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FOREWORD

The Northern Global Change Program meeting was held March 14-16, 1995, in Pittsburgh, Pennsylvania. Its purpose was to chronicle the research activities of the Northern Global Change Program over the past five years, and outline the implications of these research results for natural resource management. We thank the authors for their participation and for promptly submitting their papers in both paper and electronic form.

EFFECTS OF CALCIUM FERTILIZATION AND ACID MIST ON CALCIUM CONCENTRATION AND COLD
TOLERANCE OF RED SPRUCE NEEDLES

G. R. Strimbeck¹, David R. Vann², and Arthur H. Johnson²

Several studies have shown that exposure to acid mist impairs cold tolerance of red spruce foliage, predisposing it to winter injury, which appears to be a major factor in the decline of montane populations of the species. Other studies have shown increases in calcium (Ca) concentration in canopy throughfall in montane spruce-fir forests, and decreases in foliar Ca concentration associated with exposure to acid mist. Studies of other plant species suggest that Ca may play a role in the development of cold tolerance or in stress response to cold. These considerations have led to the specific hypothesis that the reduction in cold tolerance associated with exposure to acid mist is caused by reduction in foliar Ca. To test this hypothesis, we applied Ca fertilizer to trees in a 33 year old red spruce provenance plantation in Colebrook, NH, and used branch chambers to expose individual branches to mist of known composition.

Ca fertilizer was applied during the 1992 and 1993 growing seasons. It was applied on the ground in a 1 m diameter circle around the base of designated trees, during bud break, in two stages, two weeks apart: CaCO₃ at a rate of 200 kg ha⁻¹ Ca, and Ca Cl₂ at a rate of 800 kg ha⁻¹ Ca. To test the simple effect of fertilization, we determined Ca concentration (µg g⁻¹) and cold tolerance of foliage collected in December 1992, after the first round of fertilization. Fertilization significantly increased Ca concentration of current year foliage from a mean of 902 to 1277 µg g⁻¹ in the unfertilized and fertilized groups, respectively (n = 12 trees in each group). There were no differences in cold tolerance attributable to fertilization (Table 1).

Table 1. Statistical analysis of cold tolerance data. Probabilities from analyses of variance of critical temperature (1992) or Tm (1993-94). Separate analyses were conducted for each date. Values significant at p < 0.05 are given in boldface. * 8 degrees of freedom for February 1994; 2 trees were omitted due to severe injury.

Source	df	Dec 92	df	Oct 93	Dec 93	Feb 94
Fertilization	1	0.1082	1	0.931	0.136	0.278
Tree[Fert]	22	0.0002	10*	0.291	0.003	0.007
Mist			2	0.549	0.073	0.032
No chamber vs. chamber			1	0.302	0.318	0.825
pH 3.2 vs. pH 5.6			1	0.744	0.038	0.010
Mist * Calcium			2	0.491	0.553	0.204

The design of the final stage of the experiment was a split-plot, with trees as whole plots, fertilization as whole plot effect, and mist treatment as a subplot effect. During the 1993 growing season, branch chambers were used to expose two branches on each of 12 trees, 6 fertilized and 6 unfertilized, to either pH 3.2 or pH 5.6 mist. The following autumn and winter, we determined Ca concentration and cold tolerance of foliage from treated and untreated (no chamber) branches from each tree in October, December, and February.

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There were no significant effects of any treatment, including fertilization, on Ca concentration. Mean Ca concentration of the unfertilized group increased after the second round of fertilization (Figure 1). Trees in the plantation were closely spaced, and roots of unfertilized trees may have extended into and absorbed Ca in fertilized areas. While branches misted at pH 5.6 on fertilized trees had substantially more calcium than any other group, this difference was not significant at $\alpha = 0.05$ in either the planned analysis of variance or *a posteriori* multiple comparison procedures.

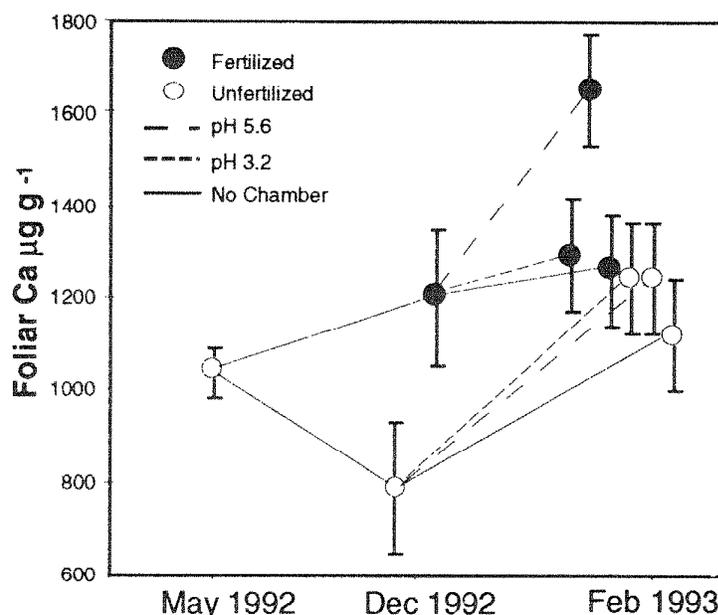


Figure 1. Changes in foliar Ca concentration over the course of the experiment. The May 1992 point is the mean \pm standard error of three branches in each of 54 trees. For the later dates error bars are standard error estimates from analysis of variance. The December 1992 points each represent 12 trees, and the December 1993 points represent 6 treated branches.

We found no significant difference in cold tolerance due to any treatment for foliage collected in October, and no significant difference attributable to Ca fertilization or its interaction with mist treatment on any date (Table 1). In the winter, branches exposed to pH 3.2 mist were significantly less cold tolerant than those exposed to pH 5.6 mist, with a mean difference of 4.4 °C in December and 5.2 °C in February (Figure 2). Simple correlation between Ca content and cold tolerance was weak ($r^2 = 0.22$) but significant ($p < 0.01$, $n = 36$), with no strong influence of either mist or fertilization on the relationship.

The clear effect of acid mist on cold tolerance lends strong support to the conclusions of earlier studies that exposure to acid mist can significantly impair cold tolerance. Our results do not support the hypothesis that this effect is mediated by foliar leaching of Ca. The lack of strong effects of mist on Ca concentration and of fertilization on cold tolerance, and the weak and inconsistent correlation between Ca concentration and cold tolerance all argue that the two responses are not directly related. However, results are uncertain because of the poor separation of Ca concentration in fertilized and unfertilized groups in the second year of the study.

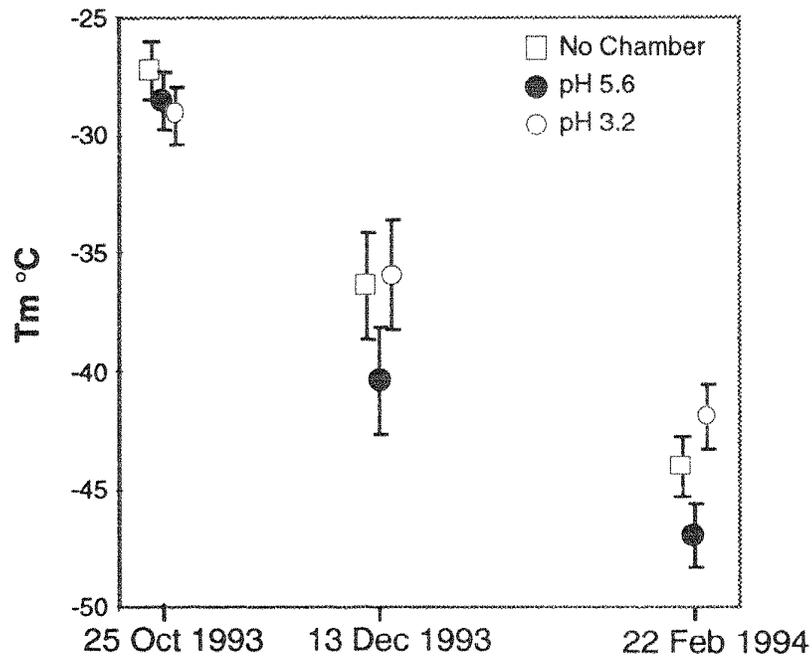


Figure 2. Mean T_m (an estimate of cold tolerance) by mist treatment on three dates. Error bars are standard error estimates from analyses of variance for each date.

OZONE-INDUCED ACCELERATED FOLIAR SENESCENCE: IMPLICATIONS FOR TOXICITY AND COMPENSATION

Eva J. Pell¹, Bryan W. Brendley², and Judith P. Sinn²

Abstract: Two-year-old seedlings of black cherry, *Prunus serotina* Ehrh., northern red oak, *Quercus rubra* L. and sugar maple, *Acer saccharum* Marsh., and ramets of hybrid poplar, *Populus maximowizii* x *trichocarpa*, clone 245 were grown in eight charcoal-filtered open-top chambers per species. Half the chambers, per species, received 0.08 $\mu\text{L L}^{-1}$ O_3 from 1000 to 1800 h each day of the growing season. Accelerated foliar senescence and associated O_3 -induced loss in Rubisco were observed in older foliage of hybrid poplar and black cherry. Younger leaves were less responsive to O_3 , and in the case of hybrid poplar actually exhibited signs of compensation to the stress. Sugar maple and northern red oak were less responsive and exhibited no signs of accelerated senescence. The relevance of the latter response is considered in the context of the indeterminate and determinate growth habits of these two groups of plant species.

INTRODUCTION

Ozone (O_3) has been associated with the induction of accelerated foliar senescence in many plant species (Reich & Lassoie, 1985; Pell, Eckardt & Enyedi, 1992). During normal leaf development, expansion of the lamina is associated with an increase in the concentration of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). Once the leaf has reached full expansion, synthesis of Rubisco becomes of less consequence and the protein levels decline until senescence (Dalling, 1987). Because Rubisco is the protein responsible for fixing CO_2 during the Calvin Cycle, decline in this enzyme is linked with a reduction in net photosynthesis. We have shown that O_3 -stressed plants exhibit a more rapid loss in Rubisco protein coupled with a more rapid decline in net photosynthesis (Pell, Eckardt & Glick, 1994); ultimately the leaf becomes chlorotic and abscises earlier than non stressed counterparts. Previously, we demonstrated that the accelerated loss in Rubisco can be attributed, in part, to enhanced degradation of the protein (Eckardt & Pell, 1994). In addition, Reddy et al. (1993) have shown that O_3 induces a reduction in mRNA for the large and small subunits of Rubisco; how this reduction in transcript relates to possible reduction in synthesis of Rubisco remains to be determined.

The accelerated loss of photosynthetic tissue has inherently negative implications for the plant. However, plants possess compensatory mechanisms to minimize the adverse effects of stress. We have associated O_3 -accelerated reduction in net photosynthesis and Rubisco content of older leaves of trembling aspen (*Populus tremuloides*) with increases in these parameters, above the level of control tissue, in younger leaves (Pell et al., 1994). The ability of plants to compensate for injury by accelerated foliar senescence as just described, is dependent on the capacity for plants to initiate new foliage. Thus, indeterminate species would have options unavailable to species that exhibit determinate or fixed growth habit. In this study we explored the ability of O_3 to induce accelerated senescence in plants with different rates of growth and with different growth habits viz. hybrid poplar, and black cherry, both indeterminate as young plants; and northern red oak, and sugar maple, relatively determinate as seedlings.

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METHODS

Cuttings of hybrid poplar clone 245, and two-year-old seedlings of black cherry, northern red oak and sugar maple were cultured as described elsewhere (Pell et al., 1995); plants received a nutrient supplement of 3.53 g l⁻¹ Osmocote (N:P:K 14:14:14; Sierra Chemical Co., Milpitas, CA) at planting. All plants were grown in open-top chambers receiving charcoal-filtered air (Pell et al., 1993); in 1993 and 1994 O₃ concentrations in these chambers averaged 0.04 μl l⁻¹ during the exposure period. For each species, four replicate chambers served as controls while an additional four chambers received supplemental O₃ from 1000 to 1800 h each day resulting in a summer average of 0.08 μl l⁻¹. Experiments were conducted in 1993 and 1994. Most of the data reported herein was collected in 1993 with the results in 1994 supporting those of 1993. In addition, in 1992 a baseline study was conducted using only plants growing in charcoal-filtered air. Ozone exposures were conducted from June 24 - September 22, 1993 and June 24 - September 27, 1994.

When hybrid poplar and black cherry plants were approximately 18 cm in height, and when the second flush of sugar maple and northern red oak was initiated, a newly emergent leaf on each plant in every chamber was tagged. Once every two weeks throughout the growing season, leaves were sampled from two plants per chamber. In the case of hybrid poplar and black cherry, a second leaf 17 and 25 leaves above the first leaf tagged, respectively, were also tagged at emergence. Subsequently these leaves were sampled along with the leaf positioned lower in the canopy.

Net photosynthesis and leaf conductance were measured by nondestructive gas exchange analysis with a Li-Cor 6200 closed-loop photosynthesis system (Li-Cor, Inc., Lincoln, NE) as described by Pell et al. (1992). After the analysis samples were harvested in the field, frozen in liquid nitrogen and stored at -80°C. Rubisco quantity was determined as described by Eckardt and Pell (1994).

Statistics

Data for each species were analyzed separately by analysis of variance (ANOVA) and significance was accepted at the P ≤ 0.05 level (SAS Institute Inc., 1985).

RESULTS

In 1992 we followed the performance of foliage of the four species in the absence of O₃. Ozone concentration from 1000 to 1800 h averaged 0.03 μl l⁻¹ for the growing season. As we have previously reported (Pell et al., 1994), as the leaves of the indeterminate species (hybrid poplar and black cherry) aged, there was a rapid increase in concentration of Rubisco followed by a precipitous decline. In contrast, northern red oak and sugar maple foliage exhibited an increase in concentration of Rubisco followed by a prolonged plateau. Gas exchange data profiles were similar in all four cases (data not shown).

The first (older) leaf of hybrid poplar to be sampled, exhibited a significant O₃-induced decline in net photosynthesis that paralleled the decline in Rubisco concentration and preceded a reduction in stomatal conductance (Fig. 1 A-C). The second (younger) leaf to be sampled, exhibited an initial increase in net photosynthesis, stomatal conductance and Rubisco content in response to O₃, followed by a decline (Fig. 1 D-F).

The older leaf of black cherry sampled exhibited a significant decline in net photosynthesis, stomatal conductance and Rubisco quantity in response to O₃. Accelerated senescence was observed in these leaves (Fig. 2 A-C). The younger leaf also sustained a reduction in net photosynthesis and stomatal conductance, but these responses were observed after a far longer O₃ exposure than was necessary to elicit a similar response in older leaves (Fig. 2 D & E). Accelerated senescence was not observed in these younger leaves, and a significant reduction in Rubisco concentration was observed only at the last sampling point (Fig. 2F).

Northern red oak did exhibit a significant reduction in net photosynthesis coupled with a drop in stomatal conductance in response to O₃ stress (Fig. 3 A & B). Neither accelerated foliar senescence nor significant changes in Rubisco content were detected (Fig. 3C).

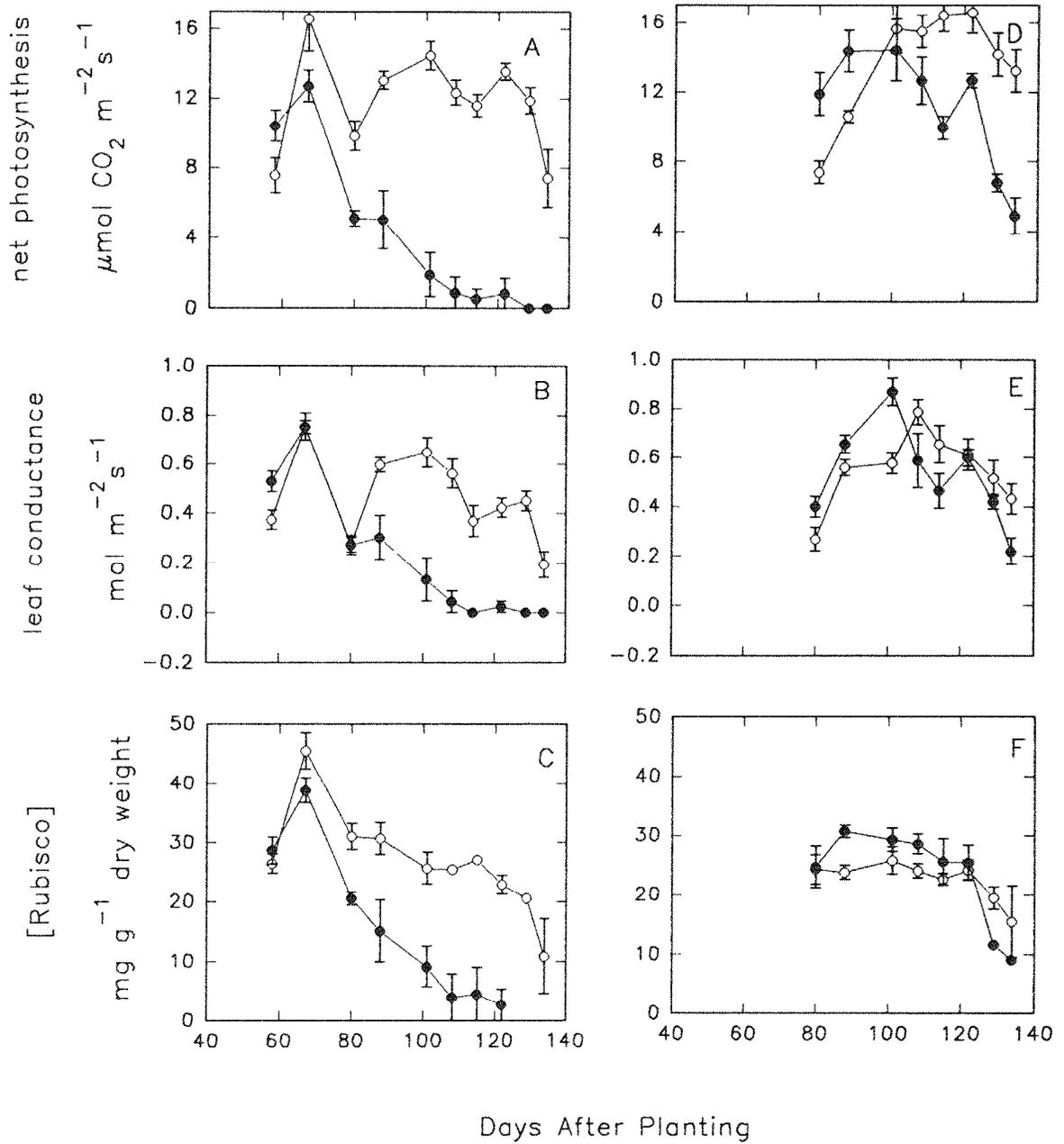


Figure 1. Effect of O_3 on net photosynthesis, leaf conductance and Rubisco content of hybrid poplar foliage, from emergence to senescence. (A-C) All samples derive from the two leaves that emerged when the plants were 18 cm tall. (D-F) All samples derive from the two leaves that emerged at a position 17 leaves above the first leaf sampled. (o) Plants grown in charcoal-filtered open-top chambers. (•) Plants grown in open-top chambers supplemented with $0.08 \mu\text{L L}^{-1} \text{ O}_3$ for 8h per day. Each value is the mean of eight and four observations \pm standard error of the mean, for gas exchange and Rubisco measurements, respectively.

