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Soil Nitrogen and Ozone Effects on Growth, Physiology, and Nutrition of Loblolly Pine and Yellow-Poplar Seedlings

M. G. Tjoelker
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O. J. Schwarz

Environmental Sciences Division
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ENVIRONMENTAL SCIENCES DIVISION

SOIL NITROGEN AND OZONE EFFECTS ON GROWTH, PHYSIOLOGY, AND NUTRITION OF LOBLOLLY PINE AND YELLOW-POPLAR SEEDLINGS[1]

M. G. Tjoelker[2], R. J. Luxmoore, and O. J. Schwarz[2]

Environmental Sciences Division
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[1]Submitted as a thesis by M. G. Tjoelker to the Graduate Council of the University of Tennessee in partial fulfillment of the requirements for the degree of Master of Science.
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LIST OF SYMBOLS AND ABBREVIATIONS

C_i	intercellular CO_2 concentration (μL/L)
D^2H	stem diameter squared multiplied by stem height (cm^3)
DMI	whole-plant dry matter increment [final minus mean initial dry weight (mg)]
FRLR	fine root/leaf dry weight ratio (g/g)
FRWR	fine root/plant dry weight ratio (g/g)
g	stomatal conductance (mmol g^{-1} s^{-1}, mol m^{-2} s^{-1})
LDR	leaf abscised/plant dry weight ratio (g/g)
LSD	Least Significant Difference test
LWR	leaf/plant dry weight ratio (g/g)
NRA	nitrate reductase activity (μmol NO_2^--N g^{-1} h^{-1})
NUE	nutrient use efficiency [DMI (g)/nutrient uptake (mg)]
PPFD	photosynthetic photon flux density (μmol m^{-2} s^{-1})
P_n	net photosynthesis (nmol CO_2 g^{-1} s^{-1}, μmol m^{-2} s^{-1})
RGR	relative growth rate (cm^{-3} cm^{-3} $week^{-1}$)
RSR	root/shoot dry weight ratio (g/g)
SWR	shoot/plant dry weight ratio (g/g)
WUE	whole-plant water use efficiency [DMI (mg)/ water use (g), instantaneous water use efficiency [net CO_2 uptake (mmol)/ H_2O transpired (mol)]

ABSTRACT

TJOELKER, M. G., R. J. LUXMOORE, and O. J. SCHWARZ. 1989. Soil nitrogen and ozone effects on growth, physiology, and nutrition of loblolly pine and yellow-poplar seedlings. ORNL/TM-11065. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 124 pp.

The effects of soil nitrogen level and chronic ozone (O_3) stress on carbon and nutrient economy were investigated in loblolly pine (*Pinus taeda* L.) and yellow-poplar (*Liriodendron tulipifera* L). The objectives of the study were: 1) to compare the growth and biomass partitioning in seedlings in response to varied low-nitrogen forest soil treatments in combination with chronic ozone levels over one growing season and 2) to investigate seedling physiological responses, including water use efficiency (WUE), photosynthesis, fine root and leaf nitrate reductase activity (NRA), nutrient concentration, nutrient uptake, and nutrient use efficiency (NUE).

One-year-old seedlings were planted individually in a forest soil in 3.5-L pots containing one of three initial soil soluble N levels of 58, 96, and 172 μg/g. The seedlings were fumigated in open-top field chambers at subambient (charcoal-filtered air), ambient, and elevated (ambient + 60 nL O_3/L) (32, 56, 108 nL O_3/L, 7 h seasonal mean, respectively) ozone levels for 18 weeks. Each of the nine ozone x nitrogen combinations had six replicates.

Growth of loblolly pine as measured by stem volume, shoot relative growth rate, and whole-plant dry weight increased with higher N level with the largest gains in new needle biomass. Higher soil N supply stimulated current needle photosynthesis and increased both instantaneous and whole-plant WUE in loblolly pine. Greater soil N level increased nutrient uptake and increased NUE for P, K, Ca, Mg, and B, whose whole-plant concentrations were diluted by new

growth. Both species were N-deficient at the lower soil N levels. Low soil N level increased the proportion of biomass partitioned to fine roots in yellow-poplar, but whole-plant growth did not respond to N treatment, suggesting other limitations on growth.

Ozone fumigation accelerated and increased leaf abscission in both species after 8 weeks of exposure. Only loblolly pine previous-year needles were abscised, and higher soil N level appeared to predispose the plants to greater abscission. Although yellow-poplar was more sensitive to ozone-induced leaf abscission, final leaf and whole-plant dry weight were not affected. Compensatory leaf growth may have ameliorated chronic ozone stress in yellow-poplar.

A significant ozone x nitrogen interaction was observed for loblolly pine new needle biomass and NRA. Higher ozone levels reduced the new needle growth response to N, whereas NRA was elevated in the high ozone and low nitrogen treatment combination. Elevated ozone exposure increased fine root NRA in loblolly pine. Chronic ozone exposure increased new needle N and S concentrations, suggesting altered N metabolism. In general, ozone exposure decreased shoot weight more than root weight, resulting in higher root/shoot ratios. At low soil N levels, particularly when N is a growth-limiting factor, N stress may counteract the effects of chronic ozone stress on biomass partitioning. Chronic ozone stress response in tree seedlings may be mediated by soil N supply.

INTRODUCTION

Ozone (O_3), the most prevalent photochemical oxidant, is considered the major phytotoxic air pollutant in North America, impairing crop yields and damaging natural vegetation more than any other gaseous air pollutant (EPA, 1985). Ozone was first identified as a phytotoxicant when Richards et al. (1958) showed that ozone was the cause of leaf "stipple" in grape. The leaves of plants damaged by ozone exposure may have a bronzed appearance or exhibit chlorotic and necrotic flecking or lesions, a consequence of damaged palisade mesophyll cells. These visual symptoms are often followed by premature leaf senescence and abscission. However, ozone damage is not limited to visible symptoms; decreased photosynthesis and growth and altered carbon partitioning often occur without visible foliar symptoms. Annual economic losses due to ozone-induced reductions in crop yields alone have been estimated at 2 to 3 billion dollars (Adams and Crocker, 1982; Shriner et al., 1982).

While the short-term effects of ozone on agricultural crop species have been well documented, the long-term effects of ozone on natural vegetation, particularly forest ecosystems are not well understood. Ozone has been implicated as a contributing stress factor in the wide spread aberrant forest declines observed in Europe, Scandinavia, and North America (McLaughlin, 1985; Jagels, 1986; Krause et al., 1986; and Grossman, 1988). The effects of ambient ozone levels on forest species have been documented in the San Bernardino National Forest (Miller, 1983). Growth reductions in jeffrey pine (*Pinus jeffreyi*) attributed to ozone exposure have been observed in Sequoia and Kings Canyon National Parks (Peterson et al., 1987). Although tree species are considered less sensitive to equivalent ozone does than annual crop species in terms of visual symptoms and reductions in net photosynthesis and growth (Harkov and Brennan, 1982; Reich, 1987), even a small reduction in net

photosynthesis or altered carbon partitioning may have significant effects on tree growth and function when integrated over the lifespan of a tree or forest stand.

In addition to air pollutant stress, other environmental factors simultaneously influence plant productivity. Variations in climate, water availability, and soil nutrient supply could interact with air pollutant stress, exacerbating or ameliorating the effects of ozone stress in forest ecosystems (McLaughlin, 1985). For example, since soil nitrogen (N) is often limiting in forest ecosystems (Kramer and Kozlowski, 1979; Tamm, 1979), it may mediate tree response to ozone stress through its effects on tree physiology and growth.

Ozone Source and Concentration

Ozone is produced naturally in both the stratosphere and troposphere through atmospheric chemical reactions involving free radicals (EPA, 1985). Tropospheric ozone concentrations near the earth's surface are increased through the photochemical reactions of NO_x and volatile organic hydrocarbons in conjunction with high levels of solar near-ultraviolet radiation (EPA, 1985; Krupa and Manning, 1988). Near urban areas this results in a typical diurnal ozone concentration profile of low ozone levels occurring at sunrise and rising to peak levels in the mid-afternoon, roughly tracking solar irradiance levels (EPA, 1985). Annual peaks in ozone concentration often occur during the summer near urban areas, however, the maximum is in the springtime for some areas (Taylor and Norby, 1985). Ozone formation is augmented by anthropogenic output of hydrocarbons or NO_x from industrial sources and motor vehicle emissions. Mean ozone concentrations during the growing season in the southeastern United States are about 60 nL/L during daylight hours with episodic concentrations exceeding 120 nL/L. Ozone concentrations reach maximum levels during sunny summer days near metropolitan areas, often exceeding the primary National

Ambient Air Quality Standard (NAAQS), currently set at 120 nL/L for one hour, while natural background ozone levels have been variably estimated between 10 and 40 nL/L (Altshuller, 1987). During 1987, for example, the NAAQS value for ozone was exceeded in over 50 metropolitan areas (Schere, 1988). High ozone concentrations area not exclusively an urban phenomenon, as large-scale air pollution episodes may span to encompass rural regions and last over a period of days, depending on meteorological conditions (Meagher et al., 1987; Schere, 1988). Unlike other air pollutants, long-term ozone concentration averages are higher at greater elevations (500 to 1500 m above sea level) far from anthropogenic pollutant sources (Taylor and Norby, 1985; Krause et al., 1986) and often with little difference between daytime and nighttime concentrations (Pinkerton and Lefohn, 1987).

Mechanisms of Toxicity and Resistance

Ozone diffuses from the ambient air into the plant leaf through open stomata in concert with photosynthetic gas exchange (i.e. gas phase uptake of CO_2) and transpiration of H_2O vapor. Ozone injury will not occur if the rate of ozone uptake is low enough that the plant can detoxify or metabolize ozone or its metabolites; or if the plant is able to repair or compensate for the effects (Tingey and Taylor, 1982). Therefore, factors which lower stomatal conductance, such as water stress or ozone itself (Tingey and Taylor, 1982), may effectively reduce ozone uptake, thereby averting potential damage. Similarly, ozone toxicity may be reduced through the protective action of enzymes and compounds such as superoxide dismutase, peroxidase, reduced glutathione, terpenoids, ascorbic acid, α-tocopherol, and carotenoids which scavenge active oxygen species (oxyradicals) generated from ozone reactions with water and other compounds within the cell (Tingey and Taylor, 1982; Halliwell, 1984 in Omielan and Pell, 1988). Interspecific and intraspecific variation in stomatal

conductance characteristics (Reich, 1987) or protective compounds and enzymes are believed to be the primary basis of ozone resistance in plants (Tingey and Taylor, 1982). Recent studies have shown that the formation of stress ethylene in plants subjected to environmental stress may be a determinant of plant sensitivity to ozone (Mehlhorn and Wellburn, 1987). Ethylene may react with ozone producing highly reactive free radicals initiating peroxidative events inside plant cells.

The primary site of ozone action is thought to be the plasma membrane (Heath, 1975; Heath, 1980; Mudd, 1982). Ozone, a strong oxidant, may react with polyunsaturated fatty acids, a component of membrane lipids (Mehlmann and Borek, 1987), ultimately altering membrane permeability. Ozone exposure causes an increase in K^+ efflux in cultured algal (*Chlorella sorokiniana*) cells (Chimiklis and Heath, 1975). Ozone inhibits the photosynthetic capacity of isolated spinach chloroplasts by disruption of membrane integrity (Coulson and Heath, 1974). Ambient ozone exposure results in thylakoid membrane damage, altering chlorophyll fluorescence kinetics, enabling early diagnosis of ozone stress on tree foliage (Strasser et al., 1987). Wheat seedlings (*Triticum aestivum*) exposed to ozone exhibit decreased foliar phospholipid content and a concomitant increase in the concentrations of free fatty acids, evidence for ozone-induced phospholipid cleavage (Mackay et al., 1987). Changes in membrane permeability may alter the ionic charge balance and osmotic water potential within the cell and organelles with potential adverse effects on a wide range of metabolic processes, including compartmentation or transport of ions or metabolites. Altered membrane permeability could also explain the increased leachability of cations from ozone-stressed foliage observed in tree species (Krause et al., 1983).

Ozone may adversely affect protein structure and function. Ozone or its free radical breakdown products may react with membrane or cytosolic proteins. This is thought to occur through oxidation of exposed sulfhydryl groups, forming disulfide linkages (Mudd, 1982). The formation of aberrant disulfide linkages within a polypeptide could alter its tertiary structure or active site, inhibiting enzyme activity or protein function. Ozone has been shown to decrease the activity of glyceraldehyde-3-phosphate dehydrogenase (NAD-linked), a key enzyme in glycolysis (Tingey, 1974; Mudd, 1982), through the oxidation of exposed cysteine and tryptophan residues (Mudd, 1982). Ozone-induced reductions in net photosynthesis of wheat seedlings have been attributed to decreased foliar content of active ribulose-1,5-bisphosphate carboxylase (Rubisco) (Lehnherr et al., 1987). Ozone has also been shown to reduce Rubisco activity in rice (Nakamura and Saka, 1978) and lower Rubisco content in alfalfa (Pell and Pearson, 1983). Using isolated *Glycine max* mesophyll cells, Omielan and Pell (1988) found that ozone exposure suppressed post-treatment photosynthetic rates equally in both light and dark treated cells exposed to ozone, and concluded that the reduction was due to a direct effect on the photosynthetic process and not due to reduced cell viability.

Ozone exposure has been shown to increase the foliar content and activity of organic compounds and enzymes, which scavenge oxyradicals and could confer protection against oxidant damage. Ozone exposure (70 nL/L) induced the synthesis of significantly greater amounts of active glutathione reductase in spinach leaves after only 2 days of exposure (Tanaka et al., 1988). The increased production of glutathione may indirectly protect other enzymes from inactivation by being preferentially oxidized itself. Similarly, two ozone-sensitive hybrid poplar (*Populus* sp.) clones showed increased peroxidase activity after fumigation with ozone (Patton and Garraway, 1986). Although peroxidase may destroy H_2O_2 produced in ozone-injured tissues, the enzyme may indirectly

contribute to injury by promoting the accumulation of cytotoxic, oxidized compounds (i.e. quinones) through its requirement for hydrogen donors (Patton and Garraway, 1986).

Ozone exposure has been shown to increase the ascorbic acid content of needles in pine species (Barnes, 1972b). Castillo and Greppin (1988) found increased extracellular ascorbic acid content and increased dehydroascorbate reductase activity in *Sedum album* leaves following ozone exposure. They postulate an integrated cycle of biochemical reactions, involving ascorbate and glutathione, leading to the destruction of ozone-induced oxidants.

Ozone and Carbon Economy

Ambient levels of ozone reduce both net photosynthesis and stomatal conductance in tree and crop species (Hill and Littlefield, 1969; Barnes, 1972; Botkin et al., 1972; Carlson, 1979; Reich and Amundson, 1985). The reduction in net photosynthesis is often accompanied by an increase in foliar respiration (Barnes, 1972; Skärby et al., 1987), although reduced respiration has also been reported (Yang et al., 1983). Skärby et al. (1987) found a 60 percent increase in accumulated carbon loss from dark respiration in field-grown Scots pine (*Pinus sylvestris*) branches exposed to ozone (range 60 to 200 nL/L) as compared to controls. Similar increases in respiration have been observed in other pine species (Barnes, 1972; McLaughlin et al., 1982). The effects of ozone fumigation on gas exchange are reversible, particularly after short term exposures of high concentration. In a study on *Picea* and *Abies* seedlings, Gross (1987) found reduced net photosynthesis, respiration, and transpiration, which were reversible when the ozone fumigations (200 to 280 nL/L) were stopped.

Ozone exposure decreases plant growth and alters carbon partitioning among plant organs (Hogsett et al., 1985; Cooley and Manning, 1988; Pye, 1988). Ozone exposure often induces premature leaf abscission (Mooi, 1980), reducing

plant biomass in addition to photosynthetic leaf area. Ozone-induced reductions in both leaf area and photosynthetic capacity result in decreased whole plant carbon gain.

Root growth is often reduced more than shoot growth after ozone exposure (Hogsett et al., 1985; Chappelka and Chevone, 1986: Cooley and Manning, 1988). The ozone-induced reduction in root growth is believed to be due to decreased translocation of photosynthate from leaves to roots, as evident in decreased root carbohydrate content and the differential ozone effects on metabolite content in roots and foliage. Ozone fumigation decreased green ash (*Fraxinus pennsylvanica*) root carbohydrate content (Jensen, 1981). Following ozone fumigation (100 nL/L) on ponderosa pine (*Pinus ponderosa*) seedlings, Tingey et al. (1976) found increased levels of soluble sugars, starch, and phenols in the shoots and decreased amounts in the roots, while root nitrogen and amino acid content increased.

Studies using ^{14}C tracer also suggest that ozone may inhibit the translocation of carbon from shoots to roots. McCool and Menge (1983) found increased amounts of photoassimilated $^{14}CO_2$ retained in leaves and less translocated to roots in tomato (*Lycopersicon esculentum*) fumigated with ozone (350 nL/L) compared to filtered air controls. Retention of ^{14}C was greater in oxidant-stressed white pine (*Pinus strobus*) foliage and branches than for non-stressed trees, providing evidence for reduced carbon export to bole and roots (McLaughlin et al., 1982).

In addition to changes in whole-plant carbon partitioning among plant organs, ozone stress may also alter the partitioning of carbon among metabolite pools within leaves. Ozone exposure (as low as 100 nL/L) decreased the ^{14}C labelled fraction of soluble sugars and increased the labelled fractions of sugar phosphates and free amino acids in white pine and loblolly pine (*Pinus taeda*) (Wilkinson and Barnes, 1973).

