesis (P_n) and needle conductance (g_n) were greatest in the tolerant individual through late September when the rates begin to decline in trees. Needle transpiration rates showed a trend similar to P_n and g_n . Ascorbate and total glutathione concentrations in current-year needles increased through the summer and fall, reached a maximum in mid-winter, and then decreased in the spring. Consistently throughout the year, ascorbate concentration was highest in the tolerant tree until the initial springtime decline began in April. The difference in needle ascorbate between the tolerant and sensitive individuals was greater in the summer months (25 to 30%) than in the winter months (8 to 19%). Glutathione content was similar, as was the ratio of oxidized/reduced glutathione, in both tolerant and sensitive trees.

Key words : Air pollution, *Pinus strobus*, Gas exchange rates, Ascorbate, Glutathione.

INTRODUCTION

Eastern white pine (*Pinus strobus* L.) is among the most ozone sensitive coniferous tree species indigenous to North America (Berry 1961, Berry and Ripperton 1963). Characteristic symptoms of injury include shortened needle length, reduced needle retention, chlor-

osis, mottling, banding and needle tip necrosis (Hayes and Skelly 1977, Blanchard *et al.* 1979). In native white pine populations, considerable genotypic variation in response to ambient oxidant concentrations has been reported (Mann *et al.* 1980, Benoit *et al.* 1982, Anderson *et al.* 1988), however, the basis for this difference in sensitivity (tolerance) has not yet been established.

Exposure of eastern white pine to ozone has resulted consistently in an inhibition of net photosynthetic rate (Barnes 1972a, Botkin *et al.* 1972, Yang *et al.* 1983a, Boyer *et al.* 1986). The severity of inhibition, in grafted scions (branches), was concentration dependent (Yang *et al.* 1983a) and correlated with the field sensitivity of the parent trees to oxidant stress (Yang *et al.* 1983b). Reich (1987) has concluded that primary ozone tolerance among plant species is related to inherent stomatal conductance and the associated control over pollutant flux into the leaf interior. However, there is no information available to support stomatal mechanism for tolerance in genotypes of white pine.

Plant antioxidant systems have the potential to react with ozone and thereby modify pollutant tolerance. The pseudocyclic electron transport pathway, especially the ascorbate-glutathione components (Halliwell 1982, Salin 1988) has the greatest capacity to interact with ozone or secondary, toxic oxygen radicals (Giamalva *et al.* 1983, Pryor 1984). An increase in needle ascorbate in several pine species after fumigation (Barnes 1972b) supports the involvement of antioxidants in the stress response to ozone. However, further investigations are necessary to establish the importance of such systems in relation to ozone metabolism and detoxification.

The present study was initiated to examine the role of gas exchange functions and selective needle antioxidant compounds as mechanisms associated with differential oxidant sensitivity in eastern white pine. As part of this research, young trees, exposed to ambient air pollution in a plantation setting, were monitored for net photosynthesis, transpiration, needle conductance (H_2O) rates, and for ascorbate and glutathione concentrations during a ten month period. Two of these saplings were used subsequently as parental grafting stock for future chamber fumigation studies. This report summarizes results obtained from field observations on the sapling trees.

MATERIALS AND METHODS

Two eastern white pine trees, differing in sensitivity to ambient oxidant pollution based on foliar characteristics, were selected for intensive physiological and biochemical studies and as source material for developing clonal grafts. The sensitive genotype displayed short needles and tip necrosis with slight chlorotic mottling late in the growing season, whereas the tolerant genotype maintained long, uniformly green needles. An additional sensitive tree was selected for periodic measurements of needle gas exchange rates. The tolerant tree carried 40 to 45% of the last year's needle flush through the winter while the sensitive trees shed the year's needle flush in early fall. Saplings were eight to ten years old