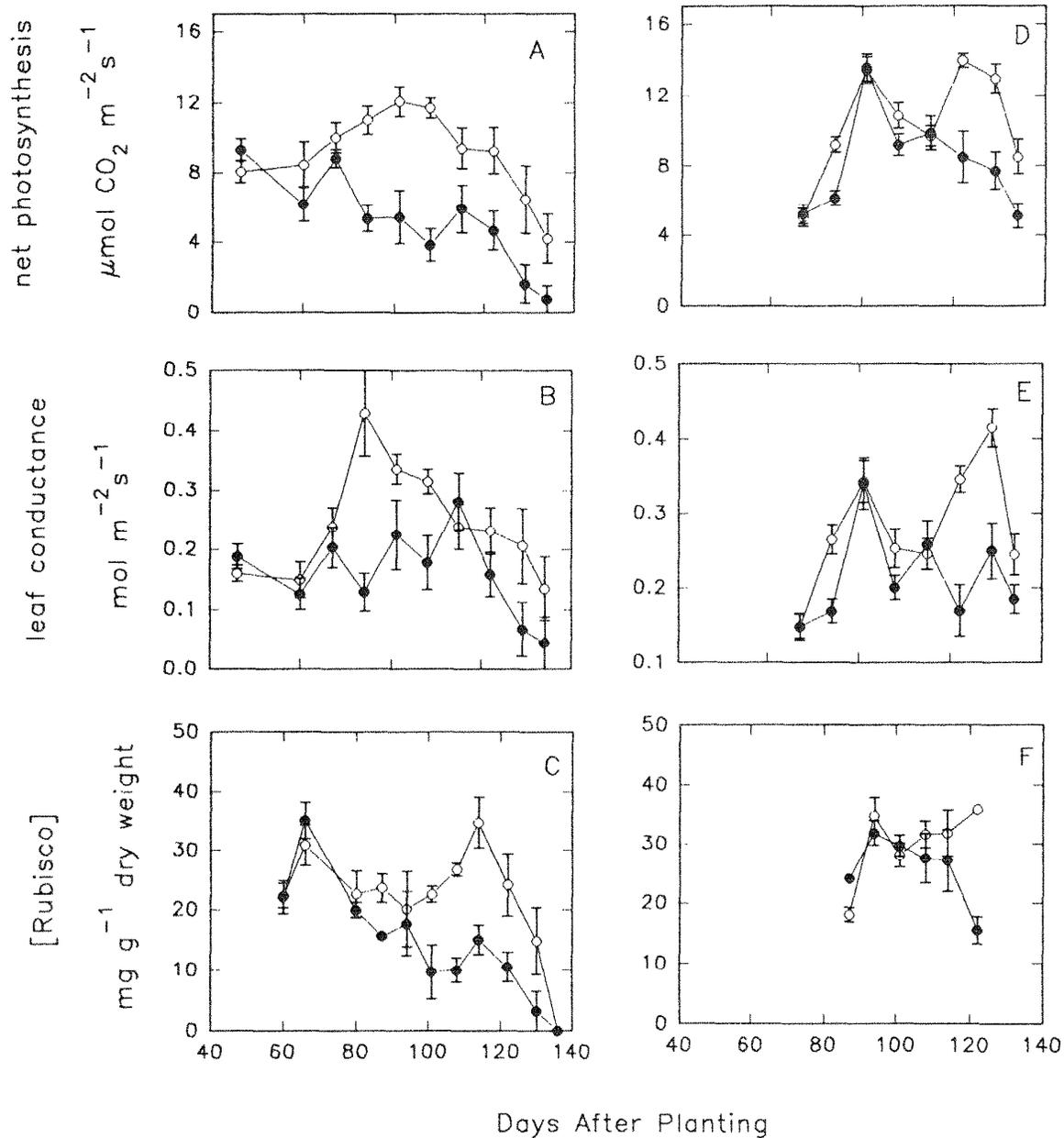


Figure 2. Effect of O_3 on net photosynthesis, leaf conductance and Rubisco content of black cherry foliage, from emergence to senescence. (A-C) All samples derive from the two leaves that emerged when the plants were 18 cm tall. (D-F) All samples derive from the two leaves that emerged at a position 17 leaves above the first leaf sampled. (○) Plants grown in charcoal-filtered open-top chambers. (●) Plants grown in open top chambers supplemented with $0.08 \mu\text{L L}^{-1} O_3$ for 8h per day. Each value is the mean of eight and four observations + standard error of the mean, for gas exchange and Rubisco measurements, respectively.

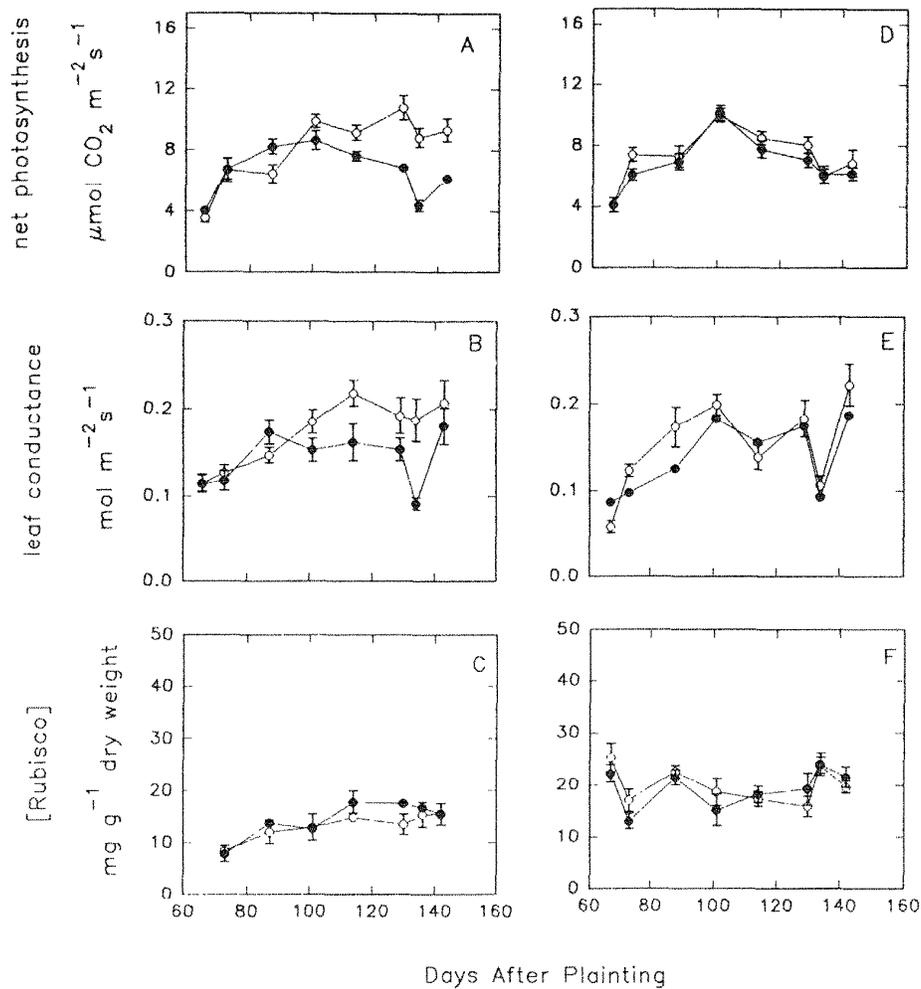


Figure 3. Effect of O₃ on net photosynthesis, leaf conductance and Rubisco content of foliage from the second flush of growth of northern red oak (A-C) and sugar maple (D-F). The leaf was analyzed from emergence to senescence. (○) Plants grown in charcoal-filtered open-top chambers. (●) Plants grown in open top chambers supplemented with 0.08 $\mu\text{L L}^{-1}$ O₃ for 8h per day. Each value is the mean of observations \pm standard error of the mean, for gas exchange and Rubisco measurements, respectively.

When sugar maple seedlings were stressed by O₃, we detected no significant effects on net photosynthesis, stomatal conductance or Rubisco content (Fig. 3 D-F). Accelerated senescence of foliage was not detected.

DISCUSSION

The resource allocation strategies of the indeterminate species were clearly different from the more determinate species examined in this study. Hybrid poplar and black cherry leaves demonstrated rapid synthesis of protein as exhibited by the brief residence time of the Rubisco peak (Figs. 1 & 2). Both species exhibited less responsive younger foliage. In fact younger leaves of hybrid poplar actually performed better in plants under O₃ stress. These

data are supported in a previous study with trembling aspen (Pell et al., 1994). Elsewhere Brendley et al. (1994) have reported that in O₃-stressed plants, younger foliage actually synthesized higher rates of Rubisco than were observed in non stressed plants. Hybrid poplar may be more effective in compensating for O₃ than is black cherry because of the higher rate of growth of the former species. Rapid rate of growth may lead to the greatest rate of senescence; it will also allow for nitrogen to be recycled most rapidly as Rubisco degrades. Thus, re utilization of nitrogen for a compensatory function in younger leaves will occur most readily in the plants with the most rapid growth rates.

Neither northern red oak nor sugar maple exhibited accelerated senescence or changes in Rubisco content following O₃ exposure (Fig. 3). Northern red oak did show a reduction in net photosynthesis; this response seemed to be closely associated with a reduction in stomatal conductance (Fig. 3).

We conclude that O₃-induced reduction in net photosynthesis may be regulated in part by a reduction in Rubisco content as shown for hybrid poplar, but it can occur without changes in this protein. Accelerated senescence seems to be tightly linked to the reduction in Rubisco content. The latter response seems to be associated with the growth habit of plants. In indeterminate species where nitrogen re utilization is a possible strategy for survival, accelerated senescence and loss of Rubisco provide a viable mechanism of compensation. For more determinate species this type of nitrogen recycling is not possible. Therefore, for plants with a determinate growth habit, accelerated senescence may be a less likely response to stress. Whether recycling of nitrogen within a leaf occurs as a result of the stress has not been determined.

ACKNOWLEDGMENTS

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ROOT GROWTH AND RESPIRATION OF ASPEN IN RESPONSE TO OZONE AND ELEVATED CARBON
DIOXIDE

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The response of tree root systems to interacting environmental stress is poorly understood in comparison to knowledge of above-ground organs. This research investigates the effect of ozone (O_3), elevated carbon dioxide (CO_2) and their combination on root system growth and respiration. Adventitiously rooted cuttings of three aspen clones (271, O_3 tolerant; 216, intermediate; 259, O_3 sensitive) were grown in 7 l pots containing peat:sand:vermiculite (2:1:1). Plants were placed in treatment chambers and exposed to either ambient air (control), ambient + 350 ppm CO_2 , 160 ppm O_3 (8 h daily), elevated CO_2 and O_3 . Root respiration was measured by sealing the entire pot into the measurement cuvette of an open-flow gas exchange system. Roots and soil were then separated; roots were dried and weighed, and soil was returned to pots and remeasured to estimate heterotrophic soil respiration. After 12 weeks, O_3 treatment caused up to a 48 percent decrease in root dry weight with a corresponding 53 percent decrease in root system respiration rate ($\mu\text{mol plant}^{-1} \text{s}^{-1}$) compared with control treatments. Changes in root dry weight and respiration rate generally followed the O_3 sensitivity rankings of the clones. In contrast, elevated CO_2 vs. control caused up to a 63 percent increase in root dry weight and a 47 percent increase in root system respiration; again there were big clonal differences in responses. Compared to the control treatment, there was no net change in root weight due to the combined O_3 and elevated CO_2 treatment yet root system respiration declined slightly. When specific root respiration rate ($\mu\text{mol g}^{-1} \text{s}^{-1}$) was calculated, there were no consistent treatment effects; however specific root respiration declined as plants aged, and interesting clonal differences were also observed. For clones 216 and 259, specific root respiration generally increased with either O_3 or elevated CO_2 in 12-week-old plants, but for clone 271 specific root respiration decreased with treatment. These results show that above-ground environmental stress affects the growth and physiology of aspen roots in complex ways, and the particular response obtained has a strong genetic component.

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EFFECTS OF OZONE AND CO₂ ON THE GROWTH AND PHYSIOLOGY OF ASPEN

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During the past three years, we have examined the effects of ozone (O₃) and carbon dioxide (CO₂), alone and in combination, on the growth and physiology of trembling aspen (*Populus tremuloides* Michx.). We have conducted several single growing season exposures of potted plants and a three growing season exposure with trees planted in the ground. All studies have been conducted in open-top chambers. Our research demonstrated that aspen is highly sensitive to ozone and that there are strong genotypic differences in response to ozone. Seasonal exposures of 70 to 100 ppm-h have a significant negative impact on height, diameter, leaf and branch retention, and above ground biomass. The O₃ sensitivity appears stable as aspen trees reach flowering age. CO₂ administered at 150 ppm above background levels did not compensate for the adverse ozone effects. Photosynthesis measurements over all growing seasons and with multiple genotypes suggest that CO₂ may increase the O₃ sensitivity of otherwise tolerant aspen clones. We are currently developing an unchambered O₃ exposure system to more closely simulate forest conditions.

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INTERACTING EFFECTS OF OZONE AND CO₂ ON GROWTH AND PHYSIOLOGICAL PROCESSES IN
NORTHERN FOREST TREES

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Globally, surface-level concentrations of both CO₂ and ozone (O₃) are increasing annually. Because many studies have shown beneficial effects of increasing CO₂, predictions have been made that elevated levels of CO₂ would compensate for growth decreases caused by O₃. For the past two years, we have been examining the interaction of O₃ and CO₂ on trembling aspen (*Populus tremuloides*) and eastern white pine (*Pinus strobus*) in open-top chamber studies involving both plants in pots and plants growing in the ground.

After two seasons of exposure to elevated ozone, alone or in combination with elevated CO₂ (ambient plus 150 ppm), soil-grown aspen and eastern white pine trees are exhibiting different response. While neither of the two pine seed sources has been negatively affected by ozone, significant negative effects of O₃ have been found for two aspen clones differing in O₃ tolerance. The negative impact of ozone was not compensated by CO₂ and for some physiological responses such as photosynthesis, stomatal conductance, chlorophyll content and leaf abscission, a significant negative interaction has been demonstrated for O₃ plus CO₂ treatment. Second-year growth and biomass measurements appear to be following our physiological measurements. Crown architecture has also been altered by the O₃ and CO₂ combination.

In addition, elevated CO₂ appears to alter the sensitivity of the tolerant aspen clone, making it more sensitive to O₃, as determined both by gas exchange and biomass measurements. The implications for these findings for modeling and response predictions will be discussed.

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THE CHRONIC RESPONSE OF YELLOW-POPLAR AND EASTERN WHITE PINE TO OZONE AND
ELEVATED CARBON DIOXIDE: THREE-YEAR SUMMARY

Joanne Rebbeck¹

Abstract: The objective of this study was to determine the long-term effects of ozone (O₃) and carbon dioxide (CO₂) on the growth and physiology of eastern white pine (*Pinus strobus*) and yellow-poplar (*Liriodendron tulipifera*) under plantation conditions. Two separate plantations of each species were established in Delaware, Ohio, in 1991 and 1992. Seedlings were fumigated from mid-May to mid-October in 1992, 1993, and 1994 in standard 3m diameter open-top chambers. The treatments, each replicated three times in a randomized block design, included charcoal-filtered air (CF), 1X ambient O₃ (1X), 2X ambient O₃ (2X), 2X ambient ozone plus 350 ppm CO₂ above ambient (2X+CO₂), and open-air (OA) chamberless plot. Monthly growth and physiological measurements taken during each growing season included stem height and basal diameter, photosynthesis, stomatal conductance, chlorophyll content, and foliar nitrogen and phosphorus concentration. Subsamples of yellow-poplar were destructively harvested in 1993. First-season exposure to O₃ plus CO₂ appeared to have a stimulatory effect on the growth of both species. In 1993, decreases in white pine height growth, though not significant, were observed for both 2X- and 2X+CO₂-grown seedlings. Biomass and growth stimulations were observed on yellow-poplar in 1993, with mean increases of 14 percent in stem diameter and 16 percent in total plant height of yellow-poplar grown in 2X+CO₂ compared with all other treatments. Although not statistically significant at p = 0.05, 2X+CO₂-grown yellow-poplar had greater leaf, stem, branch and root biomass, and total leaf area compared with all other treatments. No significant effects on the growth of white pine were observed. However in late August 1994, both total height and basal stem diameter of 2X+CO₂-grown yellow-poplar were 21 percent greater than for all other treatments. The slower growing white pine appears to be responding differently to O₃ plus CO₂ than yellow-poplar.

INTRODUCTION

In the majority of studies of the response of tree species to air pollutants, potted seedlings have been exposed to gaseous pollutants under controlled environmental conditions for one to two growing seasons. Extrapolating data on seedlings to older trees is problematic as there is very little information on the response of older trees to gaseous pollutants. Experimental approaches that have been used in scaling seedling pollutant response to older trees include the use of whole-tree or branch chambers on mature trees (Albaugh et al. 1992; Grulke and Miller 1994; Hanson et al. 1994; Heagle et al. 1989; Houpis et al. 1991; Samuelson 1994; Teskey et al. 1991); descriptive physiological studies comparing mature and sapling trees along environmental gradients (McLaughlin et al. 1990); vegetative propagation of "mature" tissue (Rebbeck et al. 1992, 1993a); and free-air chamberless exposure systems (McLeod and Baker 1988). None of these approaches is without some limitations. To date, no research group has investigated the long-term effects of exposing the same population of trees to gaseous pollutants during their development from seedlings to saplings to older trees.