Loblolly pine and yellow-poplar (*Liriodendron tulipifera*) are two important commercial forest species in the southeastern United States which are affected by ambient ozone levels (Chappelka et al., 1985; Chappelka and Chevone, 1988; Shafer et al., 1987; Adams et al., 1988). In a controlled ozone exposure experiment (50, 100, 150 nL/L for 28d), loblolly pine seedlings exhibited significant reductions (-14, -22, and -28 percent, respectively) in total dry weight and decreasing trends in stem height with increasing ozone dose as compared to controls (Kress and Skelly, 1982). Following five weeks of ozone exposure (70 nL/L), Mahoney et al (1985) observed reduced root/shoot ratio in loblolly seedlings relative to filtered air controls. Loblolly seedlings exposed to ozone (80 nL/L, 2 h/d) for 22 d showed 10 to 21 percent reductions in biomass (Chevone et al., 1984) compared to filtered air controls. Following exposure to 50 nL/L of ozone for 126 d, Barnes (1972) found a 15 percent reduction in net photosynthesis rates in loblolly seedlings compared to non-exposed controls.

In a study of the effects of genotype and ozone exposure (subambient, ambient, and ambient + 60 nL/L) on loblolly pine, Adams et al. (1988) found that elevated ozone exposure significantly decreased D^2H and secondary needle biomass relative to seedlings grown in ambient air. However, seedlings receiving subambient ozone levels exhibited an intermediate response. A significant ozone and genotype interaction was detected, suggesting that genotype influences loblolly pine response to ozone.

In an extensive field and laboratory study of the effects of ozone exposure (ambient, charcoal-filtered, ambient + 40, ambient + 80, and ambient + 160 nL/L) on loblolly pine families, McLaughlin et al. (1988) found that ambient ozone levels reduced average seedling height (-26 percent), diameter (-5 percent), and stem volume (-14 percent) growth compared to charcoal-filtered controls. Field-grown seedlings exhibited significant reductions in light-saturated photosynthesis of 13 and 25 percent after 6 and 12 weeks of exposure,

respectively. The response of loblolly pine to ambient levels of ozone was also found to be strongly dependent on genetic variation associated with family origin.

The growth response of yellow-poplar to ozone, however, is much more variable than loblolly pine. An apparent 9 percent decrease in biomass was found for yellow-poplar seedlings exposed to 100 nL/L of ozone (5 d/week) for 30 d (Chappelka et al., 1985) relative to controls. Other experiments have shown apparent increases in biomass ranging from 5 to 41 percent in response to similar ranges of ozone exposures (50 to 150 nL/L, 28 to 35 d), although none were statistically significant (Kress and Skelly, 1982; Mahoney et al., 1984). In a 6 week exposure of yellow-poplar seedlings to ozone (< 20 to 150 nL/L), Chappelka and Chevone (1988) found no significant differences in height or biomass growth, although specific leaf area (cm^2/g) increased linearly with increasing ozone exposure. Ozone also reduced the leaf area ratio and stomatal conductance, but increased the mean unit leaf rate. They also reported an apparent 10 percent decrease in net photosynthesis (not significant) in ozone treated seedlings as compared to controls. In a longer term study using open-top chambers (50 nL/L, 297 d), Duchelle et al. (1982) found a 44 percent reduction in height growth of yellow-poplar; however, again the result was not statistically significant.

Ozone and Nitrogen Economy

Ozone is a phytotoxicant that has significant effects on root processes. Studies have shown that ozone does not diffuse significantly into the soil from the atmosphere (Blum and Tingey, 1977); therefore, the impact of ozone on root processes is mediated through the exposure of plant foliage. For example, root respiration was inhibited by ozone exposure to foliage (Hofstra et al., 1981; Ito

et al., 1985). Since ozone alters root carbohydrate content, respiration, and growth, whole-plant nutrient uptake and assimilation processes may be adversely affected.

Ozone stress may affect nitrogen metabolism and nutrition in plants. In a study on kidney bean (*Phaseolus vulgaris*) plants, Ito et al. (1985) found a higher whole-plant percent nitrogen concentration with increasing ozone exposure. They attributed this to ozone-induced growth suppression rather than altered nitrogen uptake in the roots. Flagler et al. (1987) found reductions in total nitrogenase activity in field-grown soybean plants exposed to ozone relative to charcoal-filtered air controls. Foliar nitrate reductase was not significantly altered by ozone treatment regime, but exhibited generally lower activity at higher ozone levels. Although plant tissue nitrogen concentrations were unaffected, whole-plant nitrogen accumulation rates showed a significant linear decrease with increasing ozone concentration.

Ozone exposure has been found to alter nitrate reductase activity in soybean (Tingey et al., 1973). Following a short term (2 h) exposure to ozone (500 nL/L), they found significant reductions in in vivo nitrate reductase activity compared to non-exposed plants; however, in vitro (extracted) nitrate reductase activity remained unchanged. This suggests that the reduced activity was probably not due to direct action of ozone on the structure or activity of the enzyme, but rather to an interference with reactions that supply the NAD(P)H needed for nitrate reduction. Ozone exposures of 50, 150, and 300 nL/L have been shown to increase the concentration of nitrate in foliage of *Picea abies*, *Fagus sylvatica*, and *Quercus robur* relative to filtered air controls (Krause, 1988). This suggests that ozone alters nitrate metabolism. However, this result has been challenged as an artifact of the method of ozone generation (Brown and Roberts, 1988). In a study on damaged and undamaged spruce (*Picea abies*) trees in relation to forest decline, Düball and Wild (1988) found that damaged

needles had lower ammonium concentrations, increased soluble proteins, and greater glutamine synthetase activity. This may indicate high protein turnover in needles is associated with trees showing symptoms of decline.

Ozone-induced reductions in carbon allocation to roots potentially could reduce root nitrate assimilation. The enzyme nitrate reductase (EC 1.6.6.1) which facilitates the reduction of NO_3^- to NO_2^-, is substrate inducible, undergoes rapid turnover, and is considered the rate-limiting step in nitrate assimilation (Guerrero et al., 1981). A reduced supply of carbohydrates and subsequently lower root respiration rates may limit the availability of carbon skeletons as well as reducing power necessary for nitrate assimilation. Carbohydrate levels have been correlated with the capacity of leaf and root systems to reduce nitrogen (Radin et al., 1978; Oakes and Hirel, 1980). Nitrate assimilation rates may be a useful indicator of altered nitrogen metabolism in leaves and roots and may be a possible mechanism by which ozone affects the carbon and nitrogen economy of trees.

Whole-Plant Physiology

Although ozone acts directly at the level of cellular membranes and metabolism, higher-level physiological processes are inevitably affected, such as those already described: net photosynthesis, carbon translocation, metabolite synthesis, and growth. Similarly, whole-plant water use efficiency (dry matter increment/water use) may be affected by ozone stress. In addition to suppressing growth, ozone exposure may reduce stomatal conductance and transpiration and may thus increase whole-plant water use efficiency (Greitner and Winner, 1988). Alteration of root/shoot ratio could also affect drought susceptibility.

Various edaphic factors, including soil water potential or nutrient supply may mediate plant physiological response to ozone stress (Taylor, 1974; Tingey

and Taylor, 1982; Reich et al., 1987). Plants subjected to water stress may be more tolerant of ozone exposure. Studies have shown higher yields (Temple et al., 1988) or decreased foliar ozone injury symptoms (Heagle et al., 1987) in water-stressed agricultural crop species fumigated with ozone as compared to non-water-stressed plants. However, water stress itself may reduce crop yields and alter biomass partitioning (Heagle et al., 1987; Heggestad et al., 1988; Temple et al., 1988), complicating the interpretation. Nevertheless, ozone and water stress interactions have been successfully incorporated into regional soybean crop loss models (King, 1987; King et al., 1988). Water stress is believed to reduce ozone injury through a stomatal mechanism, whereby water stress reduces stomatal aperture, effectively reducing ozone uptake (Tingey and Hogsett, 1985).

Few studies exist that investigate the combination of water and ozone stress on tree species. In a study using a hybrid poplar clone (*Populus maximowiczii x P. trichocarpa*), Harkov and Brennan (1980) found that water-stressed seedlings were protected from ozone damage, which they attributed to the high diffusive resistance values in the stressed seedlings. Frasier fir (*Abies fraseri*) seedlings subjected to ozone and water stress also showed significant decreases in transpiration, conductance, and biomass as well as an increase in water use efficiency in response to increasing water stress; however, no significant interactions of ozone and water stress were detected (Tseng et al., 1988).

Ozone may act as a predisposing or mediating agent, altering cold hardiness, mycorrhizal colonization, or susceptibility to pathogens. Summertime ozone exposure may reduce cold hardiness in conifers, predisposing trees to winter frost damage and winter desiccation (Barnes and Davison, 1988; Lucas et al., 1988). Ozone stress may alter plant mycorrhizal colonization or pathogen susceptibility. Fumigation with low ozone levels (20, 70, 120 nL/L) resulted in

an increasing trend in mycorrhizal infection in northern red oak (*Quercus rubra*) seedlings (Reich et al., 1985). However, exposure to high (330 nL/L) ozone concentrations reduced mycorrhizal infection in tomato plants, and decreased the partitioning of ^{14}C to roots and increased partitioning to shoots relative to controls (McCool and Menge, 1983). Ozone damaged tomato leaves showed increased susceptibility to tomato pinworm (*Keiferia lycopersicella*) infestation, which was correlated with ozone-induced increases in foliar soluble proteins, improving host-plant suitability for insect herbivores (Trumble et al., 1987). However, ozone stress does not necessarily increase plant susceptibility to pathogens; Coleman et al. (1987, 1988) found reduced or no effect of ozone on cottonwood (*Populus deltoides*) clone susceptibility to various fungal parasites. Despite the sometimes contradictory evidence in the literature, ozone stress warrants consideration as a mediating factor in plant growth and function and as an interactive factor with other environmental stresses.

Nitrogen Stress

Aside from water availability (Kramer, 1983), nitrogen supply is often a major growth-limiting factor in forest ecosystems (Kramer and Kozlowski, 1979; Tamm, 1979). Since nitrogen deficiency has been shown to ameliorate the effects of ozone exposure in some herbaceous species (e.g. Cowling and Koziol, 1982), soil nitrogen level warrants consideration as a mediating factor in tree response to ozone stress.

Nitrogen supply has significant effects on plant physiology and growth. Plants grown under a suboptimal nitrogen supply often exhibit decreased new leaf production and lower growth rates as compared to well-fertilized plants (see review by Clarkson and Hanson, 1980). Limited nitrogen supply to leaves and the corresponding reduction in leaf nitrogen concentration has been associated with decreased protein and chlorophyll content, decreased Rubisco activity, and

increased mesophyll resistance, all of which limit net photosynthesis (e.g. Ryle and Hesketh, 1969; Bouma, 1970; Natr, 1972). Nitrogen stress may also alter carbon partitioning among plant organs, reducing shoot growth to a greater extent than root growth. Compensatory fine root growth stimulation has been shown in response to localized nitrogen supply in nitrogen-stressed barley (*Hordeum vulgare*) (Drew and Saker, 1975) as well as Douglas-fir (*Pseudotsuga menziesii*) (Friend, 1988). In addition to changes in carbon partitioning patterns, nitrogen stress has been shown to increase redistribution of nitrogen from leaves to roots in soybean (Rufty et al., 1984; Vessey and Layzell, 1987), indicating that roots may have the highest priority for nitrogen in times of nitrogen stress.

Carbon allocation to roots is necessary for maintaining water and nutrient uptake, while carbon allocation to new leaf production is essential for maintenance of carbon gain. As noted, plants grown under a suboptimal nutrient supply often exhibit decreased carbon gain and altered internal distribution of carbon. Suboptimal nutrient supply commonly results in increased proportions of carbon allocated to fine root production in crop and tree species (Clarkson and Hanson, 1980; Rufty et al., 1984; Cannell, 1986; Vessey and Layzell, 1987).

The increase in root growth relative to shoot growth may be a mechanism by which nutrient stress decreases the impact of ozone on plants. The relative strength of the root sink as affected by nutrient stress may alter the effect of ozone on the partitioning of carbon among plant organs (Cooley and Manning, 1987). Indirect evidence supporting this hypothesis comes from a study in which ozone-fumigated (70 nL/L, 5 weeks) loblolly pine seedlings, inoculated with the ectomycorrhizal fungus *Pisolithus tinctorius*, exhibited a 41 percent higher root/shoot ratio compared to non-inoculated fumigated seedlings (Mahoney et al., 1985). Possibly the mycorrhizal treatment altered root sink strength for carbohydrates, and increased relative root growth. In conclusion, a suboptimal

soil nitrogen supply may increase root sink strength for carbohydrates, counteracting the effects of ozone stress.

Ozone Stress and Nutrient Interactions

Ozone exposure has been shown to alter the concentration of elements in plant parts. However, no clear trends are evident in the literature. Ozone was shown to decrease the concentrations of Ca, Mg, K, and N in pinto bean (*Phaseolus vulgaris*) leaves and increased stem concentrations of those elements (Maas, et al., 1973). Ozone exposure decreased the concentrations of Ca, Mg, Fe, and Mn in *Phaseolus vulgaris* leaves, which was attributed to decreased transport into the leaves (Tingey et al., 1986). The concentrations of Mg, K, and P in Scots pine needles increased at the high (150 nL/L) relative to the low (0, 50 nL/L) ozone levels after a 56 d exposure (Skeffington and Roberts, 1985). Clearly, ozone-induced alterations in nutrient concentration, whether due to changes in uptake and assimilation, translocation, or increased leaching could significantly alter whole-plant nutrition.

The role of air pollutants in modifying plant nutrient status has received increased attention as a contributing factor to forest decline (Zech et al., 1985; Krause et al., 1986). Changes in foliar nutrient concentrations through increased foliar leaching or reduced uptake and assimilation processes could contribute to nutrient deficiencies, eventually resulting in impaired tree growth and function.

Nutrient supply and plant nutrient status may mediate plant response to ozone stress (Taylor, 1974; Cowling and Koziol, 1982). Many studies on the relationship of nutrition and air pollutant stress have been undertaken with greatly different results (Cowling and Koziol, 1982). In herbaceous species, ozone injury is generally increased at optimal N, P, or K levels and lessened at suboptimal or supraoptimal levels (Cowling and Koziol, 1982). In an early study on mangels (*Beta vulgaris*) and spinach (*Spinacia oleracea*), Brewer et al. (1961)

found increased incidence and severity of ozone injury with increased nitrogen and phosphorus supply. Increased phosphorus supply and foliar content enhanced ozone damage in tomato plants (Leone and Brennan, 1970). Addition of fertilizer at a moderate rate increased the sensitivity of four soybean cultivars to ozone exposure, while a higher fertilizer rate decreased ozone sensitivity (Heagle, 1979). However, there are conflicting reports in the literature; for example, the susceptibility of tobacco plants to ozone stress was shown to be increased by deficiency or excess of nitrogen (MacDowall, 1965).

Studies on deciduous tree species have revealed similar responses. Silver maple (*Acer saccharinum*) seedlings grown with optimal potassium levels showed more signs of ozone damage than did potassium deficient seedlings. The response was attributed to the effects of optimal potassium levels on guard cell aperture, resulting in increased ozone uptake and damage (Noland and Kozlowski, 1979). In a study on the effects soil fertility on the response of hybrid poplar (*Populus maximowiczii x P. trichocarpa*) seedlings to ozone exposure, Harkov and Brennan (1980) found that optimal foliar nitrogen levels predisposed the plants to the greatest amount of foliar injury, while the supraoptimal fertility regime initiated nutrient toxicity symptoms and appeared to suppress ozone injury. Optimal nutrient levels increase net photosynthesis and transpiration in trees (Keller, 1972), which may consequently predispose plants to greater ozone injury through increased stomatal conductance.

Hypotheses

The purpose of this research was to examine the effects of chronic ozone stress and soil N supply on the growth and nutrition of tree seedlings. Chronic levels of ozone and varied N availability were used to assess the effects of the combined stresses on whole-plant carbon and nutrient economy. The specific stress hypotheses examined in this study are outlined as follows:

Ozone stress hypotheses:

I. Increasing ozone stress reduces carbon allocation to fine roots, a) decreasing root dry weight growth, b) decreasing nitrate reduction, and c) resulting in decreased whole-plant nitrogen and nutrient uptake.

II. Ozone exposure lowers net photosynthesis (P_n), suppressing whole-plant carbon gain and reducing whole-plant dry matter increment (DMI) per unit nitrogen uptake, lowering whole-plant nutrient use efficiency (NUE).

III. Ambient ozone stress reduces stomatal conductance and intercellular CO_2 concentrations (C_i), when integrated over time results in increased whole-plant water use efficiency (WUE) as more CO_2 is fixed relative to H_2O transpired.

Nitrogen stress hypotheses:

I. Increasing soil N supply to N-limited seedlings results in increased P_n and whole-plant dry weight gain. In N-stressed seedlings a greater proportion of dry weight is partitioned in roots relative to shoots, which may counteract ozone effects on seedling growth and dry weight partitioning.

II. Increasing N supply to N-stressed seedlings increases DMI per unit of nutrient uptake, resulting in greater NUE.

III. Increasing N supply to N-stressed seedlings increases photosynthetic capacity and potential carbon gain per unit of H_2O transpired, resulting in greater WUE.

Research Objectives

Yellow-poplar (*Liriodendron tulipifera*), a woody dicot, and loblolly pine (*Pinus taeda*), a conifer differ in their growth and physiological characteristics. Yellow-poplar is deciduous and exhibits an indeterminate or free growth habit, permitting new leaf production throughout the growing season in contrast to the determinate growth type, which limits new growth to the preformed buds present

when dormancy is broken. Loblolly pine is intermediate between determinate and indeterminate growth forms; its growth occurs in a series of growth flushes over the course of the growing season with the first occurring in the spring and often producing most of the season's new needles. The tree species differ in gas exchange characteristics as well as nutrient requirements. Generally, stomatal conductance and net photosynthesis rates are higher in deciduous trees than in conifers (e.g. Teskey et al., 1986; Reich, 1987). Yellow-poplar and loblolly pine, both regionally important species, provide insight into interspecific differences in response to ozone stress as mediated by soil nitrogen supply. The research focused on the following objectives:

> Objective I. To compare the growth and biomass partitioning in loblolly pine and yellow-poplar seedlings grown in open-top field chambers in response to varied low-nitrogen forest soil treatments in combination with chronic ozone levels over one growing season.
>
> Objective II. To investigate the effects of combined ozone and nitrogen stress on seedling physiological responses, including water use efficiency, photosynthetic gas exchange, fine root and leaf nitrate assimilation, nutrient concentration, nutrient uptake, and nutrient use efficiency.

