

**Laboratory and Field Ecophysiological Studies
on the Impact of Air Pollution on Red Spruce and Fraser Fir**

by

Piotr Bohdan Tyszko

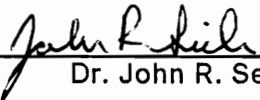
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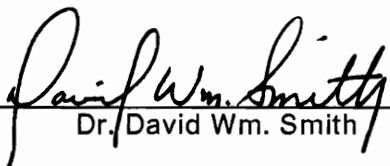
Dr. John R. Seiler



Dr. Boris I. Chevone



Dr. Peter P. Feret



Dr. David Wm. Smith



Dr. Shepard M. Zedaker

June, 1991

Blacksburg, Virginia

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(ABSTRACT)

Three studies were performed to investigate the impact of air pollution on high-elevation red spruce-Fraser fir forests in the Southern Appalachians.

In the first study, red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* (Pursh.) Poir.) seedlings were submitted to long-term (2.5 yrs), multiple growing cycle (4 and 5, respectively), intermittent ozone fumigations (0.025, 0.070, and 0.150 ppm). No effect of ozone exposure on growth and gas exchange of the seedlings was found. Net photosynthesis at saturating light intensity was reduced in both species and the light compensation point was shifted upwards in spruce when exposed to ozone. Fraser fir seedlings showed inconsistent responses of CO₂ curve parameters to ozone exposure. There were indications that ozone exposure modified cell wall modulus of elasticity in both species.

In the second study, the impact of summer exposure to ambient pollutants on winter hardiness in red spruce seedlings was examined. The seedlings were subjected to the following summertime treatments while kept in exclusion chambers on the top of Whitetop Mountain (Virginia): ambient air and clouds, ambient air with clouds excluded, charcoal filtered air, and chamberless control treatment. During the following winter the seedlings were placed in Blacksburg (Virginia), in two locations: in the open and in a shadehouse. A number of conducted tests indicated that there

were significant differences in winter damage between the chamber treatments and chamberless control, as well as between the winter exposure locations. Among the summer chamber exposure regimes, the treatment excluding clouds seemed to perform the best (although not all the evidence supports the latter statement).

In the third study, the physiology of red spruce trees of various sizes (seedlings, saplings, and overstory trees), growing on two sites on the top of Whitetop Mtn., was compared and related to ambient ozone concentration. Some seedlings were treated with an antioxidant EDU, to help evaluate the impact of ozone on their physiology. The trees of various sizes showed clear differences in gas exchange, with overstory trees photosynthesizing at the lowest rates, and seedlings - at the highest. Overstory trees also showed more negative shoot water potential and higher night respiration than smaller tree sizes. No deleterious effects of ambient ozone on red spruce physiology were detected.

Dedication

In memory of my mentor, Dr. Włodzimierz Zelawski.

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Introduction

The local point-source damage caused by industrial pollution to forests have been known since the 19th century. However, it was as late as the 1970's when scientists realized that atmospheric pollution had become a global problem. Much concern about the effects of air pollution on forests appeared in the early 1980's, when widespread weakening of tree condition was discovered in "clean air areas" in West Germany. Later, extensive forest damage symptoms were reported in other countries, particularly in Switzerland, France, East Germany, Czechoslovakia, Poland, and the United States (Prinz 1987).

There is a general consensus among scientists that air pollution is a contributing factor in many observed cases of forest decline, although there is still uncertainty as to the mechanisms of its influence on trees, and the role of particular pollutants. The problem is also complicated by natural ecological processes, such as aging of forest stands, that can be difficult to distinguish from the possible effects of pollutants.

Sulphur dioxide was the first pollutant indicted for its negative influence on vegetation. However, the symptoms of forest decline occurred also in the

areas where the concentration of SO₂ was too low to be toxic to plants. Other primary pollutants, such as HF, NO_x, and heavy metals, were found to be detrimental to forest vegetation only in close proximity to the pollution sources (Krause *et al.* 1986). Therefore, the attention of researchers was turned towards secondary pollutants, such as acid rain and ozone. Extensive studies on acid rain have not allowed scientists to conclude that its effects on forest vegetation are detrimental. In contrast, acid rain has often been shown to be beneficial to tree growth (Morrison 1984). There is little evidence for the negative impact of the acid rain on forests, and most scientists agree that this is not the major factor causing the observed damage (Prinz 1987). In some circumstances (acidic, poor soils) acidic deposition may contribute to mineral deficiency (Ulrich 1989). A more prominent role has been recently ascribed to acid mist, particularly in interaction with other pollutants (Rehfuss 1986).

Another secondary pollutant which drew researchers' attention was ozone. Most authors agree that ozone causes some damage to vegetation; however, on the basis of the existing evidence (see below), the hypothesis that ozone is the major cause of forest decline, does not seem plausible.

A decline has been found in the high elevation red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* (L) Mill.) forests in the northern Appalachians. Symptoms include the dieback of tree tops and branch tips, decreased radial growth, and increased mortality (Woodman and Cowling 1987). The causes of the decline are unclear and may vary from site to site, not to mention regional differences. Some researchers assume that this phenomenon is a natural process resulting from the aging of practically even-aged stands (Zedaker *et al.* 1987, Hornbeck *et al.* 1986). Others attribute the cause of the decline to unfavorable weather conditions that occurred in the 1960s and 1970s

(Johnson *et al.* 1988). Some authors point out inherent weaknesses of red spruce-balsam fir high elevation stands: poor organic soils (Huntington and Ryan 1988), past disturbances (Weiss and Millers 1988) and resulting diminished population size and genetic resources (Eckert and O'Malley 1988).

Another factor indicted is ozone, the concentrations of which in the affected area are supposed, by some authors, to be harmful to red spruce and balsam fir (McLaughlin 1985). Ozone may act either directly on metabolism or by impairing cold tolerance (Cumming *et al.* 1988).

High elevation coniferous forests in southern Appalachians also show considerable mortality (Dull *et al.* 1988). Decline in growth-trends during past 20 years is reported to be similar to that reported for the northern Appalachians (Adams *et al.* 1985). The history of disturbances (logging, fires - Pielke 1980) is also similar and so is expected growth decline resulting from the aging of practically even-aged stands (Zedaker *et al.* 1985). Southern spruce and fir forests suffer from pests and pathogens, most notably balsam woolly adelgid (*Adelges piceae* Ratz.) killing Fraser fir (*Abies fraseri* (Pursh) Poir.) forests in the Smokies and Black Mountains (USDA 1989). Red spruce trees in West Virginia are largely infested with cytospora canker (*Valsa kunzei* Fr.) (Mielke 1988). Also ozone levels reported from southern Appalachians may be harmful to forest trees (Chevone 1986, Lefohn 1990); however, little is known about susceptibility of these forests to ozone stress.

Many studies have been conducted to quantify the impact of ozone on plants, but the results of research are inconclusive and sometimes contradictory (Pye 1988). There are considerable differences between the results of some studies, even those done by the same scientist (e.g. Kress and Skelly 1982). The research outcomes have been found to depend heavily on species

(Reich and Amundson 1985) and even family (Hanson *et al.* 1988). Another possible source of discrepancies in the literature is the variable and often short length of exposure. Significant ozone effects may result from long-term exposure rather than from fumigations lasting no longer than one vegetative growth period. Timing of the ozone exposure may also be crucial for detecting ozone effects, with elongation and bud set being, in general, the most sensitive periods for tree growth. Heck *et al.* (1984b) noticed that changes in the growth rate of agricultural crops subjected to ozone fumigations are most likely to occur when the plants are exposed to pollutants during the early vegetative growth phase. In addition to the problems mentioned above, there are many species that have not been investigated sufficiently, including red spruce and Fraser fir.

Relatively few laboratory studies on the effects of ozone on growth and gas exchange of red spruce and Fraser fir had been published when this research was initiated. Ten weeks of noncontinuous exposure to 0.1 ppm ozone by Lee *et al.* (1988) resulted in decreased growth of red spruce seedlings, but failed to modify net photosynthesis. In contrast, Taylor *et al.* (1986) found a significant increase in root weight after fumigation with 0.12 ppm ozone. The study investigating influence of ozone exposure on growth and gas exchange of Fraser fir seedlings by Tseng *et al.* (1987) did not report any significant effects after 10 weeks of noncontinuous fumigations. However, unpublished data suggested that fumigating seedlings for more than one growth cycle may be needed before a significant growth effect occurs (Tseng 1987). The inconsistencies among the results of the previous studies suggested the need for multiple-growth cycle ozone exposure experiments.

The studies lasting for more than one growth cycle, which were conducted at the end of the eighties, did not confirm the hypothesis that ambient ozone is

harmful to red spruce (Laurence *et al.* 1989, Kohut *et al.* 1990, Thornton *et al.* 1990, Thornton *et al.* 1991).

Establishing the possible role of ozone in forest decline is complicated by our insufficient knowledge of the mechanisms of its influence on plant organisms. Several hypotheses have been put forward. Most of these assume ozone action via free radicals. The latter may interfere with photosynthetic enzymes (eg. ribulose -1,5-bisphosphate carboxygenase, Pell and Pearson 1983), or may damage cell membranes thus impairing ion equilibrium and other physiological functions (Dietz *et al.* 1988). There is evidence showing that ozone fumigated plants may develop abnormal chloroplasts (Sutinen 1987). However, none of the hypotheses explaining the mechanism of ozone's influence on plants has been shown to fully explain the damage caused by ozone in the field.

Drawing conclusions about natural forest ecosystems from the results of previous controlled ozone exposure experiments is very difficult. The environment in fumigation chambers only approximates the natural environment, and ozone concentrations used were often much greater than those measured in the ambient atmosphere. Also, most of the studies were short-term experiments, whereas the damaged forests have been exposed to polluted air for many years. Significant ozone effects may result from long-term exposures rather than from fumigations lasting no longer than one growing season. Therefore, there is a need for long-term studies on the impact of ozone on natural forest ecosystems.

In an attempt to overcome at least some of the above difficulties in investigating the impact of ozone on red spruce and Fraser fir forests in Southern Appalachians, three complementary studies were designed. The field

ecophysiological study monitored gas exchange parameters in red spruce trees of various size, growing in a natural environment. The purpose of this study was to learn about natural patterns of gas exchange and to help in scaling up from seedling to bigger trees. In the field winter hardiness experiment, the influence of ambient pollutants on winter hardiness of red spruce seedlings was investigated, using open-top chambers located in a red spruce natural habitat. Finally, a multiple growth cycle laboratory ozone exposure experiment was designed to examine possible impact of ozone on growth and physiology of red spruce and Fraser fir seedlings growing in a controlled environment. The three studies addressed the issue of the impact of ambient pollutants on spruce-fir forests in Southern Appalachians in a comprehensive manner. They attempted to treat the examined problem in a broader context of forest ecophysiology. A sequence of a laboratory controlled exposure experiment, a field exposure experiment, and a field ecophysiological monitoring study, was meant to help to remediate artificiality of environment in laboratory research on one hand, and lack of control over environmental conditions in the field studies on the other.

STUDY OBJECTIVES

The general purpose of this study is to examine whether ozone may be a significant factor in the decline of red spruce - Fraser fir high elevation stands in the Appalachians. The research consisted of three studies: a laboratory ozone fumigation experiment, a field winter hardiness experiment, and a field ecophysiological study.

The objective of the laboratory fumigation study was to determine the impact of ozone over several growing cycles on the growth and physiology (net photosynthesis, transpiration, needle conductance) of Fraser fir and red spruce seedlings.

The objective of the winter hardiness study was to determine the impact of summer pollutant exposure in the field, on winter hardiness of red spruce seedlings.

The objectives of the field study were:

1. To compare the physiology (net photosynthesis, dark respiration, transpiration, stomatal conductance, and water potential) of red spruce trees of various age (seedlings, saplings, and mature trees). Measurements were taken in a natural habitat of spruce, over three growing seasons.

2. To evaluate the physiological response of field grown seedlings sprayed with ethylenediurea (an anti-oxidant inducing chemical).

3. To determine the effect of ambient ozone exposure on the measured physiological parameters in red spruce seedlings, saplings, and mature trees.

Chapter I

EFFECTS OF LONG-TERM OZONE FUMIGATIONS ON GROWTH AND GAS EXCHANGE OF RED SPRUCE AND FRASER FIR SEEDLINGS

A B S T R A C T

Red spruce and Fraser fir seedlings from two seed sources in the Southern Appalachians (Mt. Mitchell, North Carolina, and Mt. Rogers, Virginia) were subjected to long-term (2.5 yrs) intermittent ozone fumigations (0.025, 0.070, and 0.150 ppm), while being grown through several growth cycles, in an accelerated growing regime. Following each of 4 (spruce) and 5 (fir) growing cycles, gas exchange parameters and dry weights were determined.

The ozone fumigations did not produce any effect on seedling growth in either species. The fumigation effects on gas exchange parameters were inconsistent. There was no correlation between photosynthetic rates and seedlings growth. This study highlights the need for long-term experiments on the impact of air pollution on forest trees.

INTRODUCTION

The high elevation red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* (Pursh.) Poir.) forests in the Southern Appalachians have been found to suffer growth decline (Adams *et al.* 1985) and considerable mortality (Dull *et al.* 1988) over the past few decades. Causes of the decline are often unclear and may vary from site to site. The factors implicated include pests and pathogens (USDA 1989, Mielke 1988), natural processes of aging (Zedaker *et al.* 1987), long-term climatic changes (Hamburg and Cogbill 1988), extremely cold winters (Johnson *et al.* 1988), past disturbances (Pielke 1980), and air pollution, notably ozone (USDA 1989, Chevone *et al.* 1986). Although ozone is thought to have the potential for damaging red spruce and Fraser fir forests in the Southern Appalachians, little is known about susceptibility of these populations to ozone damage.

Many studies have shown that ozone in ambient concentrations may modify growth and gas exchange characteristics of forest trees, but the results are often inconclusive and sometimes contradictory (Pye 1988). The research outcomes have been found to depend heavily on species (Reich and Amundson 1985) and even family (Hanson *et al.* 1988). Another possible source of dis-

crepancies in the literature is the variable and often short length of exposure. Significant ozone effects may result from long-term exposure rather than from fumigations lasting no longer than one vegetative growth period. Timing of the ozone exposure may also be crucial for detecting ozone effects, with elongation and bud set being, in general, the most sensitive periods for tree growth. Heck *et al.* (1984b) noticed that changes in the growth rate of agricultural crops subjected to ozone fumigations are most likely to occur when the plants are exposed to pollutants during the early vegetative growth phase. In addition to the problems mentioned above, there are many species that have not been investigated sufficiently, including red spruce and Fraser fir.

Relatively few laboratory studies on the effects of ozone on growth and gas exchange of red spruce and Fraser fir had been published when this experiment was initiated. Ten weeks of noncontinuous exposure to 0.1 ppm ozone by Lee *et al.* (1988) resulted in decreased growth of red spruce seedlings, but failed to modify net photosynthesis. In contrast, Taylor *et al.* (1986) found a significant increase in root weight after fumigation with 0.12 ppm ozone. The study investigating influence of ozone exposure on growth and gas exchange of Fraser fir seedlings by Tseng *et al.* (1987) did not report any significant effects after 10 weeks of noncontinuous fumigations. However, unpublished data suggested that fumigating seedlings for more than one growth cycle may be needed before a significant growth effect occurs (Tseng 1987). The inconsistencies among the results of the previous studies suggested the need for multiple-growth cycle ozone exposure experiments.

The present study involved fumigating red spruce and Fraser fir seedlings with ozone during bud break, elongation, and bud set for four (spruce) and five (fir) growth cycles. The seedlings were grown using an accelerated growth re-

gime allowing the completion of one growth cycle in about six to seven months. The influence of ozone exposure on the growth and physiology of the seedlings was examined after the completion of each growth cycle. Seedlings from two seed sources were examined: Mt. Mitchell, and Mt. Rogers, both located in the Southern Appalachians. The spruce-fir forest on Mt. Mitchell (North Carolina) is in a state of decline, apparently caused by infestation of Fraser fir trees by balsam woolly adelgid (*Adelges piceae*, USDA 1989). Spruce and fir trees on Mt. Rogers (Southwest Virginia) appear to be in good health (Zedaker *et al.* 1987).

In addition, the possibility of seedlings acclimating to ozone fumigations (Wellburn, pers. comm.) was evaluated. For the last growing cycle, a sample of control plants was subjected to 0.15 ppm ozone exposure, while a sample from the previous 0.15 ppm treatment was put under the control regime.

M E T H O D S

Plant material.

Half-sib (wind-pollinated) Fraser fir and red spruce seeds from two sources (Mt. Mitchell and Mt. Rogers) were stratified for 1 month and sown into 160 cc tubes (Cone-Tainer Nursery, Canby, Or.) filled with soil collected from Mt. Rogers (high-elevation organic soil). After emergence, some seedlings were transplanted or removed so that the number of seedlings per tube did not exceed three. After the first growth cycle (see below) only one seedling per tube was left.

Growing conditions

Seedlings were grown in an accelerated growing regime (Seiler and Kreh 1986, 1987) which allows the completion of one growth cycle in approximately 6-7 months. During bud break and elongation, seedlings were kept under a 16-hour photoperiod in a greenhouse environment with charcoal filtered air. The ozone fumigations began when the seedlings started breaking bud and continued under the long day conditions for six weeks. Seedlings were then subjected to an additional four weeks of intermittent ozone exposure while being exposed to an 8-hour photoperiod and decreasing temperatures in order to induce bud formation. After bud set (approximately 1 month), the seedlings were transferred to a dark cold room (2°C) or to a shadehouse (in winter) for six weeks. After the chilling treatment, seedlings were moved back to the greenhouse under a 16-hour photoperiod and following bud break were subjected to the next cycle of ozone fumigation. Beginning with the second growth cycle, seedlings were fertilized every week during the long day regime with 10 ml of 200 ppm N, 87 ppm P and 166 ppm K, supplied as 20-20-20. With five growth cycles for fir, and four growth cycles for spruce completed, the study lasted 30 and 23 months, respectively.

Treatment exposure

Seedlings were exposed to three ozone levels (0.025 - control, 0.07, 0.15 ppm) in Continuously Stirred Tank Reactors (CSTR's, Heck *et al.* 1978), three times a week, for four consecutive hours. Ozone was generated from oxygen, using a UV ozone generator (Model T-408, Welsbach Ozone Systems Corp., Philadelphia, PA). All chambers were supplied with charcoal filtered air.

Fumigations lasted ten weeks during bud break, elongation, and bud set (six weeks under long days and four weeks under short days). Due to equipment limitations only one CSTR per treatment per species was available. At every exposure, particular ozone levels were schematically assigned to different chambers. This was done to avoid confounding the effects of ozone treatments with the effects of individual chambers.

The 0.07 ppm ozone concentration approximated ozone levels occurring in the Southern Appalachians during warm and dry summers (USDA 1989). The 0.15 ppm ozone treatment roughly corresponded to the ozone concentrations occurring in the cities of the Eastern United States during severe episodes. Over the entire experiment red spruce seedlings received doses of 14.3 ppmhrs (0.025 ppm treatment - control), 34.6 ppmhrs (0.07 ppm treatment), and 70.8 ppmhrs (0.15 ppm treatment), whereas the Fraser fir seedlings received 19.4 ppmhrs, 44.5 ppmhrs, and 89.1 ppmhrs, in the respective treatments.

In order to evaluate possible acclimation to ozone exposure, a sample of 10 seedlings from the control treatment were exposed for the last last growth cycle to 0.15 ppm ozone fumigations. At the same time a sample of 10 seedlings from the 0.15 ppm treatment were exposed to control conditions.

Measurements

Following each growth cycle, two days after the last fumigation in the cycle, a sample of seedlings was harvested. Gas exchange parameters were measured using a LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska). Seedlings were clipped at the root collar and immediately put into the quarter-liter cuvette. Five replicates of two to three seedlings per replicate

were taken initially. Beginning with the fourth (fir) and third (spruce) harvest only one plant per replicate was used. Average temperature, photosynthetically active radiation, and relative humidity in the leaf cuvette were recorded during consecutive harvests (Table 1).

Leaf area of fir seedlings was determined using a LI-COR 3000 portable area meter (except for the third harvest). Spruce leaf area was obtained by measuring width and length of needles on a representative sample (30-40% of all needles) with a caliper (after the first and second cycles only). Dry weights of roots, stems, and needles were also determined after drying samples for 48 hours at 65°C.

Data analysis.

This study consisted of two parallel experiments: the one using Fraser fir and the other using red spruce seedlings. Each of the experiments was analyzed as a completely randomized two-way factorial design with two seed sources (Mt. Mitchell and Mt. Rogers) and three ozone levels (0.025, 0.07, 0.15 ppm). Individual chamber effects were averaged by rotating the treatments among the chambers. Five measurement replicates were taken per each ozone treatment and seed source combination.

The following parameters were analyzed: needle, shoot, root, and total dry weight, root:shoot ratio, net photosynthesis, transpiration, needle conductance, and water use efficiency (WUE). The latter was calculated as a ratio of needle conductance to net photosynthesis. Data were analyzed using analysis of variance to detect significant differences among the responses of seed sources to

Table 1. Temperature, photosynthetically active radiation (PAR), and relative humidity (RH) in the leaf cuvette during consecutive harvests in the multiple-cycle ozone fumigation study (means and standard deviations).

Species	harvest	date	temperature	PAR	RH
		(mo/day/yr)	(°C)	($\mu\text{Em}^{-2}\text{s}^{-1}$)	(%)
SPRUCE	1	11/17/88	27.0±0.7	580±9	10.1±1.5
	2	05/30/89	28.9±1.0	490±41	40.2±8.3
	3	11/27/89	25.3±0.3	391±22	47.8±4.8
	4	07/02/90	31.0±1.5	480±12	38.4±6.6
FIR	1	03/25/88	30.6±0.3	328±4	41.3±2.4
	2	09/28/88	30.4±0.5	508±11	34.3±4.7
	3	05/23/89	26.3±0.2	553±10	38.2±1.9
	4	11/23/89	25.9±0.6	363±15	42.8±5.9
	5	07/02/90	29.5±1.0	460±7	29.3±4.2

ozone treatments. Duncan's multiple range test at the 0.05 probability level was used to separate the response means.

Most of the measured variables were not affected by the seed source. Therefore, where appropriate, the seed sources were pooled for the analysis of ozone effects.

R E S U L T S

Growth

Total dry weights of red spruce seedlings exposed to various levels of ozone were almost identical at the last harvest (Table 2). Dry weights of fir seedlings also did not differ significantly (although the highest ozone treatment did have substantially greater biomass). None of the intermediate harvests produced significant differences among the ozone treatments in either species total dry weight. The total dry weight showed considerable random variability. Dry weights of foliage, stems, roots, or root:shoot ratio of both species also remained unaffected by the ozone exposure.

The seed source had no effect on the total dry weights; however, at the last harvest, the root:shoot ratio was significantly different between provenances of Fraser fir seedlings (Mt. Rogers: 0.64, Mt. Mitchell: 0.73).

Gas exchange

The seed source had no effect on measured gas exchange parameters.

Table 2. Total dry weights of red spruce and Fraser fir seedlings subjected to multiple-cycle ozone fumigations (means and standard deviations at intermediate and final harvests).

Total dry weight (g) by species and ozone treatment						
harvest	Red spruce			Fraser fir		
	0.025 ppm	0.070 ppm	0.150 ppm	0.025 ppm	0.070 ppm	0.150 ppm
1	.036±.013	.034±.011	.038±.010	.049±.012	.050±.009	.045±.012
2	.080±.031	.075±.030	.083±.015	.101±.031	.088±.010	.083±.012
3	.346±.433	.148±.087	.354±.554	.125±.020	.142±.029	.135±.016
4	.592±.362	.587±.255	.591±.296	.305±.278	.221±.089	.329±.178
5	-	-	-	.785±.471	.650±.224	.904±.623

No statistically significant differences due to ozone exposure at any harvest ($p=0.05$).

Table 3. Net photosynthesis of red spruce seedlings subjected to multiple-cycle ozone fumigations, expressed on dry weight and area basis (means and standard deviations in ozone treatments at consecutive harvests).

harvest	Photosynthesis/unit dry weight			Photosynthesis/unit area		
	0.025 ppm	0.070 ppm	0.150 ppm	0.025 ppm	0.070 ppm	0.150 ppm
	(nmol CO ₂ /s g)			(nmol CO ₂ /s m ²)		
1	28.3±10.1	46.5±28.7	34.5±13.6	351±113	549±281	467±196
2	26.0±6.3	25.2±7.1	24.9±6.7	325±95	319±81	306±82
3	53.7±13.7	52.0±11.7	42.3±20.9	-	-	-
4	27.0±10.0	30.9±10.4	35.0±5.2	-	-	-

No statistically significant differences due to ozone exposure at any harvest ($p=0.05$).

Table 4. Net photosynthesis of Fraser fir seedlings subjected to multiple-cycle ozone fumigations, expressed on dry weight and area basis (means and standard deviations in ozone treatments at consecutive harvests).

harvest	Photosynthesis/unit dry weight			Photosynthesis/unit area		
	0.025 ppm	0.070 ppm	0.150 ppm	0.025 ppm	0.070 ppm	0.150 ppm
	(nmol CO ₂ /s g)			(nmol CO ₂ /s m ²)		
1	52.0±5.5a	45.2±6.2b	44.1±7.5b	816±121a	704±9.9b	721±118b
2	14.7±6.3ab	18.3±6.5 a	11.7±2.8b	429±222 ab	541±246 a	311±75 b
3	26.2±8.5	24.0±10.1	29.9±6.8	536±323	429±182	498±89
4	48.0±18.8ab	57.0±10.8a	43.9±7.6b	-	-	-
5	43.1±12.5	46.4±8.7	53.6±30.3	1120±393	1153±230	1435±968

Means followed by different letters are significantly different (Duncan's multiple range test, $p=0.05$).