In this paper I summarize major findings of the first three years of long-term exposure of two important eastern tree species, yellow-poplar (*Liriodendron tulipifera*) and eastern white pine (*Pinus strobus*), to O₃ and elevated CO₂. The growth and physiological responses of these two field-planted species were studied within open-top chambers. Soil fertility was not manipulated and applications of irrigation water and pesticides were minimal. Three hypotheses were tested: (1) CO₂ enrichment ameliorates the negative effects of O₃; (2) the relative response to O₃ and CO₂ is the same in short-term studies with seedlings as with older trees; and (3) faster growing hardwood species are more sensitive to O₃ and more responsive to elevated CO₂ than slower growing conifer species.

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METHODS

In 1991, two separate but adjoining field plantations of yellow-poplar and white pine were established at the Northeastern Forest Experiment Station Forestry Sciences Laboratory at the USDA Forest Service in Delaware, Ohio, by clearing a 26- by 73-m area in a 20-year-old abandoned American elm (*Ulmus americana*) plantation. Within each plot, 12 plants were arranged in a circular pattern approximately 1.8 m in diameter. The white pine plantation consisted of seedlings from two sources: (1) northern genotype (2-0 stock) from the Upper Peninsula of Michigan (Baraga County), obtained from Dr. David Karnosky, Michigan Technological University, Houghton; and (2) southern genotype (1-0 stock) from northern Ohio (near Toledo), obtained from the State Nursery of Ohio, Marietta. Six seedlings of each source were planted within each plot. The yellow-poplar seedlings were planted on 7 May 1992, with 1-0 stock obtained from a private nursery in western Pennsylvania. Seed collections were made in southeastern Tennessee. A standard 3m diameter open-top chamber (Heagle et al. 1973) was placed over each plot after planting. No fertilizer was applied to either species. Since ambient rain was not excluded from the chambers, rain shadows sometimes resulted in the uneven distribution of water. This was corrected by supplemental watering. The frequency and duration of these waterings were based on the amount and distribution of ambient rainfall in the open-top chambers.

The study design consisted of a randomized complete block with three replications of the following treatments: charcoal-filtered air (CF), ambient O₃ concentration (1X); two times ambient O₃ concentration (2X); two times ambient O₃ concentration plus 350 ppm CO₂ above ambient (2X+CO₂); and open-air, chamberless plot (OA). The O₃ and CO₂ were dispensed automatically 24 h per day from mid-May through mid-October in 1992, 1993, and 1994. In 1994, the yellow-poplar open-top chambers were modified to increase their height to 4.6 m.

Monthly growth and physiological measurements taken during each growing season included stem height and basal diameter, photosynthesis, stomatal conductance, chlorophyll content, and foliar nitrogen and phosphorus concentration. Gas exchange rates for individual yellow-poplar leaves (node 6 to 8 or node 10 to 12 from the apex) or two sets of white pine fascicles (current-year or one-year-old needles) were measured with a LI-COR 6200 portable photosynthesis system². From these same leaves, chlorophyll was extracted from 15mm diameter discs with dimethyl sulfoxide (Rebbeck et al. 1993a). The remaining leaf tissue was digested with sulfuric acid, potassium sulfate, and copper sulfate and quantified with a Lachat QuikChem Series 4000 Automated Ion Analyzer (Lachat 1992 a, b). Subsamples were taken to assess treatment impacts on leaf ultrastructure and spectral reflectance and transmission (Carter et al. 1995; McQuattie and Rebbeck 1994). Reflected and transmitted radiances were measured with an integrating sphere (LI-COR LI-1800UW spectroradiometer with LI1800-12S integrating sphere) (Carter et al. 1995). In late September 1993, shoot systems of six yellow-poplar seedlings from each chamber were destructively harvested and leaf area and leaf and stem dry mass determined. Root systems were excavated from a fixed volume (15,625 cm³) of soil immediately surrounding the decapitated seedling and separated into tap and lateral roots. Senescent leaves harvested in October 1993 were packed in litter bags and placed in the forest floor in a hardwood stand at the Delaware Lab. Loss of leaf dry mass over time was compared among treatments (Boerner and Rebbeck 1995).

Pre-exposure stem height was used as a covariate in the statistical analysis of both the yellow-poplar and white pine growth data sets. A General Linear Model (GLM) (SAS 1988) procedure was used to test for significant treatment effects. A Least Significant Difference (LSD) means comparison test was performed on the covariate-adjusted means only when significant effects were found in the GLM analysis. The white pine data were analyzed as a split-plot design with treatment as a main plot and genotype-source as a subplot. Each monthly measurement data set was analyzed separately.

²The use of trade, firm or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

Details on plant culture and maintenance as well as treatment dispensing and monitoring are reported elsewhere (Rebbeck 1993).

RESULTS AND DISCUSSION

The cumulative ozone dose for each of the three exposure seasons (1992-1994) is shown in Figure 1. Actual ozone levels for the 2X ambient ozone treatment were 1.3X, 1.6X, and 1.4X in 1992, 1993, and 1994, respectively.

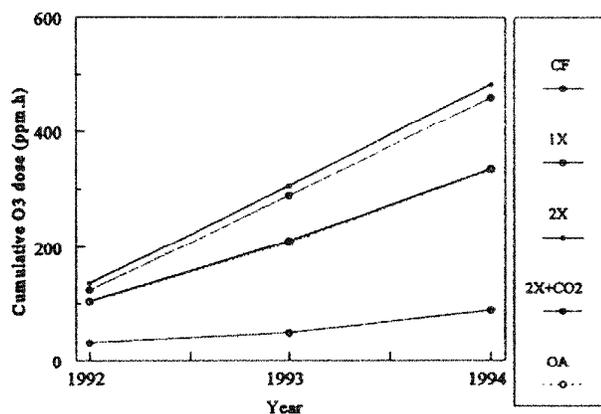


Figure 1. Cumulative ozone dose (ppm.h) of yellow-poplar grown in open-top chambers in 1992-94. The dose for white pine was comparable.

Yellow-poplar. Stimulated growth of yellow-poplar exposed to 2X+CO₂-air was observed during the three seasons of exposures (Table 1). In September 1992, stem diameter of yellow-poplar grown in 2X+CO₂ was 13, 21, and 16 percent greater than that of CF-, 1X-, and 2X-grown trees, respectively ($p \leq 0.05$). In September 1993, yellow-poplar stem diameter (that portion of the terminal stem produced in 1991, 1992, or 1993) significantly increased from 10 to 17 percent when grown in 2X+CO₂-air compared with all other treatments ($p \leq 0.05$) (Rebbeck et al. 1993b). Total height growth also was 14-18 percent greater in yellow-poplar grown in 2X+CO₂-air compared with other treatments ($p \leq 0.05$). In late August 1994, both total height and basal stem diameter of 2X+CO₂-grown yellow-poplar were 21 percent greater than for all other treatments. Further, 2X+CO₂-grown yellow-poplar tended to have greater leaf, stem, branch and root biomass, and total leaf area compared with all other treatments when harvested in September 1993; p -values ranged from 0.18 to 0.78 (Table 2) (Rebbeck and Scherzer 1994).

Table 1. Yellow-poplar total stem height and basal diameter in 1992, 1993, and 1994.*

Treatment	Total Stem Height			Basal Diameter		
	1992	1993	1994	1992	1993	1994
	(cm)			(mm)		
CF	94.85 ± 3.14a	274.97 ± 10.36a	365.9 ± 20.8a	9.85 ± 0.5a	13.99 ± 0.78a	45.17 ± 2.73a
1XO ₃	90.84 ± 4.44a	267.35 ± 8.66a	343.9 ± 20.2a	8.93 ± 0.5a	29.24 ± 1.39a	39.46 ± 3.37a
2XO ₃	96.89 ± 4.08a	285.44 ± 8.43a	382.2 ± 24.3a	9.47 ± 0.5a	29.71 ± 1.17a	43.85 ± 2.98a
2XO ₃ +CO ₂	102.88 ± 5.04b	304.70 ± 10.47b	460.0 ± 11.1b	11.33 ± 0.5b	34.41 ± 1.61b	53.94 ± 3.18b

*Each value is a mean of 36 trees ± one standard error. Means followed by different letters are significantly different at $p < 0.05$.

Table 2. Yellow-poplar biomass (g dry wt) harvested in September 1993.

Treatment	Leaves	Branches	Primary stem	Root
CF	212.1 ± 27.1 a	122.2 ± 19.3 a	284.9 ± 34.2 a	120.1 ± 10.6 a
1XO ₃	227.6 ± 38.8 a	126.3 ± 28.4 a	287.2 ± 51.9 a	110.3 ± 12.2 a
2XO ₃	215.9 ± 18.7 a	121.1 ± 15.1 a	280.4 ± 28.3 a	113.2 ± 10.1 a
2XO ₃ +CO ₂	231.2 ± 25.4 a	139.7 ± 16.5 a	356.0 ± 54.0 a	136.7 ± 10.0 a

Each value is mean of 36 trees ± one standard error. Means followed by different letters are significantly different at $p < 0.05$.

White pine. No significant growth effects attributable to O₃ or O₃ plus CO₂ were observed for white pine in any of the three growing seasons (Table 3). In September 1992, height growth of white pine in 2X+CO₂-air was 50 and 18 percent greater than for the CF- and 2X-air treatments, respectively ($p = 0.08$) (Rebbeck 1993; Rebbeck et al. 1993a). In September 1993, height growth was lower for both 2X- and 2X+CO₂-grown seedlings than for seedlings of other treatments (Rebbeck and Scherzer 1994). The two seed sources grew differently, but no genotype X treatment interactions were observed at any time.

Table 3. Stem height growth and basal diameter of white pine in 1992, 1993, and 1994.*

Treatment	Stem Height Growth			Basal Diameter		
	1992	1993	1994	1992	1993	1994
	(cm)			(mm)		
CF	3.83 ± 1.54a	27.46 ± 2.60a	49.53 ± 3.00a	8.71 ± 0.37a	16.54 ± 0.87a	27.27 ± 1.25a
1XO ₃	4.99 ± 1.55a	26.50 ± 3.23a	45.06 ± 2.65a	8.53 ± 0.39a	14.68 ± 0.90a	25.81 ± 1.23a
2XO ₃	6.32 ± 1.56a	24.13 ± 2.48a	47.93 ± 3.14a	8.94 ± 0.47a	15.94 ± 0.98a	26.56 ± 1.42a
2XO ₃ +CO ₂	7.68 ± 1.54a	20.16 ± 2.61a	50.07 ± 3.92a	8.91 ± 0.46a	15.59 ± 1.25a	30.46 ± 2.03a

*Each value is mean of 36 trees ± one standard error. Means followed by different letters are significantly different at $p < 0.05$.

Physiological Effects

Yellow-poplar. Impacts of O₃ and O₃ plus CO₂ on seasonal photosynthetic rates of yellow-poplar at saturating light were observed in 1992 and 1993 (Table 4). Additions of enriched CO₂ to twice ambient O₃ ameliorated reductions in net photosynthesis caused by O₃. In 1992, net photosynthesis was reduced by 34 percent by 2X-air and increased by 13 percent by 2X+CO₂-air compared with CF-grown trees. In 1993, 2X+CO₂ increased net photosynthesis by 31 to 45 percent compared with all other treatments. In both 1992 and 1993, respiration rates were approximately 36 percent higher in leaves of yellow-poplar trees grown in 2X O₃-air than in leaves of CF-grown trees; CO₂ enrichment to 2X O₃ had no additional effect on respiration rates. In 1992, stomatal conductance was reduced by 7 and 32 percent in 2X O₃-air and 2X+CO₂-air, respectively, compared with CF-air. In July 1993, stomatal conductance rates of 2X+CO₂-grown yellow-poplar were 20 to 45 percent lower than for all other treatments.

Table 4. Seasonal photosynthetic rates ($\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$) of yellow-poplar and white pine in 1992 and 1993 at saturating light.

Treatment	<u>Yellow-poplar</u>		<u>White Pine</u>	
	1992	1993	1992	1993
CF	13.07 \pm 1.77a	6.94 \pm 4.83a	6.85 \pm 2.06a	9.95 \pm 1.77a
1XO ₃	11.86 \pm 1.44a	8.79 \pm 3.29a	7.04 \pm 1.69a	9.11 \pm 1.99a
2XO ₃	8.61 \pm 1.25b	8.12 \pm 3.93a	7.90 \pm 1.89a	9.68 \pm 2.19a
2XO ₃ +CO ₂ *	14.82 \pm 1.88a	12.69 \pm 3.44b	6.30 \pm 0.71a	30.74 \pm 1.65b

* Measurements at 700 ppm CO₂ for 2XO₃+CO₂ versus 350 ppm CO₂ for other treatments. Values are means across sampling dates, leaf or needle age, and genotype (white pine only). Means followed by different letters are significantly different at $p < 0.05$.

In 1992, total chlorophyll content was 21 and 30 percent lower in leaves of yellow-poplar grown in 2X- and 2X+CO₂-air, respectively, than in those grown in CF-air. In 1993, total chlorophyll content was reduced by 33 percent in 2X+CO₂-air compared with all other treatments (Rebbeck et al. 1995). Total chlorophyll content was reduced by exposure to 2X O₃ only in older yellow-poplar leaves. In 1993, foliar nitrogen of yellow-poplar was reduced by 32 to 38 percent in 2X+CO₂-air compared with all other treatments; O₃ alone did not affect foliar nitrogen content in 1993 (Scherzer and Rebbeck 1995).

Litter decomposition of yellow-poplar leaves was not affected by O₃, but 2X+CO₂-grown leaves had 34 to 43 percent less decay than leaves of other treatments. These findings imply that the C:N ratio of the forest floor and soil would be raised and nitrogen limitations exacerbated by elevated CO₂.

In both 1992 and 1993, the cuticular membrane ultrastructure of yellow-poplar leaves was altered both by O₃ and elevated CO₂. McQuattic and Rebbeck (1994) reported that the cuticular membranes of 2X O₃-grown yellow-poplar leaves were 64 and 36 percent thinner than those of seedlings grown in CF-air and 2X+CO₂-grown, respectively, ($p = 0.01$). In July and August of 1994, cuticle membranes were thickest in 2X+CO₂-grown yellow-poplar leaves; CF-grown cuticles were intermediate and 2X O₃-grown were the thinnest. We hypothesize that the some of the extra carbon assimilated by the O₃ plus CO₂-grown trees was used to produce the cuticular membrane.

White pine. In 1992 and 1993, O₃ alone did not have a negative effect on the photosynthetic rates of white pine needles (Table 4). Stimulations in net photosynthesis of white pine grown in 2X+CO₂-air were not observed until late August 1992. In 1993, photosynthetic rates of current- and 1-year-old needles of trees grown in 2X+CO₂-air increased by 57 to 80 percent compared with all other treatments ($p = 0.01$).

In 1993, total chlorophyll content of 1-year-old white pine needles grown in 2X+CO₂-air was reduced 41-52 percent compared with other treated trees in 1993 (Rebbeck et al. 1995). Current-year white pine needle total chlorophyll content was not affected by 2X- or 2X+CO₂-air.

The leaf optical properties of white pine and yellow-poplar exposed to O₃ and O₃ plus CO₂ were monitored in 1992 (Carter et al. 1995). Ozone increased leaf reflectance and transmittance and decreased leaf absorbance in yellow-poplar. CO₂ added to 2X O₃ did not affect reflectance but decreased transmittance and increased absorbance within the range of 400 to 421 nm and increased transmittance and decreased absorbance in the 694 to 697 nm range. In white pine, 2X O₃ increased reflectance in the 537 to 647, 650, and 691 to 716 nm ranges. Transmittances and absorbances were not determined for white pine. The observed spectral responses were explained primarily by decreased concentrations of chlorophyll *a* as has been the case with a number of plant stressors.

SUMMARY AND CONCLUSIONS

In this three-year study, O₃ alone had no significant effect on the growth of eastern white pine or yellow-poplar. A fast-growing indeterminate hardwood species, yellow-poplar appeared more responsive to additions of CO₂ to O₃ than the slower growing determinate conifer. Yellow-poplar exhibited the typically reported responses to enriched CO₂ including increased height and diameter growth, increased shoot and root biomass production, increased photosynthesis, decreased stomatal conductance, decreased total chlorophyll and foliar nitrogen content, and decreased litter decomposition rate, even in the presence of up to 1.6X ambient O₃ (target was 2XO₃). Leaf gas exchange along with growth, biomass production, and foliar nutrient dynamics will continue to be monitored to determine if acclimation of yellow-poplar to elevated CO₂ occurs in the presence of O₃.

Although white pine exhibited increased rates of photosynthesis and decreased total chlorophyll content in response to 2X O₃ plus enriched CO₂, impacts on whole-tree growth were not observed. And although the initial growth of this white pine population was slow, it is possible that these now well-established trees will begin to exhibit more typical growth effects due to enriched CO₂.

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EFFECTS OF CARBON DIOXIDE ENRICHMENT ON RESPONSE OF PITCH PINE GROWN AT DIFFERENT
NUTRIENT LEVELS TO ALUMINUM

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The effect of elevated CO₂ on the response of pitch pine (*Pinus rigida*) to aluminum was determined in three experiments with different nutrient levels. During each experiment seedlings inoculated with the ectomycorrhizal fungus *Pisolithus tinctorius* were grown for 13 to 15 weeks in sand irrigated with a nutrient solution (pH 3.5) containing 0, 6.25, 12.5, or 25 mg/L Al (0, 0.232, 0.463, or 0.927 mM Al, respectively) in growth chambers fumigated with 350 (ambient) or 700 (elevated) $\mu\text{L/L}$ CO₂. The concentration of mineral elements in the nutrient solution of the experiment with the lowest concentration of nutrients (X) simulated that in the soil solution of a nutrient poor, sandy New Jersey Pine Barrens soil. Levels of nutrients in the two other experiments were approximately two (2X) or four (4X) times higher. Total biomass of seedlings at the higher nutrient levels was 19 percent (2X) and 172 percent (4X) higher than that at the lowest nutrient level. Growth at elevated CO₂ was significantly greater than growth at ambient CO₂ at the 2X (+24 percent) and 4X (+22 percent) nutrient levels but not at the X level. At the 2X and 4X nutrient levels, aluminum significantly reduced shoot growth (biomass, needle length) and root growth (biomass, lateral root length) at both CO₂ levels and there were no significant Al \times CO₂ interactions. At the lowest nutrient level only root growth was significantly reduced by Al. Symptoms of Al toxicity in needles differed depending on nutrient level: X, needle chlorosis at 12.5 and 25 mg/L Al at both CO₂ levels; 2X, tip chlorosis at 25 mg/L Al at ambient CO₂ only; and 4X, no needle chlorosis in any treatment. At all nutrient levels without Al, seedlings growing at elevated CO₂ had greater numbers of mycorrhizal roots than seedlings growing at ambient CO₂. In the presence of Al, mycorrhizal roots had greater Al-induced modifications (decreased numbers of bifurcate mycorrhizal roots, increased numbers of dark, stunted root tips) at the 2X and 4X nutrient levels than at the X nutrient level, especially at ambient CO₂. The CO₂ concentration did not significantly affect Al concentration in roots or needles at either 2X or 4X nutrient levels (elemental analyses for X nutrient level not available at this time). Carbon dioxide fumigation had a minor influence on the nutrition of treated seedlings. The greatest effect of CO₂ on foliar concentration of mineral elements was at the 2X nutrient level; needles at elevated CO₂ had small but significantly higher concentrations of Ca, Mg, Fe, and B than needles at ambient CO₂. Generally, Al decreased the concentration of mineral elements in roots and needles of treated seedlings. At all nutrient levels, disruption of root meristem cells and the mycorrhizal fungal mantle surrounding the short roots increased as Al concentration increased. In treatments containing 12.5 or 25 mg/L Al, aluminum was detected by energy-dispersive x-ray microanalysis in outer root cells and in dead root cells embedded in the fungal mantle.