and the two primary study specimens were growing within 15 m of each other in a landscape /Christmas tree plantation in Montgomery County, Virginia, U.S.A. The second sensitive tree was separated by about 30 m from the tolerant tree. The seed source for the plantation was wild type, collected from five mountainous counties in western Virginia. Approximately 10% of the population in the plantation had foliar symptoms similar in appearance to the sensitive study specimens. Gas exchange rates of needles were measured periodically in the field, commencing in the late spring of 1988 and continuing through April, 1989. A Li-Cor 6200 Portable Photosynthesis System (Li-Cor Inc., Lincoln, NE, U. S.A.) was used to determine net photosynthesis (P_n), transpiration (T_r) and needle conductance to water vapor (g_n) using a 250 ml cuvette. All gas exchange rates were calculated on a needle dry mass basis.

Measurements were obtained from detached fascicles within 2 min after removal from the tree, and were made during midday, under full sun, when possible, at photosynthetic photon flux densities (PPFD) of generally 1,000 to 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 1). Air temperature and relative humidity within the cuvette varied with season: ambient CO_2 concentrations displayed a summertime depression (Table 1). Ambient ozone levels were obtained from a rural field monitoring station located approximately 20 km from the plantation. Concentrations (1200 to 1800 h EST) were highest (70 to 85 nl l^{-1}) during measurement periods throughout the summer, declined abruptly in October, 1988 and began to increase again in March, 1989 (Table 1). Typical gas exchange rates were estimated from measurements on five replicate fascicles from each needle age group on the tolerant and sensitive trees at each sampling interval.

Ten fascicles from current-year needles of each tree were randomly collected in

Table 1. Environmental conditions between June, 1988 and April, 1989 in the gas exchange cuvette during field measurements of eastern white pine needles

Date	PPFD ¹ ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Temperature ($^{\circ}\text{C}$)	RH (%)	CO_2 ($\mu\text{l l}^{-1}$)	Ozone ² (nl l^{-1})
June 14	1,547	39	28	355	85
June 28	1,390	34	25	331	72
Aug. 10	1,070	45	30	311	83
Aug. 25	1,257	39	34	318	70
Sept. 7	1,522	33	28	311	59
Sept. 21	1,395	35	28	307	61
Oct. 5	1,121	25	26	314	32
Oct. 26	1,091	22	18	335	39
Nov. 29	1,202	19	22	377	23
Jan. 18	768	22	16	388	43
Feb. 10	1,479	4	18	371	32
Mar. 17	1,476	28	25	344	58
Apr. 17	1,638	30	30	378	66

¹ Photosynthetic photon flux density (400 to 700 nm)

² Mean hourly concentrations from 12:00 to 18:00 h EST

October, 1988 for dry mass and length measurements. Needles were oven-dried at 65°C for 48 h prior to measuring dry mass.

The tolerant and primary sensitive tree was sampled at monthly intervals for needle ascorbate and glutathione analysis. Tissue (500 mg) was frozen in liquid N₂ prior to grinding in a chilled mortar and pestle. The samples were further homogenized in 3 ml of cold 6% metaphosphoric acid (pH 2.8) using a polytron at maximum speed for 30 s. Homogenates were centrifuged at 30,000 × g for 10 min (4°C) and the resulting supernatants were filtered through a 0.45 μm ultrafilter. Total ascorbate (reduced plus oxidized) was analyzed in 1 ml of acid extract after addition of dithiothreitol (30 mmol final concentration) and incubation at room temperature (22°C) for 24 h. Acid extracts were stored at -20°C prior to chromatographic analysis.

Ascorbate was measured at 245 nm using a high performance liquid chromatograph (Spectra Physics model 770) equipped with a variable wavelength UV/visible detector. Separation was made using a 250 × 4.6 mm Partisphere C₁₈ octadecyl reverse-phase column (Whatman) protected with a 40 × 4.6 mm Pellicar anion exchange guard column (Whatman) and a 250 × 4.6 mm Solvecon pre-column (Whatman). The eluant was aqueous 2% NH₄H₂PO₄ (pH 3.0) at a flow rate of 1 ml min⁻¹ and a column pressure of 1,800 psi. Peak areas were calculated using a Spectra Physics System I integrator. A standard curve for ascorbate (50 to 800 μg ml⁻¹) was made using L-ascorbic acid (Fisher Scientific, 99.2% pure) in metaphosphoric acid.