Therefore, seed sources were pooled for the analysis of ozone effects on gas exchange.

Photosynthetic rates of red spruce seedlings did not differ significantly among treatments (Table 3). The patterns of photosynthetic rates shown by Fraser fir seedlings during consecutive harvests were rather erratic (Table 4). At the first harvest the highest rates were found in the control treatment, at the second and fourth harvest - in the intermediate treatment (0.07 ppm). At the third and fifth harvests the photosynthetic rates were not significantly different. The unusually low photosynthesis of the Fraser fir seedlings at the second harvest was probably linked to nutrient depletion of the soil. Fertilization started only during the second needle flush and apparently it was too late to prevent occurrence of moderate foliage chlorosis. The seedlings fully recovered during later growth cycles as shown by both the photosynthetic and growth rates (Tables 2 and 4).

The needle conductance of red spruce seedlings did not differ among the treatments, except for the second harvest, where the conductances in the 0.15 ppm ozone treatment were significantly lower than in the other regimes (Table 5). Low leaf conductance at the first harvest was probably caused by very low relative humidity during measurements (merely 10%). Water use efficiency in spruce seedlings did not differ among treatments, except during the third harvest, where the 0.15 ppm treatment produced numbers significantly lower than those of the control regime (Table 5).

The needle conductances of Fraser fir seedlings were not significantly different among ozone treatments (Table 6). Conductances tended to be lower on the harvest dates where the seedlings showed the lowest photosynthetic rates (harvest 2 and 3), while higher photosynthesis was accompanied by higher

Table 5. Needle conductance and water use efficiency in red spruce seedlings subjected to multiple-cycle ozone fumigations (means and standard deviations in ozone treatments at consecutive harvests).

harvest	Needle conductance			Water Use efficiency		
	0.025 ppm	0.070 ppm	0.150 ppm	0.025 ppm	0.070 ppm	0.150 ppm
	(nmol H ₂ O/s g dry wt.)			(mmol CO ₂ /mol H ₂ O)		
1	279±185	275±64	235±69	3.67±1.13	3.89±0.69	3.88±0.66
2	434±66a	446±74a	362±65b	2.50±0.76	2.36±0.72	2.64±0.63
3	602±177	695±156	675±82	4.70±1.12a	3.78±1.14ab	3.04±1.61b
4	520±250	551±225	720±150	1.95±0.38	2.00±0.39	1.83±0.42

Means followed by different letters are significantly different (Duncan's multiple range test, p=0.05).

Table 6. Needle conductance and water use efficiency in Fraser fir seedlings subjected to multiple-cycle ozone fumigations (means and standard deviations in ozone treatments at consecutive harvests).

harvest	Needle conductance			Water Use efficiency		
	0.025 ppm	0.070 ppm	0.150 ppm	0.025 ppm	0.070 ppm	0.150 ppm
	(nmol H ₂ O/s g dry wt.)			(mmol CO ₂ /mol H ₂ O)		
1	923±130	768±169	1455±1820	2.97±0.31a	2.88±0.47a	2.33±0.80b
2	314±78	372±72	309±42	1.38±0.29ab	1.49±0.43a	1.14±0.29b
3	280±99	269±48	314±84	4.76±1.24	3.96±1.32	4.50±0.44
4	871±468	1000±348	774±218	3.22±1.11	3.03±0.71	3.15±0.92
5	556±160	593±115	674±309	2.73±0.27	2.63±0.14	2.76±0.26

Means followed by different letters are significantly different (Duncan's multiple range test, $p=0.05$).

conductances (harvests 1, 4, and 5). Water use efficiency of fir seedlings differed significantly among treatments only during first two harvests. The pattern was similar to that of the photosynthetic rates, with 0.15 ppm ozone treatment showing the lowest WUE value. The differences in photosynthesis determined largely the WUE pattern, since the needle conductance was generally similar across treatments in both species.

Neither the control seedlings treated with 0.15 ppm ozone or 0.15 ppm treatment seedlings subjected to control treatment for the last growth cycle showed significantly different gas exchange parameters or growth from the other seedlings harvested at the end of the experiment.

DISCUSSION

The growth of red spruce and Fraser fir seedlings remained unaffected by ozone exposure lasting for four and five growing cycles, respectively. This contradicts the findings of Lee *et al.* (1988), who reported suppression of growth in red spruce seedlings exposed to intermittent ozone fumigations for ten months. Also the suggestion of Tseng (1987) that Fraser fir seedlings could be susceptible to ozone exposure over several growth cycles was not confirmed.

Most of the published research indicates that ozone exposure can increase assimilate retention in leaves (Tingey *et al.* 1976, Barnes 1972, McLaughlin *et al.* 1982). Interestingly, Reich *et al.* (1987) found that the lowest root:shoot ratio occurred in an intermediate ozone treatment, while the lowest (0.02 ppm) and

the highest (0.14 ppm) ozone treatment produced higher root:shoot ratios. In this study no effect of ozone exposure on assimilate allocation was found.

The air pollution literature concerning other tree species presents a wide range of photosynthetic responses to ozone exposure, although negative effects seem to prevail (Pye 1988). This study did not show significant effects of ozone exposure on net photosynthetic rates of the red spruce and Fraser fir seedlings. This result agrees with the findings reported by Lee *et al.* (1988) and Tseng *et al.* (1987).

Ozone was reported to both stimulate (Keller and Haesler 1984), and suppress (Vogels *et al.* 1986) leaf conductance in Norway spruce. Tseng *et al.* (1987) did not detect any effects of ozone exposure on leaf conductance in Fraser fir seedlings. In the present study no clear effect of ozone on leaf conductance was shown in either Fraser fir or red spruce. Also, water use efficiency did not seem to be affected by treatments in either species.

The switching of the treatments during the last fumigation cycle (a sample of control seedlings receiving ozone treatment and a sample of ozone treatment seedlings exposed to charcoal filtered air) did not produce any growth or gas exchange effects. Thus, it seems that the apparent resistance of the seedlings to the fumigations was due rather to inherent ozone tolerance than to acclimation to ozone exposure. Measurements made during and after fumigations (data not shown) showed that ozone exposure could temporarily suppress gas exchange; however, seedlings were able to recover between the fumigations. There are indications that this gas exchange suppression could be due partly to the exposure chamber effect.

The phenotypic appearance of Fraser fir and red spruce on Mt. Mitchell and Mt. Rogers indicates varying degree of environmental stress in both lo-

cations. Mt. Mitchell coniferous stands are severely affected by balsam wooly adelgid infestation of Fraser fir trees, while Mt. Rogers forests apparently remain healthy (USDA 1989, author's observation). Both ecosystems are subjected to relatively high concentrations of ozone, which may have potential to increase their susceptibility to other stresses (Lefohn *et al.* 1990, Chevone *et al.* 1986). The present study did not reveal any differences between seed sources in the response of the red spruce and Fraser fir seedlings to ozone. Thus, it seems that factors other than differential ozone susceptibility are contributing to the differences in forest health in both locations.

The results of this study do not indicate that red spruce and Fraser fir seedlings are susceptible to ambient level ozone fumigations. The resistance of red spruce seedlings to ambient ozone damage has also been confirmed by open-top chamber studies conducted at Boyce-Thompson Institute in Ithaca, New York (Laurence *et al.* 1989 and Kohut *et al.* 1990), and on Whitetop Mountain, Virginia (Thornton *et al.* 1990). The mentioned above studies used intermittent exposure regimes. In this experiment, due to intermittent exposure, cumulative exposure was rather low, if compared with the actual exposure in the field. The seedlings received a total exposure of from 17 ppmhrs (control) to 87 (0.15 ppm) ppmhrs, while in the natural spruce habitat, on Whitetop Mountain, trees typically receive 30 to 40 ppmhrs every month during the growing season (Lefohn *et al.* 1990). Nevertheless, this study provides valuable information on sensitivity of the examined seedlings to ozone exposure at ambient concentrations.

The outcomes of this study also draw our attention to several problems associated with the methodology of air pollution research. First, they indicate that the conclusions based on a single growth cycle experiment may be mis-

leading; different conclusions concerning the impact of ozone on photosynthesis of Fraser fir seedlings could have been drawn depending on the harvest date considered. An important argument in support of long-term, multiple growth cycle experiments is the possibility of changes in ozone sensitivity with plant's age and physiological status. Second, the statistical significance did not seem to reflect the biological significance. It often showed random character, flip-flopping among the treatments on consecutive harvest dates. Third, the differences in photosynthetic rates did not translate into differences in growth. The uncoupling of growth and photosynthesis effects is fairly common in the literature (Reich *et al.* 1986, Reich *et al.* 1987). Lee *et al.* (1988) found decreased growth of red spruce seedlings subjected to ozone fumigations, but no change in photosynthetic rates. This leads us to another conclusion: this study confirmed that instantaneous photosynthetic rates are not necessarily a reliable measure of plant's response to environmental factors. The growth performance is in any case the ultimate test of plant's performance.

C O N C L U S I O N

This study may help understand at least some of the discrepancies encountered in the literature dealing with the impact of air pollutants on forest trees. It stresses the need for long-term, multiple growth cycle experiments in air pollution research. The hypothesized negative impact of tropospheric ozone on red spruce and Fraser fir high-elevation forests in the Southern Appalachians was not confirmed.

Chapter II

EFFECTS OF LONG-TERM OZONE FUMIGATIONS ON PHOTOSYNTHESIS AND WATER RELATIONS OF RED SPRUCE AND FRASER FIR SEEDLINGS

A B S T R A C T

Red spruce and Fraser fir seedlings from two seed sources in the Southern Appalachians (Mt. Mitchell and Mt. Rogers) were subjected to long-term (2.5 yrs) intermittent ozone fumigations (0.025, 0.070, and 0.150 ppm) while being grown over several growth cycles. After completion of four (spruce) and five (fir) growing cycles, CO₂ and light photosynthesis response curves and pressure-volume curves were obtained to characterize the impact of ozone exposure on the photosynthetic apparatus and seedling water relations.

The effects of ozone fumigations on the measured parameters were not consistent across species and seed sources. Net photosynthesis at saturating light was reduced in both species (in spruce significantly) and light compensation point was shifted upwards in spruce only. Fraser fir seed sources showed differing responses in CO₂ curve parameters. There were indications that ozone exposure modified the cell wall modulus of elasticity in both species, however it was not a consistent response.

The study indicates a potential for some ozone impact on photosynthetic apparatus and seedling water relations. It is unclear, however, whether these effects can translate into any significant growth reduction under ozone concentrations occurring in the Southern Appalachians.

INTRODUCTION

The impact of ambient ozone on metabolism of forest trees has been widely studied and was reviewed recently by Pye (1988). However, few researchers have gone beyond instantaneous measurements and attempted a more in-depth analysis of photosynthesis and water relations of ozone-treated trees.

Photosynthesis light and carbon dioxide response curves can supply a wealth of information concerning the status of the assimilatory apparatus. This information could be particularly useful in case of chronic exposure to air pollutants, since point measurements of gas exchange may fail to reflect subtle changes in metabolism.

The photosynthetic response of ozone-treated loblolly pine seedlings to varying levels of CO₂ was analyzed by Sasek and Richardson (1989). The authors found that the ozonated seedlings showed lower initial slope (carboxylation efficiency) and lower CO₂ saturated carbon exchange rates than the control seedlings. The stomatal limitations, however, were not different. In an open-top chamber study by Taylor and Dobson (1989), beech (*Fagus silvatica* L.) seedlings were grown either in ambient air, or in charcoal-filtered air (the ambient air contained, among other pollutants, ozone). There were no differences in carboxylation efficiency or in the percent stomatal limitation, although the seedlings exposed to ambient air showed higher CO₂-saturated net photosynthesis. Thus, the response of photosynthetic characteristics to air pollution may depend on the exposure regime and tree species.

Photosynthetic light response curves in ozone treated loblolly pine seedlings were found to show lower light saturated photosynthesis than in control plants (Sasek and Richardson 1989, Hanson *et al.* 1988). Sasek and Richardson (1989) also reported a decrease in quantum yield efficiency upon exposure to ozone.

Apart from gas exchange, another diagnostically relevant function of a vascular plant are its water relations. There are indications that ambient ozone may interact with the plasmalemma and/or cell wall, thus changing turgor-related properties of mesophyll cells (Laisk 1990, Castillo *et al.* 1987, Halliwell 1981). Modification of the cell wall could increase the bulk elasticity modulus, making cell walls more rigid (Laisk, pers. comm.). Altered metabolism and plasmalemma functions may change osmotic relations. Ozone exposure has also been found to modify cell solute concentrations (Rehfuess and Ziegler 1986, Tingey *et al.* 1976).

The potential of ozone to modify tree water status has not been widely studied. Roberts and Cannon (1989) actually found an increase in red spruce seedling water potential upon fumigation with ozone. Barnes *et al.* (1990) found a reduction in bulk elasticity modulus in ozone fumigated Norway spruce seedlings; however, turgor and osmotic potentials were not modified significantly. Apparently, more research is needed to clarify the potential impact of ozone on tree water relations.

The objective of this study was to examine the influence of multiple-cycle ozone fumigations on the photosynthetic response to light and CO₂, and on water relations of red spruce and Fraser fir seedlings.

METHODS

Ozone exposure

Fraser fir and red spruce seedlings were subjected to multiple-cycle ozone fumigations in Continuously Stirred Tank Reactors at the Air Pollution Laboratory in Blacksburg, Virginia (see Chapter I of this dissertation for detailed description of the study). Two seed sources of spruce and fir were examined: Mt. Rogers (southwest Virginia) and Mt. Mitchell (western North Carolina). Five growth cycles lasting 30 months for fir, and four growth cycles lasting 23 months for spruce were completed. The seedlings were exposed to 0.025, 0.07, and 0.15 ppm ozone, three times a week, for four hours. Red spruce seedlings received ozone doses of 14.3 ppmhrs (control), 34.6 ppmhrs (0.07 ppm treatment), and 70.8 ppmhrs (0.15 ppm treatment). Fraser fir seedlings received doses of 19.4, 44.5, and 89.1 ppmhrs, respectively.

Photosynthetic response curves

All photosynthetic measurements were made using a LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska). The measurement dates and number of replicates are specified in Table 1. A 400 W high-pressure sodium vapor lamp (Lucalox LU400, General Electric) was used as a light source. Seedlings were allowed to acclimate for at least one hour under the light. A water filter was placed between the lamp and leaf chamber in order to prevent the cuvette temperature from rising. Uncut seedlings were inserted into leaf chamber, in some cases (larger seedlings) single branches were used.

Carbon dioxide/photosynthesis response curves were obtained using a method similar to that described by McDermitt *et al.* (1989) and Davis *et al.* (1987). Ambient CO₂ concentration was raised to about 1300 ppm by breathing into the leaf cuvette, and then gradually lowered to the compensation point, first by mixing in outside air, then, at below-ambient concentrations, with the analyser's soda lime scrubber. At various carbon dioxide concentrations measurements of net photosynthesis, and ambient as well as intercellular CO₂ (C_i) concentration were taken. Seedlings were allowed to acclimate for 1 to 1.5 minutes at every CO₂ level before a measurement was taken. It took about 15 to 20 minutes to produce data for one C_i curve consisting, on average, of 9 points. Average environmental conditions in the cuvette were: photosynthetically active radiation (PAR) $920 \pm 43 \mu\text{M}/\text{m}^2 \times \text{s}$, relative humidity $34.8 \pm 5.6\%$, and the chamber temperature $32.8 \pm 0.9 \text{ C}$.

The resulting internal and external CO₂ response curves were used to calculate several relevant parameters: carboxylation efficiency (CE), taken as the slope of the initial, linear portion of C_i curve; net photosynthesis at 350 ppm ambient CO₂ ($P_{S_{350}}$); CO₂ compensation point (CCP), which is a C_i level where

Table 1. Time schedule and number of measured seedlings (replicates) per ozone treatment and seed source combination in the photosynthetic and water relations diagnostics study of the impact of ozone fumigations on red spruce and Fraser fir.

procedure	Red spruce		Fraser fir	
	dates	replicates	dates	replicates
	(mo/day)		(mo/day)	
Last O ₃ exposure	6/30	n/a	7/1	n/a
Ps light curves	7/14	2	7/9,12	4
Ps CO ₂ curves	7/3,4	4	7/7,8	4
Pressure-volume curves	7/23	2 or 3	7/13	2

net photosynthesis is zero; and stomatal limitation fraction (SL), calculated according to Equation 1 (Sasek and Richardson, 1989):

$$SL = (P_{S_{ci}} - P_{S_{ca}}) / P_{S_{ci}} \quad (1)$$

where $P_{S_{ci}}$ and $P_{S_{ca}}$ are photosynthetic rates at 350 ppm CO₂ concentration, read from the internal and ambient CO₂ curves respectively.

Light/photosynthesis response curves were also made using the LI-6200. Various light intensities were obtained by gradually attenuating the artificial light with multiple layers of grey plastic screen. Maximum (unhindered) PAR level was 1350 $\mu\text{M}/\text{m}^2 \times \text{s}$. Seedlings were allowed to acclimate for 1 to 1.5 minutes at every light level. Relative humidity was kept at $40.0 \pm 3.6\%$, temperature at $34.3 \pm 1.1^\circ\text{C}$, and carbon dioxide concentration was 337 ± 20 ppm (after every photosynthesis measurement outside air was allowed into the system). PAR was monitored using a sensor mounted on the leaf chamber. Each light response curve took between 15 and 20 minutes to complete and was based, on average, on 11 points.

The light response curves were used to calculate the following gas exchange diagnostic parameters: quantum yield efficiency (Q), which is the slope of the initial, linear portion of the curve; light compensation point (LCP), which is the light level at zero net photosynthesis; and net photosynthesis at light saturation ($P_{S_{sat}}$).

The obtained CO₂ and light response curve parameters were analyzed using a two-way analysis of variance to investigate the effects of ozone exposure and seed source. Duncan's multiple range test was used to separate mean responses to ozone levels.

Pressure-volume curves

The free transpiration method, similar to that described in Parker and Pallardy (1987) and Ritchie and Rhoden (1985), was used. Seedlings were watered to field capacity on the previous day and allowed to rehydrate in a dark cabinet, loosely covered with wet paper towels, until the start of measurements (preliminary measurements had shown that the seedlings wrapped tightly with a plastic bag would not fully rehydrate). Shoots were clipped, immediately weighed to determine saturated fresh weight, and fitted in the pressure chamber. After the first measurement, shoots were allowed to desiccate on a laboratory bench and, periodically, fresh weight and water potentials were determined. To avoid possible xylem cavitation the rate of depressurization was kept at 0.01 MPaxs^{-1} .

Inverse water potentials were plotted against relative water content values (RWC). The plots were used to obtain the following parameters:

1. water potential (π_p) and RWC at the turgor loss point (RWC_p)
2. osmotic potential at full hydration (π_0)
3. turgor potential at full hydration (P_{tmax})
4. bulk elasticity modulus, defined as:

$$E = dP_t / dRWC$$

(2)

where:

P_t - turgor potential calculated as difference between a point on the initial, nonlinear portion of the curve and the corresponding point on the extrapolation of the linear portion towards the vertical axis;

RWC - relative water content at the same point.

The calculated modulus of elasticity values were plotted as a function of turgor potential. Linear regression was used for a particular ozone treatments, to investigate the possible impact of ozone on cell wall properties.

The pressure/volume curve parameters were analyzed using a two-way analysis of variance and, where needed, Duncan's multiple range test to separate means. The slopes of E vs. turgor potential regression lines were tested for significance using indicator variable analysis.

R E S U L T S

Carbon dioxide response

There were no significant differences or clear trends in the CO₂ compensation point, carboxylation efficiency, percent stomatal limitations, or photosynthetic rates at 350 ppm CO₂ in red spruce as a result of ozone exposure or various seed sources (Table 2).

The response of Fraser fir seedlings was more complex. There was no significant effect of ozone on the CO₂ curve parameters, as calculated for pooled seed sources. However, the response of individual seed sources to ozone exposure clearly differed (Table 3). The analysis of variance showed that the interaction between seed sources and ozone treatments was significant for carboxylation efficiency. The Mt. Mitchell seedlings increased carboxylation efficiency with increasing ozone concentration, while the Mt. Rogers plants showed an opposite trend. The effects of ozone \times seed source interaction on

Table 2. Carbon dioxide curve parameters in red spruce and Fraser fir seedlings after multiple-cycle ozone fumigations (means and standard deviations).

Species	Seed source	CCP ¹	CE ²	SL ³	<i>P</i> ₃₅₀ ⁴
		(ppm CO ₂)	((μ M g ⁻¹ s ⁻¹ /ppm) $\times 10^{-3}$)	(%)	(μ M/g s)
SPRUCE ⁵	All	86.5 \pm 19.7	0.261 \pm 0.039	54.4 \pm 6.4	0.0312 \pm 0.0063
FIR ⁶	Mt. Mitchell	96.0 \pm 39.1	0.334 \pm 0.077	50.1 \pm 10.7	0.0406 \pm 0.0113
	Mt. Rogers	69.7 \pm 8.2	0.249 \pm 0.084	40.6 \pm 9.0	0.0414 \pm 0.0142
	All	83.4 \pm 31.2	0.293 \pm 0.090	46.0 \pm 11.0	0.0410 \pm 0.0125

Explanations:

¹ CCP - internal carbon dioxide compensation point;

² CE - carboxylation efficiency, i.e. initial slope of the curve;

³ SL - percent stomatal limitations;

⁴ *P*₃₅₀ - photosynthesis at 350 ppm external CO₂;

⁵ parameters not statistically different among spruce seed sources and ozone treatments;

⁶ fir seed sources significantly different, except for *P*₃₅₀ ($p < 0.05$, t-test)

Table 3. Carbon dioxide curve parameters in Fraser fir seedlings from two seed sources, after multiple-cycle ozone fumigations (means and standard deviations).

ozone	CCP ¹		CE ²		SL ³	
	Mt. Mitchell	Mt. Rogers	Mt. Mitchell	Mt. Rogers	Mt. Mitchell	Mt. Rogers
(ppm)	(ppm CO ₂)		((μ M g ⁻¹ s ⁻¹ /ppm) $\times 10^{-3}$)		(%)	
0.025	79.7 \pm 20.2	67.4 \pm 6.6	.286 \pm .052	.303 \pm .099	46.4 \pm 8.3	42.8 \pm 2.5
0.070	84.2 \pm 18.9	69.9 \pm 11.7	.322 \pm .062	.243 \pm .075	48.8 \pm 5.5	42.5 \pm 9.7
0.150	124.0 \pm 57.1	72.7 \pm 6.5	.393 \pm .086	.186 \pm .025	57.6 \pm 15.1	35.2 \pm 13.9

Explanations:

- ¹ CCP - internal carbon dioxide compensation point;
- ² CE - carboxylation efficiency, i.e. initial slope of the curve;
- ³ SL - percent stomatal limitations;

stomatal limitations and compensation point were not statistically significant, nevertheless, these variables showed similar trends to that of carboxylation efficiency (Table 3). Compensation point in Mt. Rogers seedlings remained relatively constant, while in Mt. Mitchell seedlings it shifted upwards in the 0.15 ppm ozone treatment, with a concomitant dramatic rise in variance.

Photosynthetic rates at 350 ppm ambient CO₂ in Fraser fir seedlings tended to be the lowest in the 0.15 ppm ozone treatment.

Light response

In spruce seedlings, both ozone concentrations resulted in the suppression of the light saturated photosynthetic rate (Table 4). The light compensation point was shifted to higher light levels by both ozone treatments, with the difference between the control and 0.15 ppm treatment being statistically significant. The quantum yield efficiency was not affected. There were no significant seed source effects on light response curve parameters.

Fraser fir seedlings did not show any statistically significant effects due to either ozone exposure or seed source on any of the measured variables. However, light saturated photosynthesis and its standard deviation tended to decrease with the ozone dose (Table 4).

Pressure-volume analysis

The pressure-volume analysis parameters showed minimal variability between seed sources; the seed sources were, therefore, pooled within the ozone treatments (Table 5). In Fraser fir seedlings the maximum (saturated) turgor

Table 4. Photosynthesis light response curve parameters in red spruce and Fraser fir seedlings after multiple-cycle ozone fumigations (means and standard deviations).

Species	Ozone	$P_{S_{sat}}^1$	Q^2	LCP ³
	(ppm)	($\mu\text{M/g s}$)	($(\mu\text{M g}^{-1})/(\mu\text{M m}^{-2}) \times 10^{-3}$)	($\mu\text{M/m}^2 \text{ s}$)
SPRUCE	0.025	0.0372 \pm 0.0076 a ⁴	0.118 \pm 0.031	9.6 \pm 15.6 a
	0.070	0.0271 \pm 0.0040 b	0.090 \pm 0.030	21.2 \pm 5.6 ab
	0.150	0.0290 \pm 0.0017 b	0.132 \pm 0.026	30.8 \pm 6.5 b
	All	0.0313 \pm 0.0067	0.112 \pm 0.032	19.6 \pm 13.1
FIR	0.025	0.0481 \pm 0.0181	0.140 \pm 0.046	22.0 \pm 10.1
	0.070	0.0421 \pm 0.0112	0.157 \pm 0.037	21.4 \pm 11.1
	0.150	0.0375 \pm 0.0029	0.144 \pm 0.042	24.1 \pm 12.3
	All	0.0431 \pm 0.0132	0.146 \pm 0.041	22.6 \pm 10.6

Explanations:

¹ $P_{S_{sat}}$ - light-saturated photosynthetic rate;

² Q - quantum yield efficiency (slope of light-Ps curve)

³ LCP - light compensation point;

⁴ The means followed by different letters are statistically different (Duncan test, $\alpha=0.1$)

Table 5. Pressure-volume analysis parameters in red spruce and Fraser fir seedlings after multiple-cycle ozone fumigations (means and standard deviations).