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ELEVATED CO₂ COMPENSATES FOR WATER STRESS IN NORTHERN RED OAK

Patricia T. Tomlinson and Paul D. Anderson¹

Global climate change models predict decreased rainfall in association with elevated CO₂ in the western Lakes States region. Currently, the western edge of northern red oak (*Quercus rubra* L.) distribution coincides with the most xeric conditions of its ecological range. Decreased rainfall and water availability could alter ecological fitness and distribution. To better understand how climate change may affect this species, we are examining the interaction of CO₂ (400, 520, 700 ppm CO₂) with water stress (well-watered and water-stressed) on growth, and carbon and nitrogen metabolism of northern red oak seedlings through three flushes of development. In this report, we focus on growth, photosynthetic rate, and nitrogen responses to these stresses in three-flush seedlings.

Seedling biomass increased with increasing CO₂ and decreased with water stress. The water stress response was accompanied by a shift in relative biomass distribution from stems to roots; CO₂ did not alter the relative distribution among leaves, stems, and roots. As a consequence, shoot:root dry weight ratio was decreased by water stress but remained uniform across CO₂ treatments.

Photosynthetic rate generally increased with elevated growth CO₂ and decreased with water stress. The response to CO₂ under water stress was approximately linear; under well-watered conditions, photosynthetic rate plateaued at 520 ppm CO₂. Stomatal conductance decreased in well-watered seedlings with elevated CO₂. Although conductance was decreased by water stress, these seedlings did not respond to growth CO₂. Thus, an increased diffusional gradient for CO₂ into the mesophyll of these water-stressed seedlings would be expected with increasing growth CO₂.

Photosynthetic water-use efficiency was largely unaffected by water stress but increased with growth CO₂ to a plateau at 520 ppm. In contrast, photosynthetic nitrogen-use efficiency was decreased by water stress and the response to growth CO₂ differed between water stress regimes. In water-stressed seedlings, nitrogen-use efficiency plateaued at 520 ppm growth CO₂. However, in well-watered seedlings, efficiency increased to 520 ppm but decreased to 400 ppm levels at 700 ppm growth CO₂. Tissue nitrogen concentration was apparently involved in this decrease because photosynthetic rate did not drop to this extent.

Leaves and roots of water-stressed seedlings displayed a greater nitrogen concentration than did those of well-watered seedlings and increasing growth CO₂ generally decreased nitrogen concentrations in these tissues. In well-watered 700 ppm CO₂ grown three-flush seedlings, nitrogen concentrations in the leaves and roots were relatively low at 1.4 percent and 0.9 percent, respectively. We hypothesize that nitrogen supply was unable to meet biomass demands associated with 700 ppm growth CO₂ under well-watered conditions. Consistent with this hypothesis, between 400 and 700 ppm growth CO₂ seedling biomass increased twofold for well-watered seedlings and nearly threefold for water-stressed seedlings.

These physiological responses to the interaction between elevated CO₂ and water stress resulted in similar 3-flush seedlings grown at 400 ppm CO₂ under well-watered conditions and at 700 ppm CO₂ under water-stressed conditions. The biomass, nitrogen concentration and photosynthetic rate of these seedlings were similar. Thus, in northern red oak, elevated CO₂ compensated for water stress. However, several physiological measures differed in these seedlings suggesting mechanistic differences. For example, differences in shoot:root dry weight ratio suggested changes in root-shoot interactions; differences in water-use efficiency suggested changes in photosynthetic mechanisms among these treatments. We are currently collecting data to delineate some of these physiological mechanisms.

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MODELING IMPACTS OF CO₂, OZONE, AND CLIMATE CHANGE ON TREE GROWTH

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INTRODUCTION

Understanding the influence of ozone, CO₂, and changing climatic regimes on basic plant physiological processes is essential for predicting the response of forest ecosystems. To understand the relationships among these interacting factors, in the face of genetic and other environmental variability, requires a means of synthesis. Physiological process modeling provides one such tool: it allows the integration of diverse information from research, reflects the interactions among variables, and provides a direction for future research.

To model trace gas effects on aspen, we have adapted an existing growth process model for poplar known as ECOPHYS. ECOPHYS is a mechanistic whole-tree model that simulates growth of poplar in its establishment year (Host et al. 1990a, Isebrands et al. 1989, Rauscher et al. 1990). ECOPHYS uses the individual leaf as the primary biological unit of the model. Hourly solar radiation, temperature, and clonal (genetic) factors acting at the leaf level provide the major driving variables for plant growth. Canopy architecture is modeled by means of a three-dimensional geometric approach. By knowing leaf orientation patterns and tracking solar position over the course of the day, we calculate precise estimates of intercepted radiation, which in turn are supplied to a photosynthate production submodel. Photosynthates are distributed to various growth centers in the plant by means of a radiotracer-based model of carbon allocation (Dickson 1986). The amount of photosynthate arriving at a growth center, after respiratory losses are determined, is used to calculate biomass production and dimensional growth. The model has been subjected to extensive validations both in terms of photosynthesis (Host et al. 1990) and in regional predictions of biomass production (Host and Isebrands 1994).

Our current research has three major facets: the development of three-dimensional soil and root models to complement the existing above-ground portion of the model, the integration of existing trace gas response data into the model framework, and the scaling of the existing model in time and space; specifically to simulate the growth of an interacting population of trees for a number of years. These objectives will allow us to simulate impacts related to global change, and to provide input to models operating at larger temporal and spatial scales.

MODELING STRATEGIES AND APPLICATIONS

Soil Water and Nutrient Modeling

A three-dimensional soil water transport model was developed to provide a heterogeneous soil environment for root development. We currently simulate water movement in a 100 × 100 × 200 cm soil volume using variable-size cells (1-5 cm on a side). The use of different cell sizes allows the user to run the model under different levels of resolution. The soil model runs on an hourly times step and accounts for surface evaporation, capillary rise from a water table, water distribution from irrigation lines, and water uptake into a plant root system (Figure 1). The use of a three-dimensional model, vs. the traditional two-dimensional approach, allows us to simulate variability in moisture and nutrients in horizontal as well as vertical directions. The ability to simulate soil heterogeneity in the horizontal dimension is important not only for simulating differential growth of the canopy as a result of

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vascularization between branches and root segments, but also for simulating competition for resources among plants. This latter aspect is important as we scale from the individual tree to the patch, as described below.

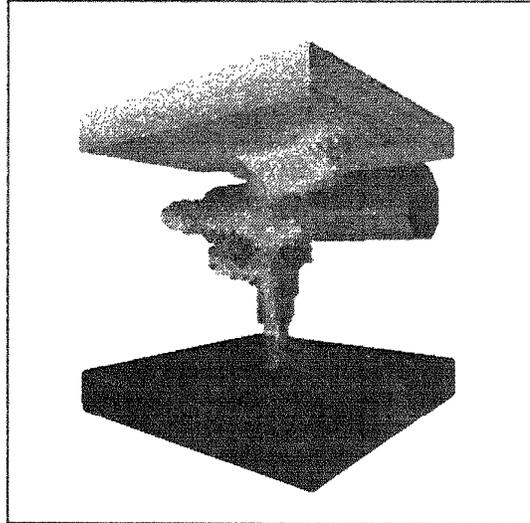


Figure 1. Visualization of a sample soil slab containing a plant root and a sub-surface irrigation line, derived from volumetric soil water content (wetter soil cells are rendered darker and drier cells lighter, while soil cells with moisture contents between 23 percent and 32 percent have been rendered transparent) following six hours of simulated soil water redistribution. The root system consists of a tap root and six lateral roots, four of which are visible as drier soil cells (lighter shade) extending left and right of the tap root. The irrigation line has wetted a cylindrical volume of soil cells (darker) located behind the tap root. The soil surface (top) has been dried by evaporation while the base of the soil slab (bottom) is being wetted by capillary rise from a water table.

Root Growth Modeling

The ECOPHYS root growth model maintains roots of various orders in three-dimensional space. Root architecture is based on a highly relaxed fractal algorithm utilizing a monopodial branching pattern and is coded using a free tree structure (Figure 2). Overall root growth is governed by hourly translocation from the shoot. Branch roots may be formed at any radial position about the main axis of a parent root for which there is a corresponding vascular bundle. Initiation angles of branch roots with respect to parent roots vary randomly along a probability distribution as do changes in direction of growth of any root apex. The growth rate of root apices and the frequency of branch initiation are regulated by soil strength. Root geotropic response in poplar is strongly correlated to root order and is taken into account in the simulation. To simulate fine root turnover, a proportion of terminal higher order roots are 'killed' and replaced by new growth at each time step.

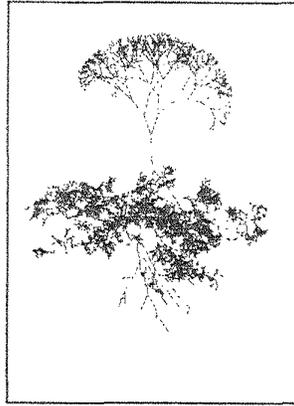


Figure 2. Two-dimensional rendering of a three-dimensional mathematical root model after 72 time steps, given a constant photosynthate input.

Trace Gas Modeling

Working with aspen and hybrid poplars, we have developed a mechanistic individual tree process model of the plant growth response to altered CO_2 and ozone concentrations. This modeling work has been done in close cooperation with field and growth chamber experiments. Specifically, we developed a mechanistic model for the A/C_i response by incorporating our existing light response model with the CO_2 model proposed by Weber et al. (1985), adding parameters for carboxylation efficiency and CO_2 saturation rates. A mechanistic understanding of stomatal conductance does not exist; we follow the assumption of Ball et al. (1982) that plants maintain a relative constant ratio of internal to ambient CO_2 levels.

Submodels have been developed to simulate the effects of ozone on leaf longevity, and effective leaf area; direct effects of ozone on photosynthesis have proven more problematic, as the interactions between ozone and CO_2 are nonlinear (Coleman et al. In review).

Scaling

Scaling ECOPHYS into a second season of growth requires the ability to simulate branch growth and development. Our strategy for growing branches is based on the concept of branch autonomy, which states that branches essentially operate like first year shoots: they translocate carbon to their own internodes, and those of the lower stem and roots, but do not translocate upwards to other leaves or branches (Isebrands 1983). In addition, leaves on branches maintain the same genetically-determined photosynthetic characteristics. Patterns of internode expansion and growth are the same as in the first year shoot. As a result, our original ECOPHYS model, which simulates the growth of a first year shoot, coupled with a branch architecture algorithm, can be used to simulate the growth of a branched poplar tree.

To scale to branches requires that ECOPHYS be translated into an object-oriented format. There are several reasons for this translation. To accommodate the exponential increase in the number of leaves and branches that occur in the second and subsequent growing seasons requires that the model run in a language that can access extended computer memory. Second, object-oriented data structures can be organized hierarchically, and relate much more to the natural organization of biological systems, rather than the array-based structure of traditional programming languages. Finally, object-oriented languages are much easier to maintain and debug than procedure-based languages.

When the recoding is complete, the current aboveground ECOPHYS model will become an 'object' within the multi-year model. This 'ECOPHYS object' will have all the components of the original model (a population of leaves and internodes, coupled with photosynthate production and transport rules), plus new information on branch location, angle, and an expanded carbon allocation matrix that describes carbon transport within a branched structure. The object-oriented ECOPHYS will thus be able to grow an individual tree into the second and subsequent growing seasons. This approach will also allow us to simulate interactions within a small population of trees.

To assess the above and below ground interactions among individual trees, we will simulate a block or patch of trees growing under uniform spacing. We envision simulating an interaction block of 16 trees in a 4 x 4 matrix, and planted under a range of typical planting densities (e.g. 2 to 4 m spacings). Trees within the block will shade each other as a function of size, leaf arrangement, branch architecture and solar angle. In addition, they will compete below ground in the three-dimensional soil matrix described previously. To scale to a field plantation level, this patch of trees can be considered a random sample within a particular soil type. To simulate growth within a plantation consisting of several soil types, the model can be run independently for each individual soil type, and area-weighted estimates of growth calculated to characterize the plantation as a whole (Figure 3).

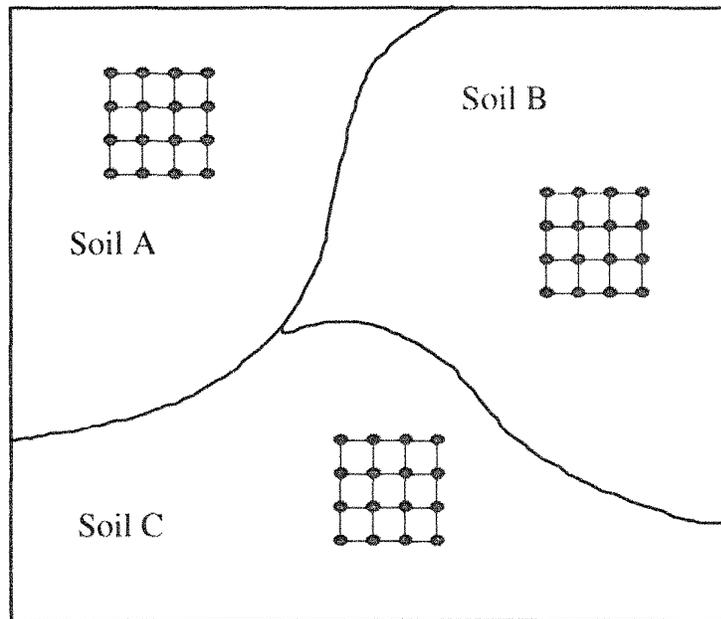


Figure 3. The scaled ECOPHYS patch model as random samples within a field consisting of three soil types.

Model Applications

One of the foremost applications of physiological process models to climate change is the prediction of growth differences, and one of the foremost challenges is the development of realistic detailed climatic change scenarios. One resolution to this challenge is the use of existing, high resolution climatic data. In the Lake States, the growing seasons of 1988 and 1992 were the warmest and coldest years in 100 years of record. We collected hourly solar radiation and temperature data from a weather station in northern Wisconsin, USA, and used these as inputs to ECOPHYS. At this site, air temperatures averaged 2 °C higher and cumulative radiation was 30 percent greater in 1988 compared with 1992. These weather data were used to simulate one growing season's photosynthesis and growth in two *Populus* clones: *P. Eugenei*, a clone characterized by a strongly vertical leaf display and a long growing season, and *P. Tristis*, a planophile clone with an early bud set date. For *P. Eugenei*, photosynthesis was 85

percent greater in 1988 compared with 1992 (Host and Isebrands, In review). Leaf area and total biomass showed increases of 70 percent and 56 percent, respectively, between these years. The patterns were similar for *P. tristis*, but the differences were less pronounced. These simulations indicate that differences in seasonal weather patterns observed over a few years can account for large but clonally-specific differences in photosynthesis and biomass production in the early growth of *Populus*.

CONCLUSIONS

Modeling at the scale of detailed physiological processes fills an important niche in the hierarchy of spatial scales required to understand global change. We have described the essential components of a modeling effort designed to understand interactions among soil, root, and shoot systems, plant response to trace gases, and the scaling of detailed physiological processes in time and space. In addition, we present an application of the model using 100 yr climatic extremes recorded over the past decade. The development and use of process models not only provides an integration of existing research, it also focuses research questions related to uncertain future conditions.

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OZONE EXPOSURE, UPTAKE, AND RESPONSE OF DIFFERENT-SIZED BLACK CHERRY TREES

Todd S. Fredericksen¹, John M. Skelly¹, Kim C. Steiner¹, and Thomas E. Kolb²

Abstract. Differences in exposure, uptake and relative sensitivity to ozone between seedling, sapling, and canopy black cherry (*Prunus serotina* Ehrh.) trees were characterized during two growing seasons in north central Pennsylvania. Open-grown trees of all sizes received a similar amount of ozone exposure. Seedlings had greater foliar ozone injury, expressed as adaxial stipple and early leaf senescence, than larger trees, which was correlated with their higher rates of stomatal conductance and greater rates of ozone uptake. The higher stomatal conductance and ozone uptake of seedlings was proportional to their higher (less negative) predawn xylem water potentials. Seedlings appeared to have some ability to compensate for injury because their free growth habit reduced exposure per unit leaf area compared to larger trees whose leaves were exposed to ozone throughout the entire growing season.

INTRODUCTION

Black cherry is a widespread deciduous tree species in eastern North America and is a highly-valuable commercial timber species in the Allegheny Mountains of Pennsylvania and West Virginia. However, black cherry also appears to be extremely sensitive to tropospheric ozone (Davis and Skelly 1992, Simini et al 1992). Ozone concentrations often reach high levels in this region during the growing season and may impact the growth of black cherry (Comrie 1994). One problem with determining the impact of ozone on large, forest-grown black cherry trees, however, is that most air pollution studies have used seedlings grown in field chambers or greenhouses (Reich 1987, Pye 1988). The objective of this study was to determine if differences in physiology and/or ozone exposure related to tree size may impact uptake of ozone and foliar injury response. More detailed results are presented by Fredericksen et al. (1995a, 1995b).

METHODS

The study site is located within the Moshannon State Forest in Clearfield County, PA within the Allegheny Plateau physiographic province. Open-grown seedlings, saplings, and 80-year-old canopy black cherry trees were identified for study during the 1993 and 1994 growing seasons. Saplings were located in a canopy gap in 1993 and in a larger forest opening in 1994. Seedlings were 1-2 year-old nursery transplants approximately 0.5-1.5 m in height. Saplings were 5-7 m and canopy trees were approximately 20 m tall. Data collected during each growing season included seasonal within-canopy ozone concentrations for each tree size, daily and seasonal patterns of leaf gas exchange, predawn xylem water potential, seasonal leaf area development, and foliar ozone injury expressed as percent adaxial stipple and early leaf senescence. Ozone uptake was calculated for each tree size class as the product of ozone exposure and stomatal conductance adjusted for differences in diffusivity between water vapor and ozone. All measurements were made within the upper crown of each tree size class.