METHODS AND MATERIALS

Seedling Stock

One-year-old dormant loblolly pine (*Pinus taeda* L.) and yellow-poplar (*Liriodendron tulipifera* L.) seedlings were obtained from commercial nurseries and placed in cold storage (3° C) until planting. Loblolly pine seedlings were purchased from Weyerhaeuser Inc., Washington, NC.(half-sib family # 8-127); the yellow-poplar were obtained from Warren County Nursery Inc., McMinnville, TN.

The seedlings were sorted by height, diameter, and extent of root development. Two uniform groups of approximately 80 individuals of each species were selected for use as planting stock, minimizing seedling size variability at the outset of the experiment. Six seedlings of each species were randomly selected for an initial harvest in which stem height and diameter were measured and foliar, stem, coarse root, and fine root (<1 mm diameter) tissues were separated and oven-dried (60° C) to a constant weight. Sixty-three of the remaining seedlings of each species were randomly chosen and assigned to one of nine ozone and nitrogen treatment combinations (initially, N = 7 per treatment combination).

Nitrogen Treatment

Bare-root seedlings (loblolly pine 7.345 $\pm$ 0.861 g, yellow-poplar 1.952 $\pm$ 0.272 g, mean ($\pm$ SE) dry weight) were individually planted in 3.5-L plastic pots filled with a low-fertility forest soil amended with either quartz sand or ammonium nitrate (NH_4NO_3) to create low, medium, and high N availabilities. A low nitrogen treatment was prepared by adding 1.25 kg of pure quartz sand to 1.70 kg of a forest soil (oven-dry weight equivalent), collected from the A horizon of a fine, loamy silicious, mesic Typic Hapludult (Lilly series), resulting

in a 40:60 percent sand/soil mixture in each pot, packed to a mean bulk density of 1.26 g/cm^3. The medium N treatment (unamended) and high N treatment (NH_4NO_3 amended) consisted of 2.70 kg of forest soil (oven dry weight equivalent), packed to a mean bulk density of 1.14 g/cm^3. Twenty-two days after the ozone exposures began 204 mg of N in the form of NH_4NO_3 (65 mM solution) was added to the pots assigned the high N treatment.

Random soil samples taken during planting for analysis of P, K, Ca, Mg, Zn, Mn, extractable NH_4 and NO_3 (Appendix A) as well as organic matter content and mechanical analysis were analyzed by the Soil Testing and Plant Analysis Laboratory of the University of Georgia Athens, Georgia. The analyses reported a mean mineral N level of 96 μg/g, therefore, the estimated low nitrogen treatment was initially 58 μg/g N. The high N (amended) treatment was estimated to result in an initial mineral N concentration of 172 μg/g.

The bottom of each pot was covered with a fine plastic mesh to retain the soil and permit water drainage. Plastic bowls placed under each pot collected any leachate which was added back to eliminate nutrient and water loss. Black nylon fabric was placed on the soil surface of each pot to reduce soil evaporation.

Ozone Exposure System

Seven days after transplanting the seedlings were transferred to nine open-top field ozone exposure chambers of the type described by Johnston et al. (1986), located at the Air Pollutant Effects Research Facility within the Oak Ridge National Environmental Research Park in Roane County, Tennessee. The cylindrical chambers (3.0 m diameter x 2.4 m height) were constructed of aluminum frames, surrounded with a transparent plastic wall. A plastic plenum encompassing the bottom half of each chamber was inflated with

air from an adjacent blower at an injection rate of approximately 70 m^3/min. The air passed through the perforated plenum inner wall into the chamber interior and was exhausted through the open top at the rate of one air change every six seconds.

The three target ozone levels consisted of a subambient, ambient, and elevated (ambient + 60 nL O_3/L) treatment. The subambient ozone level was generated by passing ambient air over charcoal filters installed in the air inlet of the blower housing. The ambient treatment chambers lacked charcoal filters, while the high ozone treatment consisted of a continuous addition of an average of 60 nL O_3/L to the ambient air stream. Using a 7 h daylight (1000-1700 h) seasonal (May through September) mean as the exposure statistic, the mean seasonal ozone concentrations were as follows: subambient, 32 nL/L; ambient, 56 nL/L; and elevated, 108 nL/L.

Ozone was generated from dried ambient air using an electric arc discharge method (Ozone Research and Equipment Corporation Model 03-SP38-A.2 ozone generator) and delivered through Teflon^R tubing to the air stream entering the high ozone treatment chambers. The ozone addition was controlled by computer regulated control valves and monitored continuously by an on-site computer data acquisition system (McEvers et al., 1988). The ozone concentration of the air in each chamber was determined and logged at fixed intervals (20 min.) through air samples constantly drawn through a Teflon^R line originating at seedling canopy height at the center of each chamber. The air samples were selected sequentially and routed to one of three co-calibrated Dasibi Model 1008-AH ozone analyzers, calibrated to NBS traceable standards at least once every two weeks.

Experimental Design

Yellow-poplar and loblolly pine seedlings were grown in pot culture in open-top field chambers during an 18 week period from May 5 to September 9, 1987. A split-plot factorial design was used with ozone level as the whole plot treatment. The whole plot treatment utilized a randomized block design with three ozone treatment levels replicated three times (one ozone concentration per chamber) for a total of nine chambers. The subplot treatment consisted of a factorial combination of three nitrogen treatments and two species replicated twice in each chamber. Each ozone-nitrogen combination was represented by six plants of each species. The number of seedlings totaled 108 (3 ozone x 3 reps x 3 N x 2 species x 2 reps). An additional 9 seedlings of each species (3 ozone x 3 N) were initially included as a replacement pool for any mortality or poor vigor among the transplanted seedlings during the first three weeks of the experiment. Final substitutions were made, and N amendments added to the high N treatments, at the end of the third week of the fumigations.

Within each chamber the seedlings were grouped by species and nitrogen levels so that each species group contained one low, medium, and high nitrogen treatment. Each group of three pots was placed in an 20 L plastic bucket which was inserted into holes in an above-ground styrofoam-block support base in each chamber. The buckets were repositioned within the chamber every six weeks in order to randomize the effects of variation in chamber microclimate.

Plant Morphology and Growth Analysis

Shoot height, the distance from the soil surface to the apex of the shoot, and stem diameter, the mean of two measurements taken at right angles from each other 5 cm from the soil surface were measured at exposure week 0, 4, 9,

and at final harvest for each seedling. Any abscised leaf tissue was collected, dried, and weighed weekly.

The yellow-poplar seedlings were harvested at the end of exposure week 16 over a two-day period (112-113 d) in which 27 seedlings (one complete rep) were removed from the chambers and taken to the laboratory, where the leaves and stem were removed, and the roots were washed from the soil with water and separated into coarse and fine root (< 1 mm diameter) classes. Leaf area was determined photometrically using a Li-Cor 3100 Leaf Area Meter (Li-Cor Inc. Lincoln, NE). The tissue components were dried to a constant weight in a forced air oven at 60° C and weighed to the nearest mg.

The loblolly pine seedlings were similarly harvested over a two-day period (126-127 d) at the end of exposure week 18. Needles were separated into current and previous-year age classes; and all needles, stems, branches, and roots (coarse and fine) were oven-dried (60° C) and weighed. The tissue dry weights were used in analyses of dry weight ratios (root/shoot, fine root/leaf, leaf/whole plant, shoot/whole plant, and fine root/whole plant) to determine the treatment effects on biomass partitioning.

Relative growth rates were calculated using stem volume data as a nondestructive measure of above-ground biomass. Stem volume, calculated as the product of diameter squared (D^2) and height (H), was used to determine the mean relative growth rate using the equation:

$$RGR = \frac{\ln D^2_2 H_2 - \ln D^2_1 H_1}{t_2 - t_1}$$

where $t_2 - t_1$ is the measurement interval in weeks and $D^2_i H_i$ is the stem volume in cm^3 transformed using the natural log. Dry matter increment (DMI) was calculated for each plant as the final plant dry weight (including abscised

leaf weight) minus the mean initial plant dry weight of the six seedlings harvested at the beginning of the experiment.

Water Use

All pots were individually watered twice weekly with known quantities of water. Measured increments of distilled water were added at the beginning of each week. All pots were weighed and watered to a predetermined water content (field capacity) at the end of each week. Weekly water use was calculated as the sum of the gravimetric water loss and intermediate watering increment minus mean evaporative water loss, calculated as the gravimetric water loss from 6 control pots similarly prepared but without plants. Since the chambers did not exclude rainfall, water use values were only calculated for the weeks in which no rainfall was recorded (week 11, 12, 13).

Whole plant water use efficiency (WUE) was calculated as the ratio of DMI (mg) to water use (g), estimated from gravimetric and evaporative water loss records over the entire experimental period. Rainfall input was calculated on a pot surface area basis using on-site rainfall data and assumed to be equally accessible to each pot.

Nitrate Reductase Activity

Intact leaves, needles, and fine root tips were assayed for potential nitrate reductase activity using a modified in vivo assay described by Jaworski (1971) and Yandow and Klein (1986). Whole leaves or needles were harvested from each plant and stored on ice until assayed (within 24 h). One day before the final harvest a mature fully expanded leaf was excised at the base of the petiole from each yellow-poplar seedling and stored on ice. A leaf punch was used to remove four leaf disks (7 mm diameter) from each leaf immediately before assaying. In loblolly pine three fascicles were randomly selected from current

year needles on the terminal leader. The needles were separated from the fascicles and cut into segments 1 cm in length. Nine segments were selected for the assay. At the final harvest 15 non-suberized fine root tips (1 cm in length) were randomly selected and cut from the intact root system of each plant and immediately assayed.

Excised leaf disks, needle segments, or fine root tip samples were placed in vials containing 3.0 ml of incubation medium comprised of 0.1 M KH_2PO_4 buffer (pH 7.5), 0.02 M KNO_3 as substrate, chloramphenicol (30 μg/ml) as an anti-microbial agent, and 1% n-propanol (v/v) as a surfactant (2% for yellow-poplar leaf disks). The vials were evacuated for approximately 30 s, facilitating rapid infiltration of the medium into the tissue. The vials were incubated in the dark for 2.0 h at room temperature (25° C).

Following the timed incubation period, a 1.5 ml aliquot was removed from the incubation vial and placed into a spectrometer cuvette. The nitrite evolved was assayed by a colorimetric reaction initiated by adding 0.75 ml of 0.1% sulfanilamide in 2 N HCl (w/v) and 0.75 ml of 0.02% N-1-naphthylethylenediamine di HCl (w/v) to the cuvette. After 15 min the absorbance was determined at 540 nm against a reagent blank. The nitrite concentration was determined from a standard curve and the enzyme activity expressed as μmol NO_2^--N g^{-1} h^{-1}, based on the oven-dry weight (60° C) of the tissue assayed.

Gas Exchange Analysis

Photosynthesis was measured in situ on a single mature fully expanded leaf of at least 3 randomly selected yellow-poplar seedlings from each treatment combination during exposure week 15 (105-106 d) and on a current-year branch of each loblolly pine during week 17 (123 and 125 d), using a portable closed infrared gas analysis system (Li-Cor 6200, Li-Cor Inc. Lincoln, NE). The gas

analysis system was programmed to record measurements based on a 5 μL/L CO_2 concentration uptake by foliage in the closed cuvette, resulting in measurement times of usually less than 1 min. The system was calibrated using a known concentration of CO_2 (530 μL/L), traceable to NBS standards. The measurements were taken on sunny days with photon flux density above saturation (> 600 μmol m^{-2} s^{-1}, PPFD).

Through use of cuvette (1 L) inserts defined areas of 13.30 or 10.64 cm^2 were used in gas exchange analysis on yellow-poplar. Single current-year branches from individual loblolly pine seedlings were selected and net CO_2 uptake measured using a 4 L cuvette. The branches were subsequently tagged for separate dry weight determination at final harvest three days later (Sept. 8-9). Photosynthesis and conductance were calculated on a needle dry weight basis for loblolly pine.

Elemental Analysis

The concentration of N, S, P, K, Ca, Mg, Mn, Fe, Al, B, Cu, Zn, Sr, and Ba were analyzed in the tissue components (leaves, abscised leaves, stem, and roots (coarse and fine)) of each seedling. The dried tissues were sub-sampled and finely ground in a Wiley mill or Cyclotec grinder and sent to the Soil Testing and Analysis Laboratory, Athens, GA for analysis. Total nitrogen concentration was determined using a block-digestion Kjeldahl method (Isaac and Johnson, 1976). Sulfur was determined using a combustion infra-red method (Leco-SC-132). The concentrations of all other elements were determined using atomic emission spectroscopy (Jones, 1975).

Whole plant nutrient uptake for each element was calculated by summing the component tissues nutrient contents (element concentration x tissue dry weight) and subtracting the mean initial whole plant content of six plants in the initial harvest. Nutrient use efficiency (NUE) values for each element were

expressed on a whole plant-basis and defined as the DMI divided by the estimated whole plant nutrient uptake (g/mg).

Data Analysis

The design was a completely randomized split plot with three replications and nine ozone x nitrogen treatment combinations. Whole plots (chambers) received ozone treatments while subplots were assigned a factorial combination of three nitrogen and two species replicated twice. All variables were analyzed using analysis of variance (ANOVA) or General Linear Models (GLM) procedures of the SAS statistical analysis software for personal computers (version 6.02, SAS Institute Cary, NC). Ozone, nitrogen, and ozone x nitrogen interactions were tested against chamber variation in separate analyses for each species (Appendix B). Means separation tests were performed on variables showing a significant response using Tukey's test or the Least Significant Difference (LSD) method.

Whole-plant nutrient responses were analyzed using the technique of Timmer and Stone (1978) and Timmer (1985). This method integrates element content and concentration with plant or tissue component dry weight in a single graphic representation. This technique facilitates diagnosis of nutrient dilution, sufficiency, deficiency, luxury consumption, and toxicity, entirely on a whole-plant or plant component basis. Following appropriate statistical analyses of the nutrient data, relative changes in nutrient concentration and absolute content were plotted in relation to treatment-induced change in biomass for ease of comparison. The direction and magnitude of the plotted response vectors were used in the interpretation of treatment effects.

RESULTS

Growth

The loblolly pine seedlings showed a significant growth response to increasing soil N level. The mean total biomass of seedlings grown at the high soil N level was 58 percent greater than seedlings grown at the lowest soil N level ($p = 0.0005$). Each tissue component significantly increased in dry weight in response to the high soil N treatment with current-year needle biomass showing the largest response, 109 percent ($p = 0.0001$, Table 1). Fine root biomass responded the least to soil N level, increasing 44 percent ($p = 0.005$) at the high soil N level relative to the low N level. The reduction in loblolly pine first-year needle biomass with increasing soil N (Table 1) was caused by greater needle abscission. No statistically significant growth response to soil N level was detected for any tissue component of yellow-poplar (Table 2).

A significant ozone x nitrogen interaction was found for current-year needle growth in loblolly pine ($p = 0.03$, Table 3). Mean current-year needle biomass increased with higher soil N level by 102, 133, and 96 percent with increasing ozone level, respectively. Needle biomass tended to decrease within the low and high soil N treatment groups by 15 and 17 percent, respectively, in response to increasing ozone concentration. Seedlings grown at the medium soil N level, however, showed an increase of 16 percent with increasing ozone exposure.

Ozone treatment alone did not result in growth responses detectable at the 5 percent level for either species. Mean final dry weight of loblolly pine seedlings grown under elevated ozone levels was not significantly different from plants grown under the ambient exposure regime ($p = 0.64$, Table 1). Mean whole-plant dry weight of yellow-poplar plants exposed to the highest ozone level was not significantly different relative to the seedlings grown under subambient ozone levels ($p = 0.48$, Table 2).

Table 1. Final harvest dry weights of tissue components of loblolly pine seedlings pooled by nitrogen and ozone treatment level. All values were tested using ANOVA. Means with the same letter or no letter are not significantly different (LSD α = 0.05, n = 18).

	Initial soil N level (μg/g)				Ozone concentration (nL O_3 /L, 7 h seasonal mean)			
Component (g)	58	96	172	% Increase[1]	32	56	108	% Increase[1]
Old needles	2.02 a	1.44 b	0.78 c	-61	1.37	1.56	1.32	-4
Current needles[2]	4.65 c	6.52 b	9.71 a	109	7.15	7.19	6.54	-9
Stem	4.96 b	5.89 b	7.85 a	58	6.10	6.49	6.08	-0
Coarse root	2.25 b	2.78 b	3.90 a	78	2.83	3.34	2.75	-3
Fine root	2.62 b	2.98 b	3.78 a	44	2.97	3.18	3.23	9
Total shoot	11.63 b	13.82 b	18.35 a	58	14.62	15.24	13.93	-5
Total root	4.86 b	5.76 b	7.68 a	58	5.80	6.52	5.98	3
Whole plant	16.50 b	19.58 b	26.02 a	58	20.42	21.76	19.91	-2

[1] Increase of third level relative to the first.
[2] Significant ozone x nitrogen interaction (p = 0.03).

Table 2. Final harvest dry weights of tissue components of yellow-poplar seedlings pooled by nitrogen and ozone treatment level. All values were tested using ANOVA (n = 18).

	Initial soil N level (μg/g)				Ozone concentration (nL O_3 /L, 7 h seasonal mean)			
Component (g)	58	96	172	% Increase[1]	32	56	108	% Increase[1]
Leaves	1.08	1.09	1.23	14	1.25	0.97	1.18	-6
Stem	1.77	1.90	2.01	13	1.93	1.54	2.22	14
Coarse root	1.57	1.64	1.72	10	1.54	1.37	2.02	31
Fine root	0.99	0.65	0.68	-31	0.75	0.74	0.83	11
Total shoot	2.86	2.99	3.24	14	3.18	2.51	3.40	7
Total root	2.56	2.29	2.41	-6	2.29	2.12	2.84	24
Whole plant	5.41	5.28	5.65	4	5.48	4.62	6.24	14

[1] Increase of third level relative to the first, no treatment effects were statistically significant.