Species	Ozone	π_0^1	P_{tmax}^2	π_p^3	RWC_p^4
	(ppm)	(MPa)	(MPa)	(MPa)	(%)
SPRUCE	0.025	-2.49±0.27	1.78±0.15	-3.04±0.22	85.2±1.6
	0.070	-2.39±0.23	1.74±0.23	-2.99±0.18	85.7±1.5
	0.150	-2.36±0.32	1.73±0.12	-2.85±0.32	87.4±2.1
	All	-2.41±0.26	1.75±0.18	-2.96±0.24	86.1±1.9
FIR	0.025	-1.98±0.07	1.20±0.22 ab ⁵	-2.35±0.14	86.0±1.1
	0.070	-1.99±0.13	1.01±0.31 a	-2.35±0.14	85.8±1.5
	0.150	-2.03±0.05	1.53±0.20 b	-2.34±0.06	86.0±2.6
	All	-2.00±0.08	1.21±0.25	-2.35±0.11	85.9±1.6

Explanations:

¹ π_0 - osmotic potential at full turgor;

² P_{tmax} - maximum turgor potential;

³ π_p - osmotic potential at turgor loss point;

⁴ RWC_p - relative water content at turgor loss point;

⁵ Means with different letters are significantly different (Duncan test, $\alpha=0.05$).

pressure (P_{tmax}) was significantly lower in the 0.07 ppm ozone treatment (1.01 MPa) than in the highest exposure treatment (1.53 MPa); the control seedlings produced an intermediate value (1.20 MPa), which was not statistically different from two other treatments (Table 5). Spruce seedlings did not show any differences among treatments in saturated turgor pressure. Relative water content at the turgor loss point (RWC_p) in spruce was higher in the highest ozone treatment (clearly, albeit insignificantly) than in the control and intermediate treatments. The latter parameter was fairly constant across treatments in fir seedlings. Osmotic potentials at full turgor (π_0) and at turgor loss point (π_p) were not modified by ozone exposure in either species.

The elasticity modulus (E), modelled as a function of turgor potential, showed different patterns for both species (Table 6). In Fraser fir, the control treatment produced the highest regression slope, followed by the intermediate, and then by the high ozone treatment. The difference in the slope between control and the 0.15 ppm ozone treatment was significant at $p = 0.1$. In red spruce the highest ozone treatment showed the highest elasticity modulus values, followed closely by the control treatment. The 0.07 ppm ozone treatment produced clearly the lowest slope, different from control and the highest ozone treatment at $p = 0.12$ and $p = 0.08$, respectively. In both species the data points, however, showed considerable variability around the regression lines and the detected patterns were not easily discernible visually.

Table 6. Slopes of regression lines modelling the relationship between modulus of elasticity and turgor potential values for particular ozone treatments. Also p-values associated with the pairwise comparisons between the slopes.

ozone (ppm)	Red spruce				Fraser fir			
	slope	p-values			slope	p-values		
		control	0.07	0.15		control	0.07	0.15
control	15.38	-	0.12	0.86	16.95	-	0.49	0.10
0.07	12.99	-	-	0.08	15.40	-	-	0.42
0.15	15.69	-	-	-	13.78	-	-	-

DISCUSSION

As described in Chapter I of this dissertation, instantaneous photosynthetic measurements, repeated several times throughout the course of the fumigations, did not show any clear pattern in response to ozone exposure. The measured photosynthesis and water potential diagnostics revealed, however, some interesting trends.

It was found that the responses of the species, and, in some cases, seed sources, were not uniform. The red spruce seedlings did not show any effect of ozone exposure on the carbon dioxide response parameters, but their light response curves were significantly modified. In contrast, Fraser fir seedlings showed only a slight effect of ozone on light saturated photosynthetic rates, and no effect on carbon dioxide response curves, when seed sources were pooled within treatments. However, carbon dioxide response curves in the particular fir seed sources responded differently to ozone exposure: the Mt. Mitchell seedlings tended to increase their carboxylation efficiency, compensation point, and stomatal limitations, while the Mt. Rogers seedlings reduced carboxylation efficiency and stomatal limitations with increasing ozone dose. The variance of compensation point values in Mt. Mitchell seedlings subjected to the 0.15 ppm ozone treatment was higher than in the remaining treatments. This could be attributed to a strong response in some particularly sensitive individuals.

The literature reporting photosynthetic diagnostics in trees subjected to ozone exposure is rather scarce. The suppression of the initial slope of the CO₂ curve in the Mt. Rogers fir seedlings confirms similar findings in loblolly pine (Sasek and Richardson 1989). On the other hand, the latter are contradicted by the stimulation of carboxylation efficiency in the Mt. Mitchell

seedlings. Carboxylation efficiency has been linked to ribulose bis-phosphate carboxylase/oxygenase (RuBPco) activity (von Caemmerer and Farquhar 1981) and ozone has been found to decrease the amount of RuBPco in alfalfa (Pell and Pearson 1983) and hybrid poplar (Reich and Lassoie 1985). The presented results suggest that ozone may either stimulate or inhibit RuBPco activity, depending on the genotype of Fraser fir.

The increase in CO₂ compensation point in the 0.15 ppm Mt. Mitchell fir seedlings probably reflects higher respiration rates in the ozone treated seedlings. The lack of the similar effect in the Mt. Rogers seedlings suggests lower sensitivity of respiration to ozone fumigations and a greater overall tolerance. Validation of this hypothesis would require closer investigation of respiration in the fir seedlings subjected to ozone exposure. No differences in photosynthesis or growth of the two Fraser fir populations were found in Chapter I of this study. It would seem, however, worthwhile to learn more about the physiological differences between the Mt. Rogers and Mt. Mitchell Fraser fir seed sources. The latter population seems to be less resistant to environmental stresses, particularly the balsam wooly adelgid (*Adelges piceae* Ratz.) infestation (USDA 1989, author's observation).

The variable effects (although insignificant) of the highest ozone treatment on stomatal limitations of the particular Fraser fir seed sources reflected trends in the needle conductance (data not shown). Mt. Rogers seedlings treated with 0.15 ppm ozone tended to increase their conductance and, thus, decrease stomatal limitations. Mt. Mitchell seedlings decreased conductance and increased stomatal limitations.

The effects of ozone exposure on the CO₂ response curve parameters in Fraser fir seedlings occurred only at 0.15 ppm ozone concentration. It suggests

that the concentrations occurring in the natural environment of Fraser fir (rarely exceeding 0.1 ppm, Lefohn 1989) should not be toxic for this species.

Reduction in the light-saturated photosynthetic rates in red spruce seedlings (and a similar trend in Fraser fir) is consistent with the reports by Hanson *et al.* (1988) and Sasek and Richardson (1989). Neither of these authors found, however, significant suppression of the light compensation point, as found in this study. Reported by Sasek and Richardson (1989) decrease in the quantum yield efficiency was not observed. Reduced light-saturated photosynthetic rate and increased LCP may result from an increase in respiration induced by ozone stress (Sasek and Richardson 1989, Reich and Lassoie (1985)

The observed effects of ozone on photosynthetic diagnostics of the red spruce and Fraser fir seedlings are not consistent. Neither do they relate to the effects on the instantaneous gas exchange and growth rates (see Chapter I). It seems that the shifting upwards of the light compensation point may have some effect on net carbon assimilation in some trees in the natural habitat. Red spruce seedlings often become established under a canopy and in the locations with frequent cloud events; thus, in an environment where light very often becomes a limiting factor. On the other hand, reduced light-saturated photosynthetic rates might have a marginal effect on the carbon assimilation in the seedlings growing in the open, on bright days. However, there is no indication that this damage potential is being realized in the natural environment.

Changes in water relations parameters do not seem to follow a clear pattern. The occurrence of the highest elasticity modulus (E) values in the control Fraser fir seedlings seems to confirm the finding by Barnes *et al.* (1990), that ozone exposure reduces E in Norway spruce. However, the spruce seedlings showed a different trend, with the 0.07 ppm ozone treatment producing the

lowest elasticity modulus values and the 0.15 ppm treatment the highest ones. The low elasticity modulus in the 0.15 ppm ozone treatment in Fraser fir seedlings suggests that the high ozone dose "loosens" the cell walls. However, an unpublished study of Sober (Laisk, personal communication) found that ozone exposure actually reduced elasticity of dwarf bean leaf cell walls. The inconsistent results of this study (and other published experiments) put in question the role of the modulus of elasticity as an universal indicator of ozone action on cell wall. An indication of ozone action in the red spruce seedlings is a increase (insignificant, but marked) in the relative water content at turgor loss point in the 0.15 ppm ozone treatment. This may result in lower drought resistance, since under water stress, foliage would lose turgor at higher water content. The possibility that ozone may decrease drought hardiness of red spruce was suggested, among others, by Chevone *et al.* (1990).

Ozone exposure has been shown to influence the solute levels in trees. Rehfuess and Ziegler (1986) reported an increase in ATP level, ATP/ADP ratio, and monosaccharides content, but decrease in starch and sucrose level in Norway spruce foliage exposed to ozone and acidic mist. In the study of Tingey *et al.* (1976), the levels of soluble sugars and starch tended to increase in tops and decrease in roots. Also Barnes (1972b) found total soluble carbohydrates and reducing sugars contents in pine needles raised by ozone exposure. Similarly, Guderian *et al.* (1988) found sugar levels in Norway spruce foliage stimulated by ozone exposure, which resulted in increased osmotic potential. In this experiment, the red spruce and Fraser fir seedling osmotic relations were not significantly modified. The osmotic potentials at full turgor and at the turgor loss point varied very little across treatments in both species. The osmotic potential at the turgor loss point in spruce seedlings tended to be least negative

in the highest ozone treatment, which may be due to the lowered water deficit at the turgor loss point. The lack of effect of ozone on osmotic relations of Fraser fir is consistent with the findings by Seiler and Chevone (1990). They reported that ozone exposure did not modify water potential and its components (osmotic and turgor potentials) in Fraser fir seedlings.

SUMMARY AND CONCLUSIONS

The effects of ozone exposure on the measured photosynthetic diagnostics were not uniform across tree species and seed sources. Carbon dioxide curves were not significantly modified in red spruce from either seed source. Fraser fir seed sources revealed opposite responses in carboxylation efficiency, compensation point, and stomatal limitations. Light-saturated photosynthesis was reduced in both species (in spruce significantly) and light compensation point was shifted upwards in red spruce seedlings. No effect on quantum yield efficiency was found in either species. There were indications that ozone exposure modified maximum turgor potential in Fraser fir seedlings and modulus of elasticity in both species. However, the patterns of the latter parameter were inconsistent between the species. The highest ozone concentration resulted in some suppression of water deficit at turgor loss point in red spruce, indicating a potential for a decrease in drought hardiness. Osmotic potential remained unchanged in both species.

The presented results indicate that ozone may have some influence on photosynthetic apparatus and water relations of red spruce and Fraser fir; however, it is unclear whether this could translate into growth effects in the long

run. In light of the growth and photosynthesis measurements shown in Chapter I of this dissertation, major deleterious effects seem unlikely.

Chapter III

EFFECTS OF POLLUTION EXCLUSION DURING THE GROWING SEASON ON WINTER HARDINESS OF RED SPRUCE SEEDLINGS

A B S T R A C T

The impact of summer ambient air and cloud water winter hardiness of red spruce seedlings was investigated. During the summer, seedlings were kept in exclusion chambers on Whitetop Mountain, VA. The seedlings were subjected to the following treatments: ambient air and clouds, ambient air with clouds excluded, charcoal filtered air, and a chamberless control treatment. During the following winter, the seedlings were placed in Blacksburg, VA, in two locations: in the open and in a shadehouse.

The chamberless control seedlings showed the lowest growth in the subsequent growing season, the highest needle loss, the highest ethylene production, the lowest chlorophyll contents, and the fastest desiccation rates. The

three chamber treatments did not differ consistently in the measured diagnostics, however, the ambient air without clouds treatment showed the greatest new growth in the following season. There were clear differences between the seedlings kept in the shadehouse and in the open, the latter showing more symptoms of stress.

INTRODUCTION

High elevation red spruce - Fraser fir forest in southern Appalachians were recently reported to suffer a growth decline (Adams *et al.* 1985) and increased mortality (Dull *et al.* 1988). Several biotic and abiotic factors have been indicted. One of the hypotheses proposes that pollution-induced susceptibility of trees to frost damage is responsible for the observed declines (Friedland *et al.* 1984). Lucas *et al.* (1988, using Sitka spruce (*P. sitchensis* (Bong.) Carr.)), Barnes and Davison (1988) and Brown *et al.* (1987) (using Norway spruce) found that summer ozone exposure may adversely affect winter hardiness of spruce.

There are a few mechanisms through which adverse winter conditions may harm an evergreen plant (Levitt 1980, Tranquillini 1982). Mesophyll cells may be damaged by desiccation caused either by intercellular freezing or intense evaporation coupled with a blocked water supply owing to frozen soil. There are studies reporting accelerated erosion of cuticular waxes under the impact of ambient ozone (Guderian *et al.* 1988). Photooxidative injury occurs when low temperatures impair the ability of green cells to effectively utilize light energy. The resulting generation of free radicals may damage photosynthetic systems; in particular bleaching of chlorophyll may occur (Steffen and Palta 1987, Barnes

and Davison 1988, Cumming *et al.* 1988). Finally, plant tissues may be killed by intracellular freezing, when ice crystals disrupt cell membranes ("pure" freezing injury, Levitt 1980). There are indications that ozone and acid mist may delay onset of winter hardiness in spruce trees (Lucas *et al.* 1988, Barnes and Davison 1988). Specific mechanisms, however, are not known. Some suggest that ozone may alter glutathione metabolism thus weakening the plant's defense against winter photooxidation (Alscher *et al.* 19..).

In contrast to the Northern Appalachians, red spruce forests in the Southern Appalachians rarely show symptoms of winter injury (Nicholas, pers. comm.). Minimum temperatures in the South do not fall below -30° C, while red spruce has been reported to be resistant to between -30° C and -40° C (Sheppard *et al.* 1989, Pukacki 1985, Thornton *et al.* 1990). Thus, occurrence of freezing injury in spruce trees is not probable in the Southern Appalachians. The potential for damage due to winter desiccation and photooxidation is not as clearly known.

This study evaluated the influence of growing season ambient air and cloudwater exposure on overall winter hardiness of red spruce seedlings. Needle loss, growth performance, and ethylene production were used to measure general effects of winter stress. The susceptibility of the seedlings to desiccation was determined via a desiccation test. Needle chlorophyll content was used to assess possible photooxidative injury.

METHODS

Half-sib red spruce seedlings were grown from seeds collected on Mt. Rogers (adjacent to Whitetop Mtn., in southwest Virginia) and planted in the 160 cc tubes (Cone-Tainer Nursery, Canby, Or.) on Nov. 10, 1988. The seedlings were kept under a long day regime (16 hours), in a greenhouse supplied with charcoal-filtered air, in Blacksburg, Virginia. In April 1989 the seedlings were induced to set buds by placing them under a short day regime (8 hours). After the seedlings set buds, they were placed in a coldroom (2°C) for four weeks in order to satisfy the chilling requirement. Afterwards, the tubes with seedlings were put into small buckets (10 per bucket) and packed with perlite in order to insulate roots. On July 2 the buckets were brought to open-top chambers in the research compound on the top of the Whitetop Mountain (36° 39'N, 81° 36'W). The seedlings were exposed, for the entire growing season, to either ambient air and clouds, ambient air without clouds, or charcoal filtered air (see Thornton 1988 for a detailed chamber description). In the ambient air and cloud treatment, seedlings were placed in chambers which lift automatically during cloud and fog events. There was also a chamberless control, protected from direct sunlight by 50% shade cloth. Filtration of the air through charcoal lowered ozone concentration by about 50%. The 12 hour ozone average during the 1989 growing season in the latter treatment was 0.022 ppm, and in the other treatments 0.045 to 0.048 ppm. 1989 was a low ozone year, with only one hourly concentration exceeding 0.100 ppm. The average pH of clouds was 3.4 (Thornton *et al.* 1990, Pier *et al.* 1990).

Following bud set and initial hardening, the seedlings were moved to a shadehouse in Blacksburg, Virginia (on Oct. 3, 1989). On Nov. 9 they were placed in one of two winter exposure treatments:

1. protected from the wind and sunlight - in a shadehouse;
2. unprotected from the wind and sunlight - in the open, adjacent to the shadehouse;

The seedlings in both overwintering locations were buried in oak sawdust and perlite up to seedling root collar. Maximum and minimum air and soil temperatures were recorded daily in both locations, using minimum-maximum thermometers. The soil temperature was measured by placing the thermometers (two per location) in 30 cm-long sections of PVC pipe, placed vertically into the sawdust. The tops of the pipes were stuffed with insulation and covered with plastic lids. To double check the soil min-max thermometers, a stick-type thermometer was inserted into the sawdust in every location. Air temperature thermometers (two per location) were placed upright on the perlite surface, unprotected. The thermometer bulbs were shielded against wind and direct sunlight only by the thermometer housing.

Before splitting into winter exposure regimes, gas exchange of the seedlings was measured to assess their condition, using the LI-COR 6200 portable photosynthesis system. In mid December a preliminary chlorophyll content determination was conducted.

Three times during the winter (mid January, mid February, late March), a sample of seedlings was removed from each treatment combination, brought into a greenhouse, and allowed to flush. These seedlings were visually examined for percent needle loss, at the harvest, and after each flush was completed, using the following categories: 1 - 0% to 10%, 2 - 11% to 40%, 3 - 41% to 70%,

4 - 71% to 100%, or dead. Chlorophyll content in last-flush needles was determined using method described by Moran (1982), using dimethylformamide as solvent.

Ethylene production by last years foliage was determined using gas chromatography (Wolfenden *et al.* 1988). A 0.2 g sample of needles was enclosed in 25 ml glass screw-capped bottles and incubated for 24 hours at a constant temperature of 25° C and under low light (around 30 $\mu\text{M m}^{-2} \text{s}^{-1}$ PAR). A sample was drawn using a 250 μl syringe. Analysis was carried out using a Varian Model 3700 gas chromatograph (6' column, filled with activated alumina F-1, 80/100 mesh; carrier gas helium, 40 ml/min; temperatures: injection port 120°C, detector 130°C, column conditions 95°C; detector: FID).

In order to examine cuticular transpiration a desiccation test was made similar to that described by Baig and Tranquillini (1980). A sample of seedlings were watered abundantly on the previous day. Last flush branchlets were clipped, immediately weighed, and set upright on a laboratory bench in a styrofoam tray. Temperature was 23-27°C, relative humidity 20-30% at various harvests (on particular measurement days temperature and humidity was fairly constant). Shoots were weighed periodically (at first every hour, later every two hours, for 7 to 9 hours) to assess the rate of cuticular transpiration (Baig and Tranquillini 1980, Eamus *et al.* 1989). Dry weight of the samples was determined after completion of the desiccation test. Fresh weights of the branchlets were then divided by dry weights and plotted against time. The slope of the linear portion of the resulting curve was taken as the measure of cuticular transpiration.

A further set of seedlings were left in their winter exposure site and allowed to flush naturally in the spring. After completion of the growth, in August,

needle loss, last-flush needle chlorophyll content, and dry weights of roots, shoots, and current-year flush were determined.

Data analysis

The study was analyzed as a completely randomized design, consisting of four summer exposure treatments, each replicated three times. Twelve seedlings in every treatment replication were split into two winter exposure treatments and three winter exposure lengths. Subsamples of four to five seedlings per summer exposure replication, per winter exposure, per harvest were taken.

Results were analyzed using analysis of variance to determine influence of summer exposure regimes on growth, chlorophyll content, ethylene production, and desiccation curve slopes. The effects of summer exposure treatments were analyzed separately within each winter exposure. Analysis of covariance was applied to account for harvest date while analysing pooled data. The Least Significant Difference (LSD) test was used to group the response means. The performance of seedlings placed in the open and in the shadehouse was compared using a t-test. The needle loss data were analyzed using nonparametric Kruskal-Wallis and Wilcoxon tests.

R E S U L T S

Winter temperatures

December 1989 was relatively cold in Blacksburg: there were five days

when minimum air temperatures fell below -20°C in the open site, and four such days in the protected site. In later months the minimum air temperature fell below -10°C only once, in late February. The minimum soil temperatures showed little variability and did not fall below -2°C . There were nine days with minimum soil temperatures below zero in the open, and fourteen days in the shadehouse, all in December. Monthly average minimum and maximum air and soil temperatures are presented in Table 1. The open site was characterized by higher amplitudes than the shadehouse location. It should be kept in mind that the air thermometers were not placed inside standard meteorological boxes and the temperature data are not directly comparable with standard meteorological data.

Net photosynthesis

The preliminary net photosynthesis survey indicated that the summer exposure had some effect on photosynthetic rates in November (Figure 1). The rates in the chamberless control seedlings were significantly lower than the rates produced by the treatment excluding only clouds.

Seedling growth

At the final, 1990 summer harvest, the chamberless control produced the lowest total dry weights and the lowest current-year flush weights (Figure 2). The seedlings that overwintered in the shadehouse were significantly smaller than the open site seedlings, but the difference in current-year flush was significant only at $p=0.15$. The greatest new growth produced in the seedlings

Table 1. Monthly average minimum and maximum air and soil temperatures (°C) in both winter locations (in the shadehouse and in the open).

month	Open site				Shadehouse			
	air		soil		air		soil	
	min	max	min	max	min	max	min	max
December	-12.2	3.5	0.9	2.8	-10.1	2.7	0.5	1.9
January	-2.8	13.7	1.9	3.4	-1.7	10.5	1.7	2.6
February	-3.9	18.3	3.6	7.7	-2.4	14.5	3.1	6.3
March	-2.3	24.2	6.0	11.3	-0.2	18.8	5.3	8.6

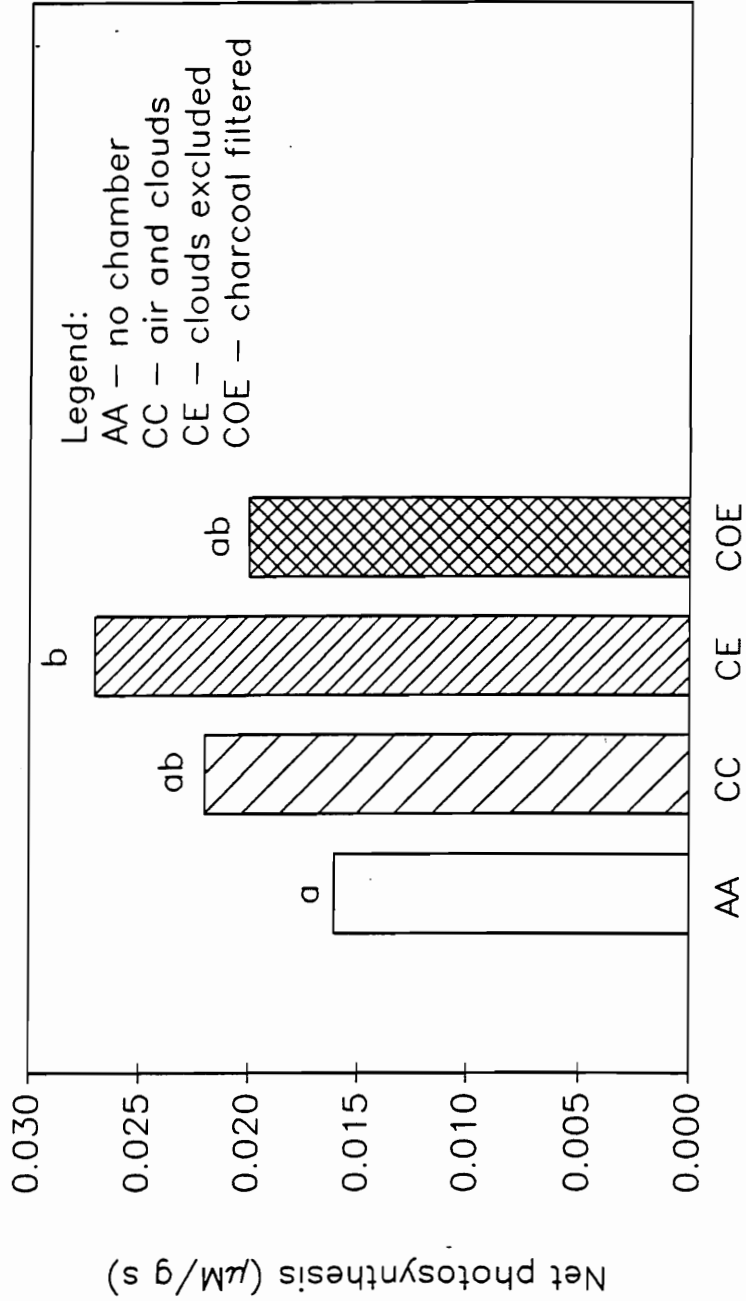


Figure 1. Net photosynthesis in red spruce seedlings before splitting them into winter exposures. Treatments: AA - no chamber, CC - ambient air and clouds, CE - clouds excluded, COE - Charcoal filtered air; see text for a detailed description. Means with different letters are significantly different (LSD, $p=0.05$).