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RESULTS AND DISCUSSION

7-hour average (0900-1600 EST) ozone concentrations were slightly higher during the 1994 growing season compared to 1993 (Table 1). Ozone concentrations were similar within the crowns of open-grown seedlings, saplings, and canopy trees, although sapling and canopy trees had lower ozone exposure than seedlings in 1993.

Ozone uptake rates tended to decrease with increasing tree size (Table 1). High seedling uptake rates were correlated with higher rates of stomatal conductance. The higher stomatal conductance and ozone uptake of seedlings may be explained by the higher (less negative) xylem water potentials of seedlings because seedlings have a shorter path length of water transport compared to larger trees, allowing for higher rates of stomatal conductance.

Table 1. Ozone exposure (nl.l^{-1} , 7-hour mean), stomatal conductance ($\text{mol/m}^2/\text{s}$), ozone uptake ($\text{umol/m}^2/\text{hr}$), predawn xylem water potential (-MPa), percentage adaxial ozone stipple (Sept. 1), and percentage early leaf abscission (Sept. 1) of seedling, sapling, and canopy black cherry trees during 1993 and 1994. Saplings sampled in 1993 were growing in a canopy gap. Saplings sampled in 1994 were open-grown. Standard errors of means are in parentheses.

Tree Size	Ozone Exposure	Stomatal Conductance	Ozone Uptake	Predawn Water Potential	% Ozone Stipple	% Early Leaf Abscission
<u>Seedlings</u>						
1993	40.3 (0.51)	0.23 (0.01)	29.6 (1.22)	0.12 (0.01)	0.6 (0.1)	6.9 (2.3)
1994	48.0 (0.51)	0.50 (0.02)	58.1 (0.79)	0.12 (0.02)	43.8 (3.5)	30.45 (10.6)
<u>Saplings</u>						
1993	42.2 (0.48)	0.16 (0.01)	19.6 (0.35)	0.22 (0.01)	0.2 (0.01)	5.2 (1.7)
1994	48.5 (0.51)	0.40 (0.02)	48.6 (0.74)	0.18 (0.02)	12.5 (1.2)	3.8 (2.0)
<u>Canopy</u>						
1993	46.3 (0.55)	0.18 (0.01)	16.1 (0.37)	0.46 (0.04)	0.1 (0.01)	6.9 (2.6)
1994	48.8 (0.53)	0.28 (.01)	33.8 (0.57)	0.36 (0.02)	19.1 (1.50)	4.4 (2.0)

Seedlings had larger amounts of foliar injury per unit ozone exposure than saplings or canopy trees during both growing seasons (Table 1). Incidence of foliar injury and leaf senescence per unit leaf area was generally low in 1993, but much greater in 1994. Seedlings had greater rates of early leaf senescence than larger trees during 1994. The higher amounts of ozone uptake and foliar injury symptoms during 1994 may be explained by a higher soil moisture content during 1994. Growing season rainfall totaled 74.5 cm during 1994 and 51.1 cm during 1993. Showman (1991) similarly observed that foliar injury symptoms were much higher during a wet year with than a drier year despite relatively higher ozone during the dry year.

During 1993, and to a lesser extent in 1994, ozone injury to older seedling leaves may have been partially compensated by the production of new uninjured leaves throughout the growing season. Early leaf abscission of

older, injured leaves and replacement with new leaves may allow for the maintenance of high rates of whole plant net photosynthesis. Canopy trees, exhibiting fixed growth, did not have this compensatory ability. Open-grown saplings exhibited some free growth, but canopy-gap saplings did not.

SUMMARY

Slight differences in ozone exposure were evident within the mature forest canopy and in forest openings. Seedlings had higher rates of ozone uptake and foliar ozone injury than saplings and canopy trees. Ozone uptake rates were positively related to stomatal conductance and xylem water potentials. Seedlings appeared to have some ability to compensate for foliar injury on older leaves by the production of new foliage throughout the growing season.

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WATER USE IN FOREST CANOPY BLACK CHERRY TREES AND ITS RELATIONSHIP TO LEAF GAS
EXCHANGE AND ENVIRONMENT

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Models of canopy gas exchange are needed to connect leaf-level measurement to higher scales. Because of the correspondence between leaf gas exchange and water use, it may be possible to predict variation in leaf gas exchange at the canopy level by monitoring rates of branch water use. Rates of water use were determined in branches of forest canopy black cherry trees (ca. 25 m) using the stem heat balance method, as were relationships between water use, leaf gas exchange, and microenvironment. Maximum rates of water flow occurred between 1200 and 1300 HRS (EST), and declined steadily throughout the afternoon hours. Total daily water use was greatest for branches within the upper crown, however, the magnitude of difference between upper and lower crown branches was less for south-facing branches than for north-facing branches. At the entire tree level, measured water flow rate (F) was correlated with stomatal conductance (g_s) ($r = 0.66$), transpiration rate (E) ($r = 0.63$), and photosynthetically active radiation (PAR) measured at the leaf surface ($r = 0.79$). The degree of association between these variables increased with stratification by crown level and increased further with stratification by branch level. Both relative humidity (RH) and PAR measured at 1 m above the canopy contributed significantly ($P = 0.0001$ and 0.01137 respectively) to a model expressing water flow rate as a function of microenvironmental variables. However, variation in RH explained a greater proportion of the total variation in measured flow rate in the lower crown than in the upper crown ($r^2 = 0.94$ and 0.60 , respectively). These results suggest that (1) at the branch level, measured rates of water use can be used to estimate variation in g_s and E , (2) much of the temporal and spatial variation in flow rate is driven by variation in incident PAR, and (3) vapor pressure deficit is probably more important to the regulation of leaf gas exchange in the lower crown while canopy boundary layer resistance is probably more important to the regulation of leaf gas exchange in the upper crown.

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EFFECTS OF ELEVATED CO₂ AND OZONE ON PHENOLIC GLYCOSIDES OF TREMBLING ASPEN

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We tested the effects of elevated CO₂ and ozone on concentrations of the phenolic glycosides salicortin and tremulacin in immature and mature foliage of the trembling aspen (*Populus tremuloides*) clones 216, 259, and 271. Elevated CO₂ increased and elevated ozone decreased concentrations of both compounds in immature foliage, and tremulacin in mature leaves. Elevated CO₂ increased and ozone had no effect on salicortin in mature leaves. The ozone tolerant clone 216 had lower concentrations of tremulacin than the other clones. Effects of CO₂ and ozone on tremulacin and salicortin in immature leaves, and tremulacin in mature leaves corresponded with effects of CO₂ and ozone on the growth of insects on the same trees (see manuscript by Herms and others)

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VARIABLE PERFORMANCE OF OUTBREAK DEFOLIATORS ON ASPEN CLONES EXPOSED TO
ELEVATED CO₂ AND O₃

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Abstract: Increasing atmospheric concentrations of ozone and CO₂ affect many aspects of tree physiology. However, their effects on tree resistance to insects have received relatively little attention. The objectives of this study were to test the effects of elevated CO₂ and ozone on the resistance of three quaking aspen (*Populus tremuloides*) clones (216, 259, and 271) to first and fourth instars of four Lepidoptera species: gypsy moth (*Lymantria dispar*), forest tent caterpillar (*Malacosoma disstria*), large aspen tortrix (*Choristoneura conflictana*), and whitemarked tussock moth (*Orgyia leucostigma*). Larval survival, growth rates, and nutritional indices were quantified. There were no treatment effects on larval survival. Elevated CO₂ decreased the growth rates of both instars of all species, except that of first instar forest tent caterpillar on aspen clone 216, which was increased. Elevated ozone increased the growth of first and fourth instars of all insect species tested. The treatment effects on growth rate were generally caused by their effects on the ability of larvae to convert digested food to biomass (ECD). Elevated ozone increased ECD. The effects of elevated CO₂ on ECD were clone dependent: elevated CO₂ decreased ECD on clones 271 and 259, but increased ECD on clone 216. Ozone had no effect on larval consumption rates. Elevated CO₂ decreased the consumption rate of large aspen tortrix but had no effect on the other species. This contrasts with other studies, in which elevated CO₂ generally increased insect consumption. There were no statistically significant interactions between the CO₂ and ozone treatments for any of the variables measured.

INTRODUCTION

Elevated concentrations of atmospheric CO₂ and ozone alter many aspects of tree physiology including gas exchange, growth, and carbon allocation (Pye 1988, Jarvis 1989). Environmentally-induced variation in tree resistance to insects and other herbivores results primarily from effects on plant nutrient and secondary metabolite concentrations, which are intimately connected to whole-plant patterns of resource acquisition and allocation (Herms and Mattson 1992). Hence, elevated CO₂ and ozone may influence interactions between plants and herbivores, and therefore their distribution and abundance (Ayles 1993, Williams and Liebhold 1995). In turn, community composition and insect-mediated ecosystem processes such as nutrient cycling may be affected (Ayles 1993, Lambers 1993). Despite this potential, few studies have addressed the effects of increased atmospheric CO₂ or ozone on tree/insect interactions, and to our knowledge, no study has examined both factors simultaneously.

Elevated CO₂ generally decreases the nutritional quality of plants for leaf-feeding insects. In some cases, this translates into decreased insect growth and survival, but in other cases insects compensate for decreased foliage

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quality by increasing their consumption rate such that elevated CO₂ has no effect on growth (Lincoln 1993, Lincoln and others 1993). The only study with forest insects suggests that effects of elevated CO₂ are likely to be dependent on the species involved (Lindroth and others 1993). The growth of gypsy moth larvae (*Lymantria dispar*) fed foliage from trees grown under elevated CO₂ was decreased on quaking aspen (*Populus tremuloides*), was unaffected on sugar maple (*Acer saccharum*), and increased on red oak (*Quercus rubra*). In the same experiment, elevated CO₂ decreased the growth of forest tent caterpillar (*Malacosoma disstria*) on quaking aspen and sugar maple, but did not affect its growth on red oak.

The effects of ozone on plant/herbivore interactions have received less attention, but some studies have found elevated ozone to enhance insect performance on crop plants (Chappelka and others 1988, Reimer and Whittaker 1989, Lin and others 1990). Only a few studies have investigated ozone effects on tree resistance to insects. In a study with cottonwood (*Populus deltoides*), ozone exposure had no effect on the aphid *Chatophorus populicola* (Coleman and Jones 1988a).

Although ozone decreased the growth and fecundity of the cottonwood leaf beetle (*Plagioderma versicolora*), this insect preferred to feed on, and consumed more, foliage exposed to elevated ozone than foliage exposed to ambient air (Coleman and Jones 1988b, Jones and Coleman 1988). Gypsy moth preferred to feed on white oak (*Quercus alba*) foliage exposed to the highest concentration of ozone (about 3X ambient), but preferred foliage exposed to ambient air over that exposed to intermediate levels of ozone (Jeffords and Endress 1984).

Objectives

Quaking aspen is the most widely distributed tree species in North America. Forest tent caterpillars, gypsy moth, large aspen tortrix (*Choristoneura conflictana*), and whitemarked tussock moth (*Orgyia leucostigma*) can be important outbreak defoliators and dominant lepidopteran folivores of aspen. Quaking aspen displays genetic variation in ozone tolerance (Berrang and others 1989). Hence, ozone effects on insect performance may be dependent on host genotype. However, to our knowledge no studies have examined genetic variation in the effects of ozone or elevated CO₂ on tree resistance to insects. The objectives of this study were to test the effects of elevated CO₂ and ozone on the nutritional suitability for these four insect species of three aspen clones (*Populus tremuloides*) that differ in their sensitivity to ozone. The aspen genotypes tested were clone 216 (Bayfield Co. Wisconsin, ozone-tolerant), clone 259 (Porter Co. Indiana, ozone sensitive), and clone 271 (Porter Co. Indiana, intermediate tolerance of ozone).

METHODS

Production of Experimental Plant Material

Softwood cuttings taken from greenhouse-grown stock plants were dipped in 5000 mg/l IBA and rooted in 65 cm³ Leach cells in vermiculite-peat (3:1) mix with intermittent misting. Rooting occurred in four weeks, after which the plants were hardened on the greenhouse bench, then transplanted into 38 cm deep x 15 cm plastic pots in a media of peat-sand-vermiculite (2:1:1), supplemented with 8 g of Sierra Osmocote (17-6-10 formulation), plus micronutrients (8-9 month release time). Two days after transplanting, plants were moved to test chambers and exposed to experimental treatments for five weeks before use in insect bioassays.

Insect Culture

Insects of all four species were obtained as newly molted fourth instars and newly eclosed first instars from Forest Pest Management Institute, Canadian Forest Service, Sault Ste. Marie, Ontario. All four species were tested as fourth instars. All but large aspen tortrix were tested as first instars. Bioassays were initiated within 24 hours of obtaining the insects.

Implementation of Ozone and CO₂ Treatments

Gaseous treatments were applied in 1.7m³ exposure chambers constructed from mylar covered aluminum angle frame. Ozone was produced using an electric discharge generator (GTC-0.25, Griffin Technics, Inc. Lodi, New Jersey) from bottled oxygen delivered through proportional control valves that were automatically adjusted to maintain 150 nmol/mol by a CR-10 datalogger (Campbell Scientific, Logan, UT). Ozone was maintained at the set point between 900 and 1700 hours each day, and returned to ambient for the remainder of the day. The ozone application resulted in 10 ppm h accumulated each week, compared to 3 ppm h for the controls exposed to ambient levels. Elevated CO₂ was manually dispensed 24 hr per day through needle valves. The ambient concentrations (approximately 400 umol/mol) were supplemented with 350 umol/mol. Ozone and CO₂ monitors were calibrated weekly. Hourly treatment concentrations were recorded for each chamber by the datalogger.

Experimental Design

Elevated and ambient ozone and CO₂ were applied to four chambers in factorial combination. Each CO₂/ozone treatment combination was replicated twice, for a total of eight chambers in the experiment. Five replicate individuals of all three aspen clones were grown in each chamber, for a total of 15 trees in each chamber. Thus, the experiment constituted a 2 × 2 × 3 × 4 nested factorial, with two levels of ozone (ambient and elevated), two levels of CO₂ (ambient and elevated), three aspen clones (216, 259, 271), and four insect species (three in the case of first instars with large aspen tortrix omitted from the test). We measured the growth of four individual larvae (fourth instars) or four groups of larvae (first instars) within each four-way treatment combination. Each of the four replicate larvae or larval groups received leaves from a separate tree so that individual trees were the experimental unit. First and fourth instar bioassays were treated as separate experiments.

Foliage Sampling and Leaf Assignments

To control for effects of tree ontogeny on leaf chemistry and insect performance, larvae were assigned leaves of a specific physiological age as determined by their leaf plastichron index (LPI) (Larson and Isebrands 1971). Leaves were numbered sequentially with LPI one designated as the youngest leaf on the tree that was at least 3 cm in length. Assignments were based on judgements of leaf ages that larvae were likely to feed on under field conditions. First instar forest tent caterpillar, gypsy moth, and whitemarked tussock moth were fed LPI two, four, and seven, respectively. Gypsy moth and forest tent caterpillar both were fed LPI 10 and 11, whitemarked tussock moth were fed leaf plastichron 13. Each large aspen tortrix replicate was randomly assigned either LPI 10, 11, 13, or 15.

First Instar Bioassay Procedures

Ten larvae were confined to petri dish an (8.5 cm diam. by 2 cm deep) with a base of plaster-of-Paris and charcoal. Water added to the plaster base provided a high humidity environment that maintained the turgor of detached leaves fed to larvae. Simultaneous measurements of leaves from trees exposed to the same treatments but not fed to insects showed no main or interacting effects of any of the treatments on fresh weight or respiratory losses of dry mass of leaves over the 48-hour bioassay period. Larvae were reared in a growth chamber at 25°C, under an 18:6 light:dark cycle.

All 10 larvae from each dish were weighed as a group prior to initiation of the bioassay. After 48 hours of feeding the bioassay was terminated and the number of surviving larvae was recorded. Duration of the bioassay was quantified to the nearest 15-minute interval. Surviving larvae were then flash frozen, oven-dried to constant weight, and then weighed as a group. In order to estimate initial weights, twenty 10-larvae samples were weighed for each of the three species, then immediately frozen at -40°C, dried to constant weight and reweighed. The relationship of fresh mass to dry mass of these insects was determined from these data using linear regression. These regression equations were then used to estimate initial dry mass of the bioassay insects based on their initial fresh masses. We calculated percent survival and relative growth rate for each of the three species.

Fourth Instar Bioassay Procedures

Bioassays with fourth instars were conducted as above, with the following alterations. Only one larva was used per petri dish, and the petri dishes were larger: 11.5 cm diam. by 2.5 cm deep. The initial dry mass of each insect was estimated from initial fresh mass as above, except that regression equations were developed from 20 individual larvae. Total leaf area consumed during the bioassay was determined by measuring the area of the leaf with a digital image analyzer before and after the bioassay period. Frass was collected, dried to constant weight, and weighed. The portions of the leaves not consumed were collected, and their cumulative area and dry mass determined. Initial dry mass was then calculated as:

$$\text{initial dry mass} = (\text{initial leaf area} * \text{final dry mass}) / \text{final leaf area}$$

The amount of foliage consumed was estimated by subtracting final leaf dry mass from initial leaf dry mass. Relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) were calculated from gravimetric measurements following Avrcs and McLean (1987) (note: AD=the percentage of food consumed that is digested; ECD=the percentage of digested food that is converted to biomass; RGR=RCR*AD*ECD).

Data Analysis

The data were analyzed by ANOVA (SAS, Proc GLM; Type III sum of squares). Aspen clone and insect species were nested within the CO₂ and ozone treatments. CO₂, ozone, aspen clone, insect species, and chamber were all treated as fixed effects, and were tested over residual error (MSE). Chamber was considered a fixed effect because the chambers used in the study were not selected at random from a larger population of chambers. Treating chamber as a fixed effect increases the power of the experiment for detecting treatment effects, but limits the scope of inference from the analysis to this study (see Bennington and Thayne 1994 for a discussion of fixed vs. random effects in ANOVA). Means were separated using the protected LSD test.

RESULTS

Larval Survival

First instar survival over the 48-hour bioassay period was high. 84 percent, 92 percent, and 99 percent for first instar gypsy moth, forest tent caterpillar, and whitemarked tussock moth, respectively, and over 90 percent for fourth instars of all species. Survival was not affected by any of the treatments.