Table 3. Current needle growth of loblolly pine seedlings in response to ozone and nitrogen treatment combinations. Means (± SE) with the same letter are not significantly different (Tukey $\alpha = 0.05$, $n = 6$, $p = 0.03$).

Ozone level (nL/L)	Current needle dry weight (g/plant)		
	Initial soil N level (μg/g)		
	58	96	172
32	5.41 ± 0.97 cd	5.11 ± 0.61 cd	10.93 ± 0.75 a
56	3.93 ± 0.29 d	8.49 ± 1.04 abc	9.15 ± 0.82 ab
108	4.61 ± 0.61 d	5.95 ± 0.81 cd	9.04 ± 0.88 ab

Leaf biomass showed the largest decreasing trends in response to ozone fumigation in both species, however, these decreases were not statistically significant. Final harvest leaf biomass in yellow-poplar was 6 percent less in the elevated compared to the subambient ozone treatment ($p = 0.67$). This was the only dry weight component exhibiting a lower mean at the high ozone level relative to the low. Loblolly pine showed a similar trend. Current-year needle biomass, pooled by ozone level, showed an apparent 9 percent decrease at the highest ozone exposure level relative to the subambient treatment ($p = 0.53$).

Nitrogen fertilization significantly stimulated above-ground pine growth, as measured by D^2H, at exposure week 9 and at final harvest ($p = 0.01$ and $p = 0.001$, respectively, Fig. 1). Yellow-poplar D^2H growth did not respond to increasing soil N levels; the differences among the N treatments were not statistically significant due to high seedling variability (Fig. 2). Yellow-poplar stem volume gained only about 5 cm^3, 6 times less than loblolly pine stem volume, which increased nearly 30 cm^3 over the experimental period. Yellow-poplar shoot relative growth rate (RGR) was significantly suppressed in seedlings grown at the lowest N level during the 9 to 16 wk interval ($p = 0.05$, Table 4). Increasing soil N level produced significant increases in shoot RGR of loblolly pine over the entire season ($p = 0.0001$, Table 4), beginning during the 4 to 9 wk exposure interval ($p = 0.01$) and continuing through 9 to 18 wk ($p = 0.002$), with significant gains at the highest soil N level. Ozone exposure did not significantly alter shoot RGR in either species (data not shown).

Biomass Partitioning

Elevated ozone exposure significantly altered biomass partitioning patterns in both species, indicating changes in whole plant carbon economy. Final harvest root/shoot dry weight ratio (RSR) was 8 percent greater in loblolly pine seedlings grown at the high ozone level ($p = 0.04$, Table 5) relative to the

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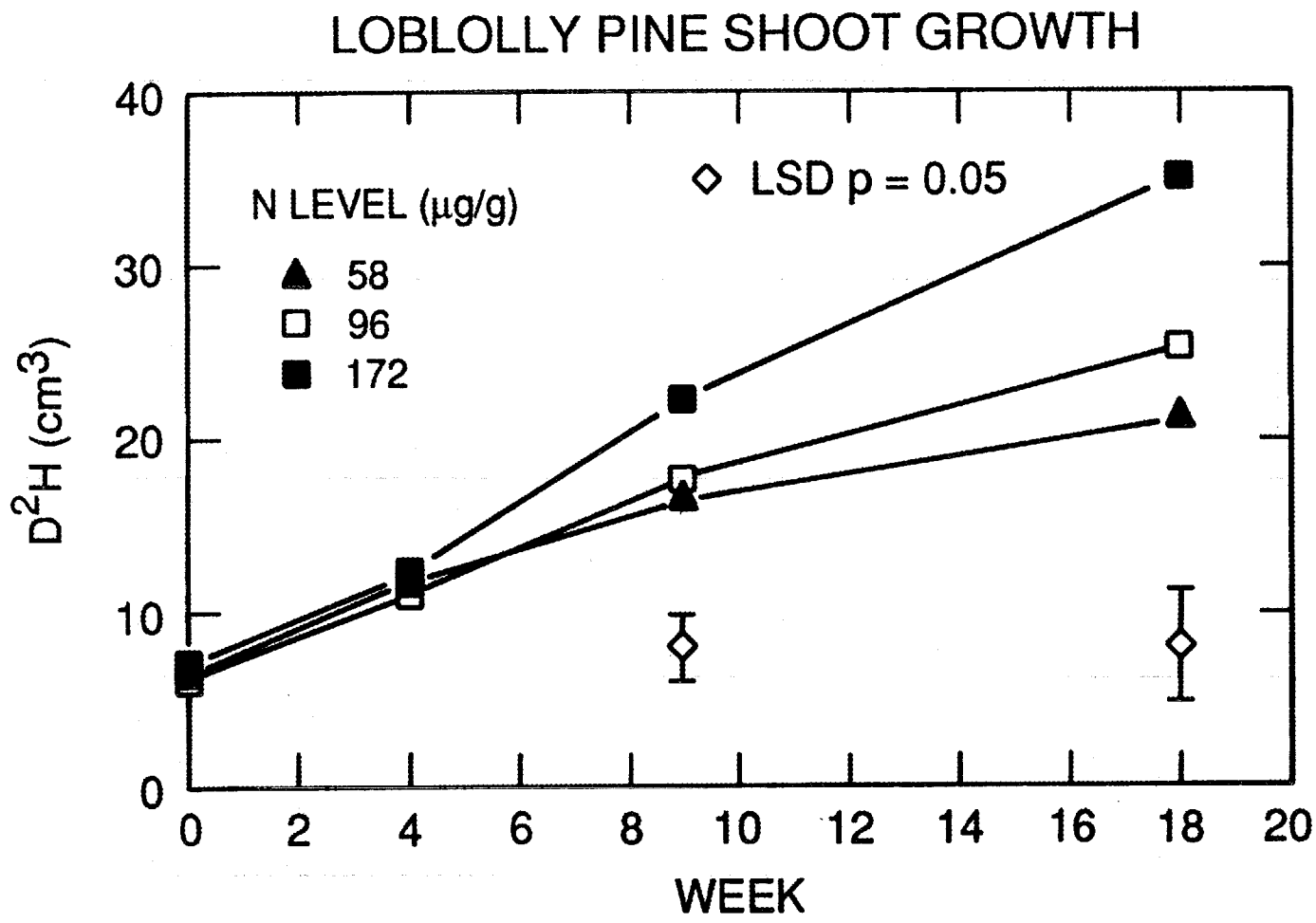

Fig. 1. Mean cumulative stem volume growth (D^2H) of loblolly pine seedlings grown under three soil nitrogen levels. Each point represents the mean of 18 plants.

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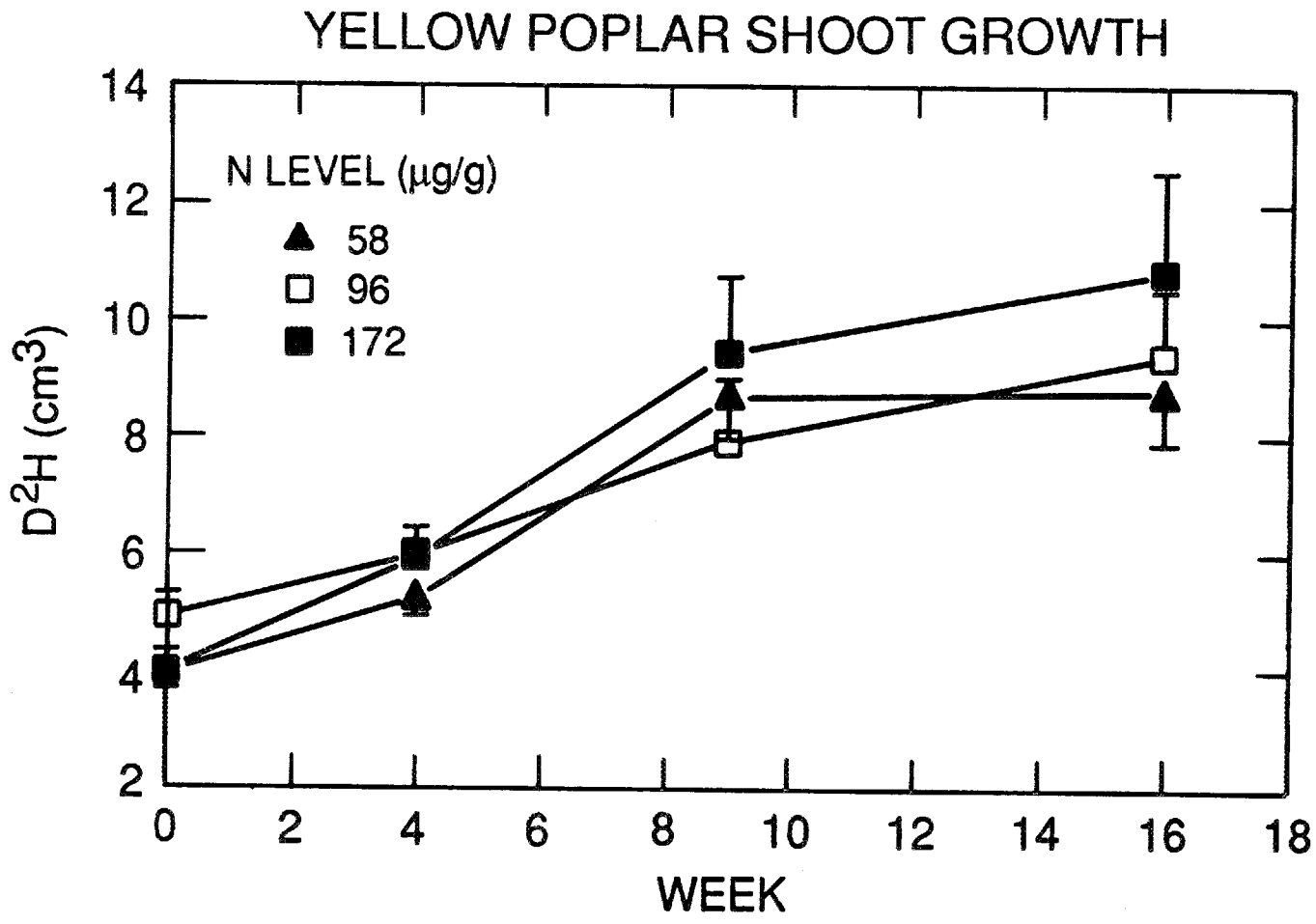

Fig. 2. Mean (± SE) cumulative stem volume growth (D^2H) of yellow-poplar seedlings grown under three soil nitrogen levels. Each point represents the mean of 18 plants.

Table 4. Mean relative growth rates (RGR) of seedlings in relation to soil nitrogen treatment. Interval means were calculated using stem volume data (diameter squared x height). All values were tested across N levels within each interval using ANOVA. Means with the same letter are not significantly different (Tukey α = 0.05, n = 18).

	RGR (cm^3 cm^{-3} $week^{-1}$)		
	Initial soil N level ($\mu g/g$)		
Interval (week)	58	96	172
		Loblolly pine	
0-4	0.13 a	0.14 a	0.14 a
4-9	0.07 b	0.10 ab	0.12 a
9-18	0.03 b	0.04 b	0.05 a
0-18	0.03 c	0.05 b	0.06 a
		Yellow-poplar	
0-4	0.07 a	0.06 a	0.09 a
4-9	0.10 a	0.06 a	0.08 a
9-16	0.00 b	0.04 a	0.01 ab
0-16	0.05 a	0.04 a	0.05 a

Table 5. Biomass partitioning in seedlings pooled by ozone and nitrogen treatments. All values were tested using ANOVA and $p \leq 0.05$, unless otherwise stated. Means with the same letter or no letter are not significantly different (n = 18, LSD α = 0.05). LWR = leaf/plant weight ratio, SWR = shoot/plant weight ratio, FRWR = fine root/plant weight ratio, FRLR = fine root/leaf weight ratio, RSR = root/shoot ratio.

	Ozone concentration (nL O_3 /L, 7 h seasonal mean)			Initial soil N (μg/g)		
Ratio	32	56	108	58	96	172
			Loblolly pine			
LWR[1,2]	0.42 a	0.40 ab	0.39 b	0.40	0.41	0.40
SWR	0.72 a	0.70 b	0.70 b	0.71	0.71	0.71
FRWR	0.15 b	0.15 b	0.17 a	0.16	0.16	0.15
FRLR	0.36 b	0.37 b	0.42 a	0.40	0.38	0.37
RSR	0.40 b	0.42 ab	0.43 a	0.42	0.42	0.42
			Yellow-poplar			
LWR	0.22	0.21	0.17	0.19	0.19	0.20
SWR	0.59	0.56	0.55	0.56	0.57	0.57
FRWR[3]	0.13	0.14	0.13	0.16 a	0.12 b	0.12 b
FRLR[4]	0.60 b	0.69 ab	0.78 a	0.84 a	0.64 b	0.59 b
RSR	0.70	0.81	0.83	0.82	0.77	0.76

[1] Ozone effect (p = 0.056).
[2] Significant ozone x nitrogen interaction (p = 0.051).
[3] Significant ozone x nitrogen interaction (p = 0.030).
[4] Ozone effect (p = 0.069).

subambient ozone level. Root/shoot ratio showed a similar, but statistically weaker, trend in yellow-poplar seedlings. Yellow-poplar RSR was 19 percent greater in plants grown at the elevated ozone level compared to the subambient level ($p = 0.20$, Table 5). Fine root/leaf ratio (FRLR) showed the largest response across ozone levels, increasing by 17 percent in loblolly pine ($p = 0.004$) and 30 percent in yellow-poplar ($p = 0.069$) at the high ozone exposure level as compared to the subambient ozone treatment group. The decrease of 7 percent ($p = 0.056$) in leaf weight ratio (LWR) as well as the 13 percent increase ($p = 0.010$) in fine root weight ratio (FRWR) in response to the elevated ozone level resulted in the increased in FRLR in loblolly pine in the elevated compared to the subambient ozone treatment.

Soil nitrogen treatment altered biomass partitioning patterns in yellow-poplar, but not in loblolly pine. Yellow-poplar seedlings grown under the low N level increased the proportion of biomass partitioned to fine roots (FRWR) by 33 percent compared to plants grown at the medium and high N levels ($p = 0.003$, Table 5); however, an ozone and nitrogen interaction was detected ($p = 0.03$, Table 6). Elevated ozone exposure decreased the proportion of biomass allocated to fine roots by 13 and 23 percent within the low and high N treatment groups, respectively, but increased it by 27 percent within the medium soil N group. FRWR was also decreased at the high soil N level relative to the low within each ozone level. A marked nitrogen treatment effect was observed for FRLR, which dropped by 30 percent from a mean of 0.84 at the low N treatment to 0.59 at the high N treatment ($p = 0.003$).

Leaf Abscission Dynamics

Elevated ozone exposure caused a significant increase in leaf senescence and abscission. Foliage of both species fumigated at the high ozone level displayed sporadic chlorotic flecking and necrotic lesions, symptoms characteristic

Table 6. Biomass partitioning to yellow-poplar fine roots in relation to ozone and nitrogen treatment combination. Ratios were tested using ANOVA. Means (± SE) with the same letter are not significantly different (LSD α = 0.05, n = 6, p = 0.03).

	Fine root/plant dry weight ratio		
	Initial soil N level (μg/g)		
Ozone level (nL/L)	58	96	172
32	0.15 ± 0.06 ab	0.11 ± 0.04 bc	0.13 ± 0.03 bc
56	0.19 ± 0.05 a	0.12 ± 0.03 bc	0.12 ± 0.04 bc
108	0.13 ± 0.05 bc	0.14 ± 0.03 abc	0.10 ± 0.02 c

of ozone damage; however, visible injury was more apparent in yellow-poplar. Visual assessments of yellow-poplar foliage indicated that ozone injury was concurrent with foliar senescence.

Final cumulative leaf loss, expressed as the percentage abscised of the total leaf biomass produced over the experimental period, rose from 12 to 37 percent in yellow-poplar ($p = 0.03$) with increasing ozone concentration, but was not affected by ozone exposure (from 6 to 8 percent) in loblolly pine (Table 7). Although loblolly pine needle abscission increased from 4.2 percent to 11.0 percent with increasing soil N level ($p = 0.0001$), a significant ozone x nitrogen interaction was found for leaf loss ratio (LDR), the abscised leaf weight divided by total plant weight ($p = 0.04$, Table 8). This ratio assesses abscised foliar biomass loss on a whole-plant basis. The abscised tissue consisted of first-year needles only, no current-year needles were abscised. Within the low and elevated ozone treatment groups, higher soil N level increased the proportion of biomass lost through abscission while the ambient ozone level showed a similar trend. Increasing soil N level increased needle abscission, suggesting greater leaf abscission with improved whole plant N nutrition. Cumulative leaf loss of yellow-poplar was not significantly affected by N treatment ($p = 0.42$, Table 7).

In addition to increasing the amount of leaf biomass abscised, elevated ozone exposures altered the timing of leaf abscission. Loblolly pine seedlings grown under the elevated ozone exposure regime exhibited earlier leaf senescence and abscission, beginning at week 8 of fumigation, than those exposed to the ambient and subambient ozone exposure regimes (Fig. 3). Ozone appeared to induce and accelerate foliar senescence. Final mean cumulative leaf loss per plant in loblolly pine was affected by ozone and nitrogen combination ($p = 0.016$, Fig. 3). In general, the highest soil N level predisposed the seedlings to greater leaf loss throughout the experimental period. Yellow-poplar seedlings exposed to elevated ozone concentrations also showed earlier as

Table 7. Leaf abscission in seedlings in response to ozone fumigation and soil nitrogen level. Means with the same letter or no letter are not significantly different (LSD α = 0.05, n = 18). LDR = Leaf loss (g) divided by whole plant dry weight (g). % Loss = percentage of leaf biomass abscised (g) of total leaf production (g).