Total and reduced glutathione concentrations in needle tissue were analyzed in acid extracts according to the procedure of Brehe and Burch (1976). Extracted samples were diluted with 5% Na₂HPO₄ (pH 7.0) prior to assay at 412 nm using an LKB Ultraspec II (model 4050 UV/visible) spectrophotometer.

RESULTS

Prior to the initiation of gas exchange measurements, needle tipburn was evident on the oxidant sensitive trees. This injury was characteristic of ozone-induced necrosis on eastern white pine and approximately 15 to 20% of the needle tip tissue was killed. Two ozone episodes occurred before June 14, 1988 during which hourly concentrations ranged between 75 and 115 nl l⁻¹ from 1200 to 1800 h EST. Concentrations in excess of 90 and 100 nl l⁻¹ occurred for 25 and 12 h, respectively, which represented 59 and 28% of the total hours between 1200 and 1800 h during the episodes.

In addition to needle injury, fascicle length and dry mass differed between the sensitive and the tolerant trees (Table 2). Dry mass and length in current-year needles was approximately 60 and 45% less, respectively, in the primary sensitive tree than in the tolerant specimen. An even greater comparative dissimilarity in current-year needle dry mass (75%) and length (59%) occurred between the secondary sensitive and tolerant trees.

Table 2. Mean fascicle dry mass and length in oxidant sensitive and tolerant eastern white pine trees during the 1988 growing season (n=10).

Tree class / needle age	Dry mass (mg)	Length (cm)
Tolerant		
current (1988)	61.2±6.5 ^a	9.88±0.47 ^a
1-yr-old (1987)	54.3±8.7	9.22±1.12
Sensitive (p) ^b		
current	24.6±6.0	5.39±0.09
1-yr-old	26.5±5.7	6.37±1.51
Sensitive (s) ^b		
current	15.6±2.4	4.07±0.75
1-yr-old	20.3±0.4	4.91±0.64

^a represents one standard deviation

^b p denotes primary sensitive tree, s denotes secondary sensitive tree

Comparable differences occurred in one-year-old needles also. When gas exchange measurements were initiated on June 14, P_n in current-year tolerant needles ($39 \text{ nmol g}^{-1} \text{ s}^{-1}$) was about 50% greater than in primary sensitive needles ($21 \text{ nmol g}^{-1} \text{ s}^{-1}$). Net photosynthesis in both individuals continued to increase through mid-September, although the rate was always greater in the tolerant tree (Fig. 1A). When P_n begin to decline in early October and then rise again in mid-March, CO_2 exchange rates were similar between the two trees. In one-year-old needles, P_n was generally lower than in the younger flush, although tolerant needles maintained a higher rate than sensitive needles. Net photosynthesis in the secondary sensitive tree was exceedingly low ($4 \text{ nmol g}^{-1} \text{ s}^{-1}$) when measurements were first made in June (Table 3). By October, P_n in this specimen had attained rates comparable to the tolerant tree and net CO_2 exchange was similar in all three individuals in mid-March. The sensitive trees shed the one-year-old flush by early November, whereas the tolerant tree carried more than 40% of one-year-old needles through the winter.

Stomatal conductance in current-year needles also was 50% greater in the tolerant tree compared to the primary sensitive tree at the initiation of gas exchange measurements on June 14 (Fig. 1B). A difference in g_s continued until early September when the rate begin to decline in both individuals, paralleling the decrease in P_n . Conductance in both trees again began to increase in March, following the pattern of P_n . Stomatal conductance in the secondary sensitive tree was only half that of the tolerant tree in mid-June (Table 3); however, by early fall, g_s was similar in the two trees. Through the fall and into spring, g_s was somewhat higher in the secondary sensitive individual.