CO₂ Effects on Insect Performance

Elevated CO₂ decreased the growth of first instars of all three species, but only on two of the three aspen clones tested (Table 1, significant CO₂*clone interaction). Overall, elevated CO₂ decreased the relative growth rates of first instar forest tent caterpillar, gypsy moth, and whitemarked tussock moths by 65 percent, 32 percent, and 48 percent, respectively (Figure 1). However, the growth rates of none of the insect species were decreased by elevated CO₂ when feeding on aspen clone 216, and elevated CO₂ actually increased the growth rate of forest tent caterpillar by 80 percent on this clone (Table 1, significant CO₂*clone interaction). Elevated CO₂ decreased the relative growth rates of fourth instars of all species on all clones (Table 1, Figure 2). Percent decreases in growth rate ranged from 51 percent for gypsy moth to 26 percent for large aspen tortrix.

Table 1. F-values from ANOVA of performance of first and fourth instar gypsy moth, forest tent caterpillar, whitemarked tussock moth, and fourth instar large aspen tortrix on three aspen clones exposed to two levels of CO₂ and ozone. Statistical significance is shown as follows: **** indicates $P \leq 0.0001$; *** indicates $P \leq 0.001$; ** indicates $P \leq 0.01$; * indicates $P \leq 0.05$.

Source	1st Instar		4th Instar				
	df	RGR	df	RGR	RCR	ECD	AD
CO ₂	1	25.8 ****	1	34.3 ****	7.0 ***	1.7	5.1 *
O ₃	1	31.4 ****	1	14.8 ****	0.2	14.4 ***	35.5 ****
CO ₂ *O ₃	1	2.9	1	0.4	0.1	0.1	0.6
chamber(CO ₂ *O ₃)	4	10.4 ***	4	5.9 ***	2.0	0.7	5.9 ***
aspen clone	2	19.0 ****	2	0.7	0.1	2.9	13.5 ****
CO ₂ *clone	2	5.5 ***	2	3.7 *	2.1	5.1 ***	2.1
O ₃ *clone	2	0.4	2	3.8 *	1.2	0.8	5.0 **
CO ₂ *O ₃ *clone	2	0.9	2	5.5 **	3.3 *	1.2	2.1
clone*chamber(CO ₂ *O ₃)	8	1.2	8	1.9	1.6	0.8	1.0
insect species	2	27.4 ****	3	69.5 ****	192.0 ****	9.7 ***	19.4 ****
CO ₂ *insect	2	0.7	3	0.8	2.9 *	1.2	1.6
O ₃ *insect	2	1.3	3	0.5	0.1	0.2	2.0
CO ₂ *O ₃ *insect	2	3.9 *	3	0.2	0.1	1.2	0.8
insect*chamber(CO ₂ *O ₃)	8	1.4	12	0.4	0.4	0.6	1.5
clone*insect	4	2.8 *	6	0.5	0.2	0.3	3.7 **
CO ₂ *clone*insect	4	2.8 *	6	0.5	1.4	1.7	0.6
O ₃ *clone*insect	4	1.3	6	1.3	1.2	1.2	0.1
CO ₂ *O ₃ *clone*insect	4	1.3	6	1.1	2.4 *	0.4	1.0
clone*insect*chamber(CO ₂ *O ₃)	16	1.5	24	1.5	1.7 *	1.1	1.5
error	215		286				

The consumption rate (RCR) of large aspen tortrix was decreased 20 percent by elevated CO₂. Consumption rates of the other species were not affected (Figure 3, Table 1, significant CO₂*insect interaction). The unusually high consumption rates for large aspen tortrix are artefacts resulting because the image analyzer used to measure leaf area was unable to differentiate skeletonized foliage (characteristic of large aspen tortrix feeding) from completely consumed leaves. Hence, consumption was overestimated. This prevents direct comparisons with the other species, but will not affect relative comparisons of large aspen tortrix performance on the different CO₂ and ozone treatments. Elevated CO₂ had a minor but statistically significant effect on the ability of larvae to digest food, decreasing AD by only 5 percent (Table 1, Figure 4). The effect of elevated CO₂ on larval ability to convert digested food to biomass (ECD) was dependent on the aspen clone fed upon (Table 1, significant CO₂*clone interaction). Elevated CO₂ decreased ECD by 51 percent and 32 percent on clones 259 and 271, respectively, but ECD was increased 55 percent on clone 216 (Figure 5).

Ozone Effects on Larval Performance

Elevated ozone increased the growth rates of first instars of all three species (Table 1). Forest tent caterpillar larvae were affected most dramatically, with their growth rate increasing 10-fold on ozone exposed foliage. Elevated ozone increased the growth rates of gypsy moth and whitemarked tussock moth by 70 percent and 60 percent, respectively (Figure 6). The growth rate of fourth instars of all species was also increased by elevated ozone, with percent increases ranging from 27 percent for forest tent caterpillar to 59 percent for gypsy moth (Figure 7). Ozone had no effect on relative consumption rate (Table 1, Figure 8). However, elevated ozone decreased the ability of larvae to digest food (Figure 9), with the magnitude of the effect dependent on clone (Table 1, significant ozone*clone interaction). Elevated ozone decreased AD 29 percent on clone 259, 14 percent on clone 216, and 6 percent on clone 271. Ozone increased the ability of larvae of all four species to convert digested food to biomass (ECD) (Table 1), with the percent increases ranging from 35 percent for forest tent caterpillar to 230 percent for large aspen tortrix (Figure 10).

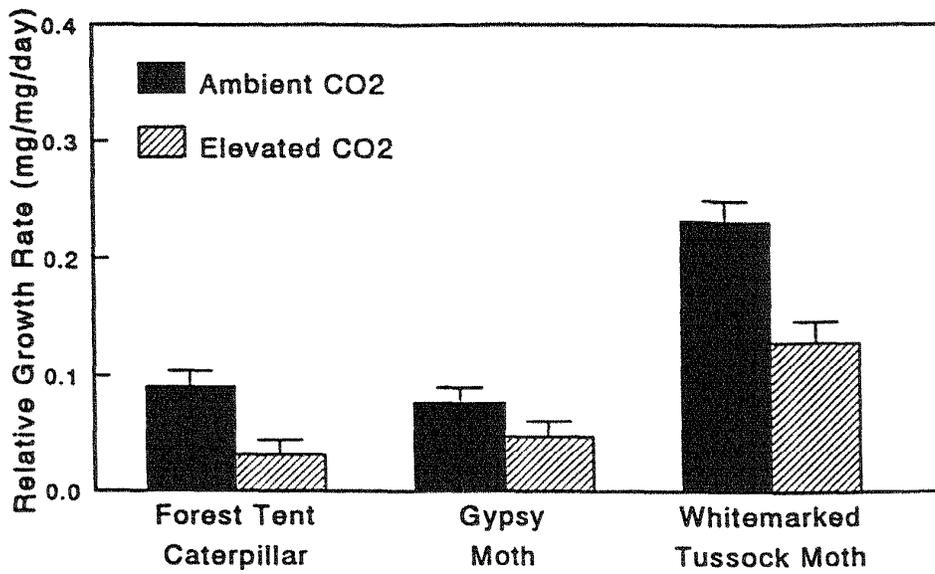


Figure 1. Effect of ambient and elevated CO₂ on the relative growth rate (RGR) of first instar forest tent caterpillar, gypsy moth, and whitemarked tussock moth feeding on quaking aspen. Data are expressed as least square means \pm one standard error.

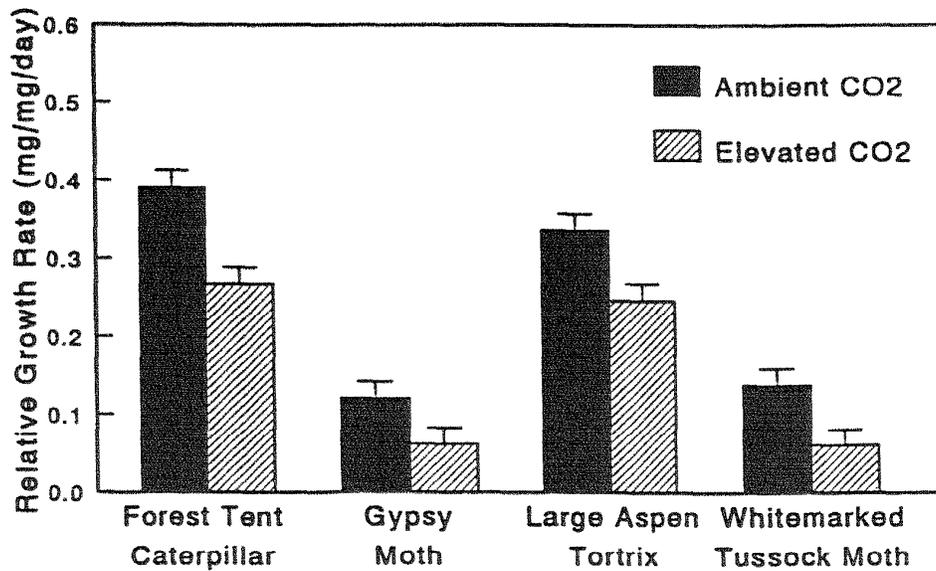


Figure 2. Effects of ambient and elevated CO₂ on the relative growth rate (RGR) of fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth, feeding on quaking aspen. Data are expressed as least square means \pm one standard error.

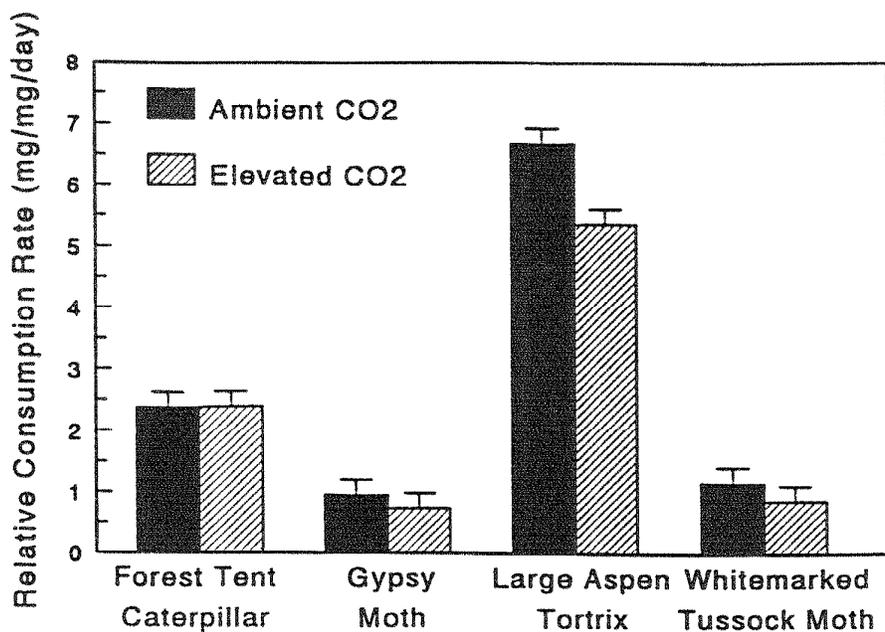


Figure 3. Effect of ambient and elevated CO₂ on the relative consumption rate (RCR) of fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on quaking aspen. Data are expressed as least square means \pm one standard error.

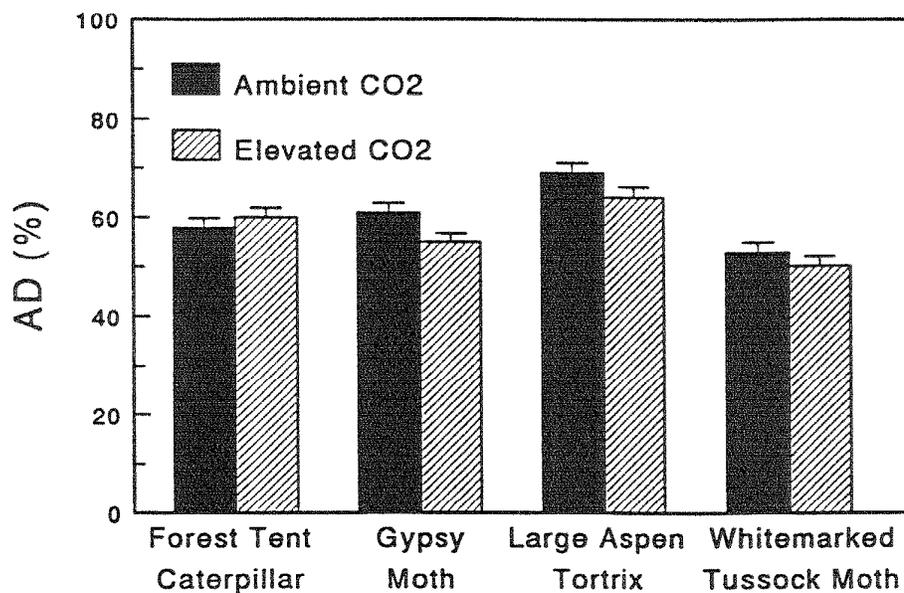


Figure 4. Effect of ambient and elevated CO₂ on the percentage of consumed food digested (AD) by fourth instar forest tent caterpillar, gypsy moth, whitemarked tussock moth, and large aspen tortrix feeding on quaking aspen. Data expressed as least square means \pm one standard error.

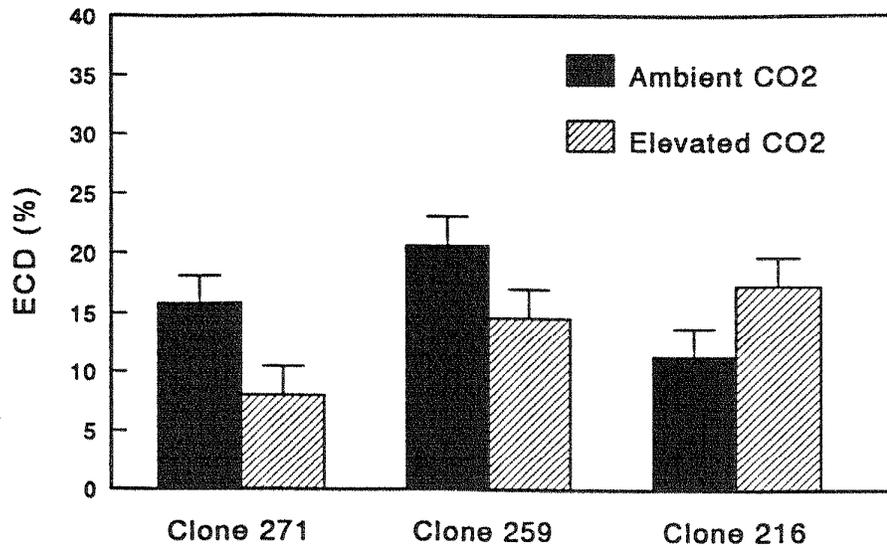


Figure 5. Effect of ambient and elevated CO₂ on the percentage of digested food converted to biomass (ECD) by fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on three clones of quaking aspen. Data are expressed as least square means of all insects combined \pm one standard error.

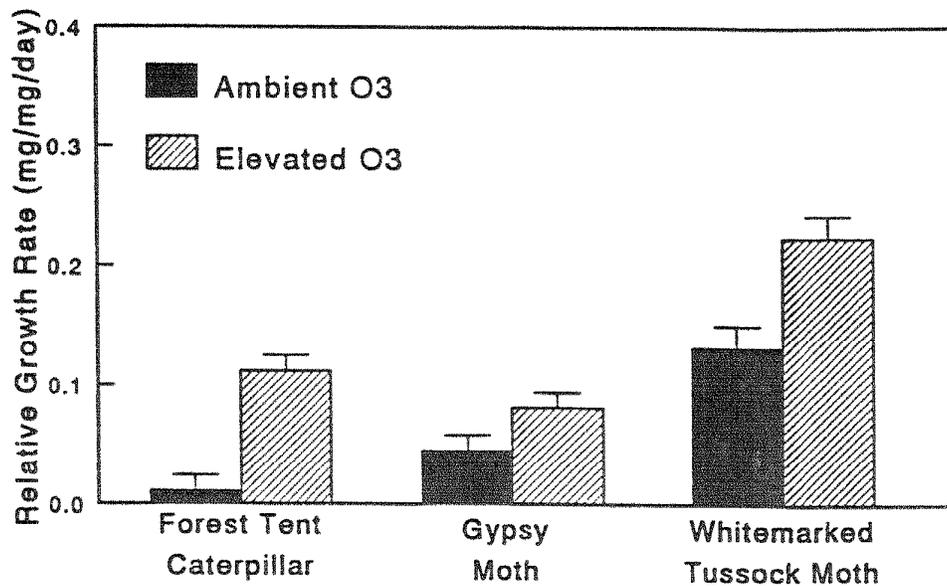


Figure 6. Effect of ambient and elevated ozone on the relative growth rate of first instar forest tent caterpillar, gypsy moth, and large aspen tortrix feeding on quaking aspen. Data are expressed as least square means \pm one standard error.

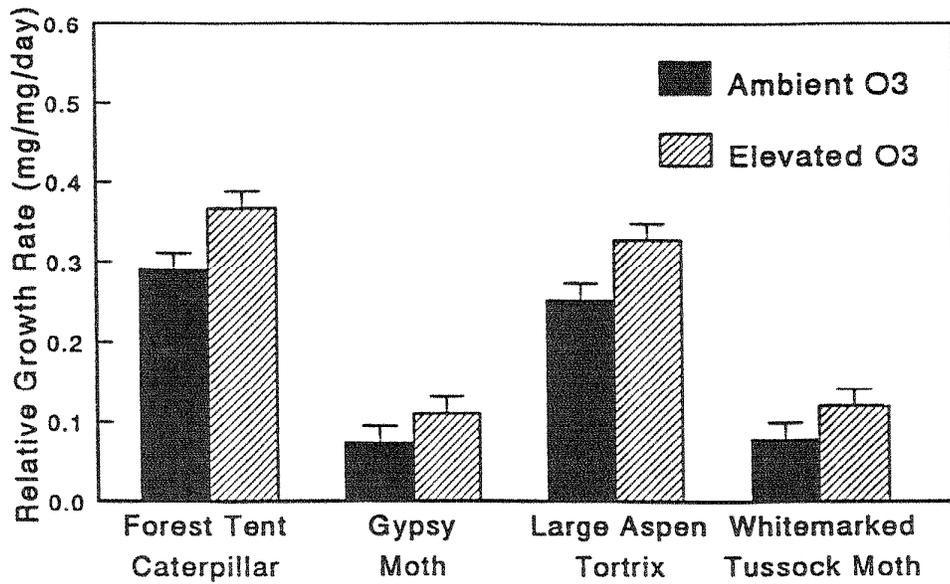


Figure 7. Effect of ambient and elevated ozone on the relative growth rate (RGR) of fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on three clones of quaking aspen. Data are expressed as least square means \pm one standard error.