	Ozone concentration (nL O_3 /L, 7 h seasonal mean)			Initial soil N (μg/g)		
Ratio	32	56	108	58	96	172
			Loblolly pine			
LDR[1]	0.03	0.03	0.04	0.02 b	0.02 b	0.05 a
% Loss	6.8	6.3	8.2	4.2 b	6.1 b	11.0 a
			Yellow-poplar			
LDR	0.04 b	0.04 b	0.12 a	0.06	0.05	0.09
% Loss	12.0 b	15.1 b	36.6 a	19.7	18.5	25.5

[1] Significant ozone x nitrogen interaction (p = 0.04).

Table 8. Needle abscission in loblolly pine seedlings in response to ozone and nitrogen treatment combinations. Leaf death ratio (LDR) is the proportion of foliar biomass abscised relative to whole-plant dry weight. Ratios were tested using ANOVA. Means (± SE) with the same letter are not significantly different (Tukey $\alpha = 0.05$, $n = 6$, $p = 0.04$).

Ozone level (nL/L)	LDR Initial soil N level (μg/g) 58	96	172
32	0.013 ± 0.004 c	0.027 ± 0.011 abc	0.054 ± 0.009 ab
56	0.012 ± 0.005 c	0.033 ± 0.009 abc	0.037 ± 0.005 abc
108	0.029 ± 0.010 abc	0.020 ± 0.004 bc	0.058 ± 0.011 a

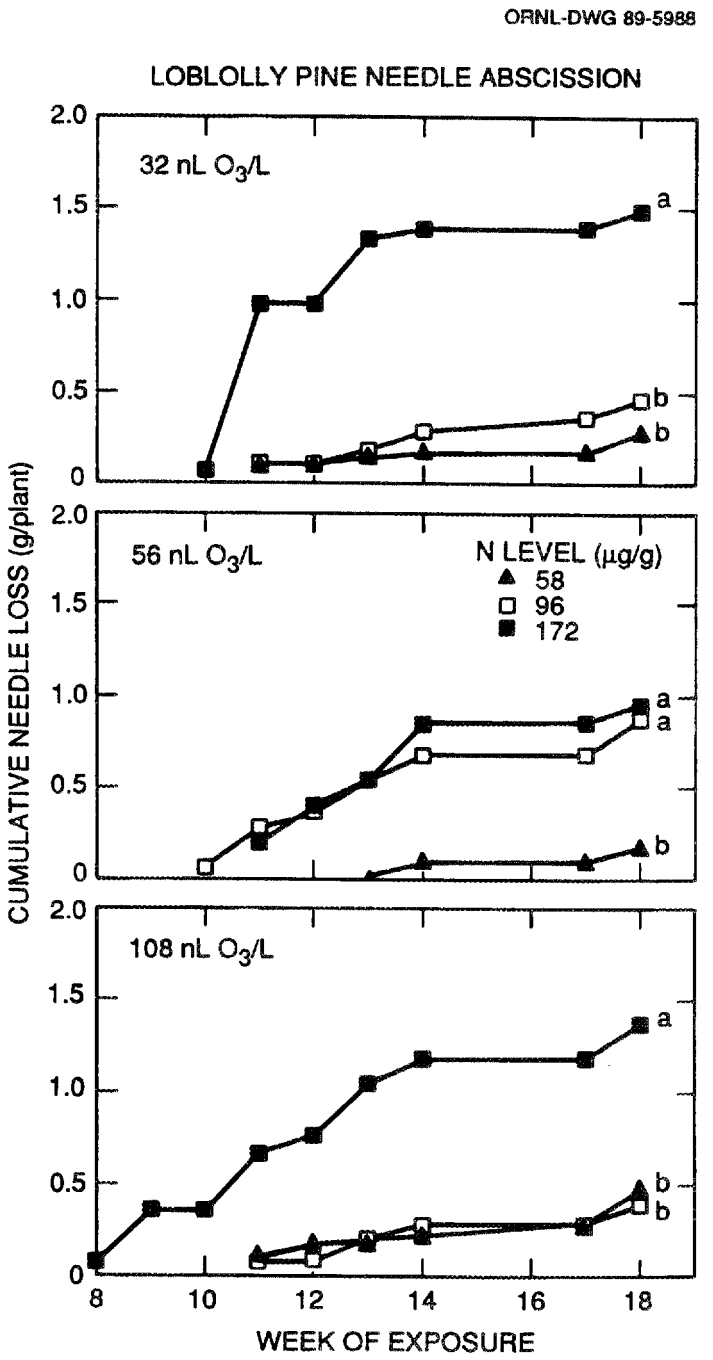

Fig. 3. Mean cumulative leaf abscission per plant for loblolly pine seedlings grown under three ozone and soil nitrogen levels over 18 weeks of exposure. Each point represents the mean of 6 plants. Final cumulative means with the same letter within each ozone level are not significantly different (LSD α = 0.05).

well as greater cumulative leaf abscission (Fig. 4). Ozone-induced biomass losses through leaf abscission were 2 to 5 times greater for yellow-poplar than for loblolly pine seedlings. It is of note that although ozone significantly increased leaf abscission in yellow-poplar, final harvest leaf biomass was not significantly reduced.

Water Use

Whole-plant water use was significantly greater at higher soil N levels in both species. Mean whole-plant water use rates, calculated during a midseason period (exposure week 11-13) in which no rainfall was recorded, were higher in loblolly pine seedlings grown in the medium and high soil N levels ($p = 0.0001$, Fig. 5). Yellow-poplar seedlings also exhibited greater water use at the higher N levels in spite of the lack of significant whole plant biomass differences across the N treatment levels ($p = 0.04$, Fig. 6).

Loblolly pine WUE calculations based on gravimetric water use estimates for the entire experimental period and whole-plant DMI showed that WUE increased with improved N nutrition. Nitrogen fertilization significantly increased the WUE by 81 percent as compared to seedlings grown under the low N level ($p = 0.0008$, Table 9). Mean loblolly pine plant WUE did not statistically differ across ozone levels. However, loblolly pine current-year foliage showed non-significant declining trends in mean g, and C_i, and increasing trends in instantaneous WUE (2.11, 2.36, 2.85 mmol CO_2 /mol H_2O, respectively, $p = 0.23$) with higher ozone level. Yellow-poplar whole-plant WUE differences were not statistically significant for either ozone or nitrogen treatments (Table 9).

Nitrate Reductase Activity

Foliar and fine root tissue potential nitrate reductase activity (NRA) were assayed, and in both species fine root NRA was approximately one order of

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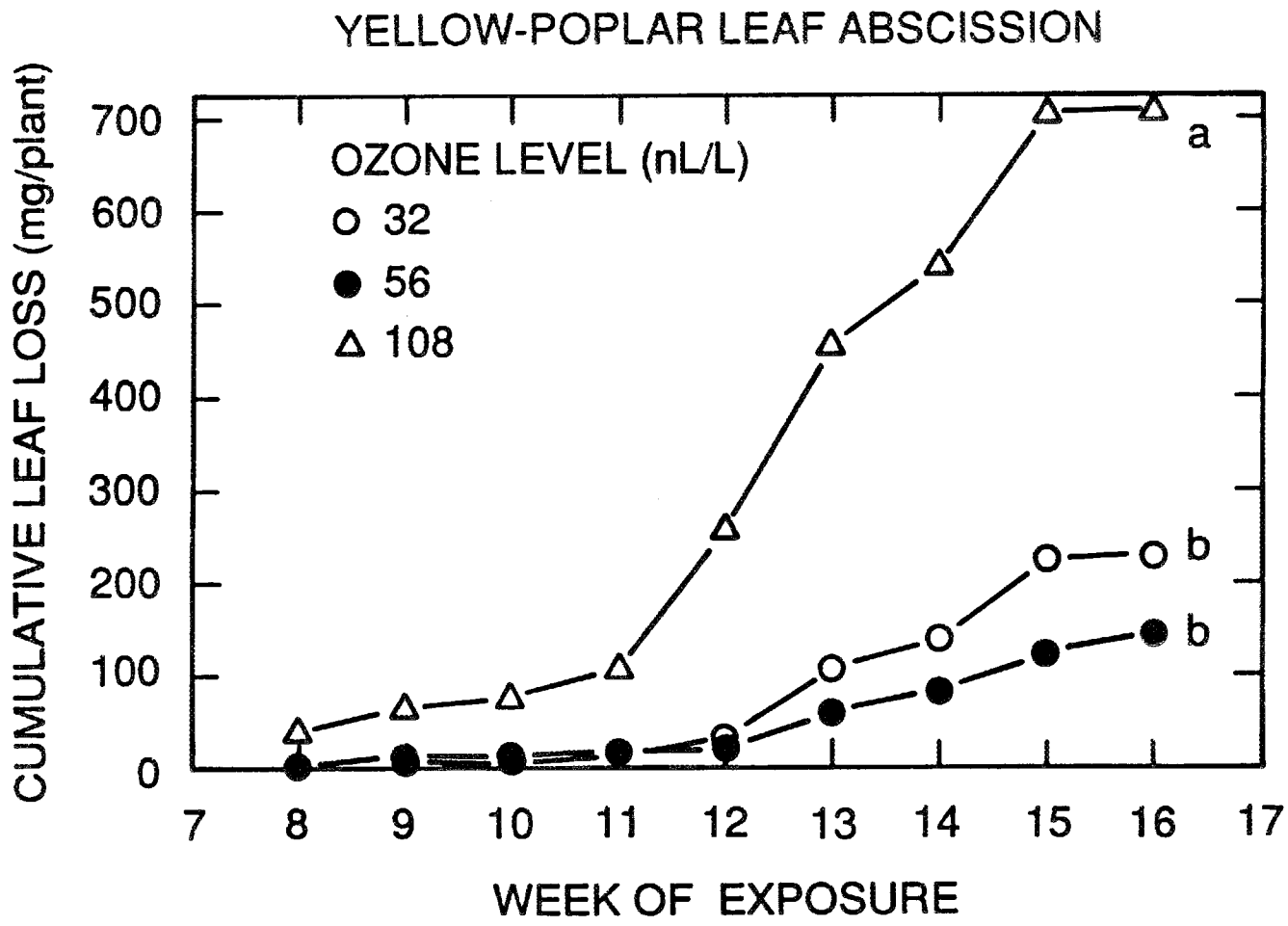

Fig. 4. Yellow-poplar mean cumulative leaf abscission per plant in seedlings pooled by ozone level over 16 weeks of exposure. Each point represents the mean of 18 seedlings. Final cumulative means with the same letter are not significantly different (LSD α = 0.05).

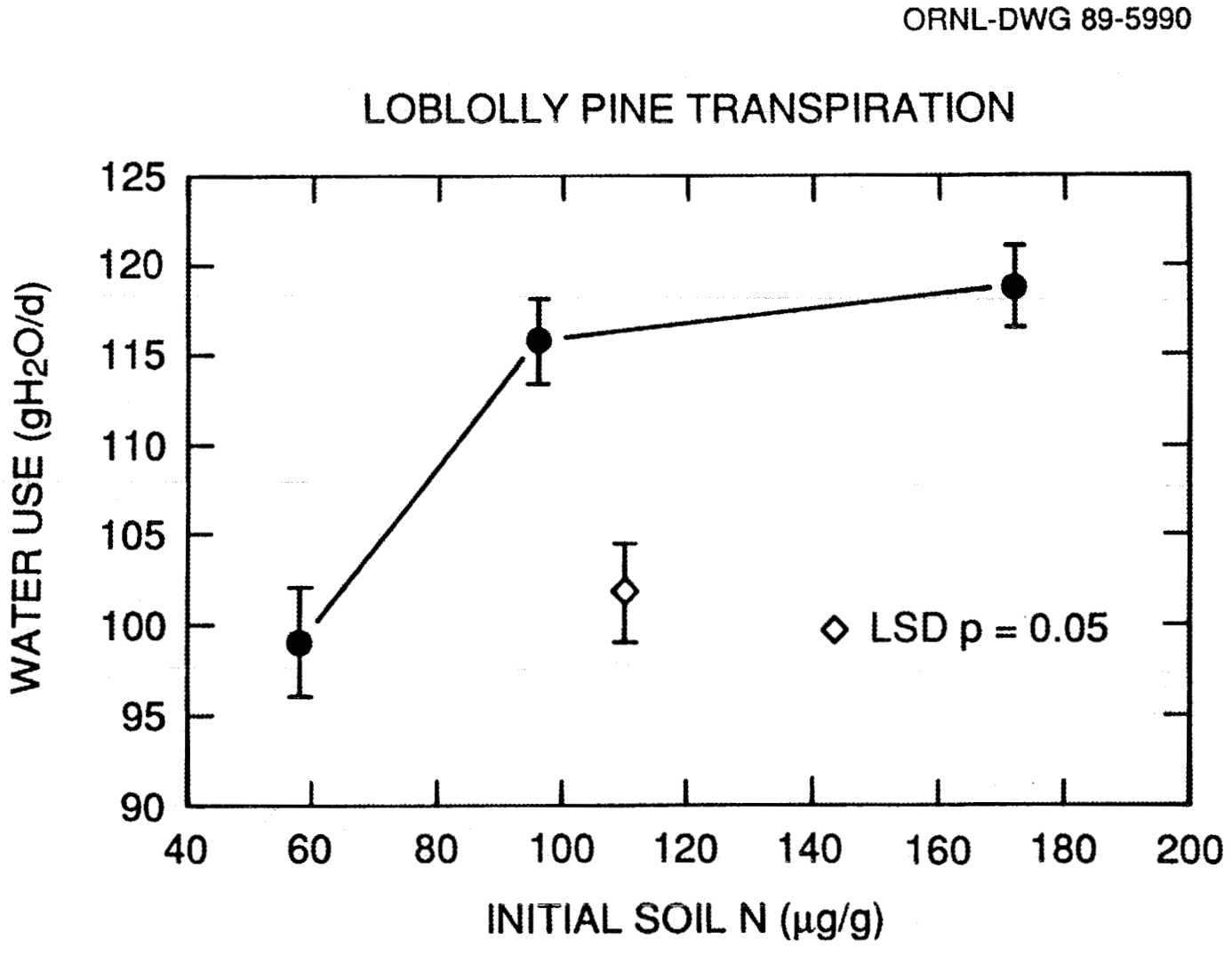

Fig. 5. Loblolly pine midseason whole-plant transpiration rate in seedlings pooled by nitrogen treatment. Means (± SE) calculated from week 11-13 water use data are shown (n = 18).

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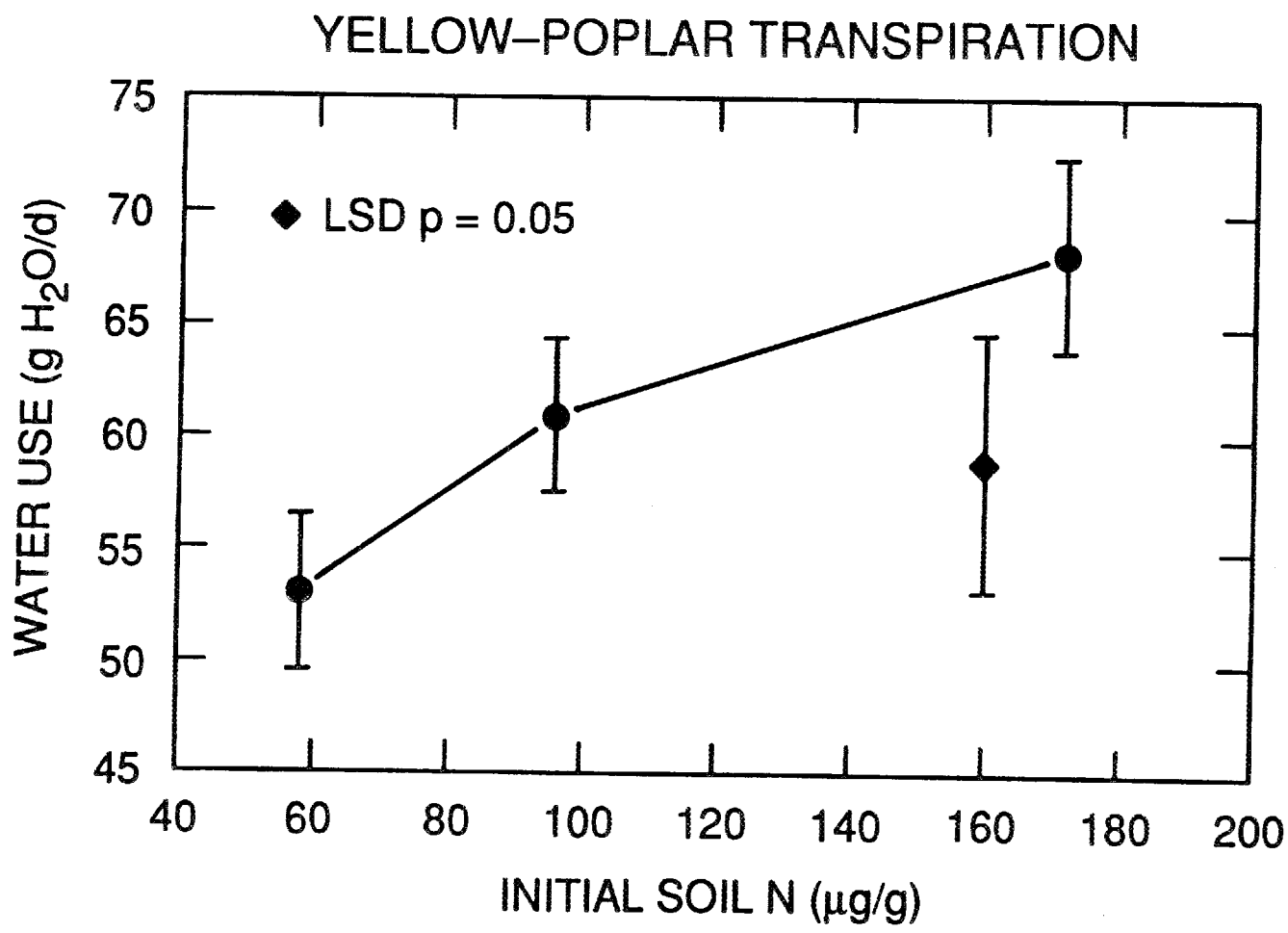

Fig. 6. Yellow-poplar midseason whole-plant transpiration rate in seedlings grown at three soil nitrogen levels. Means (± SE) calculated from week 11-13 water use data are shown (n = 18).