Needle transpiration was approximately 20% greater in current-year needle of the tolerant tree in mid-June, and this difference continued until mid-August (Fig. 2). Transpiration rates were similar in both trees throughout the remainder of the measurement period.

Ascorbate concentration in current-year needles was about $1,000 \mu\text{g gfw}^{-1}$ in mid-June and increased through the autumn months to a winter maximum of 3,000 to $3,500 \mu\text{g gfw}^{-1}$ (Fig. 3A). At all sampling period, more than 95% of the ascorbate was in the reduced form. By early April, ascorbate content began to decrease again toward summer levels. Consistently throughout the year, ascorbate concentration was highest in the tolerant tree until the initial wintertime decline began in April (Fig. 3B).

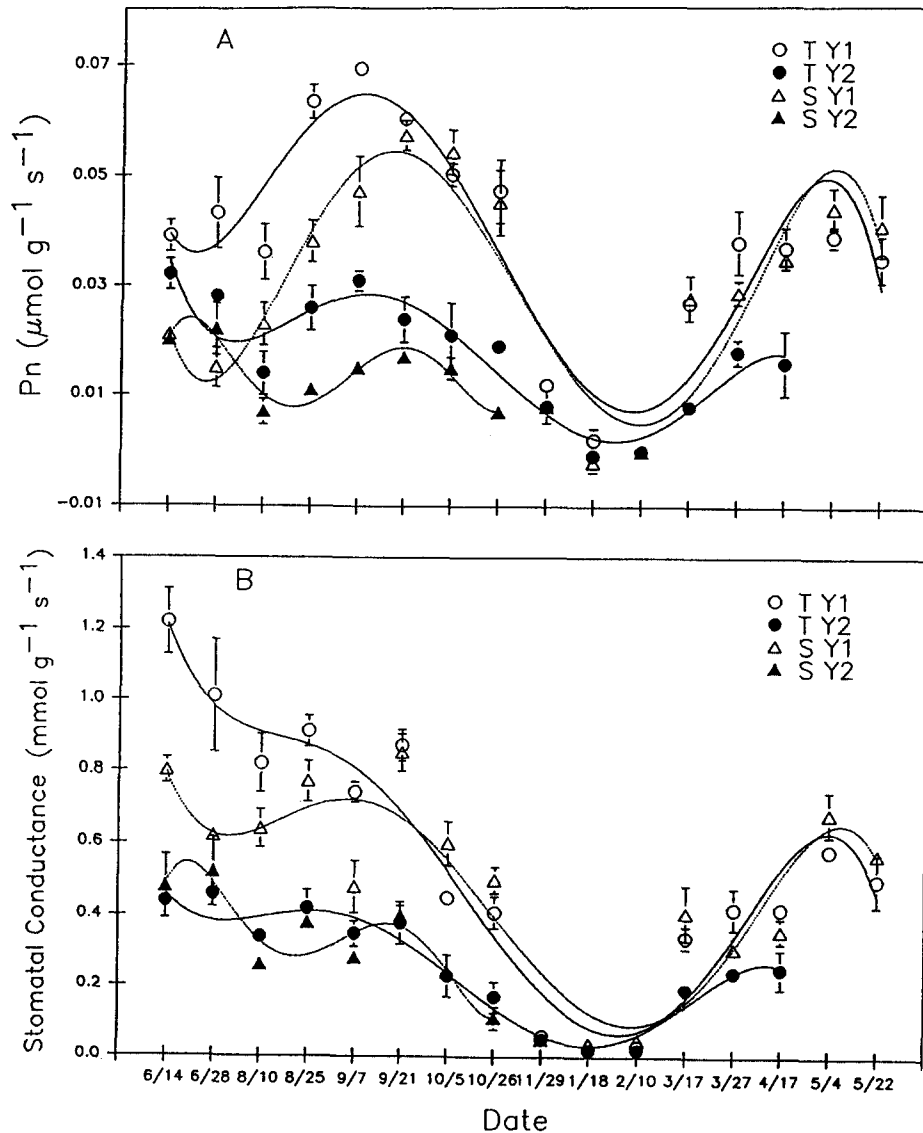


Fig. 1. Net photosynthesis (A) and stomatal conductance (B) in oxidant tolerant and sensitive eastern white pine trees during the 1988 growing season and through the 1988~89 winter months. Error bars are one standard deviation of the mean ($n=5$) and, where not apparent, are contained within the symbol. TY1, TY2, SY1, SY2 represent current-year and year-old needles in the tolerant (T) and sensitive (S) tree, respectively.