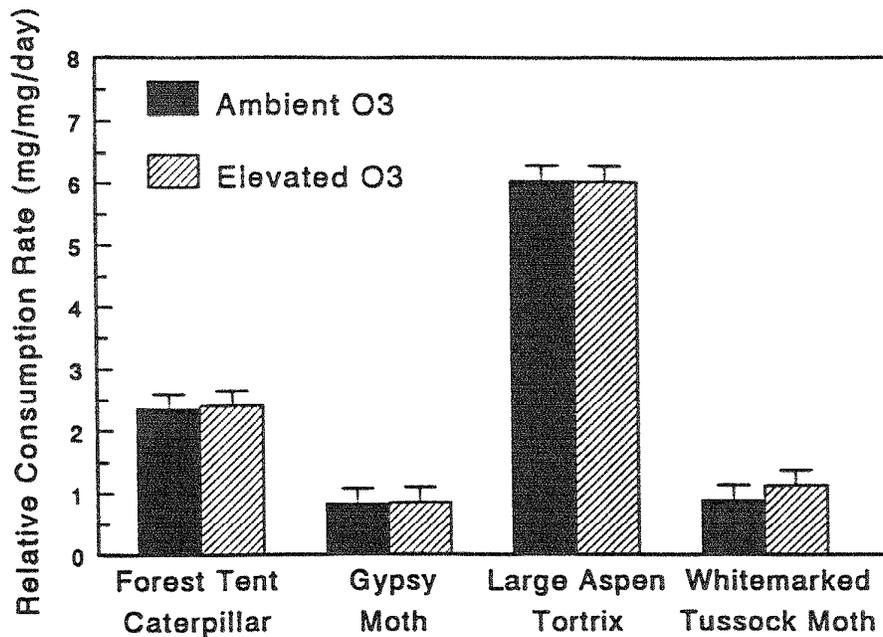


Figure 8. Effect of ambient and elevated ozone on the relative consumption rate (RCR) of fourth instar forest tent caterpillar, gypsy moth, whitemarked tussock moth, and large aspen tortrix feeding on quaking aspen. Data are expressed as least square means \pm one standard error.

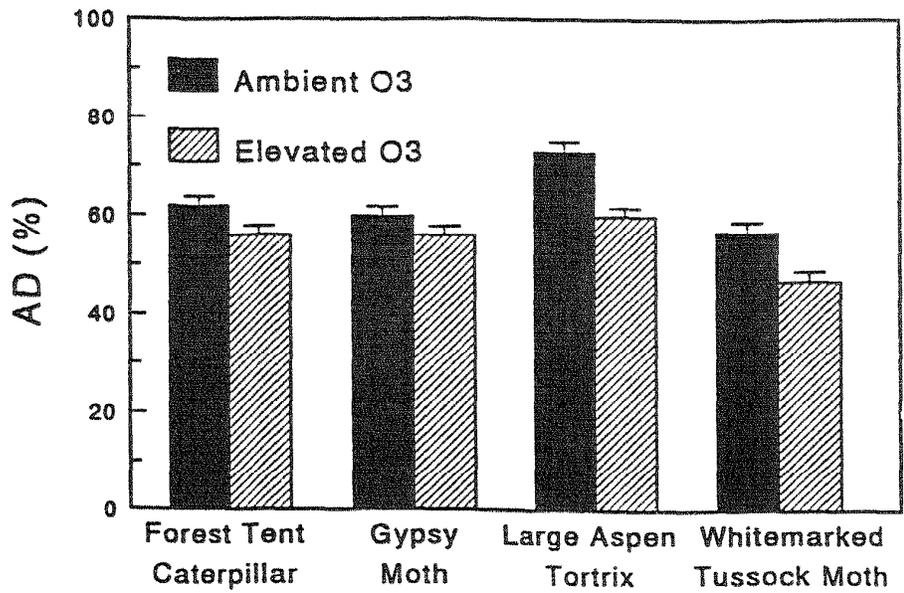


Figure 9. Effect of ambient and elevated ozone on the percentage of consumed food digested (AD) by fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on three clones of quaking aspen. Data are expressed as least square means \pm one standard error.

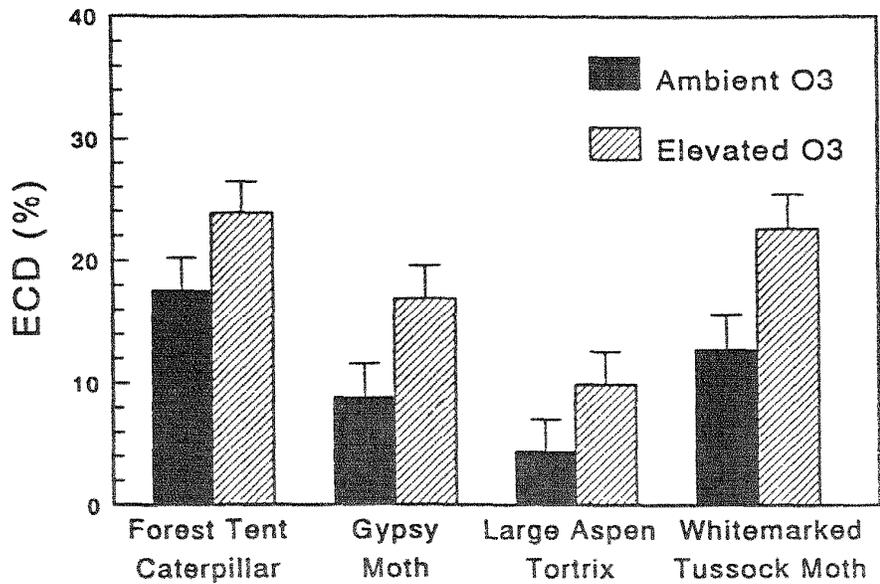


Figure 10. Effect of ambient and elevated ozone on the percentage of digested food converted to biomass (ECD) by fourth instar forest tent caterpillar, gypsy moth, whitemarked tussock moth, and large aspen tortrix feeding on quaking aspen. Data are expressed as least square means \pm one standard error.

DISCUSSION

CO₂ Effects on Insect Performance

Most studies have found elevated CO₂ to decrease the nutritional quality of plants for insects, in some cases resulting in decreased growth and survival (Lincoln 1993, Lincoln and others 1993). In other cases, however, insects increased their consumption rates, compensating for decreased nutritional quality (Lincoln 1993, Lincoln and others 1993). Lindroth and others (1993) in the only published study of the effects of atmospheric CO₂ on forest insects found that consumption rates of gypsy moth and forest tent caterpillar increased substantially, but their growth rates declined.

In this experiment, elevated CO₂ decreased the growth rates of fourth instars of all species. However, the results of elevated CO₂ on the growth of first instars were dependent on the host genotype. Elevated CO₂ decreased the growth rate of first instars on two of the three clones tested. However, the growth rate of first instar forest tent caterpillar increased dramatically on clone 216, and the growth of the other two species were not affected on this clone.

Previous studies have found that insects frequently increase consumption rates to compensate for elevated CO₂-induced reductions in foliage quality (Lindroth and others 1993, Lincoln and others 1993). In this experiment, however, elevated CO₂ decreased the consumption rate of large aspen tortrix and had no effect on the other species. The small reduction in the ability of larvae to digest food caused by elevated CO₂ was not enough to account for the dramatic decreases observed in growth rate. Rather, decreased growth was due to decreased ability of larvae to convert digested food to biomass (ECD), and in the case of large aspen tortrix, decreased consumption rate. However, the effect varied by clone. Elevated CO₂ decreased the ECD of larvae feeding on clones 259 and 271, but increased the ECD of larvae feeding on 216 (the same clone on which first instar forest tent caterpillar growth rate increased).

Lindroth and others (1993) found that elevated CO₂ decreased the ECD of gypsy moth and forest tent caterpillar on aspen. Decreased ECD is consistent with increased concentrations of toxins in the diet. In a companion study using the same plants, we did see a corresponding increase in the phenolic glycosides tremulacin and salicortin in response to elevated CO₂ (see abstract by Nitao et al.). Tremulacin in particular has been shown previously to decrease growth and survival of gypsy moth and forest tent caterpillar (Lindroth 1991). Tremulacin concentrations were lowest in clone 216, the clone on which ECD and first instar forest tent caterpillar growth rate were increased. However, effects of CO₂ on other secondary metabolites and nutrients also may have affected growth. Elevated CO₂ almost universally decreases foliar N concentrations (Lincoln and others 1993), which frequently limits insect growth (Mattson 1980). Analyses of condensed tannins and N are currently underway.

Ozone Effects on Insect Performance

To our knowledge, this study represents the first report on the effects of ozone on the growth and nutritional physiology (as opposed to feeding preferences) of Lepidoptera larvae feeding on woody plants. The growth rates (RGR) of first and fourth instars of all species fed foliage exposed to elevated ozone were increased. Growth rates were increased because elevated ozone increased the ability of larvae to convert digested food to biomass (ECD), which overcompensated for the smaller negative effect of elevated ozone on their ability to digest foliage. Ozone had on effects on consumption rates. The enhanced quality of foliage exposed to elevated ozone may be due to the observed decrease in foliar concentrations of tremulacin (see abstract by Nitao et al.).

No Interactions Between CO₂ and Ozone

To our knowledge, this is the first study that simultaneously tested the effects of atmospheric ozone and CO₂, and there were no interactions between them. The ozone and CO₂ treatment levels used in this experiment had roughly equal and opposing effects on insect performance, with elevated ozone decreasing and elevated CO₂ enhancing aspen resistance to the four species.

SUMMARY AND CONCLUSIONS

The effects of elevated atmospheric CO₂ and ozone on the resistance of three aspen clones to four species of Lepidoptera larvae were tested. Elevated ozone generally increased, and elevated CO₂ generally decreased insect growth. However, elevated CO₂ increased the growth rates of first instar forest tent caterpillar feeding on aspen clone 216. Most previous studies have found elevated CO₂ to increase insect consumption rates. However, in this study, elevated CO₂ decreased the consumption of large aspen tortrix and had no effect on the other species. Ozone also had no effect on consumption. Effects on insect growth occurred primarily because of treatment effects on the ability of larvae to convert digested food to biomass, but in the case of elevated CO₂, the effect was clone dependent. Elevated CO₂, while having an overall negative effect on ECD, increased the ECD of larvae feeding on clone 216. Effects of CO₂ and ozone on insect performance corresponded with ozone and CO₂ effects on foliar concentrations of the toxic phenolic glycoside tremulacin. There were no interactions between the ozone and CO₂ treatments.

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GENETIC AFTEREFFECTS OF INCREASED TEMPERATURE IN LARIX

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Abstract: We tested the hypothesis that temperature during gametogenesis and embryogenesis can affect progeny genotype and phenotype. Identical crosses were made among cloned parents of Larix spp. inside and outside a greenhouse, where the temperature inside averaged 4°C above the outside temperature. Significant growth differences as a function of crossing environment were observed. When the crosses were grown in the same environment the phenotypes of crosses made inside tended to resemble more southern ecotypes. In addition, segregation distortion at the chlorophyll- a/b-protein locus as a function of crossing environment was observed. These results support the hypothesis that progeny phenotype and genotype can exhibit aftereffects that are a function of crossing environment.

INTRODUCTION

Modification of progeny performance as a function of parental environment has been reported for both Picea abies (L.) Karst. and Pinus sylvestris L. (Lindgren and Wang 1986, Johnsen 1989a and 1989b, Johnsen and others 1989, and Dormling and Johnsen 1992). For both species, the same crosses were made on cloned parents (of northern origin) in seed orchards about 5-8° latitude apart and the resulting progenies were planted on the same site. Full- and half-sibling progeny produced at the more southern locations consistently grew more than their siblings produced in the north. In addition, progeny produced in the south exhibited less cold hardiness and overall seemed to behave like southern ecotypes. These modifications due to parental environment have been called aftereffects. Similar changes in growth habits of inbred lines of Pisum and Linum were induced by temperature or fertilization regime (Highkin 1958, Durrant 1971), which also appear to be heritable in these two species.

In general, clonal seed orchards located in more southerly regions of a species range flower better, which has resulted in the common practice of locating clonal seed orchards as far south within the range as possible (Schmidting 1987). In addition, consistently good flowering can be obtained using indoor, potted breeding orchards (e.g. Philipson 1983, Ross 1985, Adams and Greenwood 1992). These practices require further understanding of the effects of breeding environment on progeny performance by forest tree species. In addition, the possibility of rapid global warming raises questions about the ability of long-lived woody perennials to adapt to such changes. Are there short-term, as yet uncharacterized genetic strategies so that better adapted progeny can be selected before seed is even shed? The purpose of this paper is to compare the effect of adjacent indoor (greenhouse) and outdoor environments on height growth and allele frequencies of progeny from identical crosses Larix spp.

MATERIALS AND METHODS

Fourteen parents were selected from a potted, indoor clonal breeding orchard of Larix spp. for creation of identical indoor and outdoor breeding populations. The orchard was established by grafting in 1987. Details of establishment and culture are described by Eysteinnsson and Greenwood (1993). The parents, representing three species (five from Larix laricina (DuRoi) K. Koch, five from L. decidua Mill., and four from L. leptolepis Gord.), were each represented by at least four ramets, all of which remained in the greenhouse until mid-November, 1991. At that time two randomly chosen ramets of each parent were moved outside to a pad immediately adjacent to the greenhouse. Crossing began in the spring of 1992 and continued in 1993 (Table 1). The greenhouse remained unheated most of

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the winter, so that shoots went through winter dormancy. Temperatures were recorded simultaneously at hourly intervals at both locations using thermocouples (two inside and two out) attached to an ECD model 50 data logger (ECD Inc., Milwaukie, OR 97222-8825).

Twelve pairs of identical indoor-outdoor crosses (nine made in 1992 and three in 1993) yielded enough viable seed for a common garden progeny test. One cross was repeated in 1992 and 1993. Most crosses were intraspecific (four among eastern larch parents, three among European larch, three among Japanese larch) with two interspecific crosses between Japanese and European larch. Pollen always came from the same location as the female parent (see Eysteinnsson and Greenwood 1993 for details on pollen collection and pollination). A total of five of the 14 parents were involved in two to three crosses each, but there were no reciprocal crosses between inside and out.

In early January, 1994, seed was germinated in 150x15mm petri dishes on moistened filter paper (Whatman #42) in a growth chamber under a 16h photoperiod (incandescent light only), 28°C day, 20°C night. Germinants (radicle emerged) were transplanted to 65 cm³ plastic tubes (Ray Leech cells, Stuewe and Sons, Inc. 2290 SE Kiger I. Drive, Corvallis, OR 97333) containing peat moss:builder's sand:vermiculite:perlite (8:4:3:3) with Osmocote 17.6:12 (four mo. timed release) fertilizer at 4g·l⁻¹ (Grace-Sierra, 1001 Yosemite Dr., Milpitas, CA 95035). Percentage of seed germinating was recorded for each family. Seedlings continued to grow in a heated greenhouse (20-25° day, 15-20° night) under a 16h photoperiod (natural daylight supplemented with high pressure sodium lamps for 16h). Each family was grown as a single block, and trays containing the blocks were shuffled weekly to even out the effects of environmental variation.

In late May 1994, seedlings were transplanted into 11.4 l plastic pots containing peat moss:vermiculite:builder's sand (2:1:1) and Osmocote as described above, and moved into the same fiberglass-covered greenhouse used for the indoor breeding orchard with no supplemental photoperiod. The seedlings were arranged in four blocks containing four replicates, the latter consisting of full-sibling pairs (one sibling from outside, one from inside were placed side by side) of 12 families, for a total of 384 trees. The heights of all the seedlings were measured immediately after repotting. All the seedlings were watered via drip irrigation, assuring that the potting medium was close to field capacity most of the time. The trees were measured again in late September 1994.

In the spring of 1992 and again, in 1993, embryos were carefully excised from seeds gathered in the respective year. DNA was extracted from the seeds as described by Doyle and Doyle (1990). Inheritance of specific alleles at one of the loci encoding a chlorophyll-*a/b*-binding protein (*cab*) protein was determined by PCR amplification of the genomic sequence, followed by analysis using single strand conformational polymorphisms (SSCP) (Orita and others 1989).

Statistical analysis was performed using SYSTAT software (Systat, Inc., 1800 Sherman Ave., Evanston, IL 60201-3793).

RESULTS

Temperatures averaged about 4°C warmer inside than out in 1992 but the difference was greatest between January and September (Figure 1). The lack of difference in the fall is probably due to greater cloud cover during the day, which is typical of fall and early winter in the northeastern US. Similar temperature profiles were observed in 1993.

A significant block effect developed during the growing season, probably due to uneven temperature in the greenhouse (Table 1). The effect of cross was highly significant at both measurement times. The effect of crossing environment was not significant at either time, but the cross by location interaction was highly significant at both dates, indicating that the crosses grew differently as a function of crossing environment. In general, crosses made outside among eastern larch grew more, while crosses made inside among the other species tended to grow more (Figure 2). The ANOVA of the height differences between crossing environments reveals that the effect of cross on height difference between crossing environments is highly significant (Table 2). Height difference due to crossing environment was significantly different from zero to five of the 12 crosses at each measurement date ($p < 0.05$).