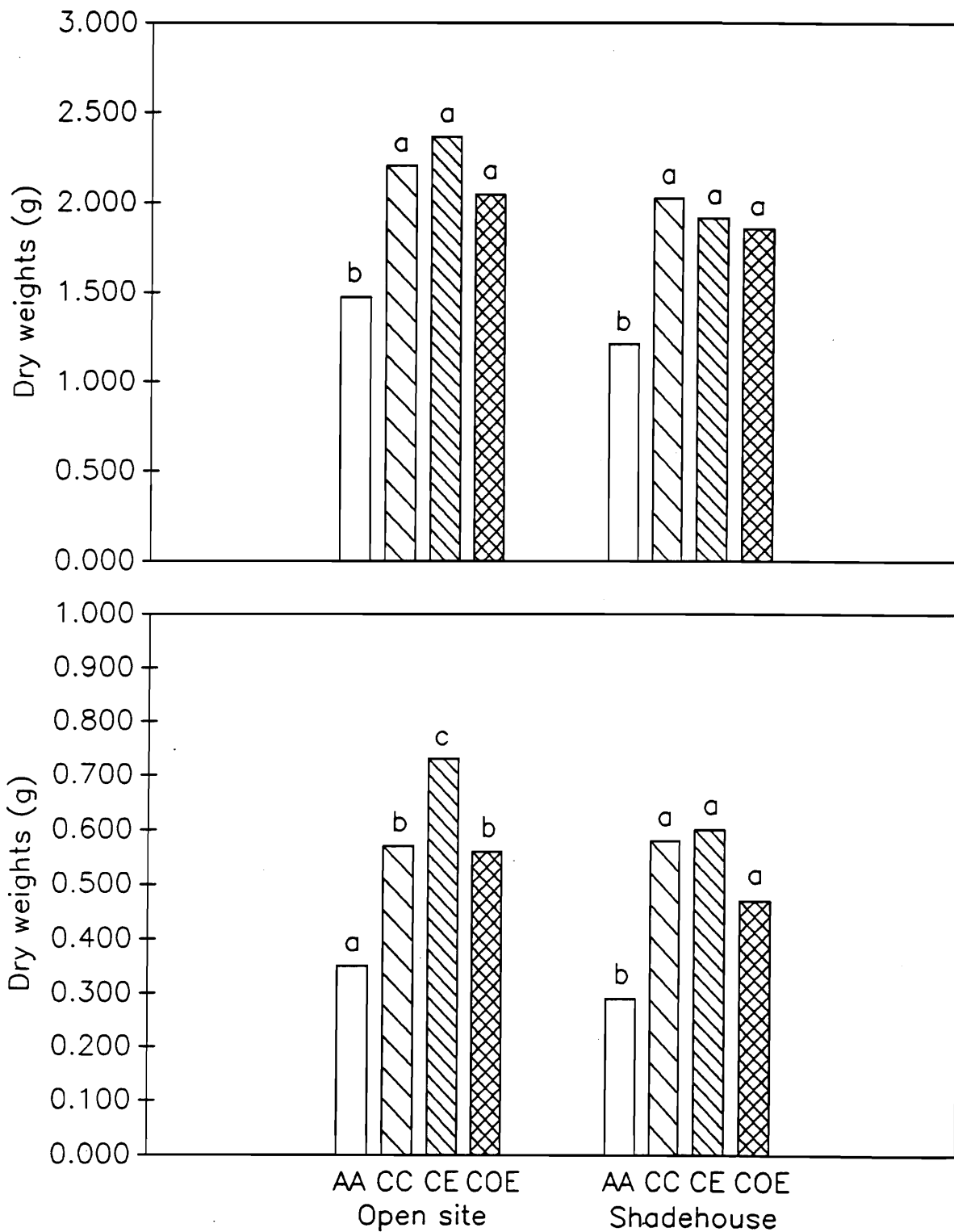


Figure 2. Total (above) and new flush (below) dry weights in red spruce seedlings at the final (summer 1990) harvest. Treatments: AA - no chamber, CC - ambient air and clouds, CE - clouds excluded, COE - charcoal filtered air; see text for a detailed description. Means with different letters are significantly different (LSD, $p=0.05$).

overwintered in the open was in the chamber treatment supplied with ambient air with clouds excluded. New growth and total weights of the seedlings grown in charcoal filtered air tended to be second lowest in both winter locations.

Needle loss

At the final harvest in the open overwintering site, the chamberless control showed significantly higher damage than the ambient air chamber treatment (Figure 3). Other treatments produced intermediate results, not significantly different from either extreme. The needle loss in the shadehouse seedlings did not significantly differ among the summer exposure regimes; however, the chamberless control tended to be the worst damaged. The latter treatment consistently produced the highest needle loss during the three winter harvests in both locations. The damage was higher in the open overwintering site than in the shadehouse at final harvest.

Ethylene production

The ethylene production patterns and amounts were not consistent during the consecutive harvests (Figure 4). At the first harvest (January) there were no differences either among chamber treatments or between overwintering locations. At the second and third harvests the open site seedlings produced significantly more ethylene than the shadehouse seedlings, and among the latter the chamberless control showed the highest ethylene production. The ethylene production in the open site seedlings never showed significant differences among treatments.

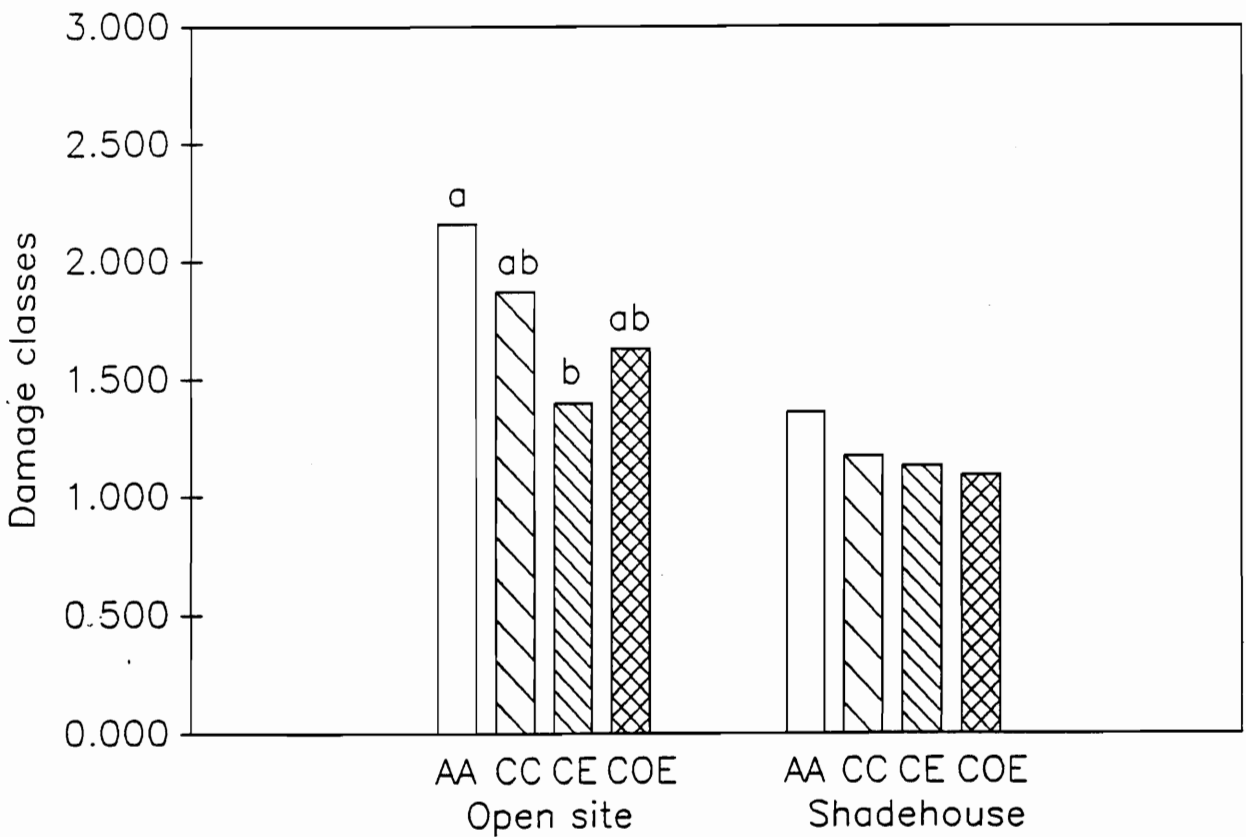


Figure 3. Needle loss in red spruce seedlings at the final (summer 1990) harvest. Treatments: AA - no chamber, CC - ambient air and clouds, CE - clouds excluded, COE - charcoal filtered air; see text for a detailed description. Means with different letters are significantly different (LSD, $p=0.05$, overall significance confirmed using Wilcoxon test, $p=0.05$).

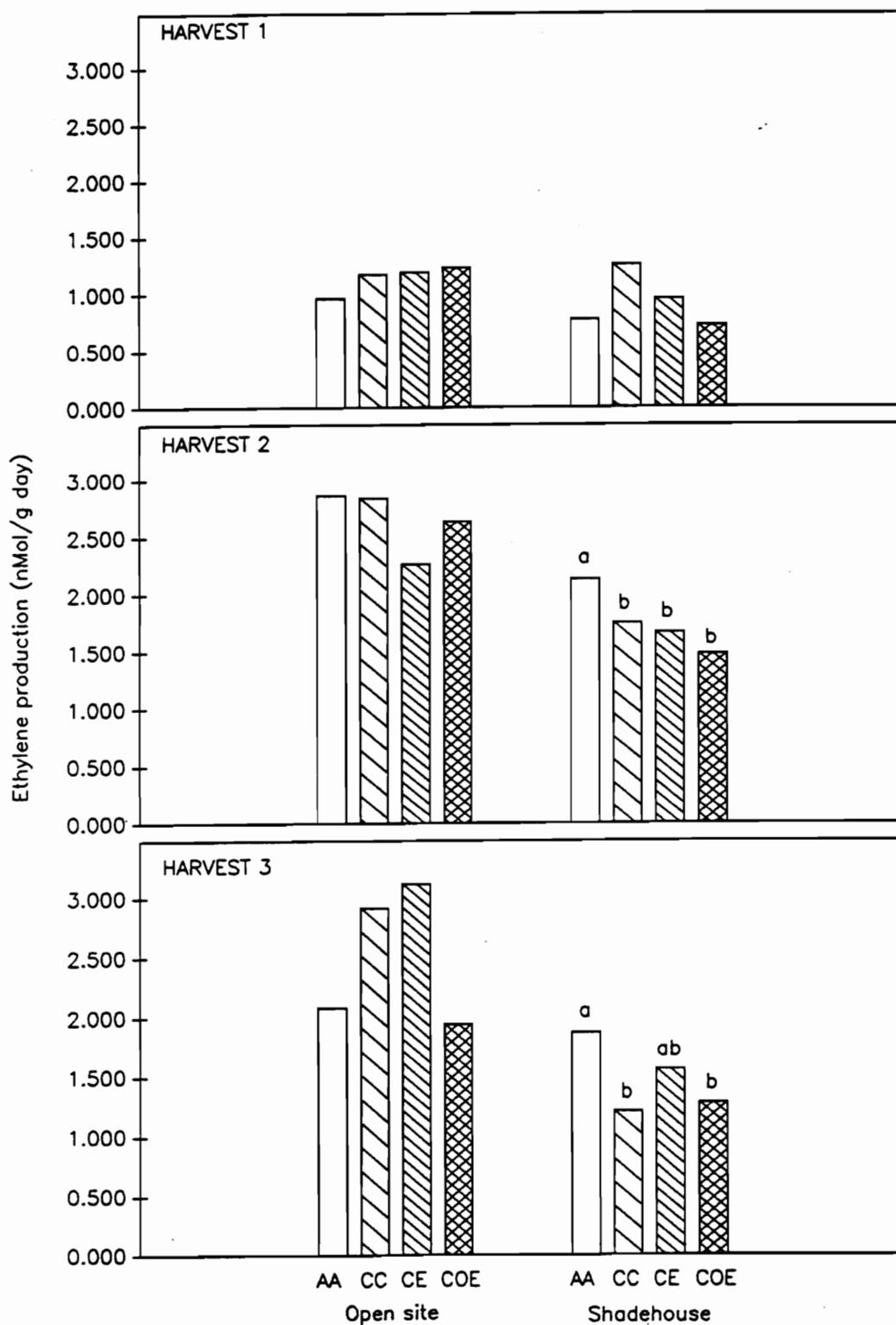


Figure 4. Ethylene production in red spruce foliage during consecutive harvests. Treatments: AA - no chamber, CC - ambient air and clouds, CE - clouds excluded, COE - charcoal filtered air; see text for a detailed description. Means with different letters are significantly different (LSD, $p=0.05$).

Chlorophyll content

The patterns of chlorophyll content at the final harvest were different from those at wintertime harvests. The pooled data for the wintertime harvests (Figure 5) show the chamberless control as the treatment which resulted in the lowest chlorophyll content in both locations. In contrast, at the final summer harvest the chamberless control produced the highest chlorophyll levels. Other treatments did not differ significantly one from another at any harvest.

Average chlorophyll contents in protected and unprotected seedlings at consecutive harvests tended to decrease with time (Table 2). The values at the final, summer harvest, were clearly lower than those measured during winter. One should point out that the 1989 flush was investigated in winter, while in the summer the new 1990 flush was used for chlorophyll determination.

The chlorophyll content differed significantly between locations only at the final harvest, with the shadehouse seedlings showing much higher average concentration than the open site seedlings.

Desiccation test

The chamberless control seedlings from the open overwintering site were losing too many needles during the desiccation trial, and therefore could not be reliably tested. The chamber treatments did not result in different slopes of desiccation curves, neither did the overwinter locations (Figure 6). The only effect present was that of the exposure chamber in the shadehouse seedlings: the chamberless control branchlets were desiccating at the highest rates.

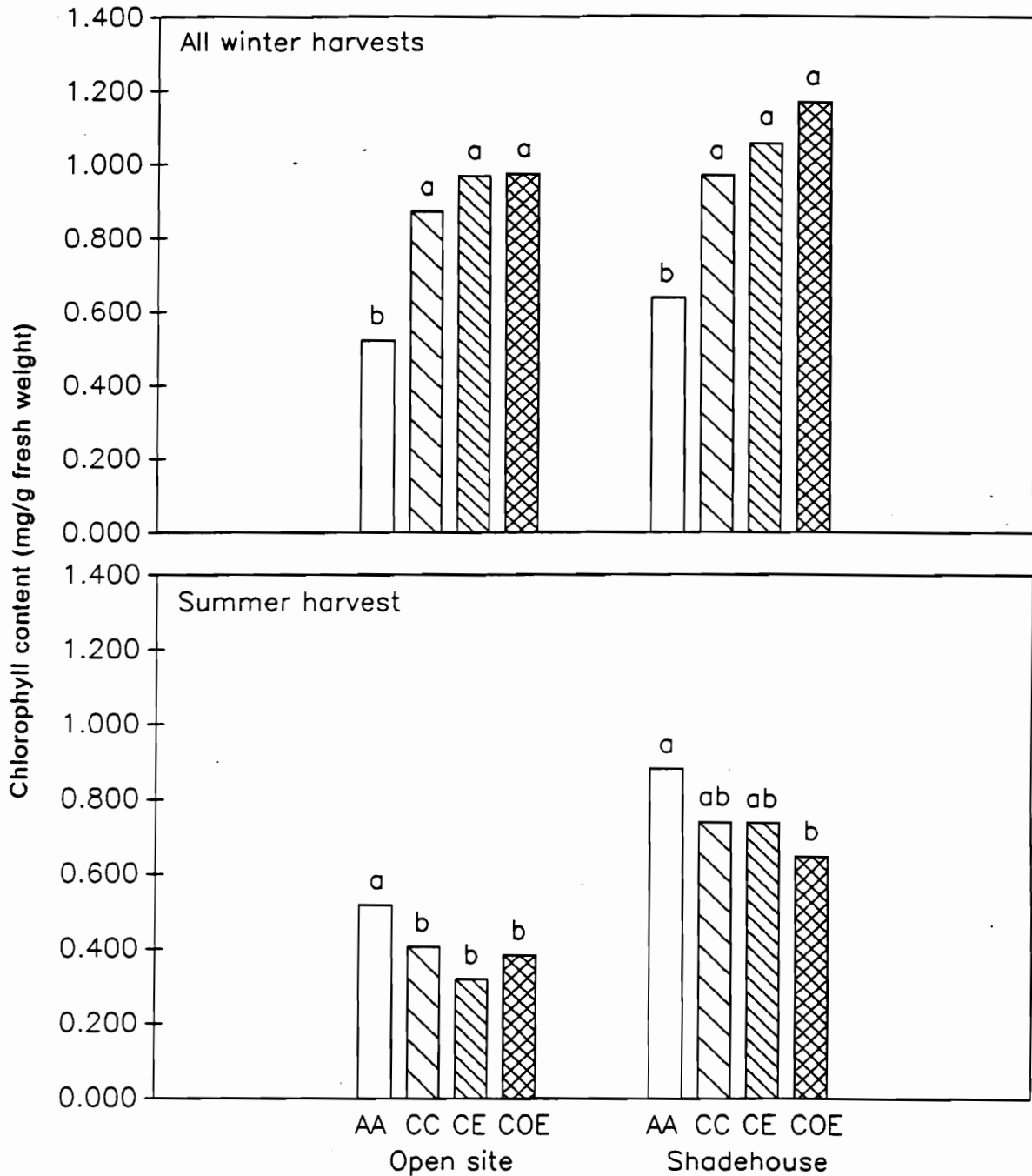


Figure 5 Needle chlorophyll content: pooled data from all winter harvests (above) and results from summer harvest (below). Treatments: AA - no chamber, CC - ambient air and clouds, CE - clouds excluded, COE - charcoal filtered air; see text for a detailed description. Means with different letters are significantly different (LSD, $p=0.05$).

Table 2. Average chlorophyll contents during consecutive harvests in both winter locations (in the shadehouse and in the open).

Harvest	Winter location	
	Open	Shade
	(mg/g fresh weight)	
December	0.863	1.166
January	0.810	0.954
February	0.921	0.805
March	0.779	0.916
Summer	0.406	* 0.751

Asterisk denotes a pair of means significantly different (t-test, $p=0.0001$).

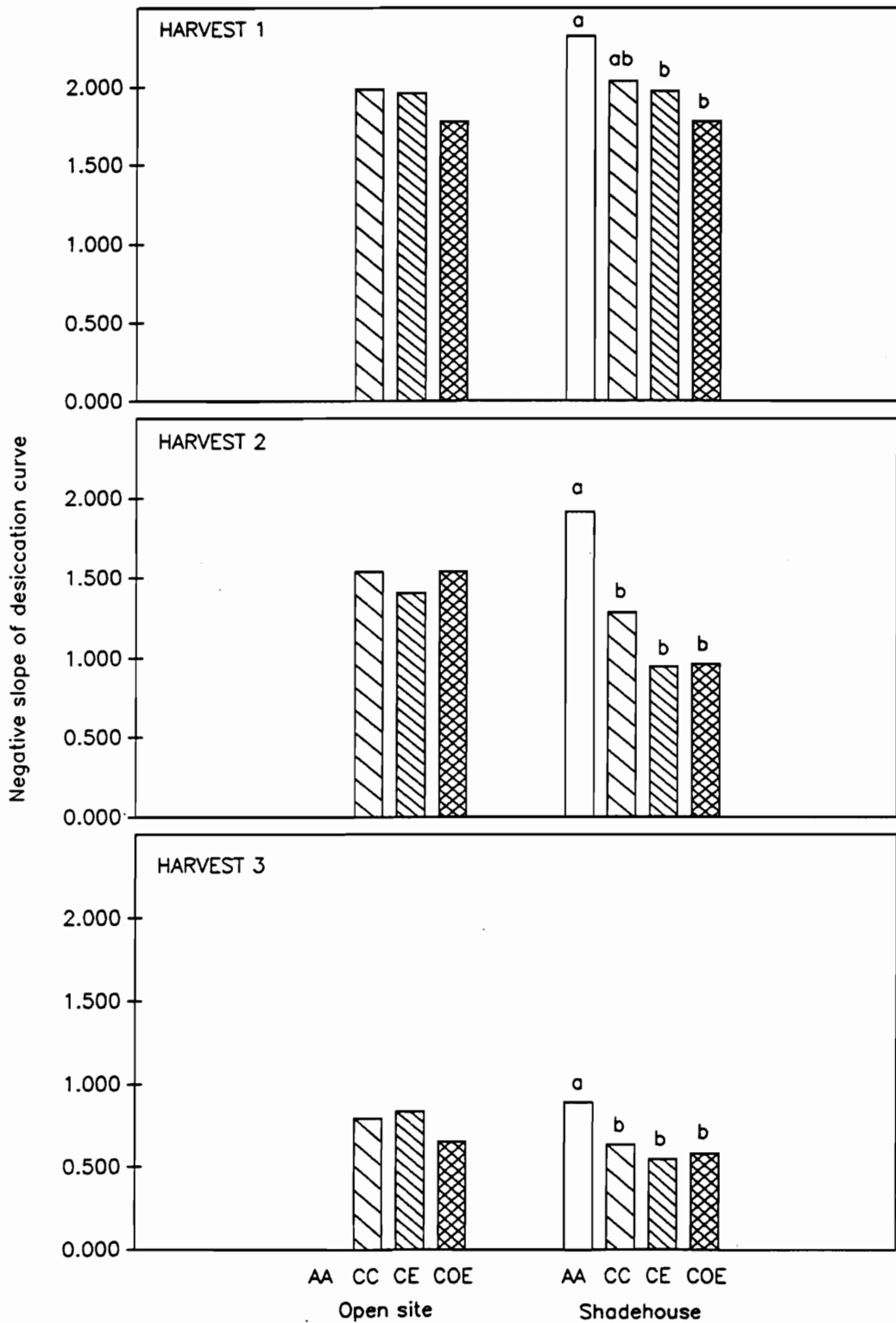


Figure 6 Negative slopes of spruce foliage desiccation curves during consecutive winter harvests. Treatments: AA - no chamber, CC - ambient air and clouds, CE - clouds excluded, COE - charcoal filtered air; see text for a detailed description. Means with different letters are significantly different (LSD, $p=0.05$).

DISCUSSION

The photosynthetic rates of seedlings grown under different air regimes, on Whitetop Mtn., were not statistically different when measured in November, one month after the transfer of the seedlings to Blacksburg. The pollutant regimes also did not result in differences in total weights of the seedlings measured after the following winter and next growing season. These results confirm other reports showing that net photosynthesis and growth of red spruce seedlings are not adversely affected by the ambient levels of ozone and precipitation acidity (Taylor *et al.* 1986, Laurence *et al.* 1989, Alscher *et al.* 1989, Barnes *et al.* 1990, Deans *et al.* 1990). Kohut *et al.* (1990) reported that two-year exposure to acidic precipitation increased photosynthetic rates in red spruce seedlings; however, growth remained unchanged. The long-term ozone fumigation experiment presented in the Chapters I and II of this dissertation did not produce any consistent pattern of net photosynthesis and growth of red spruce seedlings as affected by ozone. Most importantly, Pier *et al.* (1991) and Thornton *et al.* (1990), using the same open-top chambers on Whitetop Mountain, did not find consistent effect of ambient pollutants on photosynthesis and growth of red spruce seedlings over two seasons of exposure.

The conclusion that the red spruce seedlings were not significantly affected by ambient pollution on Whitetop Mountain is further supported by the results of chlorophyll concentration determination, desiccation test, and ethylene production assessment. The only measured parameter which showed significant differences among treatments was the new flush dry weight. The seedlings grown in the air deprived of cloud water in 1989 and overwintered in the open produced in the 1990 growing season more above-ground biomass

than the seedlings in the other treatments. This is supported by some trends in the other parameters. The seedlings grown in the chambers excluding clouds but not ozone, showed the highest photosynthetic rates in November and tended to be less damaged when kept in the open site. However, these trends were not consistent enough to allow one to draw firm conclusions.

Not surprisingly, winter exposure in the open resulted in a considerable needle loss, particularly in the chamberless control seedlings. Both last and first flush needles were affected, although the younger needles seemed to have survived in better shape. There was a marked spatial variability, resulting probably from the wind flow patterns over the seedlings. Fincher *et al.* (1989) found that summer-long exposure to ozone did not influence the degree of winter injury in red spruce seedlings, as evaluated in the spring. However, there are also reports describing a direct effect of either ozone or acid mist on foliar injury in spruce (Leith *et al.* 1989, Mengel *et al.* 1990,).

The chlorophyll content in the needles was not related to chamber treatment. Studies investigating influence of ozone and/or acid mist exposure on chlorophyll content have not produced consistent results. Pier *et al.* (1991) reported mixed results for red spruce seedlings kept over the period of two seasons in the same chambers on Whitetop Mtn. Cummings *et al.* (1988) found a decrease in chlorophyll content in red spruce seedlings upon fumigation with ozone, while Alscher *et al.* (1989) did not detect any differences in a follow-up study.

The chlorophyll content in the chamberless control seedlings was found to be lower than in the chamber treatments during winter. Interestingly, the pattern reversed in the following summer: the chamberless control seedlings

showed the highest chlorophyll content in the newest-flush needles. This may reflect compensation for higher needle loss in this treatment.

Ethylene production was not affected by the ambient air regime. The chamberless control showed the highest rates, but only in the seedlings overwintered in the shadehouse. The seedlings overwintered in the open showed overall higher ethylene production reflecting the higher level of stress they were subjected to. The stress imposed on the unprotected seedlings seemed to have overwhelmed possible chamber effect. The described above differences appeared only at the second and third harvests, when the ethylene production was clearly higher than at the first harvest. This suggests that the seedlings survived the December cold in relatively good shape and only after longer time showed clear symptoms of stress. This study confirms the value of ethylene production as a measure of stress in red spruce (Chen and Wellburn 1989); however, it suggests caution when dealing with interacting multiple stresses. Other stresses (in this case a set of winter stresses) may overshadow the effects of stresses under consideration (in this case a putative air pollution stress).

The published literature does not offer a clear picture of the impact of ozone and acid mist (the suspected pollutants on Whitetop Mountain) on protective function of cuticle in spruce trees. Eamus *et al.* (1989) found no significant effect of mist pH on cuticular resistance in red spruce seedlings. Barnes *et al.* (1990) reported that ozone and acid mist effects on cuticular transpiration depended on soil nutrient status and clone of Norway spruce seedlings. Based on the desiccation results in this study, red spruce cuticle seems to be resistant to one-season exposure to ambient air on Whitetop Mountain.