The difference in needle ascorbate between the two trees was greater in the summer months (25 to 30%) than in the winter months (8 to 15%).

Total needle glutathione content generally increased through the summer and fall, reaching a maximum in January of approximately $300 \text{ nmol gfw}^{-1}$ (Fig. 4A, B). The in-

Table 3. Net photosynthesis ($\text{nmol g}^{-1} \text{s}^{-1}$) and stomatal conductance ($\text{mmol g}^{-1} \text{s}^{-1}$) to water vapor in current-year needles of eastern white pine trees displaying different foliar sensitivities to ambient oxidant pollution.

Tree Class	Jun. 14	Sep. 21	Oct. 26	Jan. 18	Mar. 17
Tolerant					
P_n	39 ± 2.8^a	60 ± 2.7	47 ± 5.6	2 ± 2.1	27 ± 1.2
g_s	1.22 ± 0.092	0.87 ± 0.044	0.41 ± 0.044	0.02 ± 0.012	0.34 ± 0.032
Sensitive (p) ^b					
P_n	21 ± 1.0	57 ± 2.7	45 ± 5.8	-2 ± 1.8	28 ± 4.1
g_s	0.80 ± 0.036	0.85 ± 0.052	0.50 ± 0.036	0.04 ± 0.002	0.41 ± 0.076
Sensitive (s) ^b					
P_n	4 ± 0.7	49 ± 3.5	42 ± 4.5	1 ± 1.4	24 ± 2.3
g_s	0.68 ± 0.147	0.90 ± 0.081	0.54 ± 0.055	0.10 ± 0.011	0.43 ± 0.059

^a represents one standard deviation

^b p denotes primary sensitive tree, s denotes secondary sensitive tree

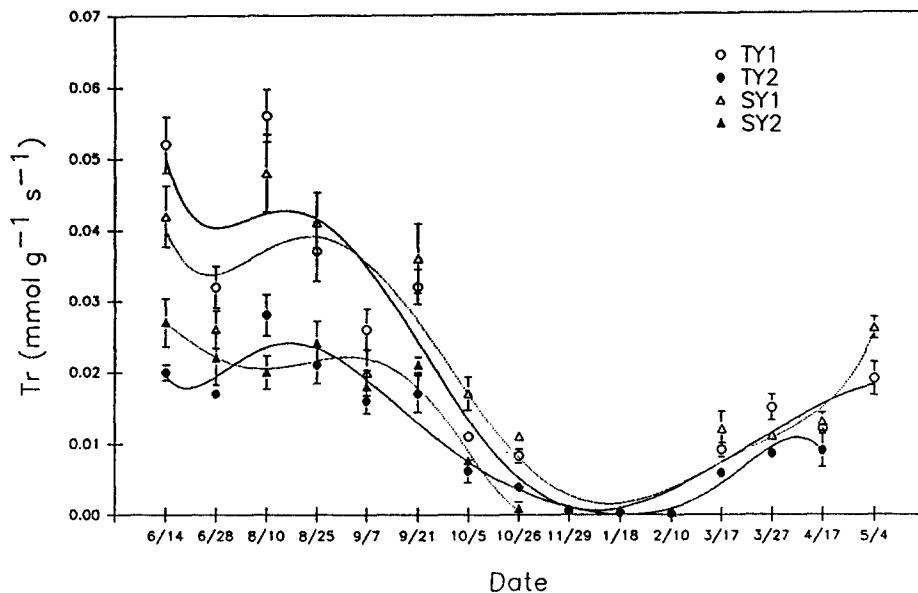


Fig. 2. Transpiration rates in oxidant tolerant and sensitive white pine trees during the 1988 growing season and through the 1988~89 winter months. Figure legends are as in Fig. 1.