Table 9. Whole-plant water use efficiency (WUE) values in response to ozone and nitrogen treatment. Means with the same letter or no letter are not significantly different (Tukey $\alpha = 0.05$, n = 18).

WUE [DMI/water use (mg/g)]					
Initial soil N level (μg/g)			Ozone concentration (nL O_3 /L, 7 h seasonal mean)		
58	96	172	32	56	108
Loblolly pine					
1.07 b	1.25 b	1.94 a	1.41	1.51	1.34
Yellow-poplar					
0.574	0.483	0.489	0.487	0.412	0.609

magnitude greater than foliar NRA. Ozone fumigation significantly altered in vivo NRA of loblolly pine fine root tissue assayed at the end of the 18 week experiment. Seedlings grown under the elevated ozone exposure regime exhibited 80 percent higher NRA as compared to the ambient and subambient ozone levels ($p = 0.03$, Fig. 7). Yellow-poplar fine root NRA showed no significant response to the ozone or nitrogen treatment combinations (data not shown).

Loblolly pine current-year needle NRA, assayed after 13 weeks of ozone exposure, responded to ozone and nitrogen treatment combination ($p = 0.05$, Fig. 8). At both the subambient and ambient ozone levels mean NRA was greater at the higher soil N levels; however, in the elevated ozone treatment the NRA response was altered, showing significantly greater activity at the low N level relative to plant grown under low N and ambient ozone. Yellow-poplar leaf NRA, assayed after 16 weeks, was more than doubled in plants grown at the lowest soil N level relative to the higher soil N levels ($p = 0.008$, Fig. 9).

Gas Exchange Characteristics

Soil nitrogen supply limited photosynthesis in loblolly pine. Seedlings grown under the low N level exhibited a 25 percent reduction in net CO_2 uptake as compared to the high N level ($p = 0.002$, Fig. 10a). Stomatal conductance (*g*), however, was significantly affected by both ozone and nitrogen treatments, exhibiting a significant ozone x nitrogen interaction ($p = 0.0057$, Fig. 10b). Elevated ozone exposure decreased *g* by an average of 35 percent among the three soil N treatment groups relative to the subambient ozone level. Mean *g* of seedlings pooled by ozone level ($n = 18$) showed a declining trend from 1.36 mmol g^{-1} s^{-1} in the subambient treatment to 0.85 mmol g^{-1} s^{-1} in the elevated ozone level. Increasing ozone exposure reduced *g* by 3, 6, and 16 percent, respectively, among seedlings grown at the high soil N level as compared to the

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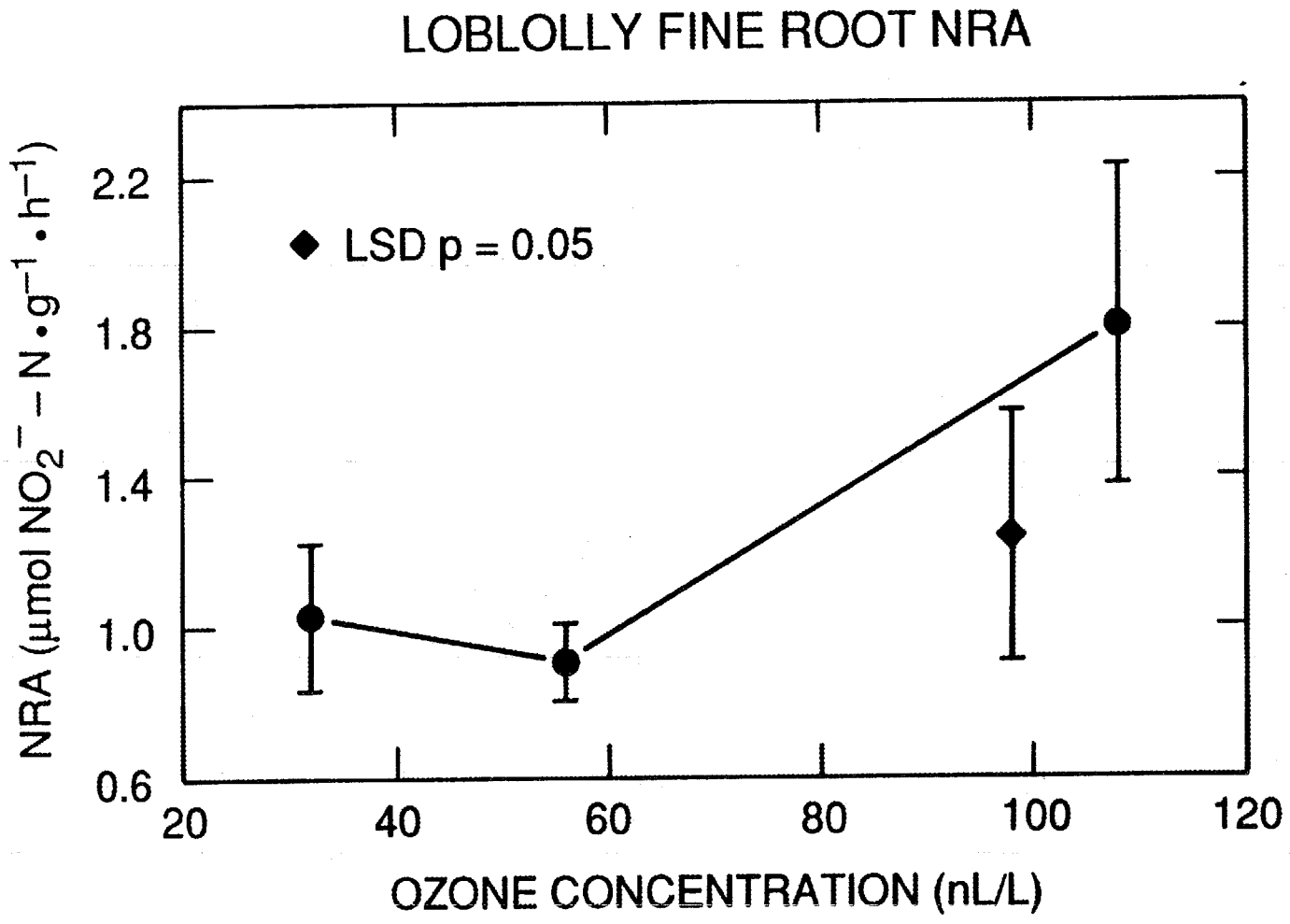

Fig. 7. Loblolly pine fine root tip in vivo nitrate reductase activity in seedlings pooled by ozone fumigation level. Each point represents the mean ± SE of 18 plants.

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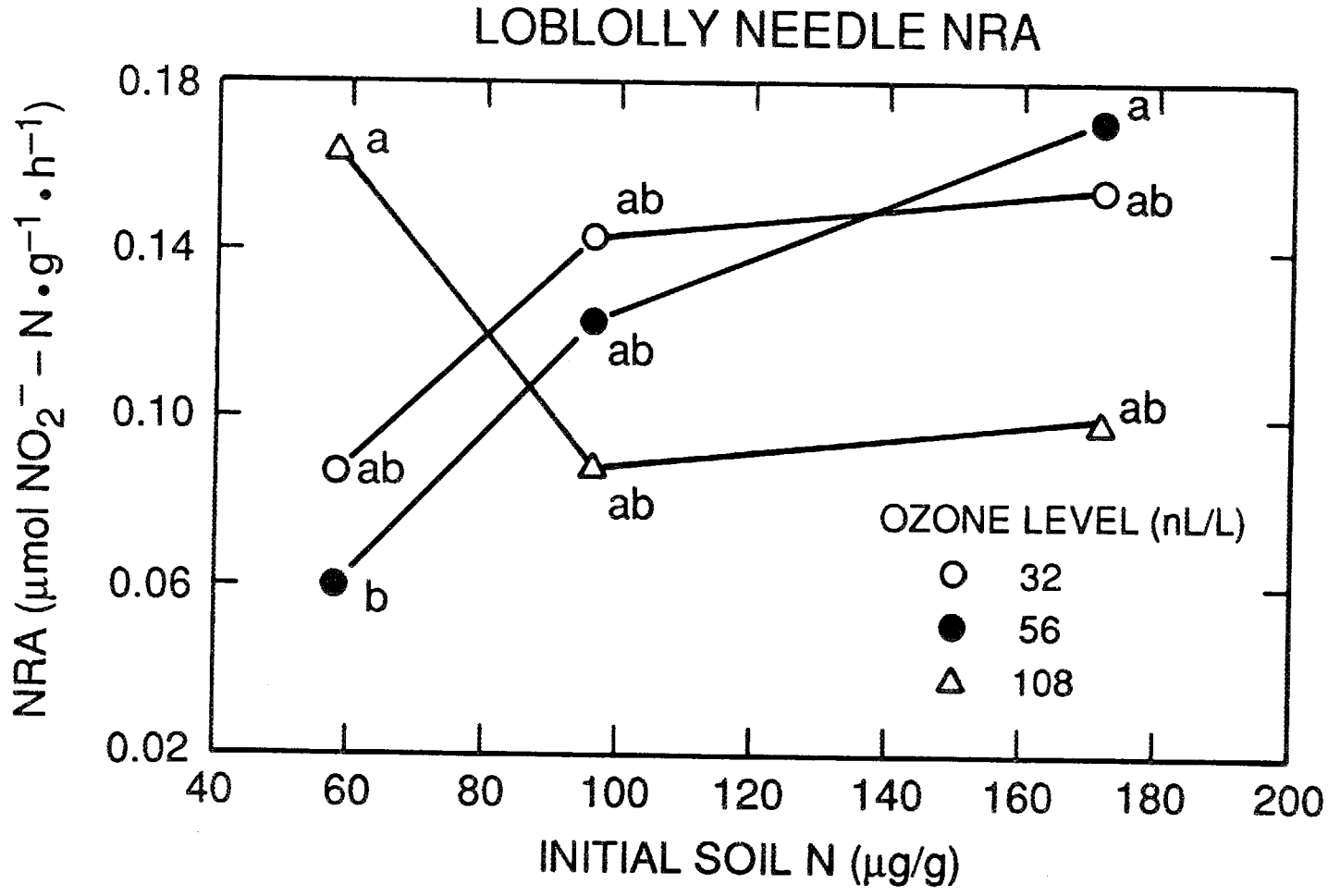

Fig. 8. Loblolly pine current-year needle in vivo nitrate reductase activity in seedlings grown under nine ozone x nitrogen treatment combinations, assayed after 13 weeks of exposure. Each point represents the mean of 6 plants. Means with the same letter are not significantly different (LSD α = 0.05).

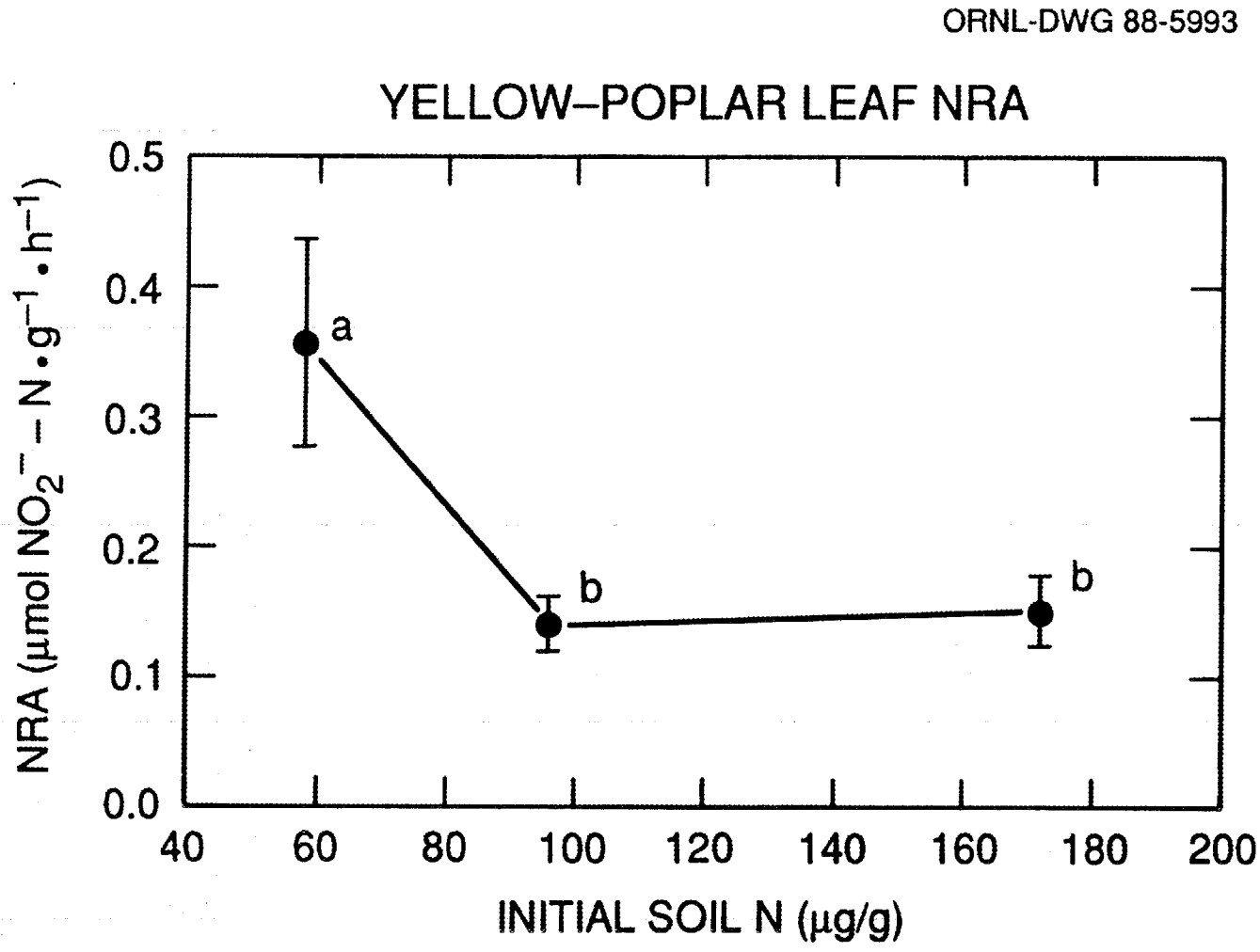

Fig. 9. Yellow-poplar leaf in vivo nitrate reductase activity assayed at week 15 in seedlings grown under three soil nitrogen levels. Means (± SE) with the same letter are not significantly different (LSD α = 0.05, n = 18).

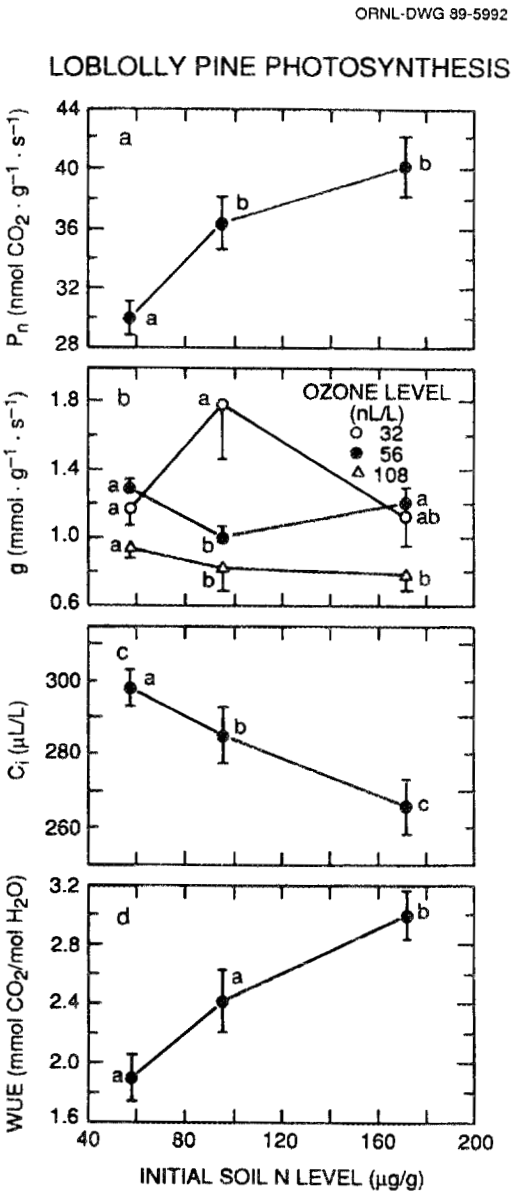

Fig. 10. Late season gas exchange characteristics of current-year foliage of loblolly pine seedling grown at three soil nitrogen levels. Means with the same letter are not significantly different (LSD α = 0.05). a) Mean (± SE) net photosynthesis (P_n) of seedlings pooled by nitrogen level (n = 18). b) Mean (± SE) stomatal conductance (*g*) by ozone and nitrogen treatment combination. Each point represents the mean of 6 plants, and means within each nitrogen level with the same letter are not significantly different (LSD α = 0.05). c) Mean (± SE) internal CO_2 concentration (C_i) (n = 18). d) Mean (± SE) instantaneous water use efficiency (WUE) values, calculated as P_n divided by transpiration rate (n = 18).

low. Internal CO_2 concentration (C_i) was 11 percent lower at the high soil N level relative to the low, dropping from 298 to 266 μL/L (p= 0.0001, Fig. 10c).

The transpiration response while similar to the response of *g*, also exhibited a significant reduction with increasing soil N level (main effect). Instantaneous transpiration rates were 17 percent lower at the high relative to the low soil N level (p = 0.03), dropping from a mean (± SE) of 16.91 ± 1.79 μmol g^{-1} s^{-1} at the low N level to 14.02 ± 1.85 μmol g^{-1} s^{-1} at the high N level; however, a significant ozone x nitrogen interaction was found (p = 0.018, Table 10). Transpiration was lower at the high soil N level relative to the low among the three ozone levels, while among N levels increasing ozone exposure had little effect except for a higher value at the medium N level in seedlings exposed to subambient ozone concentrations.