Almost all of the measured variables reveal a strong chamber effect. Thornton reported that the temperatures were higher in the Whitetop Mtn. chambers than outside and, therefore, the seedlings within chambers required more watering than those in the open-air plots. This also resulted in the higher soil Ca and Mg concentrations in the chamber treatments (Thornton *et al.* 1991). However, Thornton in his experiment did not find any chamber effect on growth of red spruce seedlings. Nevertheless, the finding of a significant chamber effect on the desiccation rates was indirectly corroborated by Thornton *et al.* (1991). They reported that the seedlings from the chamberless control on Whitetop Mtn. had significantly lower epicuticular wax concentrations than the seedlings from the open-top chambers. The authors suggested that this was due to the growth of the chamberless control plants under shade cloth. Since light stimulates epicuticular wax deposition, the seedlings in the chambers were thought to be advantaged due to better light conditions. However, it does not seem possible to fully explain the observed chamber effect with the differences in light regimes alone (50% shade cloth vs. fiberglass domes allowing 70% of PAR). The chamberless seedlings were also exposed all the time to wind and rain. It seems that lower wax content and higher cuticular transpiration rates may be also due to mechanical erosion of wax in the chamberless seedlings. Clarifying the role of specific factors in the chamber effect would require more research.

Different environmental conditions in the two winter exposure locations resulted in marked differences in the measured parameters. Higher needle loss, ethylene production, and lower chlorophyll contents suggest a higher level of environmental stress outside the shadehouse than inside. Yet, the total weights of the seedlings grown in the open were higher than in the protected

site. This was probably due to better light supply, unhindered by the slathouse roof.

S U M M A R Y

In light of the presented data, and discussed literature, the hypothesis that air pollution affects adversely red spruce seedlings in Southern Appalachians by impairing their hardiness to winter desiccation is not plausible. A recent report by Thornton *et al.* (1991) confirms earlier findings that minimum temperatures in the discussed region do not fall below the tolerance limit of red spruce to freezing injury (Sheppard *et al.* 1989, Pukacki 1985). There are also no reports of occurrence of winter injury in this region.

Overall, red spruce seems to be well adapted to the stresses occurring in its habitats in Southern Appalachia, including pollution stress.

Chapter IV

SEASONAL COURSE OF GAS EXCHANGE IN RED SPRUCE TREES OF VARIOUS SIZE FROM TWO SITES ON WHITETOP MOUNTAIN, VIRGINIA

A B S T R A C T

The purpose of this study was to compare the physiology of red spruce trees of various size growing in their natural habitat, and relate it to ambient ozone concentration. The study was conducted on Whitetop Mtn. (elev. 1682 m) in Southwest Virginia, on two sites. One site was located near the summit, on the edge of a declining mature red spruce stand. The second site was on a side slope with a visually healthy mature spruce stand. The xylem water potential and gas exchange of foliage of various age from red spruce seedlings, saplings, and overstory trees was measured throughout three growing seasons, every two weeks, weather permitting. Some seedlings were treated with an

antioxidant chemical EDU to help evaluate the impact of ambient ozone on the physiology of red spruce. Ambient ozone concentrations were obtained from the Whitetop Mtn. Mountain Cloud Chemistry Project monitoring station.

Overstory trees showed the lowest photosynthetic rates and needle conductance, the highest night respiration, and the most negative shoot water potentials. Seedlings had the highest net photosynthesis and needle conductance, and intermediate night respiration. Saplings showed intermediate photosynthesis and needle conductance and the lowest night respiration rates. Shoot water potential in saplings and seedlings did not differ. In general, net photosynthesis decreased with needle age.

No deleterious effects of ambient ozone on red spruce physiology were detected.

INTRODUCTION

The recent interest in the Eastern coniferous high-elevation ecosystems was prompted by reports that the red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* (L.) Mill.) forests in the northern Appalachians are undergoing a decline. Reported symptoms include the dieback of tree tops and branch tips, decreased radial growth, and increased mortality (Woodman and Cowling 1987). The causes for this decline are unclear and may vary from site to site, not to mention regional differences. Some researchers assume that this phenomenon is a natural process resulting from the aging of practically even-aged stands (Zedaker *et al.* 1987, Hornbeck *et al.* 1986, Van Deusen *et al.* 1991).

Others attribute the cause of the decline to unfavorable weather conditions that

occurred in the 1960s and 1970s (Johnson *et al.* 1988). Some authors point out inherent weaknesses of red spruce-balsam fir high elevation stands, such as poor organic soils (Huntington and Ryan 1988), past disturbances (Weiss and Millers 1988), and resulting diminished population size and genetic resources (Eckert and O'Malley 1988).

Another factor indicted is air pollution, specifically ozone and acidic precipitation (mostly cloudwater), which are supposed, by some authors, to be harmful to red spruce and balsam fir in this area (McLaughlin 1985, Joslin *et al.* 1988). Ozone may act either directly on metabolism or by impairing cold tolerance (Cumming *et al.* 1988). Acidic cloudwater may leach out nutrients from needles (Joslin *et al.* 1988).

High elevation red spruce and Fraser fir (*Abies Fraseri* (Pursh) Poir.) forests in southern Appalachians also show considerable mortality (Dull *et al.* 1988). Decline in growth-trends during past 20 years was reported to be similar to that for the northern Appalachians (Adams *et al.* 1985). The history of disturbances (logging, fires - Pielke 1980, Pyle and Schafale 1988) is also similar and so is the expected growth decline resulting from the aging of the practically even-aged stands (Zedaker *et al.* 1987). Southern spruce and fir forests also suffer from pests and pathogens such as the balsam woolly adelgid (*Adelges piceae* Ratz.) which is responsible for the death of Fraser fir trees in the Smokies and Black Mountains (USDA 1989). Red spruce trees in West Virginia are largely infested with cytospora canker (*Valsa kunzei* Fr.) (Mielke 1988). Ozone levels reported in the southern Appalachians may also be harmful to forest trees (Chevone *et al.* 1986, Lefohn *et al.* 1990); however, little is known about the susceptibility of these forests to ozone stress. The possible impact

of cloudwater on the health of high-elevation forests in this region is also unclear.

One of the most challenging problems of air pollution research is scaling up the experiments conducted on seedlings to mature trees and, ultimately, whole stands. Many studies have been done on the impact of ambient ozone on forest trees (Pye 1988); however, almost all of them utilized seedlings. We know very little about the differences in basic physiological processes between trees of various age, not to mention differences in response to environmental stress. This study describes the key metabolic parameters (gas exchange and water potential) in red spruce trees of various size (seedlings, saplings, and overstory trees), and relates them to ambient ozone concentration. The measurements were made over the course of three growing seasons, every two to three weeks. In addition, some seedlings were sprayed with an antioxidant inducing chemical (EDU), in order to evaluate possible impact of oxidative stress on the measured physiological parameters.

METHODS

Site description

The study was conducted at the Whitetop Mountain Research Site located at the summit of Whitetop Mountain (1682 m) in southwest Virginia. Red spruce trees of three sizes were selected: overstory trees (15-20 m tall), saplings (1-1.5 m tall), and seedlings (approx. 0.3 m tall). Trees were located at the edge of a throughfall study (conducted by Dev Joslin, Tennessee Valley Authority Air Quality Branch; Joslin *et al.* 1988), on two sites: on the top of the mountain and

on the northern slope, approximately 100 m away from the upper site (on the lower site there were no seedlings). The upper site seedlings and saplings were located outside a retreating edge of a mature stand, approximately 20 to 30 m away. The overstory trees were located inside the stand, some 15 to 30 meters from the edge. During the period of the study, the edge of the old stand retreated, so the measured overstory trees became gradually much more exposed to the stress of the open space. The canopy at the lower site was essentially intact, with occasional gaps.

Measurements.

Gas exchange (photosynthesis, dark respiration, leaf conductance) and environmental parameters (temperature, relative humidity) were measured with a LI-6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). Gas exchange measurements were taken approximately every two weeks throughout three growing seasons (see Table 1 for the dates of the measurements). In seedlings and mature trees, only the last two growth flushes were measured. In saplings 2-year-old, 1-year-old, and current-year needles were measured (current-year needles become one-year-old in the second year of measurements and two-year-old in the third year). During the third season, four flushes were sampled in saplings on the upper site. Branchlets were cut and within five minutes placed into the LI-6200 cuvette. Preliminary measurements showed that detached red spruce shoots maintained their usual gas exchange levels for approximately twenty minutes after clipping. Sample dry weight was determined in the laboratory at VPI&SU (Blacksburg, Virginia). Except for days with clear sky, a portable light (General Electric EYF bulb, 75W 12V) was used to as-

Table 1. Days when measurements were performed on Whitetop Mountain and corresponding ozone concentrations (from MSCP monitoring station, means of hourly means from 10:00 to 14:00 hrs).

Year	Calendar date	Julian date	Ozone conc.
	(month/day)	(day)	(ppb)
1988	6/06	158	81
	6/20	172	66
	7/06	188	-
	8/02	215	71
	8/16	229	50
	8/31	244	50
	9/14	258	-
	9/27	271	53
	10/25	299	-
1989	4/27	117	64
	5/18	138	66
	6/02	153	53
	6/22	173	58
	7/10	191	70
	8/03	215	68
	8/17	229	59
	8/30	242	60
	9/19	262	-
	10/03	276	50
	10/25	298	55
1990	5/09	129	49
	5/18	138	52
	6/01	152	56
	6/18	169	55
	7/05	186	62
	7/18	199	36
	8/02	214	51
	8/13	225	54
	8/30	242	45
	9/26	269	54

sure a constant saturating light level (PAR 800-1000 $\mu\text{M}/\text{m}^2 \times \text{s}$). Shoot water potential was determined using a pressure chamber. In seedlings and saplings, sample branchlets were taken usually from a zone approximately 1/3 from the top. To access foliage of the mature trees, scaffolding was erected in the Summer of 1988. Therefore, the data for mature trees were collected from the Fall of 1988 on.

Measurements started at the upper site at 1000 to 1100 hours and lasted for 1.5 to 2 hours. The lower site measurements started at 1200 to 1300 hours and took usually up to 1.5 hour to complete.

Several times during the study, night respiration and predawn shoot water potential were measured. The night measurements were taken beginning at 2200 to 2300 hours. A full set of measurements took up to four hours to complete. However, only in the upper site was a sufficient number of measurements completed (three for respiration, four for water potential); therefore, the analysis will not include any data from the lower site.

The LI-6200 analyzer was calibrated with standard gases in the laboratory in Blacksburg, Virginia, on the night before each trip. On each site, the CO_2 analyzer and flow meter were zeroed, and LI-COR K-test (a measure of cuvette water absorption/deabsorption, LI-COR 1987) was performed before the measurements started.

Ambient ozone concentrations (hourly averages) were obtained from the Whitetop Mountain MSCP monitoring station (Lefohn *et al.* 1990).

Data analysis.

The upper and lower site data were analysed separately using a com-

pletely randomized design. The analysis accounted for the fact that individual trees were nested within tree sizes. There were three tree sizes: mature trees (5 on both locations), saplings (5 at both locations), and seedlings (on upper site only: 8 were used in the EDU study - see below, and 5 were used in other analyses).

The data were analyzed using analysis of covariance to examine the role of tree size and foliage age on measured plant parameters on the particular measurement days and over the three seasons. Measurement date, temperature, and shoot water potential (as a measure of water stress) were used as covariates. It should be pointed out that the needle generation was pseudoreplicated (nested within individual trees). Therefore, testing this variable was not formally correct and cannot be treated rigorously. The upper and lower sites could not be compared using statistical analysis, since the sites were not replicated and were confounded by the time of measurement. Simple comparisons were therefore made between the sites. Multiple regression techniques were used to determine the impact of ozone concentration during the measurements on net photosynthesis and needle conductance in trees on the upper site. Average of hourly concentrations from 1000 to 1400 hrs (during the measurements) were used. Time (dates of measurements), water potential (as a measure of water stress), photosynthetic chamber temperature and relative humidity, and needle age were used as additional variables. Separate regressions were fitted for each tree size and for pooled tree sizes. Pressall Macro on SAS (a program ordering all possible models according to PRESS, Cp, and R² statistics) was used to select the best models. The models were checked for significance of variables and collinearity.

EDU study

An additional study was conducted to evaluate the response of red spruce seedlings to EDU (N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea). Four field-grown seedlings received a foliar application of EDU every second week, for two years. EDU was applied as a 500 ppm water solution, 250 ml per seedling, using a hand-held, household-type pump sprayer. Four control seedlings were sprayed with pure water. The first EDU applications in particular seasons took place after the second measurement series in 1988 and after the first series in 1989.

The gas exchange measurements with a the LI-6200 were taken every two weeks (as in the main study), before application of the next EDU treatment. The results were analyzed using an analysis of covariance to ascertain the possible influence of EDU treatment and interaction between EDU treatment and ambient ozone concentration on gas exchange parameters. Measured values before the treatment started and chamber temperature were used as covariates.

R E S U L T S

Daytime measurements.

Tree sizes

Net photosynthesis of foliage coming from trees of various size differed significantly throughout the duration of the study. On the upper site, the net photosynthesis in one-year-old foliage was the highest in seedlings and the

lowest in overstory trees (Figure 1). The differences were significant on most of the measurement days. When the data pooled over three seasons were analyzed (Table 2), both current-year and one-year-old foliage photosynthetic rates differed significantly among tree sizes. During the year 1989, photosynthetic rates in overstory trees declined markedly relative to those in seedlings and saplings (Figure 1). This was probably due to one tree which showed little or no net photosynthesis during that time and did not survive the winter of 1989/1990.

Measurements conducted at the lower site confirmed the trend from the upper site. Overstory trees showed significantly lower photosynthetic rates than saplings in both needle generations (Figure 2, Table 2).

The differences between overstory trees and younger trees were more pronounced in current-year foliage than in one-year-old needles (Figure 3, 4, Table 2). Buds on seedlings tended to flush earlier in the season than those on saplings, while overstory trees tended to break buds the latest (Figures 3 and 4, author's observations).

On the upper site, seedlings tended to show the highest needle conductance rates, while overstory trees showed the lowest (Table 3). However, these differences were not entirely consistent throughout the season (Figure 5). Saplings on the lower site produced conductance rates significantly higher than those in overstory trees. The LI-6200 was not able to produce reliable measurements of water vapor-related parameters (conductance, transpiration, internal CO₂ concentration) at very low (below 25%) and very high (above 75%) humidities. Therefore, the overall number of conductance data points (measurement days) available for analysis was smaller than in the case of photosynthesis (23 days vs. 29).

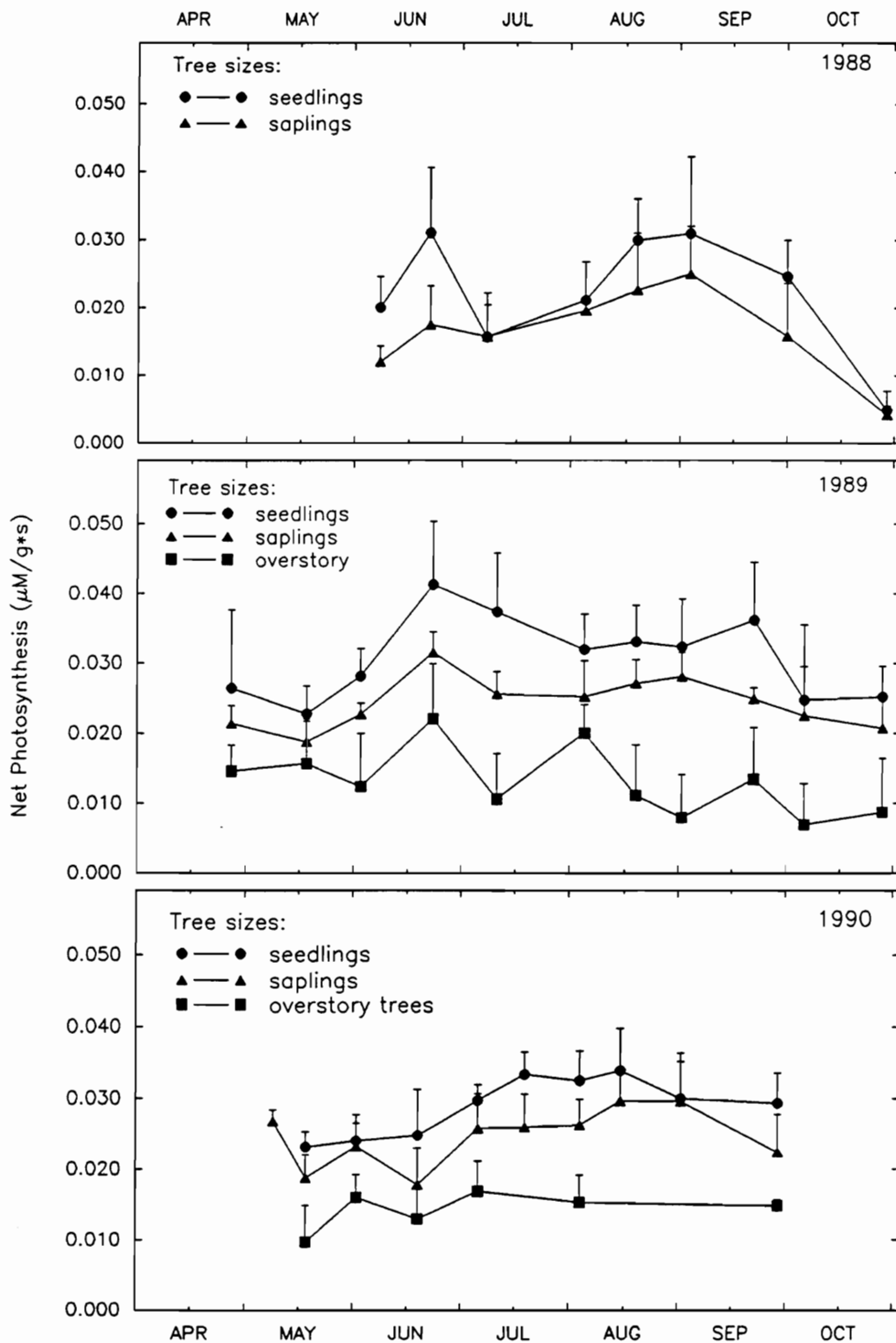


Figure 1. Net photosynthesis throughout three growing seasons in red spruce one-year-old needles from seedlings, saplings, and overstory trees, upper site. Bars indicate standard deviations.

Table 2. Photosynthetic rates in various tree sizes and needle generations of red spruce, on two sites on Whitetop Mountain, Virginia.

		Net photosynthesis			
Site	Foliage	Seedlings	Saplings	Overstory trees	Mean ¹
($\mu\text{Mol/s}\times\text{g dry weight}$)					
Upper	current	0.0360 aA	0.0271 bA	0.0134 c	0.0202
	1-year	0.0279 aB	0.0222 aB	0.0134 b	0.0178
	2-year	-	0.0202 B	-	-
	Mean ²	0.0319	0.0246	0.0134	0.0190
Lower	current	-	0.0245 a	0.0101 bA	0.0184
	1-year	-	0.0221 a	0.0139 bB	0.0187
	2-year	-	0.0195	-	-
	Mean ²	-	0.0233 a	0.0120 b	0.0186

¹ only saplings and overstory trees included

² only current-year and one-year-old needles included

Means followed by different letters are not significantly different (LSD test, $p=0.05$). Capital letters denote differences within columns, lower case letters denote differences within rows.

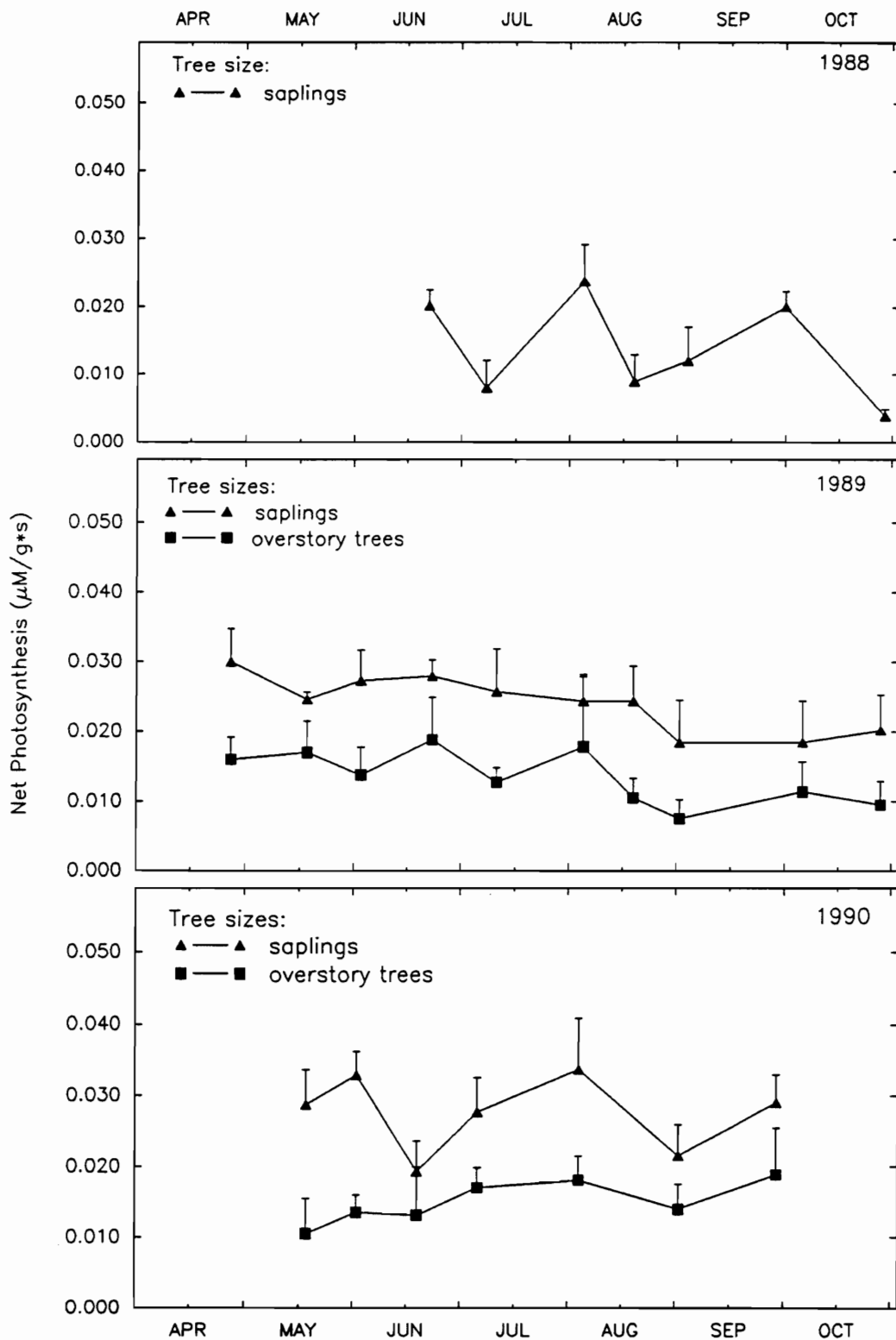


Figure 2. Net photosynthesis throughout three growing seasons in red spruce one-year-old needles from saplings and overstory trees, lower site. Bars indicate standard deviations.

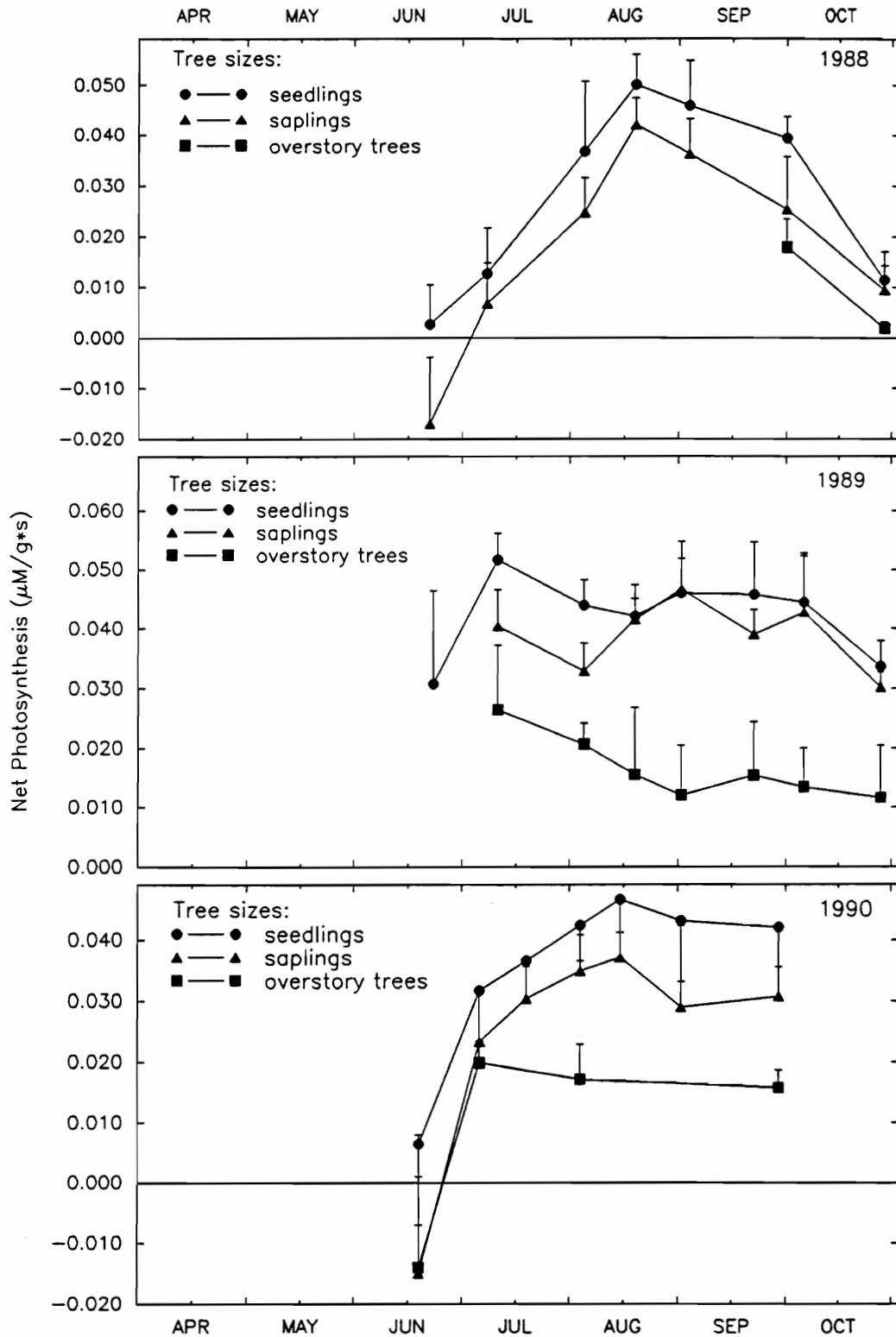


Figure 3. Net photosynthesis throughout three growing seasons in red spruce current year needles from seedlings, saplings, and overstory trees, upper site. Bars indicate standard deviations.