crease was more uniform in the tolerant tree, especially during the August through December period, than in the sensitive tree. By March, glutathione concentration had decreased to near the summer minimum of 50 to 100 nmol gfw^{-1} . Except in young tissue (June and July), reduced glutathione (GSH) was the predominate form and comprised 85 to 95% of the total glutathione present in the needles (Fig. 4A, B). There were no apparent differences in glutathione, either oxidized or reduced, between the tolerant and sensitive trees.

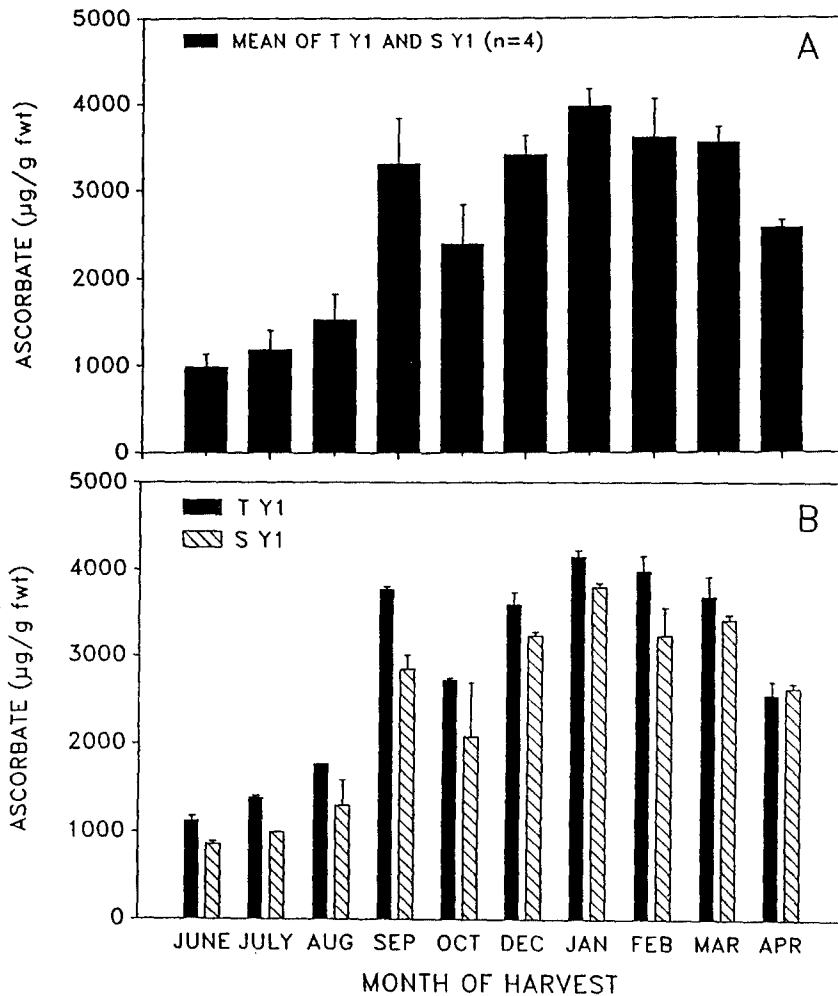


Fig. 3. Monthly needle ascorbate concentrations in oxidant tolerant and sensitive eastern white pine trees as a mean of both genotypes(A) or individually in each tree(B). Error bars represent one standard deviation.