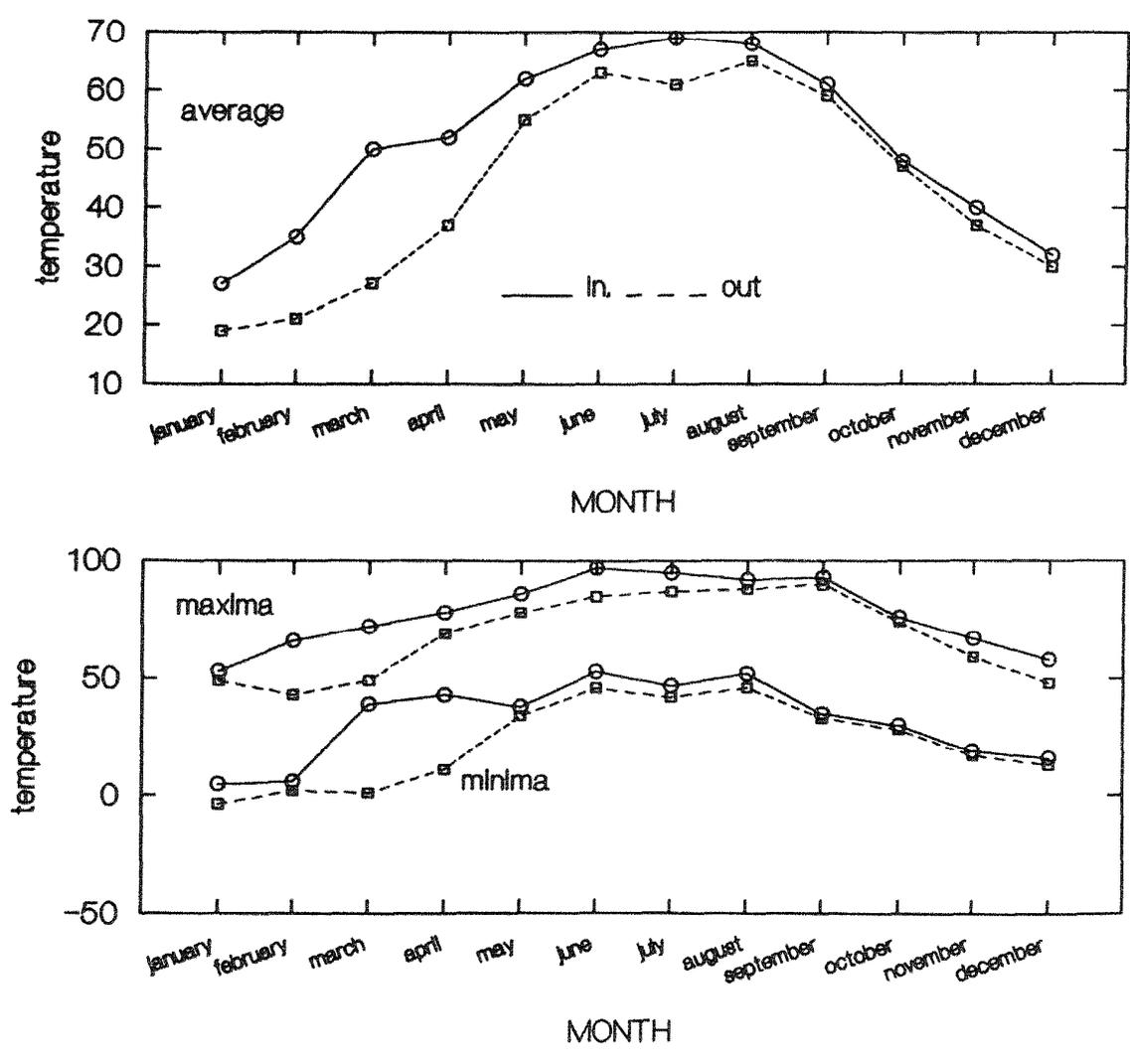


Figure 1. Mean monthly temperatures (1992) at breeding orchards located inside and outside the greenhouse.

Table 1. ANOVAs for heights in May and September.

Source of Variation	DF	May Height		September Height	
		Mean Square	P	Mean Square	P
Block (B)	3	1.91	0.912	2518	0.031
Cross (C)	11	139.43	0.000	23,984	0.000
Location (L)	1	11.80	0.297	644	0.381
BxC	33	11.17	0.422	772	0.591
BxL	3	6.56	0.611	2483	0.032
CxL	11	47.64	0.000	3034	0.000
BxCxL	33	8.36	0.810	888	0.380
Error		10.8		835	

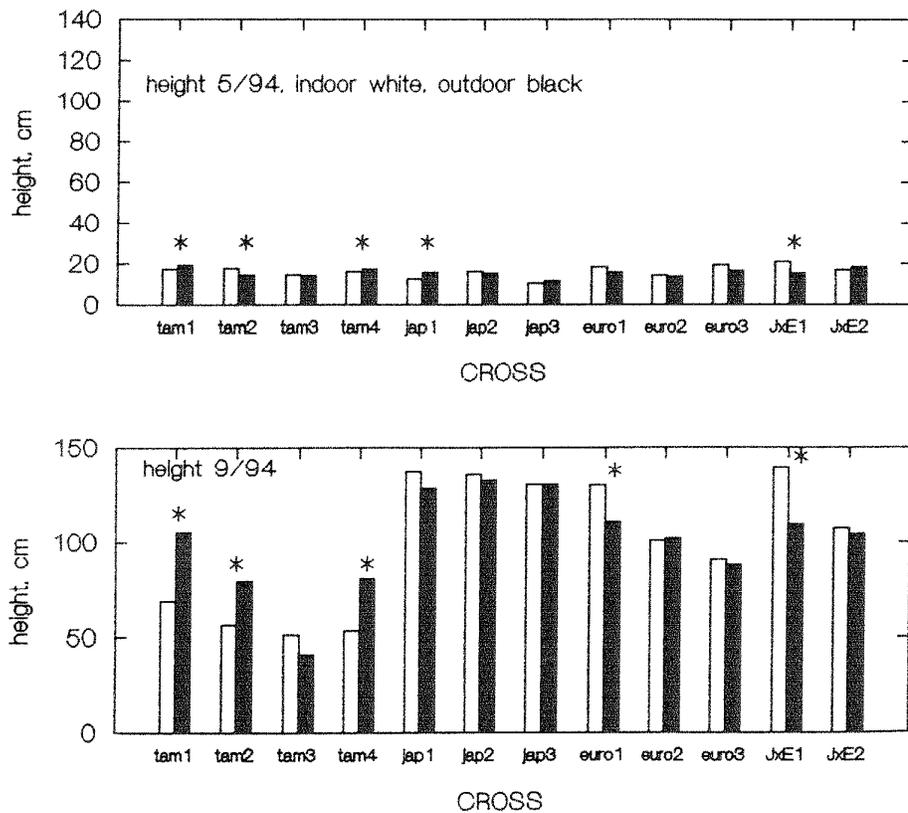


Figure 2. Heights of indoor (white) and outdoor (black) siblings of 12 identical controlled crosses made inside and outside the greenhouse. Abbreviations: tam = *Larix laricina*, jap = *L. leptolepis*, euro = *L. decidua*. Asterisks indicate crosses where indoor-outdoor difference was significantly > zero at $p < 0.05$.

Table 2. ANOVAs for height difference between indoor and outdoor full-sibling heights by cross.

Source of Variation	DF	Mean Square	P	Mean Square	P
Block (B)	3	16.93	0.424	5533	0.011
Cross (C)	11	93.14	0.000	5778	0.000
BxC	33	17.20	0.547	1629	0.305
Error		18.04		1437	

Height differences due to crossing environment caused by differences in seed vigor must be considered because weight and percent germination of seed from crosses made inside tended to be slightly greater. However, there was no significant correlation between percent germination and height at either measurement date (Pearson $r=-0.150$ in May, $r=0.193$ in September). Also, height growth rank between crossing environments changed for four of the 12 crosses between the May and September measurements. May and September heights were not significantly correlated (Pearson $r=-0.081$), providing further evidence that residual effects of seed vigor on seedling height, if they exist at all, are not persistent.

The opposite response to crossing environment by *L. laricina* crosses may be a function of bud set following repotting. The relatively poor growth of these crosses was due to a tendency to set bud relatively easily after repotting: by midsummer, less than one third of these crosses had been growing continuously (see Table 3). The observation that a slightly higher percentage of the crosses made outside grew continuously explains in part why the crosses made outside grew more. In contrast, most of the trees from the crosses involving the other species grew continuously, with the percentage slightly higher for crosses made inside. The bud set probably occurred because ambient photoperiod at the time of repotting was about 14h, about 2h shorter than the trees had been receiving prior to repotting.

Table 3. The percentage of trees by species on 7-19-94 that grew continuously following repotting the previous May.

Species	# Trees		Continuously Growing	
	In	Out	In	Out
Eastern larch	91	87	27%	32%
Japanese larch	53	53	98%	96%
European larch	70	79	69%	63%
Hybrid (JxE)	42	43	68%	67%

Environmental aftereffects could be the result of either epigenetic or genetic changes. In the latter category, changes could arise either as a result of changes in the mutation rate or in selection for particular alleles or haplotypes within the genome. Selection should be detectable as segregation distortion, or non-Mendelian inheritance. Previous observations while constructing a genetic map for larch suggested that one of the cab loci protein would be a candidate marker for detecting segregation distortion (Hutchison et al. 1995, Unpublished). As shown in Table 4, allele 1 was found at significantly higher frequency in the progeny of an indoor grown *L. decidua* cross in 1992. Progeny from the outdoor grown clone was not available. This observation was repeated the following year. Though the significance of the segregation distortion for the indoor grown trees was decreased, the trend remained

the same. Of even greater interest is that segregation distortion reverses in the progeny of the clonal replications that were grown outdoors (Table 4).

Table 4. Frequency of occurrence of two alleles at the *cab-3* locus in *L. decidua* grown inside (1992 and 1993) and outside (1993), including χ^2 for deviation from expected 1:1 segregation of the two alleles.

1992-Indoor				1992-Indoor				1993-Outdoor			
Allele 1	Allele 2	χ^2	p	Allele 1	Allele 2	χ^2	p	Allele 1	Allele 2	χ^2	p
37	19	5.79	0.016	34	25	1.37	0.24	18	39	7.74	0.005

DISCUSSION

Significant environmental aftereffects on height growth of five of the 12 full-sib families created inside and outside the greenhouse have been demonstrated, which are not a function of seed vigor. The most likely cause of the aftereffects observed here is temperature, which was consistently higher inside the greenhouse throughout the periods of both gametogenesis and embryogenesis in larch. However, differences in light quality due to the fiberglass covering of the greenhouse were also present. These effects appear to be similar to those demonstrated for *Pinus sylvestris* and *Picea abies*, in that crosses made inside the greenhouse behaved like southern ecotypes. The crosses made between *L. decidua* and *L. leptolepis* inside tended to grow slightly more, and the majority of the trees among these crosses grew continuously after repotting. In the two crosses where this difference was significant at $p < 0.05$, all the trees were growing actively in mid-July. In contrast, the majority of trees among the eastern larch crosses set bud after repotting, and had not resumed growth by mid-July, so the full growth potential of these crosses was not realized. Three of the four larch crosses exhibited significantly more height growth when made outside, and among these crosses, 40 percent of the trees from the outside crosses had been growing actively most of the summer, versus 29 percent for the crosses made inside. The greater frequency of resumption of active growth by the outdoor crosses is consistent with demonstrations that more northerly ecotypes at *Picea abies* require smaller heat sums before bud burst occurs (e.g. Beuker 1994). Observations on frost hardiness as a function of crossing environment have also been made, but await confirmation by further observations in the spring of 1995.

Environmental aftereffects could potentially be explained by selection for specific alleles or regions of the genome. This selection could take place either during gametogenesis or in the developing embryo. Segregation distortion of the *cab* locus could be due to linkage to a generalized lethal allele at another locus. Alternatively, it could be due to environmental selection of a specific allele in the elevated temperatures of the greenhouse. Data from inheritance of allele 1 and allele 2 in the indoor vs. outdoor plants provides at least a partial answer to these two possibilities. In the indoor population allele 1 was the 'favored' allele (Table 4). However, the outdoor population of trees showed a strong segregation distortion in the opposite direction, that is, selection for the alternative allele (Table 4). These data strongly suggest that the segregation distortion is not due to a general lethal allele at a locus near the *cab* locus. If that were the case, the selection should have been for the same allele in both populations. Rather, it appears that environment affect which allele is selected in these two populations.

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PHYSIOLOGICAL AND GENOTYPIC RESPONSES OF CENTRAL HARDWOOD SPECIES TO
ALLELOCHEMICALS, OTHER STRESSES, AND THEIR INTERACTIONS

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In response to increasing carbon dioxide levels, most general circulation models (GCMs) predict increasing temperatures and decreasing precipitation for the central hardwood region of the United States. Plants in this region will need to adapt to these changes as well as to other stress agents if they are to germinate, grow, and reproduce. For the last five years, our research program under the global change initiative has been designed to increase our understanding of the physiological and genetic mechanisms used by plants to respond and adapt to multiple stresses in forest ecosystems. To achieve this objective, we have conducted a series of field, greenhouse, and laboratory studies aimed at understanding how plants respond to allelochemicals, drought, and competition.

Rangewide provenance trials provide us with information about the amount of the genetic variation that exists within a species as well as information on the effects of moving local populations to new environments. Recently, we remeasured several rangewide provenance tests for black walnut (*Juglans nigra* L.) and white ash (*Fraxinus americana* L.) that had been established in the 1960s to mid 1970s. These studies continue to provide new information about the genetic variation that exists within these species as they enter their mature growth phase. In addition, we can continually test previous recommendations about the effects of moving seed to new environments. The up-to-date information will help us predict how local populations might respond to altered climatic conditions.

Bresnan and others (1994) recently confirmed our previous recommendations that collecting black walnut seed from 160 to 320 km (100 to 200 miles) south of a planting site results in increased growth of black walnut with acceptable survival for most of the central hardwood region. Similar movement of seed from sources along the edges of the natural range for black walnut, however, resulted in reduced survival or growth. Except in the test plantings along the western edge of the natural range, seed collected east of a planting site tended to show faster tree growth than seed collected west of the planting sites. Southward movement of seed to warmer climates and westward movement of seed to drier sites may simulate the effects of a warmer, drier climate such as that predicted by various GCMs. Few correlations, however, were found between latitude or longitude of the provenance source and subsequent height or diameter growth. These results suggest that sufficient genetic variation exists in black walnut populations throughout most of the central hardwood region to enable them to adapt to changes predicted by the various GCMs.

Recommendations similar to those for black walnut have also been made for white ash. With white ash, the movement of seed or seedlings up to 320 km (200 miles) northward also results in growth increases over local plant material for the central hardwood region. Recently, Roberds and others (1991) found that white ash seed from the southern part of the natural range of white ash tended to be more broadly adapted and had acceptable growth considerably north of their origin. On the other hand, moving seed from the northern parts of the natural range led to

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reduced tree growth. Rink and Kung (1991) confirmed earlier findings by using response surface regression modeling of new data to estimate genotypic responses of white ash to different environments. Latitudinal (north - south) movement of seed had a significant effect on tree growth at 10 of 12 test sites, while longitudinal (east - west) movement of white ash seed had a significant effect at only 4 of the 12 plantings. These results suggest that white ash adaptation to temperature gradients predicted by current GCMs will be more sensitive than its adaptation to decreased precipitation in the central hardwood region.

Greenhouse studies using a modified stair-step approach have also been done to evaluate the genetic and physiological response of black walnut, white ash, and white oak (*Quercus alba* L.) to drought and other stresses. In these studies, progenies of four to six local trees (half sib families) of each species were evaluated for their response to moisture stress, altered nutrient regimes, and leachates produced by a tall fescue sod (*Festuca arundinaceae* Schreb.). Interference by tall fescue on growth of hardwood trees is thought to involve reduced nitrogen cycling and the production of allelochemicals (Van Sambeek 1989, Van Sambeek and others 1989). In our greenhouse studies, 36 to 40 seedlings of each species were grown in 170 L, 60 cm deep wooden boxes filled with a mixture of 1:1 sand:silt-loam topsoil. A vacuum system was used to control available soil moisture at either 0.01 MPa (field capacity) or between 0.04 and 0.05 MPa (McBride and Rink 1986). Half the boxes were watered as needed with leachates collected from under a tall fescue sod. The remaining boxes were watered as needed with distilled water or water collected from under a sand bed. Depending on the species tested, nutrient regimes were altered by the addition of 6N-24P-24K fertilizer or growth of bluegrass (*Poa pratensis* L.) between the rows of seedlings.

Significant differences existed among the half-sib families of white ash, black walnut, and white oak in response to drought and/or allelochemicals produced by tall fescue sod. Leachates from tall fescue had a greater effect on height growth of black walnut seedlings when soils were near field capacity than when available soil moisture was limiting (Rink and Van Sambeek 1985). These observations confirm results from a field planting in which irrigated walnut trees with a tall fescue ground cover had slower growth than the non-irrigated walnut trees (Van Sambeek and McBride 1993). Conversely, tall fescue leachates reduced white ash seedling growth more when the seedlings were under moisture stress than when they were under adequate soil moisture (Rink and Van Sambeek 1987). In two separate trials, height growth of white oak seedlings was unaffected by reduced available soil moisture, tall fescue leachates, or altered soil nutrient levels (Van Sambeek and Rink 1990). Several demonstration plots at our Hardwood Research Demonstration Area confirm that black walnut and white ash trees are more responsive to cultural treatments for controlling tall fescue than are white oak trees (Van Sambeek and Walters 1989).

A subsequent study using dittany (*Cunila origanoides* (L.) Britton) confirmed how difficult it is even under controlled conditions in a greenhouse to separate the factors controlling interference by one plant on neighboring plants (Kobe and others 1992). Although the stair-step method was used, height growth of dittany may have differed in response to either rapid uptake of added nutrients in the recycled leachate or the production of allelochemicals by seedlings of either white oak, sugar maple (*Acer saccharum* Marsh.), flowering dogwood (*Cornus florida* L.), pawpaw (*Asimina triloba* (L.)Dunal), or black walnut. Although dittany is more commonly found in white oak stands than in sugar maple stands, leachates from white oak seedlings reduced dittany growth more than leachates from sugar maple. As with many other field and greenhouse studies on allelopathy, genotypic growth differences among the dittany plants apparently masked any response to allelochemicals.

Recent developments in hardwood micropropagation have made it possible to efficiently vegetatively propagate white ash (Navarrete and others 1989). In addition, the system used for in vitro culture provided a highly controlled environment for testing response of hardwood plantlets to allelochemicals and other stresses. The main advantage of the in vitro studies over greenhouse or field studies is the use of chemically defined growth medium eliminating possible growth inhibition from phytotoxic chemicals naturally occurring in soil that may mask effects of allelochemicals.

Preece and others (1991) recently described and tested the suitability of using cloned white ash plantlets as part of an in vitro bioassay to test for allelochemicals. They found significant differences in root growth among three ash clones grown on Murashige and Skoog (MS) medium, a high salt medium containing all essential macronutrients and micronutrients, organics, sucrose, and a gelling agent. No differences in root growth were found within clones when

roots were allowed to elongate on one-quarter to three-quarter strength MS medium. Thus, small aliquots of plant extracts or soil leachates could be added to one-quarter strength MS medium without creating osmotic potentials that would inhibit root growth of the ash microplants. Navarrete (1993) used the bioassay to confirm that leaf extracts from tall fescue contain one or more phytotoxic compounds at low concentrations, which can inhibit root growth of cloned ash microplants. By altering the macroenvironment, this bioassay could also be used to test for effects of increased temperature or elevated carbon dioxide on microplant growth along with their potential interaction with other stress factors including moisture stress, allelochemicals, pesticides, and air pollutants.

In conclusion, the high-value tree species in the central hardwood region have the ability to respond and to adapt to multiple stresses and their interactions. Our research program will continue to evaluate the physiological and genetic mechanisms used by trees to adapt to changes in their environments as well as options for adaptation or mitigation management.

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