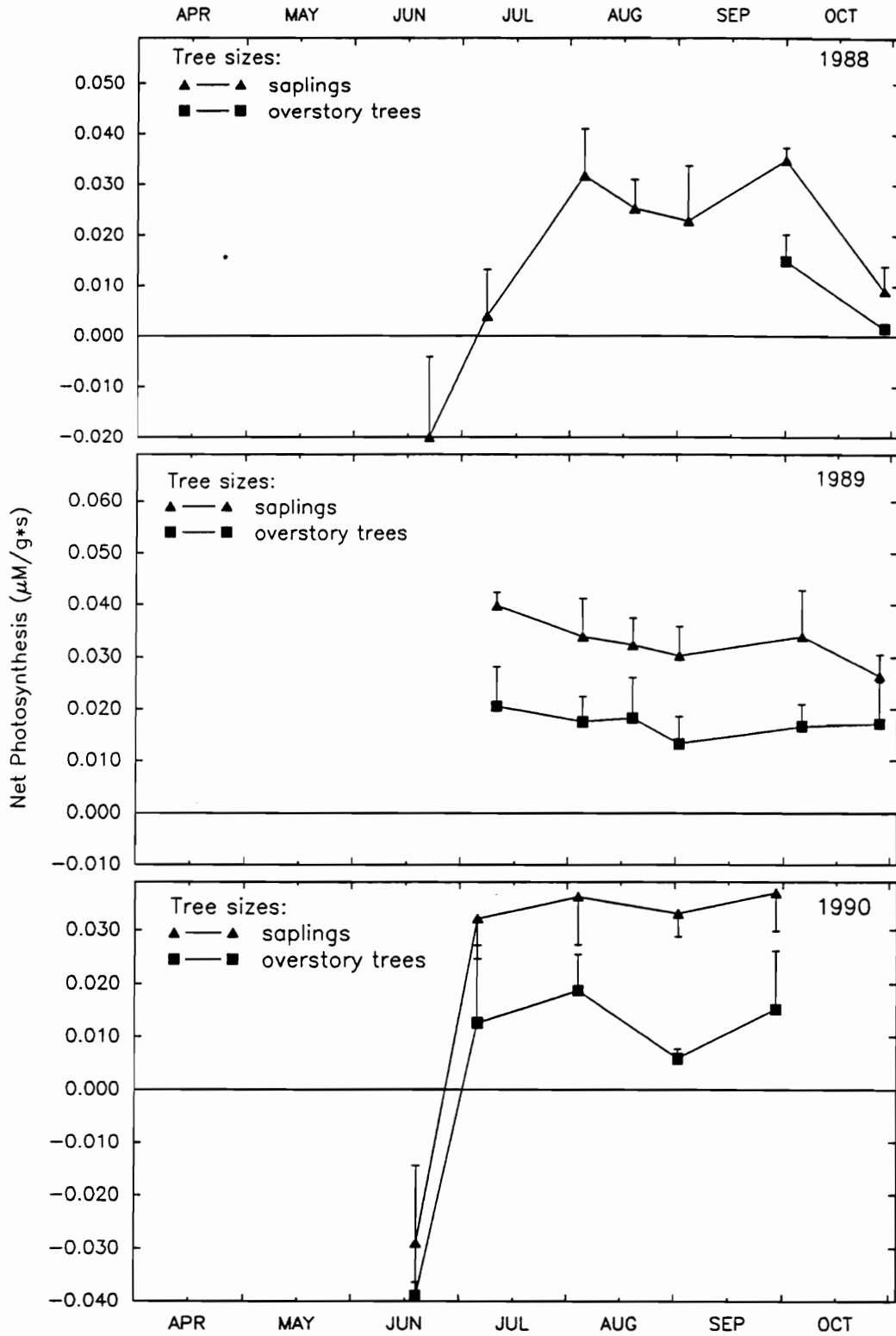


Figure 4. Net photosynthesis throughout three growing seasons in red spruce current year needles from saplings and overstory trees, lower site. Bars indicate standard deviations.

Table 3. Needle conductance rates in various tree sizes and needle generations of red spruce, on two sites on Whitetop Mountain, Virginia.

		Needle conductance			
Site	Foliage	Seedlings	Saplings	Overstory trees	Mean ¹
(nMol H ₂ O/s×g dry weight)					
Upper	current	1662	699 A	553	626
	1-year	574	526 B	461	493
	2-year	-	512 B	-	-
	Mean ²	1118	612	507	560
Lower	current	-	901 a	756 b	828
	1-year	-	686 a	587 b	636
	2-year	-	724	-	-
	Mean ²	-	793 a	671 b	741

¹ only saplings and overstory trees included

² only current-year and one-year-old needles included

Means followed by different letters are significantly different (LSD test, p=0.05). Capital letters denote differences within columns, lower case letters denote differences within rows.

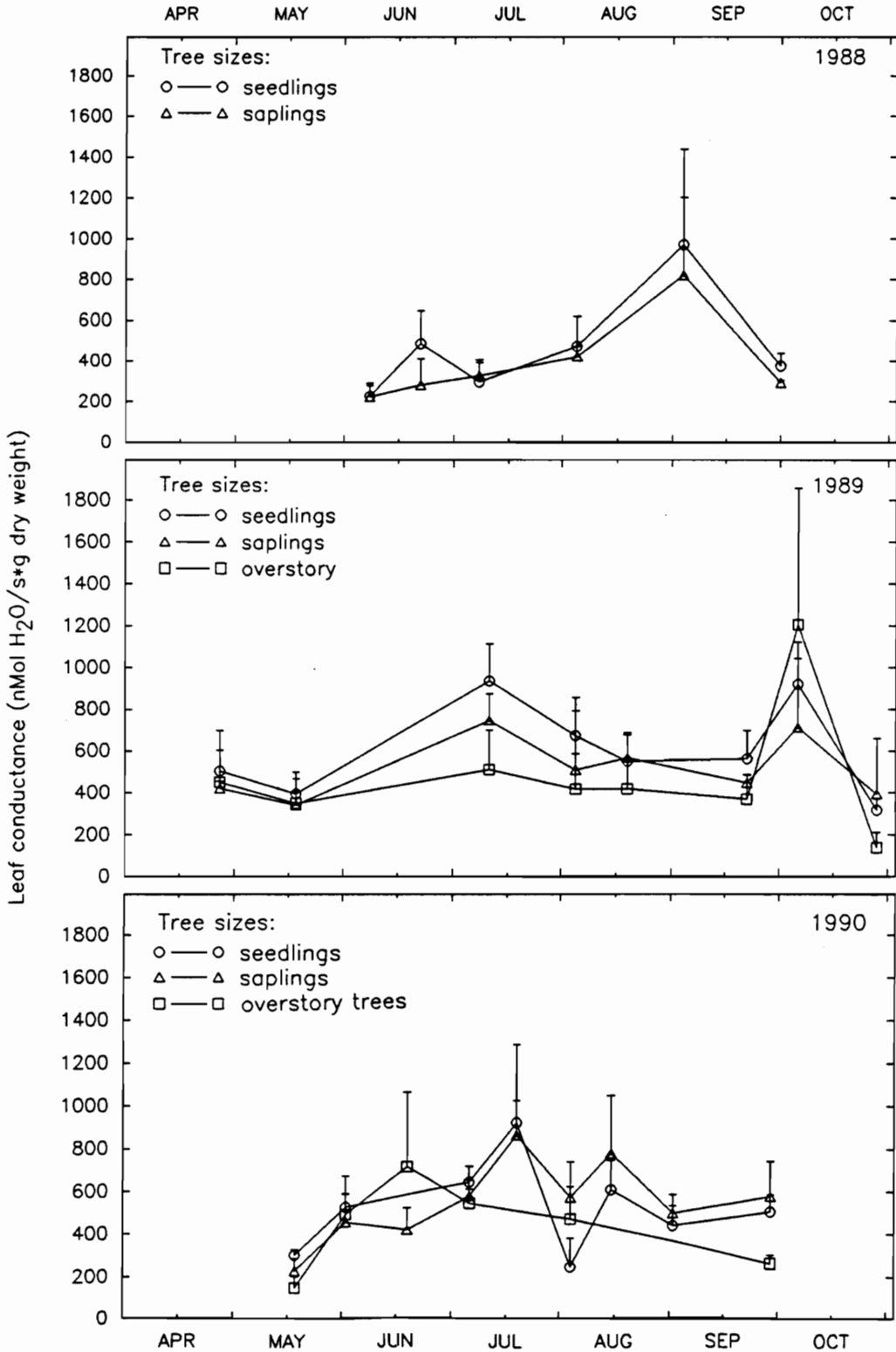


Figure 5. Needle conductance throughout three growing seasons in red spruce one-year-old needles from seedlings, saplings, and overstory trees, upper site. Bars indicate standard deviations.

At both sites overstory trees tended to show the most negative water potentials (Table 4); however, on many measurement days there were no differences due to tree size (Figures 6 and 7). There were virtually no differences in shoot water potential between saplings and seedlings. The increase in variability in overstory tree water potential on the upper site, in the second half of the 1989 season, was due to the mentioned above decline of one of the trees (Figure 6).

Needles of various age.

Net photosynthetic rates generally declined with needle age (Figures 8,9,10,11,12). Current-year needles, after initial higher respiration during needle expansion, showed clearly the highest rates. These rates tended in some cases to converge with the rates of older needles towards the end of a growing season. As mentioned above, in the overstory trees, the gap between current-year and one-year-old foliage was less than in the smaller trees. The differences among the older than current-year foliage were less marked than current-year and one-year-old needles. Analysis performed on data pooled over three growing seasons confirmed these trends with respect to seedlings and saplings (Table 2). In overstory trees on the upper site, the average photosynthetic rates were identical in current-year and one-year-old needles. On the lower site, the current-year foliage in the old trees showed significantly lower mean photosynthetic rate than that in one-year-old needles. It is apparent from Figure 12 that this was due mainly to low current-year foliage photosynthetic rates in year 1990, as well as to one series of measurements taken when the new flush was still vigorously respiring.

Table 4. Shoot water potential in various tree sizes of red spruce, on the two sites on Whitetop Mountain, Virginia.

Site	Shoot water potential			Mean ¹
	Seedlings	Saplings	Overstory trees	
	(MPa)			
Upper	- 0.99	- 1.02	- 1.30	- 1.16
Lower	-	- 0.92 a	- 1.12 b	- 0.99

¹ only saplings and overstory trees included

Means followed by different letters are significantly different within rows (LSD test, p=0.05).

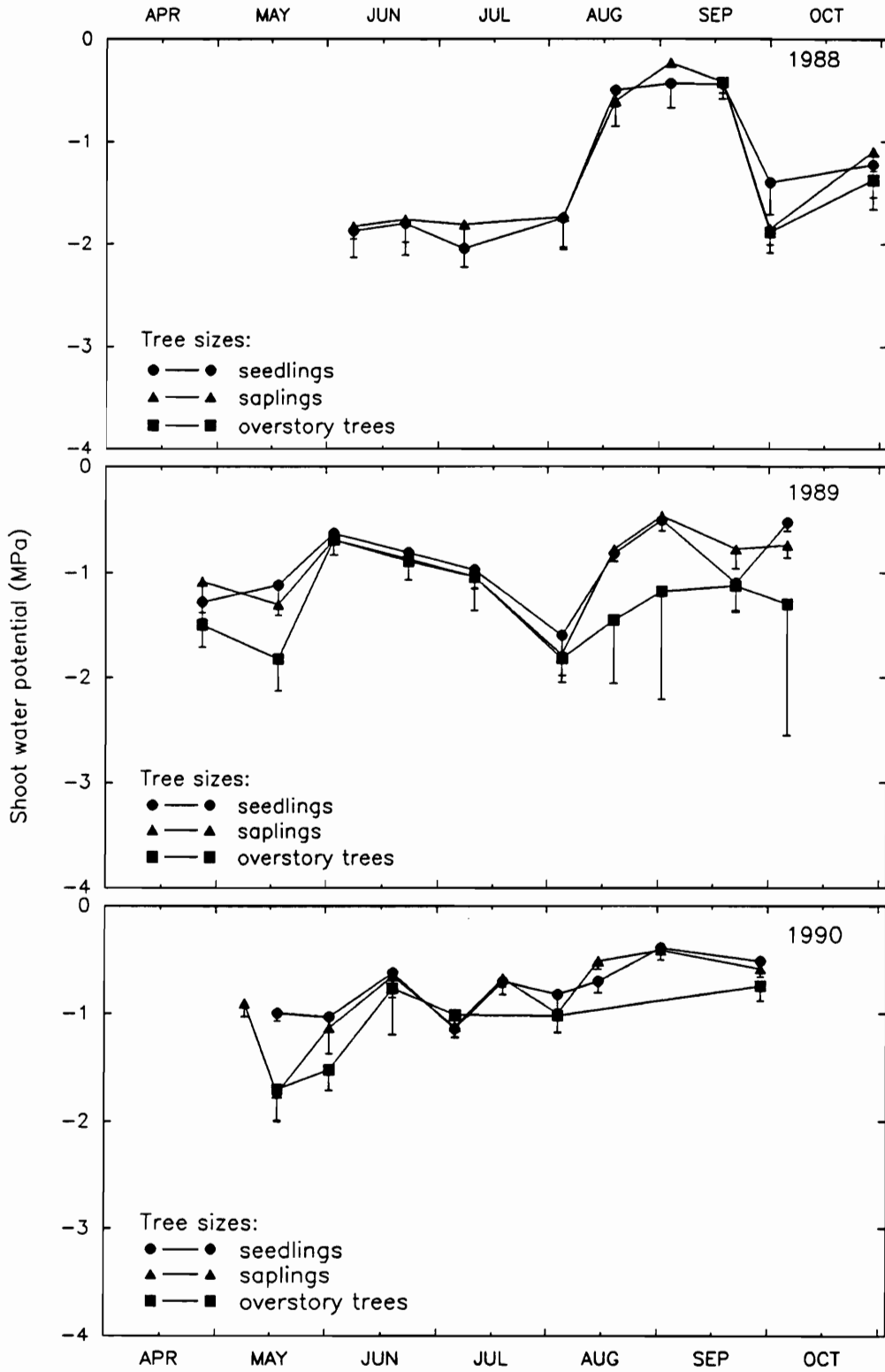


Figure 6. Shoot water potential throughout three growing seasons in red spruce seedlings, saplings, and overstory trees, upper site. Bars indicate standard deviations.

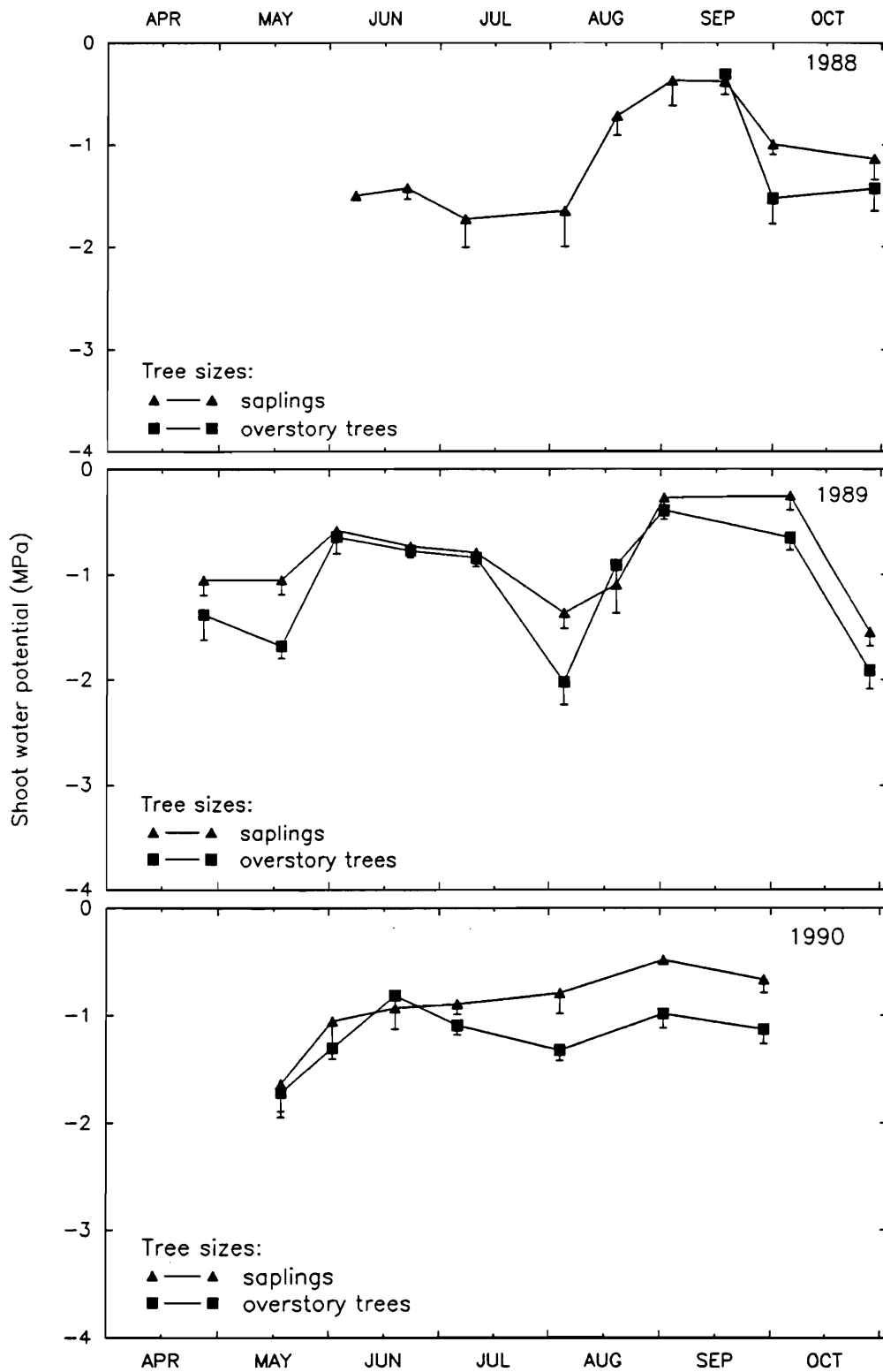


Figure 7. Shoot water potential throughout three growing seasons in red spruce saplings and overstory trees, lower site. Bars indicate standard deviations.

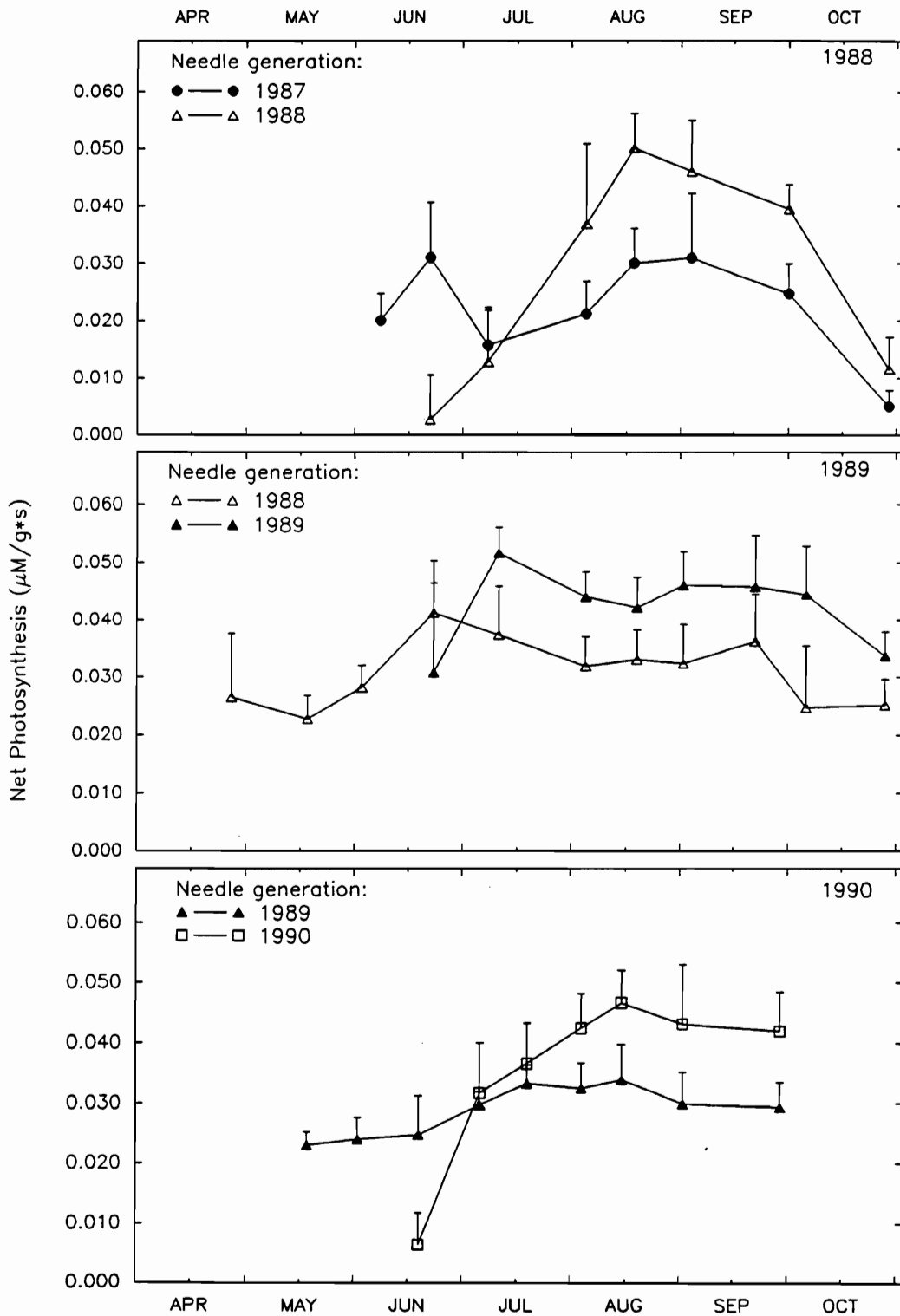


Figure 8. Net photosynthesis throughout three growing seasons in red spruce seedling needles of various age, upper site. Bars indicate standard deviations.

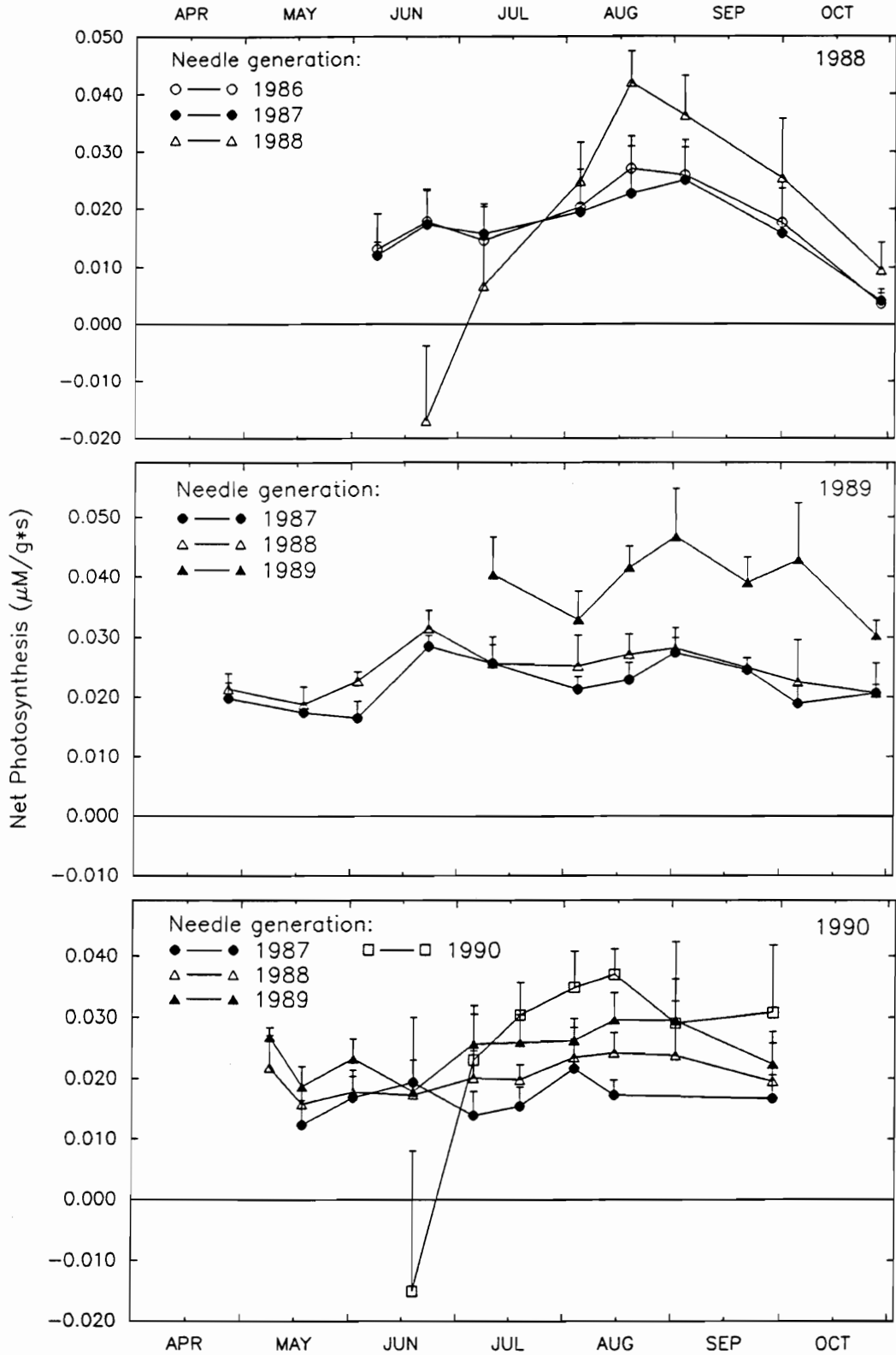


Figure 9. Net photosynthesis throughout three growing seasons in red spruce sapling needles of various age, upper site. Bars indicate standard deviations.