DISCUSSION

Oxidant sensitive and tolerant eastern white pine trees were selected as study specimens based on foliar characteristics (Berry 1961, Benoit *et al.* 1982, Anderson *et al.* 1988). Tipburn, short needle length and premature needle senescence were evident on the sensitive trees, whereas long, uniformly green needles were present on the tolerant tree. While these characteristics are indicative of differential sensitivity to ozone, controlled experiments in fumigation chambers and open-top field chambers are necessary to confirm that a variation in ozone response exists between the genotypes. Such studies are

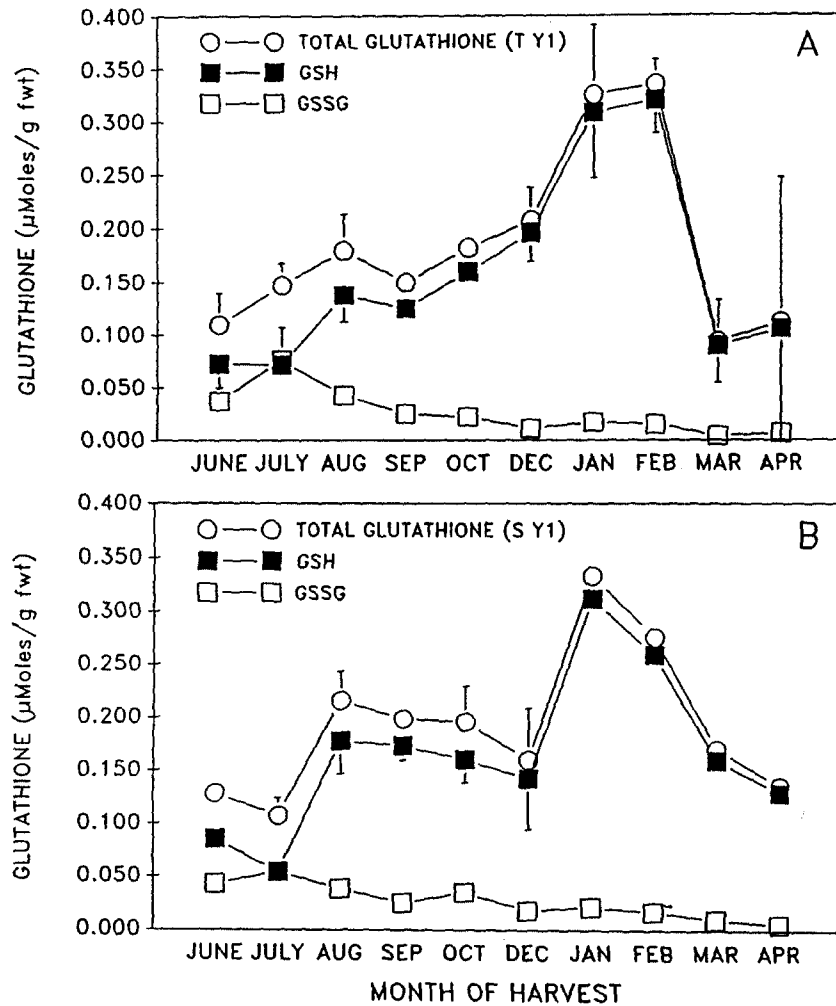


Fig. 4. Monthly needle concentrations of total, oxidized (GSSG) and reduced (GSH) glutathione in oxidant tolerant (A) and sensitive (B) eastern white pine trees ($n=2$). Error bars represent one standard deviation and, where not apparent, are contained within the symbol. TY1, SY1 represent current year needles in the tolerant and sensitive tree, respectively.

presently in progress.

Ozone exposure for several days or weeks can cause a reduction in P_n in eastern white pine (Barnes 1972a, Botkin *et al.* 1972, Yang *et al.* 1983a,b). Hourly ambient concentrations during gas exchange measurement periods in June and August averaged 70 to 85 nl l^{-1} and when intense ozone episodes occurred in late June and early July, hourly concentrations were 100 to 120 nl l^{-1} . Such ozone levels were sufficient to inhibit P_n in sensitive white pine genotypes in controlled environment fumigations (Yang *et al.* 1983b).