Nitrogen fertilization significantly increased instantaneous WUE, calculated as CO_2 assimilation rate divided by instantaneous transpiration rate (p = 0.005, Fig. 10d). Instantaneous WUE values pooled by N level increased from means of 1.9 and 2.4 mmol/mol in seedlings grown at the low and medium N level to a significantly greater mean of 3.0 mmol/mol at the high N level. The response of instantaneous WUE to soil N treatment was quite comparable to whole plant WUE values, which were lower (Table 9). No significant main effects or interactions were observed for any gas exchange component of the yellow-poplar seedlings (Data not shown).

Nutrition

Ozone fumigation significantly increased the concentrations of N and S in current-year needles of loblolly pine (p = 0.0017 and 0.0002, respectively, Table 11). Mean N concentration was 18 percent higher in needles of seedlings grown under elevated ozone relative to the subambient treatment. Needle S

Table 10. Late season instantaneous transpiration rates of loblolly pine current-year needles in relation to ozone and nitrogen treatment combinations. Values were tested using ANOVA. Means (± SE) with the same letter are not significantly different (LSD α = 0.05, n = 6, p = 0.018).

Ozone level (nL/L)	Transpiration rate (μmol g^{-1} s^{-1}) Initial soil N level (μg/g)		
	58	96	172
32	16.34 ± 1.67 ab	21.58 ± 1.75 a	13.42 ± 1.17 b
56	18.72 ± 1.90 ab	14.60 ± 1.15 b	14.98 ± 1.27 b
108	15.67 ± 1.86 b	14.20 ± 3.16 b	13.68 ± 2.89 b

Table 11. Loblolly pine current needle nutrient concentrations in relation to ozone exposure. Means with the same letter or no letter are not significantly different (LSD α = 0.05, n = 18).

	Nutrient concentration (mg/g)		
	Ozone concentration (nL O_3 /L , 7 h seasonal mean)		
Nutrient	32	56	108
N	7.6 a	6.9 a	9.0 b
S	0.58 a	0.63 a	0.79 b
P	0.85	0.94	1.01
K	8.0	8.1	8.7
Ca	2.5	2.7	2.7
Mg	1.1	1.2	1.3

concentration was 30 percent higher in plants grown under elevated ozone levels relative to the ambient and subambient ozone levels.

Soil N level significantly altered whole plant N status. Loblolly pine seedlings grown at the high soil N levels had significantly higher N concentrations in new needle ($p = 0.002$), stem ($p = 0.003$), coarse root ($p = 0.0001$), and fine root ($p = 0.0001$) tissue components, resulting in higher whole-plant N concentration (Fig. 11). Yellow-poplar tissue N concentration was significantly greater in leaf ($p = 0.023$) and fine root ($p = 0.0004$) components at the higher soil N supply relative to the low. The shifts in nutrient concentration and content relative to mean whole-plant dry weight indicate that N was a growth-limiting nutrient for both species (Figs. 11 and 12); however, the yellow-poplar seedlings did not significantly increase dry weight growth in response to soil N fertilization.

Higher soil N level also decreased the concentrations of some nutrients. Loblolly pine new needle P, K, Ca, and Mg as well as stem P, Ca, and Mg tissue concentrations were significantly lower with increasing soil N level. These concentration changes were evident at the whole-plant level in a dilution-type response in plants grown at the high soil N level as compared to the low (Fig. 11). Although not statistically significant, Sr and Cu showed a similar whole-plant dilution response in seedlings grown at the high relative to the low soil N level.

In yellow-poplar seedlings Ca, Mg, Ba, Cu, and Zn showed similar dilution-type trends. Mean Mg concentration was significantly lower in all yellow-poplar tissue components with higher soil N level and is reflected in a lower mean whole-plant content relative to seedlings grown under the low soil N level (Fig. 12).

Increasing soil N level significantly increased loblolly whole-plant nutrient uptake estimates for all elements (Table 12). No significant soil N or ozone

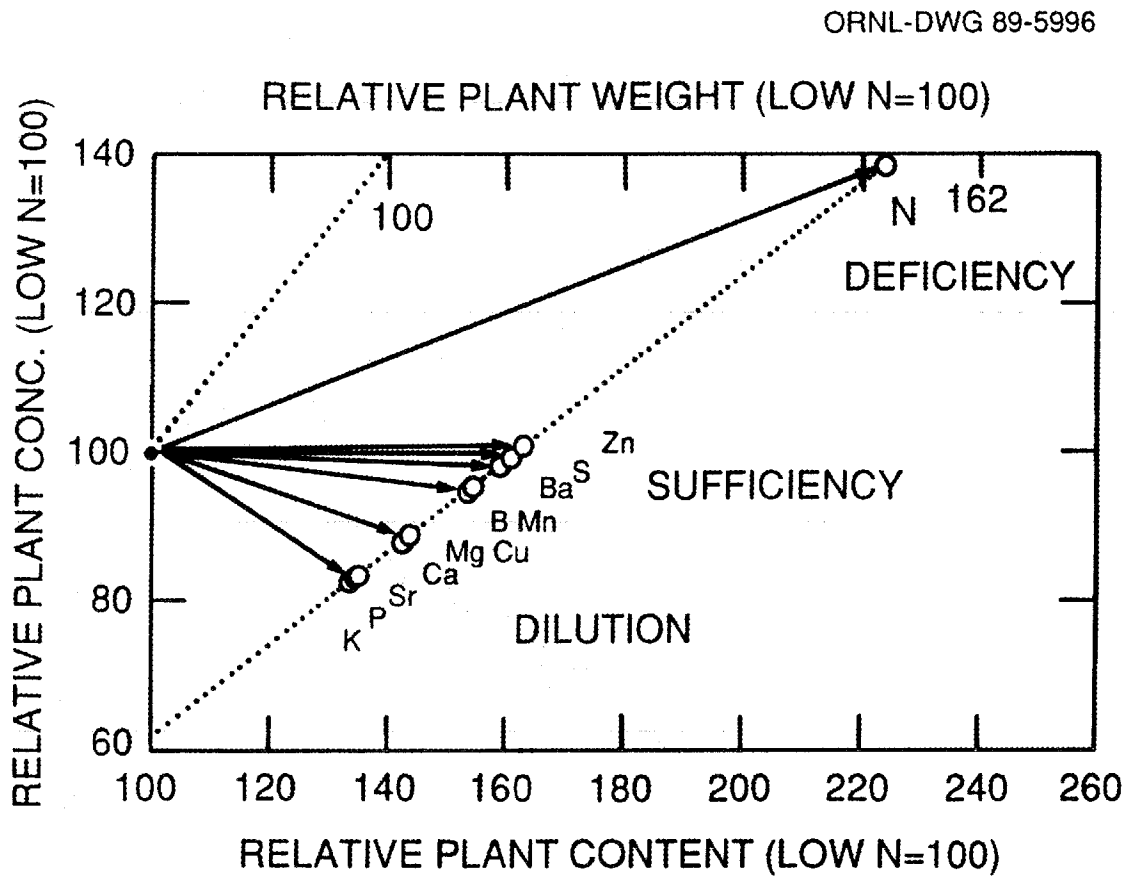

Fig. 11. Loblolly pine whole-plant nutrient status in response to soil nitrogen treatment-induced changes in plant biomass. The mean plant dry weight of the low N treatment group was set to a relative value of 100. The mean N treatment-induced increase in plant dry weight is shown for the high N group (162), and is shown as a dotted line. The mean nutrient content and concentration for the high N group relative to the low N treatment are plotted. The N treatment induced simultaneous changes in relative nutrient content, concentration, and dry weight as indicated by arrows.

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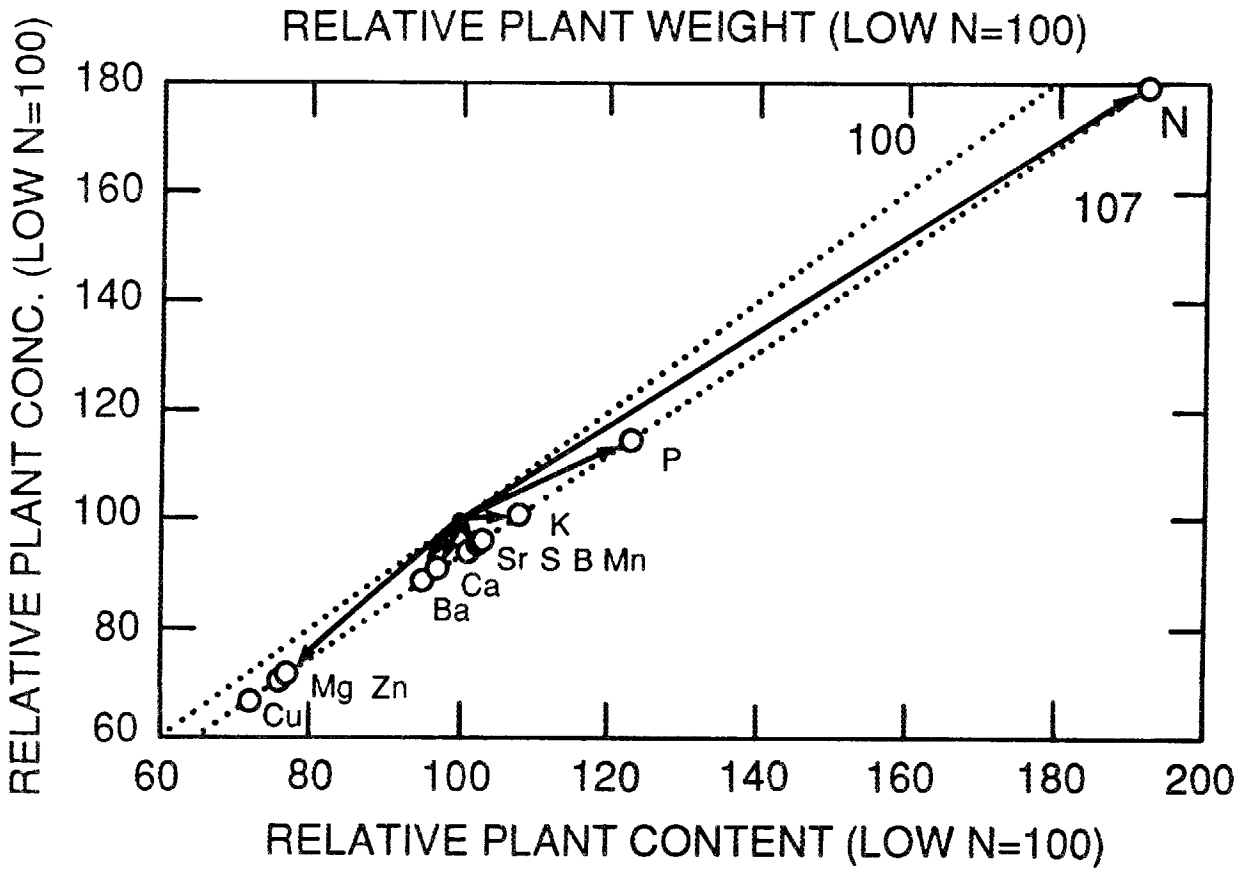

Fig. 12. Yellow-poplar whole-plant nutrient status in response to soil nitrogen treatment-induced changes in plant biomass. The mean plant dry weight of the low N treatment was set to a relative value of 100. The mean whole-plant dry weight for the high N group (107) is shown as a dotted line on which relative nutrient relationships are plotted. The N treatment induced changes in relative nutrient content, concentration, and dry weight as indicated by arrows.

Table 12. Loblolly pine whole-plant nutrient uptake estimates for seedlings grown under three soil nitrogen levels. Nutrient uptake values were calculated as final content minus mean initial content for each element and tested using ANOVA. Means with the same letter are not significantly different (Tukey α = 0.05, n = 18).

		Nutrient uptake (mg/plant)			
		Initial soil N level (μg/g)			
Nutrient		58	96	172	% Increase[1]
Macro	N	16.9 b	39.1 b	129.1 a	660
	S	4.45 b	5.44 b	9.99 a	120
	P	6.12 b	8.24 ab	11.3 a	85
	K	40.3 b	57.1 a	68.8 a	71
	Ca	22.0 b	25.9 b	40.1 a	82
	Mg	10.2 b	11.9 b	17.8 a	75
Micro	Mn	5.43 b	6.88 b	9.36 a	72
	B	0.198 b	0.245 b	0.324 a	64
	Cu	0.067 ab	0.061 b	0.141 a	110
	Zn	0.079 b	0.156 b	0.408 a	420
	Sr	0.100 b	0.127 b	0.197 a	97
	Ba	0.143 b	0.179 ab	0.242 a	69

[1] Increase of high N relative to low N treatment.

treatment effects or interactions were detected for nutrient uptake estimates in yellow-poplar (Table 13).

Soil N level altered NUE. Loblolly pine seedlings grown at the highest soil N level exhibited significantly higher NUE for P, K, Ca, Mg, B, and Ba (Table 14) relative to the low soil N level, while the mean NUE for N was 60 percent lower at the high relative to the low soil N level. Neither nitrogen nor ozone treatment significantly altered yellow-poplar NUE (Table 15).

Loblolly seedlings fumigated with the elevated ozone level showed apparent declining trends of whole-plant NUE (Table 16). With the exception of N and Mn, all elements exhibited apparent lower use efficiency at the elevated ozone level relative to the subambient level. The significantly higher new needle N and S concentrations indicate a type of decreased NUE (i.e. inverse of concentration) for these elements in foliage produced under ozone stress (Table 11). While not statistically significant at the whole-plant level, the declines in whole-plant NUE suggest possible trends which could be further amplified over successive growing seasons.

Table 13. Yellow-poplar whole-plant nutrient uptake estimates for seedlings grown at three soil nitrogen levels. Nutrient uptake values were calculated as final content minus mean initial content for each element and tested using ANOVA. Means ± SD are shown.

		Nutrient uptake (mg/plant)		
		Initial soil N level (μg/g)		
	Nutrient	58	96	172
	N	27.6 ± 29.6	44.0 ± 47.6	65.6 ± 56.1
	S	5.21 ± 6.40	3.47 ± 4.43	5.27 ± 4.81
Macro	P	1.43 ± 2.97	1.54 ± 2.51	2.15 ± 3.87
	K	32.6 ± 29.8	38.2 ± 27.0	36.2 ± 36.2
	Ca	21.8 ± 23.0	22.8 ± 24.2	20.8 ± 26.0
	Mg	8.80 ± 9.96	6.50 ± 6.68	6.06 ± 7.93
	Mn	1.89 ± 1.63	1.77 ± 1.16	1.96 ± 1.72
Micro	B	0.070 ± 0.073	0.070 ± 0.057	0.071 ± 0.085
	Cu	0.081 ± 0.081	0.057 ± 0.040	0.047 ± 0.063
	Zn	0.172 ± 0.240	0.105 ± 0.100	0.123 ± 0.142
	Sr	0.147 ± 0.143	0.150 ± 0.139	0.148 ± 0.167
	Ba	0.138 ± 0.134	0.197 ± 0.250	0.122 ± 0.219

Table 14. Loblolly pine whole-plant nutrient use efficiency values (DMI/uptake) for selected elements in response to nitrogen treatment level. Means with the same letter are not significantly different (Tukey α = 0.05, n = 18).

		Nutrient use efficiency (g/mg)			
		Initial soil N level (μg/g)			
Nutrient		58	96	172	% Increase[1]
Macro	N	0.439 a	0.180 a	0.168 a	- 62
	P	1.57 b	1.55 b	1.85 a	18
	K	0.226 b	0.217 b	0.291 a	29
	Ca	0.436 b	0.501 a	0.515 a	18
	Mg	0.900 b	1.06 a	1.13 a	26
Micro	Mn	2.11 a	2.12 a	2.78 a	32
	B	45.4 b	50.8 b	61.3 a	35
	Ba	64.7 b	69.6 b	86.3 a	33

[1] Increase of high N relative to the low N treatment.

Table 15. Yellow-poplar whole-plant nutrient use efficiency values (DMI/uptake) for selected elements in response to nitrogen treatment level. Means are shown (n = 18).

		Nutrient use efficiency (g/mg)		
		Initial soil N level (μg/g)		
Nutrient		58	96	172
	N	0.198	0.256	0.362
Macro	P	0.527	2.54	1.21
	K	0.076	0.101	0.056
	Ca	2.30	0.444	0.094
	Mg	0.154	0.477	0.468
	Mn	2.07	2.96	2.11
Micro	B	58.5	95.3	65.1
	Ba	17.8	12.8	18.9

Table 16. Loblolly pine whole-plant nutrient use efficiency values (DMI/uptake) in response to ozone treatment level. All ratios were tested using ANOVA. Means are shown (n = 18).

	Nutrient use efficiency (g/mg)			
	Ozone concentration (nL O_3/L, 7 h seasonal mean)			
Nutrient	32	56	108	Percent change[1]
N	0.219	0.334	0.233	6.4
S	2.30	2.28	1.87	-19
P	1.83	1.61	1.53	-16
K	0.242	0.259	0.234	-3.3
Ca	0.509	0.460	0.483	-5.1
Mg	1.08	1.03	0.984	-8.9
Mn	1.64	2.75	2.63	60
B	54.0	51.6	52.0	-3.6
Cu	226	102	147	-35
Sr	100	98.9	97.7	-2.3
Ba	78.1	75.2	67.3	-14

[1] Percent change of high ozone level relative to the low.

DISCUSSION

Growth and Biomass Partitioning

The loblolly pine seedlings responded to increasing N fertilization with significant increases in growth. While all tissue components responded to N fertilization, the largest gains occurred in new needle biomass (Table 1). Shoot growth responded more than root growth to increased N supply. Shoot relative growth rates were larger with higher soil N levels throughout the growing season (Table 4). These responses indicated that N was a growth-limiting nutrient in plants grown at the two lower soil N levels.