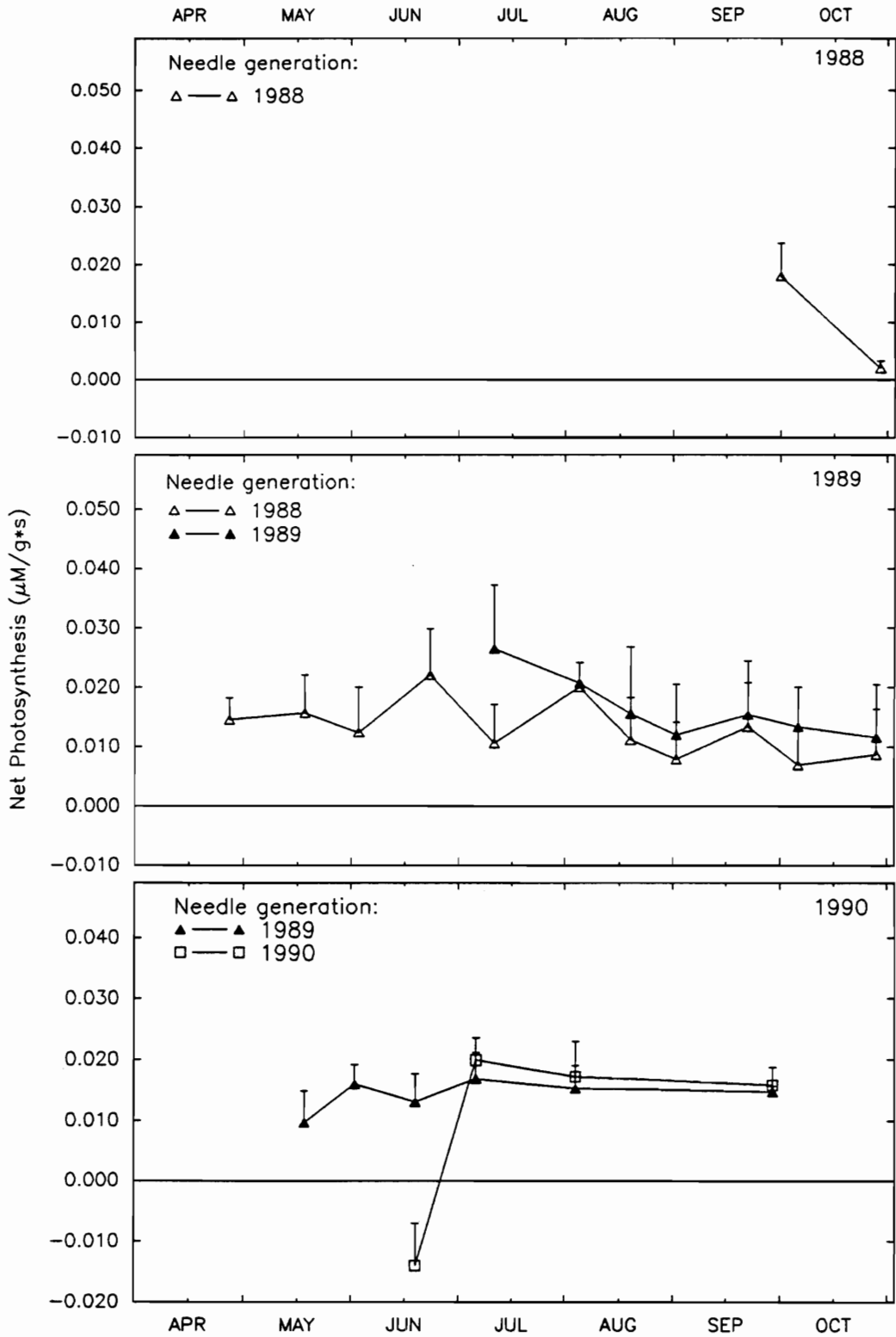


Figure 10. Net photosynthesis throughout three growing seasons in red spruce overstory tree needles of various age, upper site. Bars indicate standard deviations.

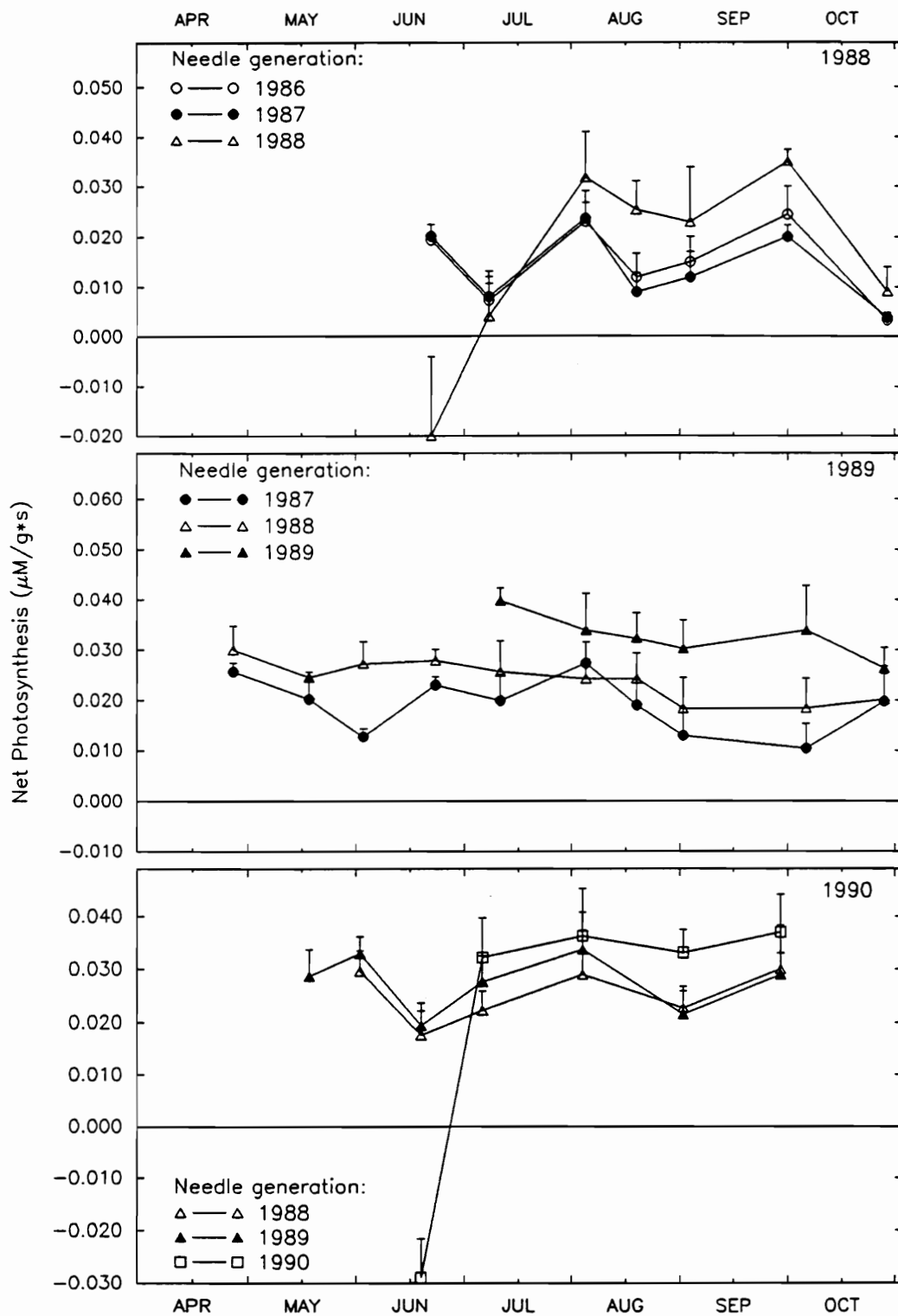


Figure 11. Net photosynthesis throughout three growing seasons in red spruce sapling needles of various age, lower site. Bars indicate standard deviations.

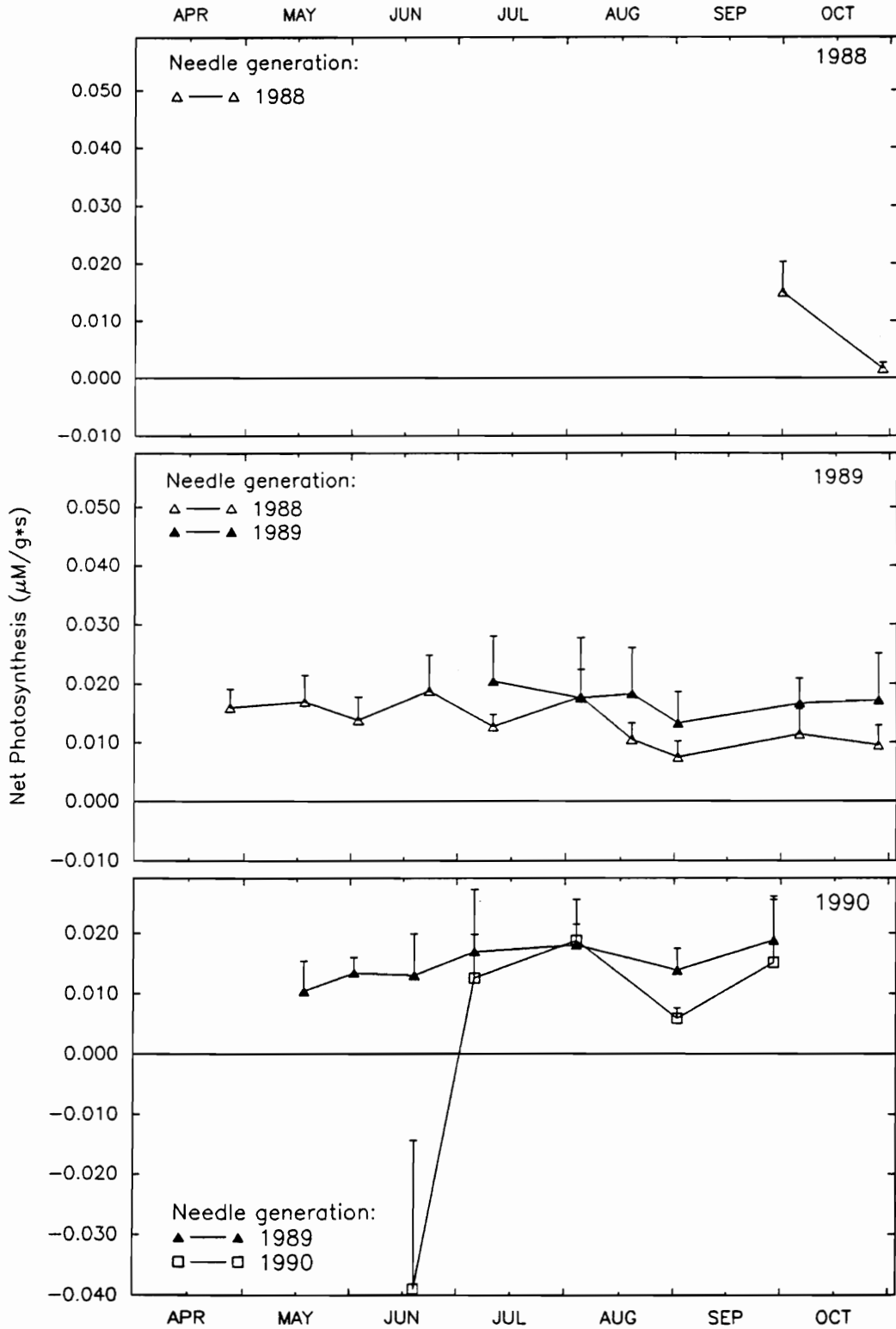


Figure 12. Net photosynthesis throughout three growing seasons in red spruce overstory tree needles of various age, lower site. Bars indicate standard deviations.

Needle conductance rates appeared higher in current-year needles than in one-year-old needles (Table 3, Figure 13). However, only in saplings on upper site was this difference significant.

Comparison between sites.

Average photosynthetic rates on both sites did not differ (Table 2, Figure 14 and 15). Needle conductance rates were apparently higher on the lower site (Table 3). Shoot water potential tended to be on average slightly more negative on the upper site (Table 4), although Figure 16 shows that there were no consistent differences on particular measurement dates.

Night respiration and predawn water potential

One-year-old foliage night respiration rates in trees of various sizes on the upper site differed significantly, with saplings showing the lowest rates and overstory trees - the highest rates (Table 5). Respiration rates in current-year needles were not significantly different, although a similar trend was apparent.

There were no significant differences between current-year and one-year-old foliage in the analyzed dataset. However, in data not used in the analysis (because it did not include mature trees), collected earlier in the 1989 growing season (mid-July vs. late August), current-year needles produced much higher respiratory rates than older needles.

Nighttime shoot water potential in overstory trees was significantly more negative than in saplings and seedlings (-0.56 MPa vs. -0.23 and -0.25 MPa).

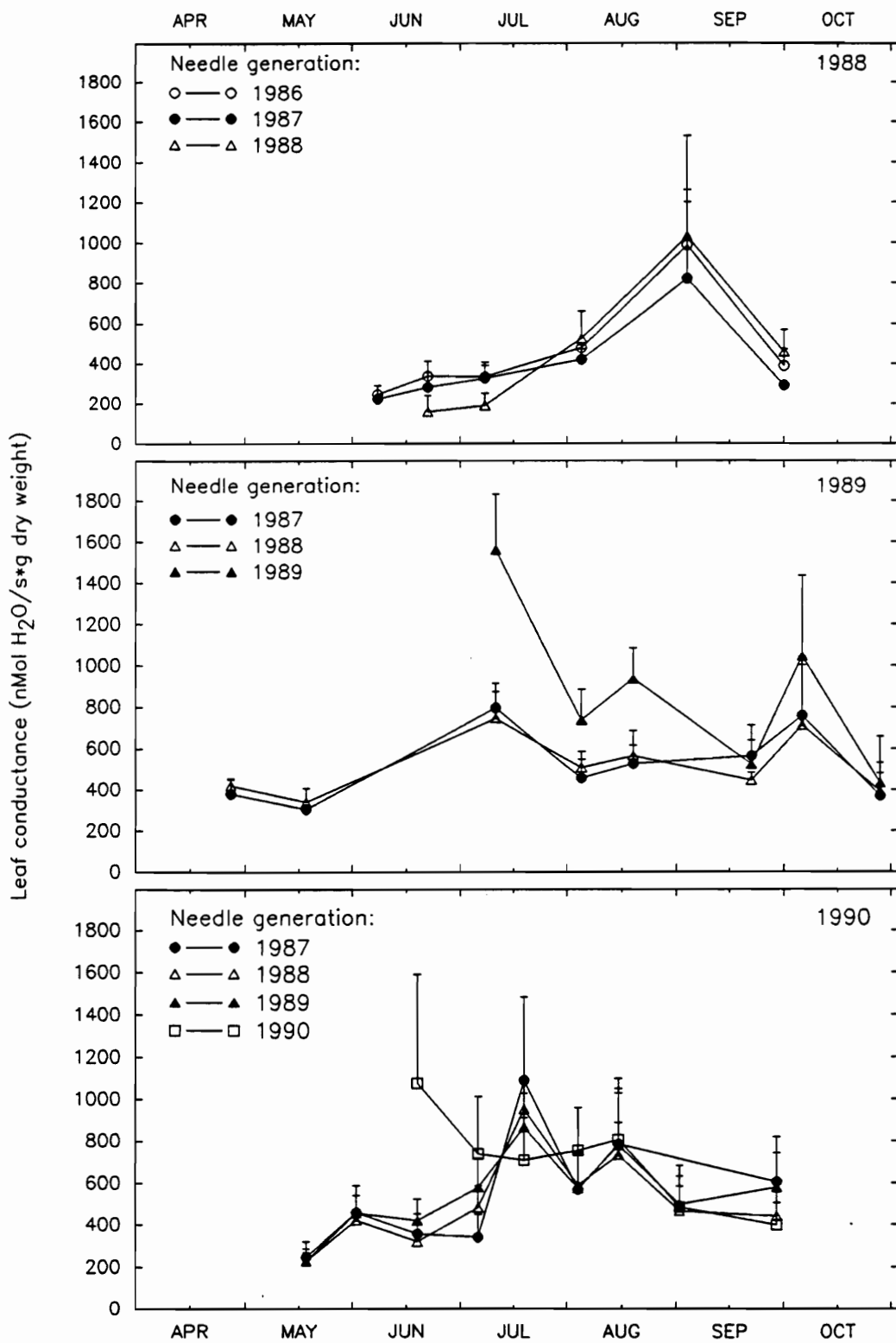


Figure 13. Needle conductance throughout three growing seasons in red spruce sapling needles of various age, upper site. Bars indicate standard deviations.

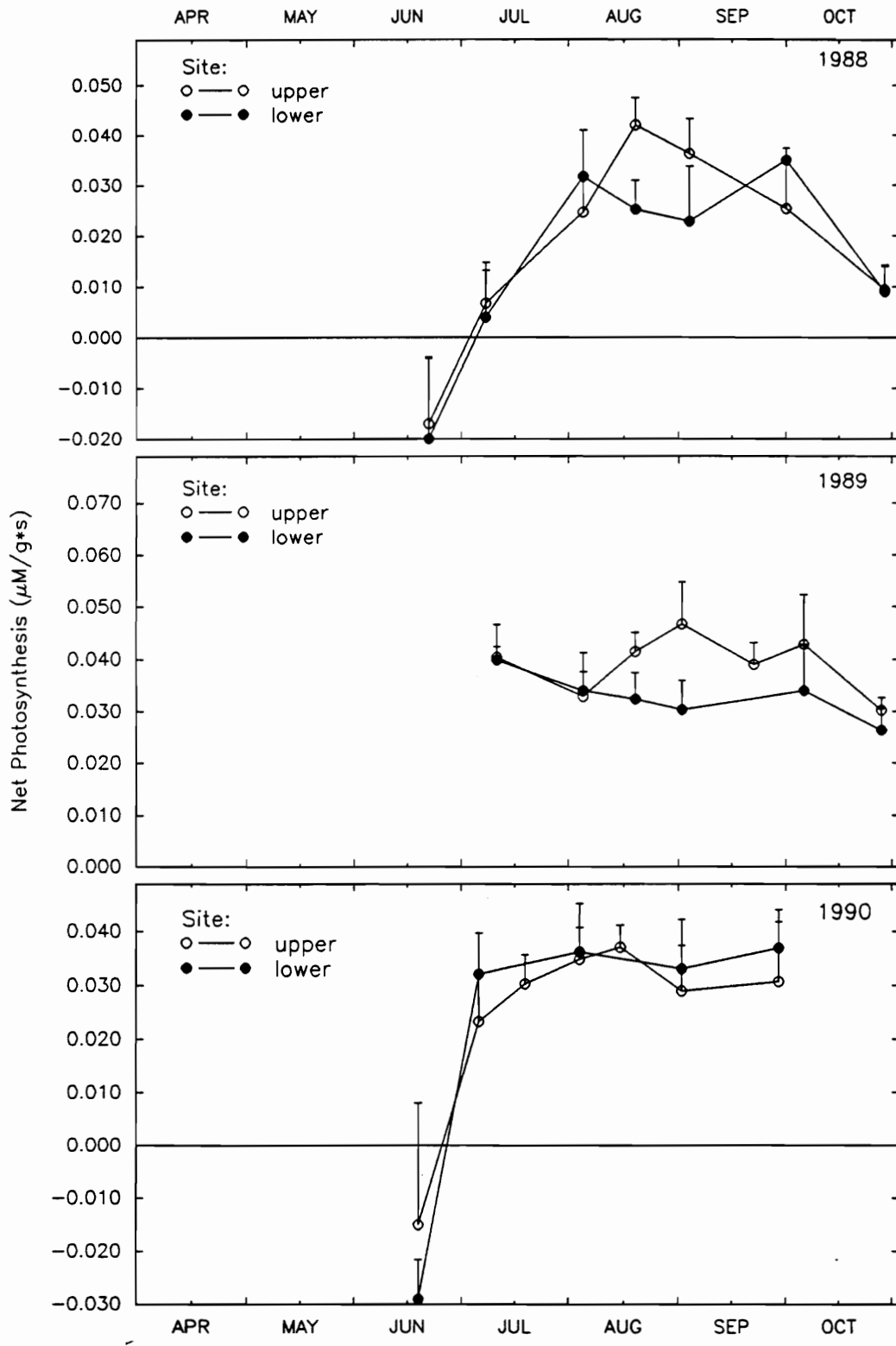


Figure 14. Net photosynthesis throughout three growing seasons in red spruce sapling current year needles on upper and lower sites. Bars indicate standard deviations.

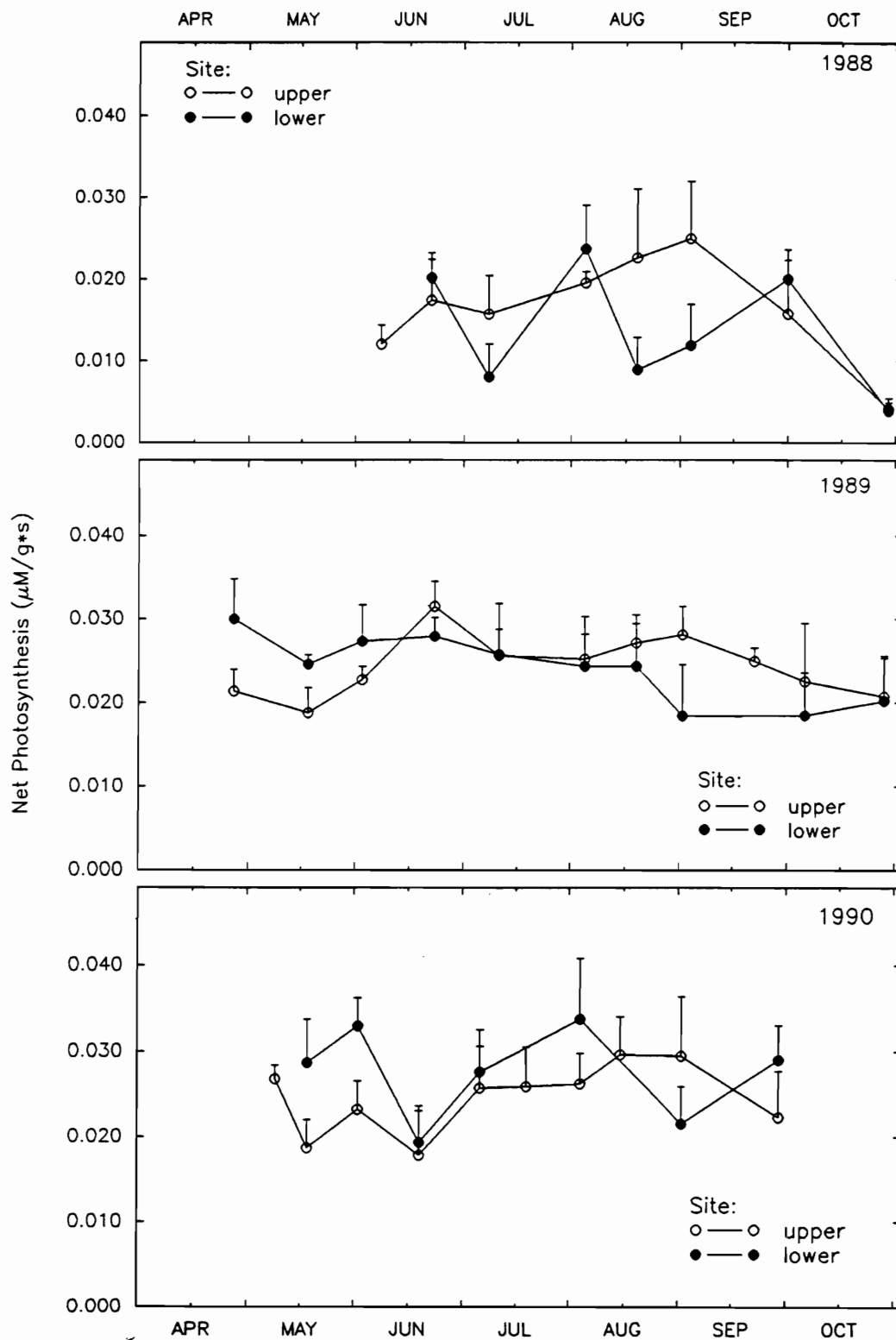


Figure 15. Net photosynthesis throughout three growing seasons in red spruce sapling one-year-old needles on upper and lower sites. Bars indicate standard deviations.