Throughout most of the growing season, current-year needles of the tolerant tree

maintained the highest P_n and g_n . Coyne and Bingham (1981, 1982) reported that stomatal conductance (g_s) and gross photosynthesis in one- and two-year old needles of ozone tolerant ponderosa pine exceeded rates measured in ozone sensitive trees. However, during early growth of current-year needles, g_s in sensitive pines was greatest and may have contributed initially to more severe ozone damage in this ecotype. In all needle age classes, maximum gross photosynthesis was always highest in tolerant trees. Whether ambient ozone concentrations in early June, prior to the initiation of gas exchange measurements, affected g_n in the developing needles of sensitive eastern white pine is not known. However, when ozone levels declined in September and October, gas exchange rates in all trees were similar, suggesting no permanent impairment of needle physiology as was observed in ponderosa pine (Coyne and Bingham 1982).

The maintenance of a high P_n and g_n , and presumably a greater ozone uptake rate, in the tolerant tree indicates a more effective mesophyll capacity to withstand oxidative stress compared to the sensitive trees. The needle concentration of the antioxidants, ascorbate and glutathione, increased through the autumn months in both tolerant and sensitive trees. The increase in glutathione normally occurs in temperate climate conifers (Esterbauer and Grill 1978) and may be associated with the winter hardening process. Throughout most of the year, ascorbate concentrations were greatest in the tolerant tree, and comparative differences between tolerant and sensitive study specimens were maximum during June, July and August when ozone concentrations were highest. An increase in ascorbate was observed in several pine species after ozone fumigation (Barnes 1972b) and the ascorbate content in the tolerant tree may be associated with a reduced sensitivity to ozone in this genotype. The low antioxidant concentrations in young tissue may be related to the phenomenon of emergence of tipburn in eastern white pine, a condition that is considered to result from ozone exposure (Berry and Ripperton 1963).

A difference in glutathione content between the tolerant and sensitive tree was not observed. However, oxidized ascorbate is enzymatically reduced in the chloroplast with glutathione as the immediate electron donor (Halliwell 1982, Salin 1988). The results suggest that in eastern white pine under oxidative stress, endogenous glutathione levels are sufficient to reduce an increased amount of oxidized ascorbate, even though chloroplast glutathione concentration normally is 1 to 5 mM, whereas ascorbate content is 25 to 40 mM (Salin 1988). Hence the flux of reducing equivalents required to maintain reduced glutathione may be greater in the tolerant tree. An alternative explanation is that an increase in glutathione reductase activity occurs in needles of the tolerant tree and thereby maintains a high ratio of reduced to oxidized glutathione to accommodate the higher ascorbate concentration.

적 요

대기의 오존농도에 대한 민감도가 다른 스트로브 잣나무의 가스교환과 아스코르브산, 글루타치온의 농도변화를 1988년 6월부터 시작하여 10개월간 측정하였다. 오존에 의해 가시적 피해를 입은 나무의 당년잎의 건중량과 길이는 저항성이 있는 나무에 비해 각각 60~75%와 45~60% 작았다. 순광합성량과 잎의 전도율은 전체적으로 가스교환이 감소하기 시작하는 늦은 9월까지 저항성이 있는 나무에서 높은 값을 보였다. 증산작용도 비슷한 양상을 보였다. 당년잎의 아스코르브산과 글루타치온 농도는 여름, 가을동안 계속 증가하여 겨울에 최고치를 나타내다가 봄에 감소하기 시작했다. 아스코르브산 농도는 4월에 감소하기 시작할 때까지 일년 내내 저항성이 있는 나무에서 높은 농도를 나타내었다. 예민한 나무와 저항성이 있는 나무사이의 아스코르브산 농도차이는 겨울동안보다 (8~19%) 여름에 (25~30%) 훨씬 컸다. 글루타치온의 농도는 예민한 나무와 저항성이 있는 나무에서 비슷한 결과를 보였다.

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