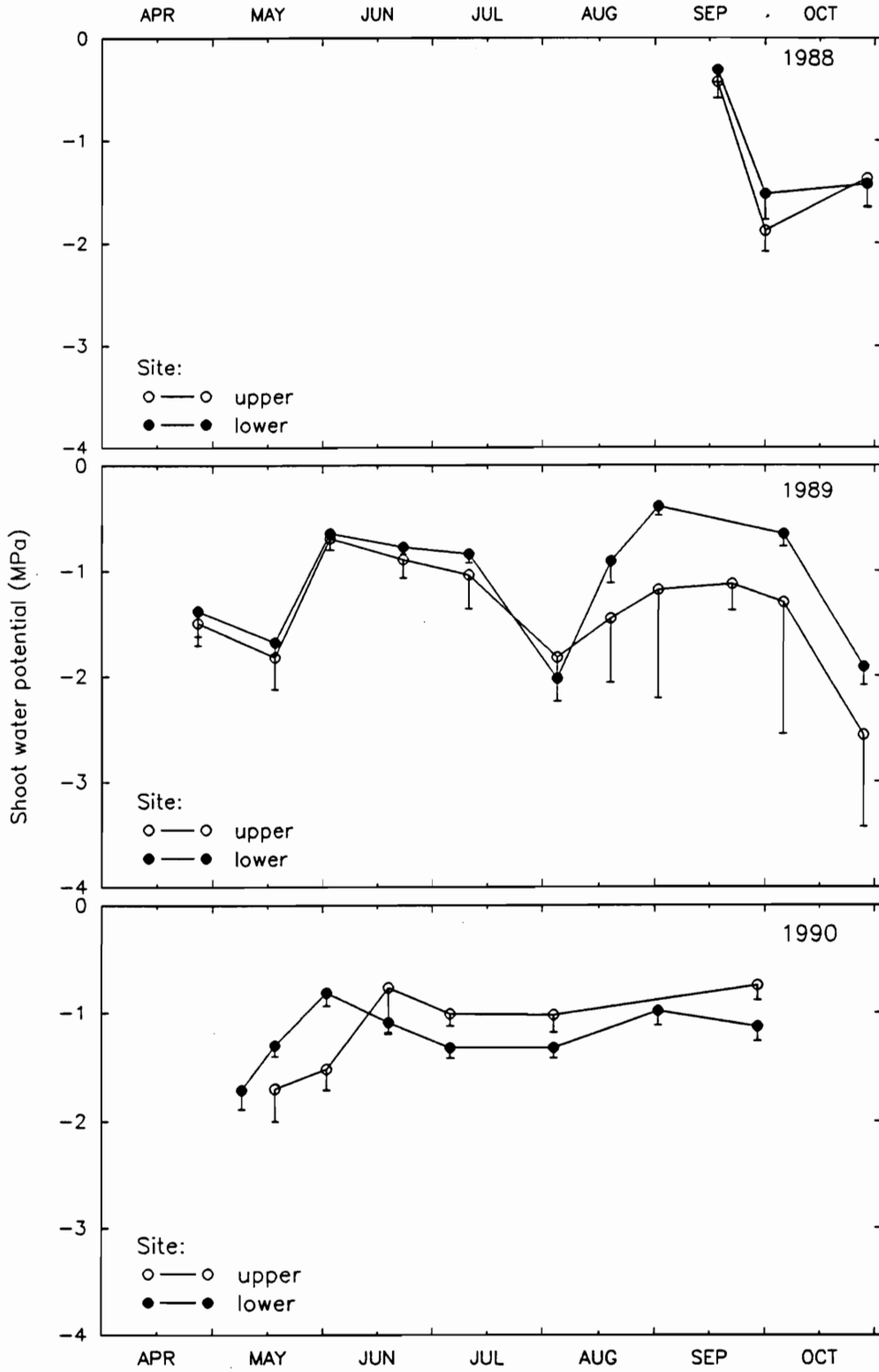


Figure 16. Shoot water potential throughout three growing seasons in red spruce overstory trees on upper and lower sites. Bars indicate standard deviations.

Table 5. Night respiration in various tree sizes and needle generations of red spruce, on the upper site on Whitetop Mountain, Virginia.

Foliage	Night respiration			Mean ¹
	Seedlings	Saplings	Overstory trees	
	($\mu\text{Mol/s}\times\text{g dry weight}$)			
current	0.00240	0.00231	0.00307	0.00259
1-year	0.00255 a	0.00178 a	0.00410 b	0.00281
2-year	-	0.00173	-	-
Mean ²	0.00247 a	0.00207 a	0.00360 b	0.00270

¹ only saplings and overstory trees included

² only current-year and one-year-old needles included

Means followed by different letters are not significantly different (LSD test, $p=0.05$).

Impact of ambient ozone on gas exchange parameters

The average ozone concentrations on measurements days (Table 1) showed little variability. Most of the values fell between 0.050 and 0.065 ppm, with extremes of 0.036 and 0.081 ppm.

No significant effect of ozone on photosynthesis and needle conductance was found when data pooled across tree sizes were analysed using regression analysis. However, the particular tree sizes did not behave uniformly.

Photosynthesis was significantly influenced by ambient ozone concentration only in overstory trees. This relationship can be described using the following equation:

$$Ps = 0.000246 \times O_3 + 0.000434 \times T + 0.00437 \times WP - 0.00263 \times NG$$
$$R^2 = 0.88$$

Where:

Ps - net photosynthesis

T - temperature inside cuvette (°C)

O₃ - ambient ozone concentration (ppb)

WP - shoot water potential (MPa)

NG - needle generation

Collinearity between cuvette temperature and ozone concentration was a concern, however, collinearity diagnostics indicated that it was not damaging. Correlation between ozone concentration and temperature was not very strong ($R^2 = 0.24$).

Only saplings showed significant effect of ozone on needle conductance, which can be described by the equation:

$$\text{Cond} = 591 - 3.18 \times O_3 + 7.16 \times RH - 86.9 \times NG$$

$$R^2 = 0.24$$

Where:

Cond - needle conductance ($\mu\text{Mol H}_2\text{O/s}\times\text{g dry weight}$)

RH - relative humidity inside cuvette (%)

O₃ - ambient ozone concentration (ppb)

NG - needle generation

Analysis of residuals revealed that negative photosynthetic rates (in flushing foliage) had a damaging impact on prediction and variance in regression analysis. Therefore, the above equations were obtained using data without these values. Dropping the new flush respiration data was legitimate, since their gas exchange was determined rather by plant phenology than current environmental conditions.

EDU study

Photosynthetic rates in seedlings treated with EDU were not significantly different from those in the control plants (Table 6). As demonstrated by the analysis of covariance using pre-treatment gas exchange data as a covariate, a tendency of the EDU seedlings to show higher rates was due not to the treatment effect, but to the inherent differences between the seedlings assigned to both groups (Fig. 17). Photosynthetic rate pattern after the EDU applications began, was not different from the pattern before the treatment started. Analysis of covariance revealed that there was no interactive effect of EDU treatment and ambient ozone concentration (average from 1000 to 1400 hrs) on net

Table 6. Photosynthetic rates and needle conductances in various needle generations of red spruce seedlings, sprayed with EDU or with pure water (control treatment), on Whitetop Mountain, Virginia.

Net photosynthesis			
Treatment	current-year	1-year-old	mean
($\mu\text{Mol/s}\times\text{g dry weight}$)			
EDU	0.0386	0.0289	0.0337
control	0.0337	0.0251	0.0294
mean	0.0362	0.0270	0.0315
Needle conductance			
($\text{nMol H}_2\text{O/s}\times\text{g dry weight}$)			
EDU	860	577	718
control	774	518	646
mean	817	548	682

No statistically significant differences were detected between the two treatments (t-test, $p=0.05$).

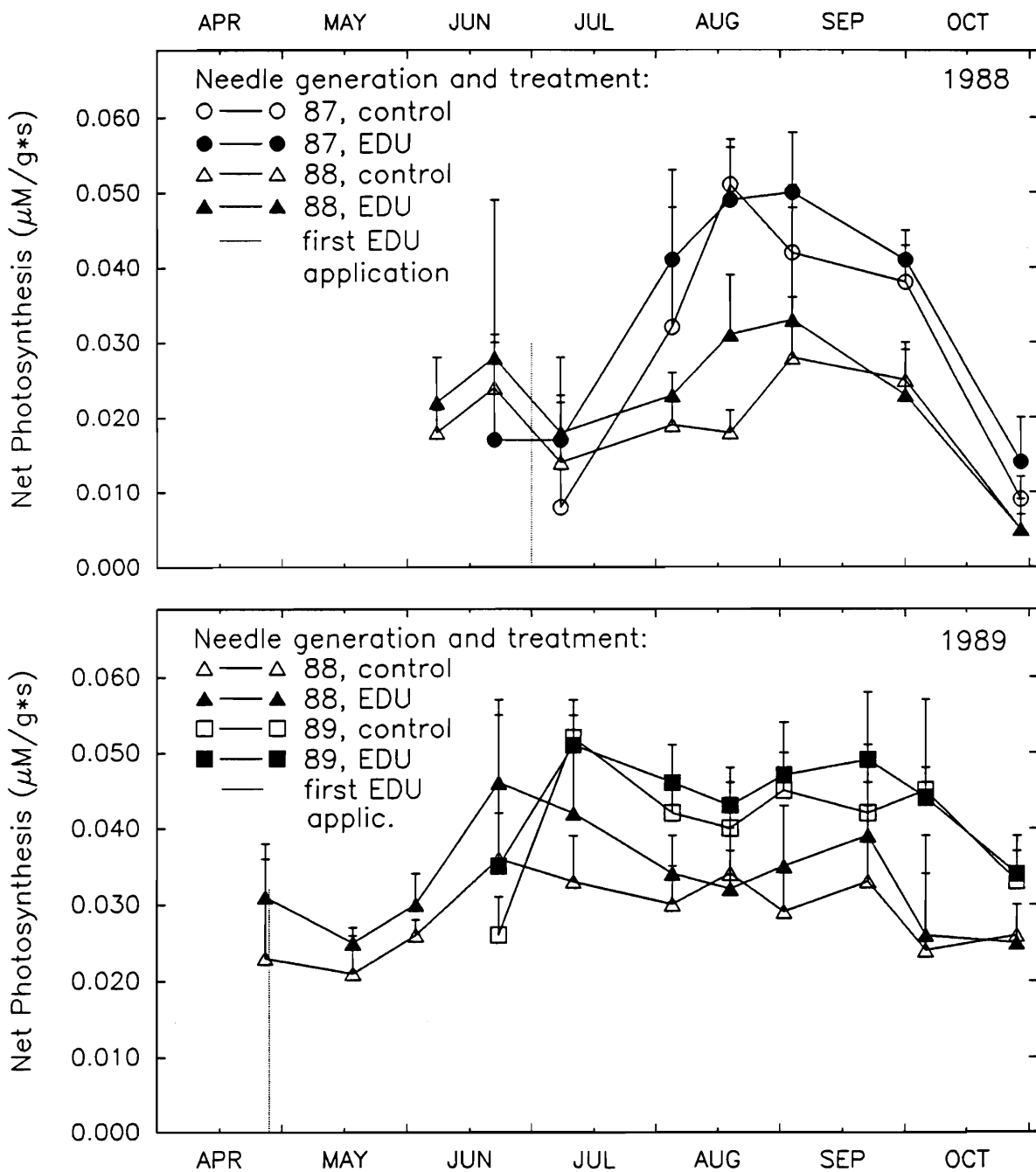


Figure 17. Net photosynthesis throughout two growing seasons in red spruce seedlings treated with EDU and in control seedlings, in current year and one-year-old needles. Bars indicate standard deviations.

photosynthesis. Needle conductance also remained unaffected by EDU treatment and EDU \times ozone interaction (Table 6).

DISCUSSION

The presented results reveal considerable differences in metabolism in red spruce trees of various age. Overstory trees showed the lowest photosynthetic rates, needle conductance, and the highest respiratory rates, which may be due to high degree of stress (e.g. increased exposure to wind) these trees are exposed to, as well as natural aging processes. Seedlings showed the highest photosynthetic and needle conductance rates. This is undoubtedly related to their vigorous growth. Night respiration in seedlings was lower than in overstory trees but higher than in saplings; possibly because of intensive metabolism of the small but rapidly growing plants. Saplings produced intermediate photosynthetic rates and the lowest respiratory rates. They are under much less exposure than the overstory trees are, yet do not undergo as fast a development as the seedlings do. Relative growth rate (RGR) is the highest in the early stages of tree's life. In absolute terms, the observed annual growth of branches was much smaller in overstory trees (1 to 4 cm) than in seedlings and saplings (6 to 10 cm). Hutcheson (personal communication) found that these lengths on the upper site averaged 2 cm and 10 cm, respectively.

The more negative average water potentials in overstory trees than in seedlings and saplings (difference from 0.2 MPa on the lower site to 0.3 MPa on the upper site - Table 3) can be largely explained on the basis of height dif-

ference. Zimmermann and Brown (1971) reported a maximum rate of decrease in shoot water potential with tree height of 0.015 to 0.020 MPa/m. The measured overstory trees were 15 to 20 meters tall, which could produce a drop in water potential of the mentioned above magnitude. The factors which can cause a higher drop (as was observed in some trees) are higher water stress in crowns of the overstory trees (wind, sun) and increased xylem hydraulic resistance. The magnitude and variability in shoot water potential measurements were similar to those reported by Andersen and McLaughlin (1991).

The particular tree sizes also differed in phenology, with seedlings breaking bud the earliest and overstory trees flushing the latest. This confirms a common observation that lower layers of forest vegetation break buds earlier than upper layers do.

Similar differences in gas exchange and water status parameters between trees of various sizes were found by Halpin *et al.* (1990). The authors compared twelve-year-old loblolly pine trees with seedlings and found that the seedlings showed higher needle conductance and less negative shoot water potentials than the big trees. The differences in photosynthetic rates were not consistent.

Variability in metabolic parameters in young and old trees may be due to the mentioned above environmental differences. However, inherent differences between them, e.g. different levels of growth regulators, may also play a role. Addressing this hypothesis would require, however, extensive and difficult studies, since little is known about changes in growth regulator levels with age in trees.

There were marked morphological differences between foliage in young and old trees. Overstory tree needles were more succulent and appeared to have a thicker cuticle than those in seedlings and saplings. Thus, it seems

that the specific leaf area was lower in overstory trees. If the gas exchange rates were expressed on a surface area basis instead of per unit of dry weight, the photosynthetic rates in overstory trees and young trees (seedlings and saplings) might differ less, if any.

Dry weight seems to be a practical basis to express gas exchange characteristics in the conifers with 'tridimensional' needles, such as red spruce. Precise determination of leaf area in such species is very time consuming. McLaughling *et al.* (1990) found that in comparison of red spruce trees growing on two sites, the outcome depended on whether dry weight or shoot length was used as a basis for calculating gas exchange parameters. Expressing gas exchange parameters per shoot length is not only simple, but also eliminates the influence of seasonal variability in carbohydrate levels. However, it may not be a very good basis for comparing trees with different needle dimensions.

Declining photosynthetic rates in older age classes may result from natural needle aging. Weikert *et al.* (1989) found that photosynthetic capacity in Norway spruce (*Picea abies* Karst.) one-year-old needles was higher in the branches where buds were removed than in the branches which were allowed to flush. The authors attributed this phenomenon to mineral deficiencies resulting from the transfer of nutrients from older needles to flushing new needles. In this study, the differences between net photosynthesis in needles of various age in saplings (only in saplings were needles older than one-year measured) were most pronounced between current flush and one-year-old needles. The differences among older needle generations were generally smaller. McLaughlin *et al.* (1990) found, to the contrary, that photosynthetic rates did not differ between current-year and one-year-old needles. In three-year-old needles it was, however, clearly lower. Unfortunately, the number of measurements taken by

McLaughlin and colleagues was rather small (five measurements, and only two of them included three-year-old needles). Hutcheson (pers. comm.) found that current-year needles showed higher net photosynthesis than older needles on the upper site, but did not differ on the lower site (in the same mature trees on Whitetop Mtn. as in this study).

The two sites, upper and lower, had different microclimatic conditions. The upper site was more exposed (located on the top, with a tree canopy which was breaking up) and this was reflected in a more negative shoot water potentials and lower needle conductance rates than on the lower site. Net photosynthesis, however, was not different. Hutcheson (pers. comm.) also found that needle conductance in mature trees tended to be higher on the lower site (although not consistently). Photosynthetic rates basically did not differ (except for current-year needles, which showed higher rates on the top). Interestingly, Hutcheson found that chlorophyll contents, cuticle contact angles, and cuticular wax contents did not differ between the sites.

The presented results provided an ecophysiological perspective on red spruce stand processes. One of the overstory trees on upper site showed decreasing photosynthetic rates (down to negative values) and shoot water potentials (down to - 3.5 MPa) during 1989 season. This tree, in fact, was dying, despite of apparently green needles, and it did not survive the following winter. As mentioned before, the upper site overstory trees were undergoing a decline, as the stand edge was retreating towards the experimental plot. Hutcheson (pers. comm.) found that flush length in overstory trees was much lower on the upper site than on the lower site. What was likely happening on the upper site, was in fact a regeneration process. Vigorously growing seedlings and saplings were replacing an old growth stand which was declining due to exposure and

age. On the lower site, the canopy was still basically intact and undergrowth was sparse. The above observations are consistent with what is known about dynamics of the high-elevation coniferous forests. Regeneration in these ecosystems follows disturbance, usually resulting from exposure of weakened old trees. It often proceeds in a wave-like pattern (Sprugel and Bormann 1981).

EDU has been shown to be an effective antioxidant protecting plants against ozone injury, possibly through stimulation of antioxidant enzyme systems (Whitaker *et al.* 1990, Brennan *et al.* 1990, Nowak and Craker 1989). However, this study did not detect any effect due to EDU treatment; either EDU was not effective, or the putative ozone stress was negligible.

As shown by regression analysis, net photosynthesis was significantly correlated with ambient ozone only in overstory trees, and the corresponding coefficient was positive. The possibility that photosynthetic apparatus is stimulated by moderate ozone concentrations (0.060 to 0.080 ppm) cannot be ruled out. Increase in net photosynthesis upon ozone fumigation has been reported in literature (Mahoney *et al.* 1984). It also may be due to the fact that higher ozone concentrations are associated with high temperature and irradiance (Krupa and Manning 1990), both factors conducive to high carbon assimilation. However, the conducted analysis suggests that the effect of ozone concentration was largely (although not entirely) independent from the effect of photosynthetic chamber temperature.

Needle conductance was found to be negatively correlated by elevated ambient ozone concentration in saplings. This was not, however, followed by any changes in photosynthesis and it is unclear whether this reduction would have any physiological consequences.

The ability to detect an effect of ozone on gas exchange in red spruce trees on Whitetop Mountain was reduced by a small range of ambient ozone concentrations. The bulk of ozone mean concentrations fell between 0.050 and 0.065 ppm, which is fairly representative for this location (Lefohn 1989). High ozone episodes may occasionally drive these levels above 0.100 ppm; however, in this study the highest average ozone concentration barely exceeded 0.080 ppm.

S U M M A R Y

Red spruce seedlings, saplings, and overstory trees, growing on Whitetop Mountain, were found to differ in their physiological characteristics. Overstory trees showed the lowest photosynthetic rates and needle conductance, the highest night respiration, and the most negative shoot water potentials. Seedlings produced the highest net photosynthesis and needle conductance, and intermediate night respiration. Saplings showed intermediate photosynthesis and needle conductance and the lowest night respiration rates. Shoot water potential in seedlings and saplings did not differ. In general, net photosynthesis decreased with needle age. On the upper site, a regenerative process was observed: stand canopy was declining, while young trees - seedlings and saplings were growing rapidly.

No deleterious influence of ozone on red spruce physiology was detected. Results presented in Chapters 1, 2 and 3 suggest that red spruce is resistant to the ambient concentrations of ozone in Southern Appalachians. The outcomes of this study do not supply any evidence to the contrary.

Summary and Conclusions

The present studies were undertaken to test the hypothesis of the deleterious impact of pollution on high-elevation red spruce-Fraser fir forests in Southern Appalachians. No effect of ozone exposure on growth and gas exchange of the seedlings was found in the laboratory fumigation study. However, net photosynthesis at saturating light was reduced in both species, and light compensation point was shifted upwards in spruce. Fraser fir seedlings of various seed sources showed different responses of CO₂ curve parameters to ozone at 0.150 ppm. There were indications that ozone exposure modified the cell wall modulus of elasticity in both species, although not consistently. It is difficult, however, to attribute any biological significance to these results in the absence of major effects of ozone on seedling growth. This experiment also indicated that conclusions based on a single growth cycle experiment may be misleading. Different conclusions concerning the impact of ozone on net photosynthesis in Fraser fir seedlings could have been drawn depending on the harvest date considered.

The winter hardiness study did not detect any effects of summer ozone exclusion on winter hardiness and subsequent growth of red spruce seedlings. It indicated that exclusion of cloudwater may benefit seedling growth in the following season, but only when the seedlings were overwintered in an exposed site. However, exclusion of both ozone and cloudwater did not result in improved performance of the seedlings. Keeping the seedlings in the exclusion chambers over summer clearly benefitted their winter hardiness and subsequent growth (strong chamber effect). The seedlings which overwintered in the open showed more symptoms of stress than the seedlings kept in the shadehouse, however, their growth in the subsequent growing season was not less than in the shadehouse seedlings. This experiment did not, however, reveal any strong and consistent effects of summer pollutant exposure on red spruce seedlings.

In the Whitetop Mountain ecophysiological study, the trees of various size showed clear differences in gas exchange, with overstory trees photosynthesizing at the lowest rates, and seedlings - at the highest. Overstory trees also showed more negative shoot water potential and higher night respiration than smaller tree sizes. Net photosynthesis tended to decrease with needle age. No deleterious effects of ambient ozone on red spruce physiology were detected in either the EDU experiment or from regression analysis of gas exchange and ozone monitoring data.

The Whitetop Mountain study also showed that direct scaling up from results of experiments conducted on seedlings to older trees is not legitimate, at least in the case of red spruce. When extrapolating from seedlings to saplings and mature trees, one needs to take into account the differences between physiology of trees of various developmental stage.

There were indications in the literature that ambient ozone may be harmful to high-elevation coniferous forests in the Appalachians (McLaughlin *et al.* 1985, Chevone *et al.* 1986, Lee *et al.* 1988). However, recent open-top chamber studies conducted both in the Northern Appalachians (Laurence *et al.* 1989, Kohut *et al.* 1990) and in the Southern Appalachians (Thornton *et al.* 1990, Pier *et al.* 1990, Thornton *et al.* 1991) did not provide evidence supporting the hypothesis that ozone is a major detrimental factor in the high-elevation spruce stand in the region. Fraser fir was not investigated except for the study by Tseng *et al.* (1987), which did not produce clear evidence of ozone's toxicity to this species.

The results presented in this dissertation also concur with the above findings, and it appears unlikely that, at current ambient ozone levels, the high-elevation red spruce and Fraser fir forests in Southern Appalachians are being affected. However, the budget and time constraints usually do not allow to test the role of pollution over the entire life-span of forest trees, and such chronic, very long-time effects cannot be ruled out.

The gas exchange data collected on Whitetop Mtn. provided an ecophysiological perspective on stand processes and may help to model the stand responses to environmental stresses. As confirmed by the ozone fumigation study, there is no direct link between the instantaneous photosynthetic rates and tree growth. (although trees with high growth rates may have higher photosynthetic rates than trees with low growth rates - compare seedlings and mature trees).

Conducting a set of studies - from a laboratory controlled ozone exposure experiment, through a field exposure experiment, to a field monitoring study,

provided a more comprehensive perspective on the investigated problem. The laboratory experiment allowed to test the ozone sensitivity of red spruce and Fraser fir seedlings under controlled conditions. The winter hardiness study tested the possible impact of oxidative stress and cloud water on red spruce seedlings in open-top chambers located in a natural habitat. Finally, the field gas exchange monitoring study provided a background picture to compare the exposure studies with. The regression analysis of correlation between field gas exchange rates and ambient ozone concentration was meant to verify the findings of the exposure studies concerning the impact of ozone on red spruce. No correlations were found which would indicate negative impact of ozone on gas exchange, thus confirming the findings of the exposure studies.

The presented results underscore a need for long-term, ecophysiological studies on the impact of air pollution on forests. Our knowledge about both stand processes and physiological mechanisms of pollutant action is still insufficient, and more basic research is needed. In author's opinion, forest science was not equipped to answer all the questions asked by the public, legislators, and administrators, in connection with forest declines (perceived or real) of the eighties. This was true both in Europe and North America. However, our body of knowledge was considerably expanded during these years and we have good foundations to build on in the future.

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Vita

Piotr (Piotrek) Bohdan Tyszko was born on January 9, 1960, in Warsaw, Poland. He completed his secondary education in the Juliusz Slowacki High School (nr. VII) in Warsaw. In 1979 he enrolled to the Warsaw Agricultural University (Szkola Glowna Gospodarstwa Wiejskiego), Department of Forestry. He graduated with a degree of Master of Science in Forestry in year 1986. From 1985 to 1987, Piotrek worked as a laboratory technician at a tree physiology division of the Institute of Plant Biology at Warsaw Agricultural University. In 1987 he undertook doctoral studies in forestry at Virginia Tech (Virginia Polytechnic Institute and State University) in Blacksburg, Virginia.