These findings generally concur with those from similar studies in conifers. Nitrogen fertilization of balsam fir increased both needle dry weight and N content (Timmer and Stone, 1978). Nitrogen fertilization increased needle weight and subsequent stem growth in both loblolly pine (Wells, 1970) and jack pine (Weetman and Algar, 1974). After N fertilization, significant gains in stem and branch growth were found in Douglas-fir that were associated with increased needle N concentration, content, and surface area (Brix and Ebell, 1969).

The growth responses of yellow-poplar to the treatment combinations were highly variable, which limited the ability to discriminate significant growth responses. This was likely due to low replication (n = 6 per treatment combination) and high variability in root development and vigor or genetic variability among the seedling stock, despite the attempt to select a uniform group and initial randomization. Mean shoot RGR was not affected by N fertilization, except for a significant growth suppression in the low N group during the last 2 months of the growing season as compared to the medium N group (Table 4). Biomass partitioning patterns may also suggest that N supply limited growth. The proportion of biomass allocated to fine roots and FRLR were significantly increased in seedlings grown at the lowest soil N level, a

common tree growth response to a limited nutrient supply (Cannell, 1985; Friend, 1988). The seedlings also exhibited symptoms of foliar chlorosis among the lower soil N levels, suggesting N-deficiency.

Ozone exposure alone did not significantly affect biomass growth in either species; however, there was evidence that ozone affected carbon gain in loblolly pine current needle growth over the 18 week exposure period in an ozone and nitrogen interaction (Table 3). Elevated ozone concentrations reduced new needle biomass in both the low and high soil N groups as compared to the subambient exposure level. There was no evidence that root growth was impaired more than shoot growth in loblolly pine or yellow-poplar with increasing ozone stress, contrary to previous reports (Hogsett et al., 1985; Chappelka and Chevone, 1986; Cooley and Manning, 1988). Instead, reductions in LWR, SWR, and a concomitant increase in RSR (Table 5) suggest that shoot growth was more impaired relative to root growth in the loblolly pine seedlings. Biomass partitioning patterns in yellow-poplar responded similarly with apparent reductions in LWR and SWR, resulting in increases in both FRLR and RSR (Table 5).

The seedlings were grown in a low-fertility forest soil at suboptimum nutrient levels, which could account for overriding effect of the soil N treatments on seedling growth response. Low plant N status increases root sink strength for carbon relative to the shoot (Drew and Saker, 1975; Cannell, 1985; Friend, 1988). Therefore, the N stress response may have precluded ozone-induced reductions in carbon allocation to roots in these seedlings exposed to chronic ozone levels.

Leaf Abscission

Ozone exposure both accelerated and increased leaf abscission in these species. The elevated ozone exposure regime significantly stimulated leaf

abscision in yellow-poplar (Fig. 4; Table 7). Ozone fumigation increased the percentage of leaf biomass abscised relative to total leaf production, but did not significantly alter live leaf area or biomass at the end of the growing season; evidence for greater leaf turnover. The free growth habit of yellow-poplar permits new leaf production throughout the growing season. These results suggest that at least for seedlings grown in a high light environment, ozone induced leaf losses may be compensated for through increased new leaf production. Similarly, Mooi (1980) found a 60 percent increase in cumulative leaf abscission in ozone-fumigated hybrid poplar cultivars after 5 months; however, final leaf and stem dry matter were also not significantly affected. Reich and Lassoie (1985) also found accelerated leaf senescence and evidence for increased leaf production in hybrid poplar exposed to ozone.

Classification of yellow-poplar as an ozone-sensitive species based on visual injury symptoms or leaf abscission data alone is inadequate. Compensatory leaf production or increased leaf turnover could help explain the lack of significant ozone effects on yellow-poplar growth over short term exposures (one growing season or less), observed in many studies (Kress and Skelly, 1982; Mahoney et al., 1984; Chappelka and Chevone, 1988).

Loblolly pine seedlings abscised only older (previous year) needles, which may suggest a greater sensitivity of the mature needles to ozone-induced abscission. Ozone exposure has been shown to affect older, mature foliage more than younger leaves in a range of species, including bean (McLaughlin and McConathy, 1983; Okano et al., 1984) and white pine (McLaughlin et al., 1982). The significant main effect of soil N level on needle loss (Table 7) suggests that N fertilization may have predisposed the seedlings to greater needle abscission. Elevated ozone exposure appears to have induced or accelerated needle abscision in the high N treatment group, which exhibited needle loss two weeks earlier than the ambient and subambient ozone groups (Fig. 3). Ozone did not

affect net needle loss. Neither final cumulative percent needle loss nor the proportion of biomass abscised were significantly altered by ozone treatment level alone (Table 7). These results are similar to those of Harkov and Brennan (1980), who found that increased soil fertility, resulting in optimal leaf N concentrations, predisposed hybrid poplar (*Populus maximowiczii x P. trichocarpa)* trees to the greatest amount of foliar ozone injury. Mineral nutrition has been shown to modify the sensitivity of crop plants to foliar ozone injury, often resulting in greater ozone injury with improved nutrient status (Cowling and Koziol, 1982).

Overall, loblolly pine foliage was less sensitive to ozone-induced leaf abscission than was yellow-poplar on a percentage leaf biomass loss basis (Table 7). Interspecific variation in ozone sensitivity among trees has been correlated with differences in leaf conductance and concomitant potential ozone uptake (Reich, 1987). In the present study yellow-poplar late-season instantaneous leaf conductances were roughly three times higher than loblolly pine (0.3 and 0.1 mol m^{-2} s^{-1} approximate means); which, if representative of values occurring over the entire growing season, could have permitted increased ozone uptake and subsequently greater leaf abscission in yellow-poplar. The cumulative effects of ozone-induced leaf loss compounded over many growing seasons could significantly reduce tree growth and further alter carbon and nutrient allocation patterns.

Gas Exchange Processes

Soil N level significantly altered photosynthetic gas exchange processes in loblolly pine. The seedlings grown under the low soil N level were visibly N-deficient, exhibiting symptoms of foliar chlorosis. Late-season net photosynthesis was limited by soil N supply (Fig. 10a). The lack of a significant effect of soil N level on *g* combined with the evidence of increasing C_i with

decreasing soil N level suggests that carbon fixation metabolism rather than leaf *g* limited photosynthesis (Fig. 10c). Photosynthetic capacity was probably limited by Rubisco content (or any other biochemical component in CO_2 fixation) in the N-stressed seedlings, resulting in higher mesophyll C_i. Previous studies have demonstrated decreased net photosynthesis in response to limited N supply relative to non-stressed plants (Natr, 1972; Moorby and Besford, 1983).

Ozone exposure affected late-season instantaneous *g* and transpiration (Fig. 10b, Table 10), as evident in significant ozone and nitrogen interactions. These results suggest that ozone and nitrogen treatment combination may independently affect both *g* and transpiration without altering P_n. Although ozone-induced reductions in *g* and transpiration have been recorded for many species, they are usually coincident with significant reductions in P_n (Hill and Littlefield, 1969; Reich and Amundson, 1985).

Water Use Efficiency

Whole-plant midseason water use rates were significantly increased in response to soil N supply in both species (Figs. 5 and 6). The response in loblolly pine was likely due to the increase in leaf biomass in response to soil N supply. The significant yellow-poplar response may have been due to temporal differences in leaf biomass across N treatments during exposure weeks 11 to 13, which were later masked by increased ozone-induced abscission.

The significant increase in P_n in combination with no differences in transpiration rate in response to higher soil N level lead to significantly higher instantaneous WUE in loblolly pine seedlings grown under the high soil N level (Fig. 10d). The response of the late-season instantaneous WUE values to soil N supply was similar to the integrated whole-plant WUE estimates, calculated for the entire growing season (Table 9). Therefore, the late-season instantaneous estimates of WUE served as a reliable indicator of trends in integrated whole-

plant WUE. The whole-plant WUE estimates were lower than the instantaneous leaf WUE values, since the instantaneous measure did not take into account integrated whole-plant level carbon losses in respiration or carbon partitioning dynamics.

These findings are similar to those of Conroy et al. (1988), who found that higher P supply increased the whole-plant transpirational water loss and needle photosynthesis relative to P-deficient *Pinus radiata* seedlings, and that the concomitant plant dry weight increase was sufficiently large to increase the plant WUE. With the addition of a limiting nutrient, an increase in photosynthetic capacity per unit of leaf tissue increases WUE. For example, an increase in the ratio of mesophyll cell surface (or mesophyll photosynthetic capacity) to leaf surface would increase WUE by increasing photosynthesis more than it increases transpiration (Nobel, 1983).

There was no evidence that chronic ozone exposure affected WUE in either the late-season instantaneous or whole-plant water use calculations (Table 9). These results are comparable to those of Tseng et al. (1988), who also found no significant chronic ozone effects on needle WUE in Frasier fir. In contrast, leaf WUE was found to decrease in hybrid poplar in response to chronic ozone stress (Reich and Lassoie, 1984). However, through use of $^{13}C/^{12}C$ ratios as a measure of integrated WUE, Greitner and Winner (1988) concluded that WUE improved in radish (*Raphanus sativus*) and soybean plants exposed to ozone. Further studies are needed to clarify the effects of chronic ozone stress on integrated WUE in both coniferous and deciduous trees.

Whole-Plant Nutrition

Soil N level had significant effects on whole-plant nutrient status in loblolly pine. Following the interpretation of Timmer and Stone (1978) and Timmer (1985), N was determined to be the major growth-limiting nutrient. Seedlings

grown at the highest soil N level exhibited a 40 percent increase in N concentration which was associated with a 62 percent increase in whole-plant dry weight growth relative to the low N treatment (Fig. 11). Similar increases in needle N composition and dry weight were found in balsam fir in response to soil fertilization (Timmer and Stone, 1978). Annual N fertilization of a loblolly pine plantation resulted in significant biomass increases which also corresponded to significant increases in foliar N content (Johnson and Todd, 1988).

The whole-plant concentrations of S, Ba, Zn, Mn, and B remained relatively unchanged in seedlings grown at the high soil N level relative to the low, suggesting that uptake was sufficient to keep pace with increased growth. These nutrients were considered non-limiting. The dilution-type response of P, K, Ca, Mg, Sr, and Cu signified decreasing concentration due to higher plant dry weight, hence the supply was diluted by new growth, suggesting that they also were not major limiting nutrients. This finding is comparable to the findings of Timmer and Stone (1978), who found a similar dilution-type response of foliar P, K, Ca, and Mg with increased N fertilization of balsam fir. Although the whole-plant concentrations of these elements decreased in loblolly pine, the absolute whole-plant uptake values for all nutrients showed significant increases in response to increased soil N supply (Table 12). From the gas exchange data (Fig. 10) it is clear that increased N availability stimulated photosynthesis such that the concentrations of the other nutrients in the foliage and whole-plant were diluted by growth and accumulation of carbohydrates.

By definition, increased whole-plant NUE implies a dilution-type response, since NUE was defined in terms of biomass gain per unit nutrient uptake (DMI/uptake). Nutrient use efficiency was used strictly as an index of nutrient economy in terms of whole-plant carbon gain, and does not imply alteration of specific plant mechanisms such as nutrient retranslocation or shifts to or from storage forms. Loblolly pine seedlings grown under the highest soil N level

exhibited greater whole-plant NUE for P, K, Ca, Mg, Mn, B, and Ba, increasing between 18 to 35 percent as compared to the low soil N group, while N use efficiency appeared to decrease (Table 14). Increased supply of N has been shown to result in its decreased use efficiency in plants (Ingestad, 1979; Lajtha and Klein, 1988), while the NUE for other nutrients increases as growth dilutes internal plant concentrations (Timmer and Stone, 1978; Lajtha and Klein, 1988).

The yellow-poplar seedlings showed a relative increase in N and P concentration and content in seedlings grown at the high relative to the low soil N treatment, but no significant growth response (Fig. 12). This suggests that growth may have been nutrient-limited even at the high soil N level. Soil N fertilization did not preclude growth limitations due to inadequate supply of other nutrients, since only N was added to the soil comprising the high N treatment. The relative whole-plant concentration of P showed a 15 percent increase, suggesting that P may also have been a limiting nutrient in seedlings grown at the low soil N level. Higher soil N supply may have significantly altered nutrient/N weight proportions relative to seedlings grown at the low soil N level. For example, the decline in Mg concentration and content in plants grown at the high relative to the low soil N level constitutes an antagonistic response to N fertilization. This effectively resulted in a lower Mg/N ratio, which may have prevented a significant growth response. Nutrient weight proportions in plant tissues have been correlated with growth response (Ingestad, 1979b; Adams and Allen, 1985).

The current-year foliage of loblolly pine seedlings fumigated with elevated ozone levels exhibited increased N and S concentrations (Table 11). This is evidence that ozone fumigation altered needle N metabolism. Similarly, Krause (1988) found significant increases in foliar nitrate-nitrogen concentrations in both coniferous and deciduous trees following ozone fumigation. However, these findings have recently been attributed to an artifact of ozone generation using

ambient air (Kogelschatz and Baessler, 1987; Brown and Roberts, 1988). In experiments with Norway spruce fumigated with purified and unpurified ozone, Brown and Roberts (1988) concluded that the rapid accumulation of nitrate in the needles arises from the deposition of dinitrogen pentoxide (N_2O_5) and nitric acid vapor formed from N_2 in air-fed electric discharge ozone generators (the type used in this experiment). Therefore, the seedlings exposed to the elevated ozone treatment may have received additional N through foliar deposition of nitric acid vapor relative to the ambient and subambient ozone treatments, which may have contributed to the significant increase in needle N concentration (Appendix C).

The increased N concentration was limited to loblolly current-year foliage only, and ozone had no statistically significant effect on the whole-plant N concentration or uptake estimates. The concomitant increase in foliar S concentration in loblolly pine needles with ozone fumigation is evidence that the N was present in reduced form, such as free amino acids or proteins, of which S is a constituent, rather than solely as nitrate.

Reduction of nitrate is facilitated by the enzyme nitrate reductase. Foliar deposition of nitrate at the high ozone level may have contributed to the significant second order interaction of needle NRA to the ozone and nitrogen treatment combinations (Fig. 8). Needle NRA activity, assayed after 13 weeks of ozone exposure, was higher in the most N-deficient seedlings in response to the elevated ozone level as compared to the ambient level. Although needle NRA was statistically the same across all soil N levels at the high ozone level, the response pattern was different from the ambient and subambient ozone responses. The significant increase in fine root NRA (Fig. 7) in response to elevated ozone exposure suggests that ozone may indirectly affect root nitrate assimilation metabolism, although NRA induction through increased soil nitrate levels from throughfall leaching of plant surface-deposited N and direct soil

deposition cannot be ruled out (Appendix C). Further studies are necessary to elucidate both foliar and root NRA response to chronic ozone stress as well as the identity of nitrogenous compounds comprising the increased needle N concentration. The present study suggests that ozone exposure may alter N metabolism in loblolly pine seedlings.

Summary and Conclusions

The following are a review of the ozone and nitrogen stress hypotheses:

Ozone stress:

I. Ozone stress did not significantly reduce biomass partitioning to fine root growth or lower whole-plant nitrogen or nutrient uptake. Fine root nitrate assimilation was not decreased by ozone stress. Hypothesis I is rejected.

II. Ozone stress did not significantly reduce P_n or whole-plant carbon gain or alter whole-plant nutrient status, decreasing whole-plant NUE. Hypothesis II is rejected.

III. Although ozone and nitrogen treatment combination did affect *g* and transpiration without changing P_n, elevated ozone exposure did not significantly increase either late-season or integrated whole-plant WUE estimates. Hypothesis III is rejected.

Nitrogen stress:

I. Increasing soil N supply increased P_n and whole-plant growth and altered biomass partitioning patterns in yellow-poplar. N was the major growth-limiting factor, which may have precluded the effects of chronic ozone stress. Hypothesis I cannot be rejected.

II. N stimulated P_n and growth which diluted the whole-plant concentrations of most nutrients, increasing NUE. Hypothesis II cannot be rejected.

III. With increased soil N supply, loblolly pine seedlings significantly increased both late-season instantaneous WUE and integrated WUE. Hypothesis III cannot be rejected.

Loblolly pine and yellow-poplar seedlings were exposed to both varied soil nitrogen supply and chronic ozone stress levels for one growing season. Increased soil N level significantly increased shoot growth and whole-plant biomass in loblolly pine, whereas yellow-poplar growth was highly variable. Ozone and nitrogen treatment combination affected loblolly pine new needle growth. N-deficiency was the overriding stress factor and may have counteracted ozone effects on growth and biomass partitioning through increased root/shoot ratios. Yellow-poplar was more sensitive than loblolly pine to ozone-induced leaf abscission; however, increased new leaf growth and greater leaf turnover may have ameliorated the effects of leaf abscission on growth. Increased soil N level may predispose older needles to abscission in loblolly pine. Nitrogen was the major growth-limiting nutrient in both species. Increased N availability increased photosynthesis in loblolly pine, increasing growth and effectively diluting most other nutrients, increasing NUE. Ozone-induced increases in new needle N and S concentrations, suggested altered nitrogen metabolism. It is clear that not only ozone stress but the interaction between ozone and nitrogen stress is important to the growth, physiology, and nutrition of trees.

REFERENCES

Adams, M. B., and H. L. Allen. 1985. Nutrient proportions in foliage of semi-mature loblolly pine. Plant Soil. 86:27-34.

Adams, M. B., J. M. Kelly, and N. T. Edwards. 1988. Growth of *Pinus taeda* L. seedlings varies with family and ozone exposure level. Water, Air and Soil Pollut. 38:137-150.

Adams, R. M., and T. D. Crocker. 1982. The economics of acid deposition in economically relevant response information. *In* Crocker, T. D. ed. The Case of Acid Deposition. Ann Arbor, Michigan.

Altshuller, A. P. 1987. Estimation of the natural background of ozone present at surface rural locations. J. Air Pollut. Control Assoc. 37:1409-1417.

Barnes, J. D., and A. W. Davison. 1988. The influence of ozone on the winter hardiness of Norway spruce [*Picea abies* (L.) Karst.]. New Phytol. 108:159-166.

Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:133-138.

Barnes, R. L. 1972b. Effects of chronic ozone exposure on soluble sugar and ascorbic acid contents of pine seedlings. Can. J. Bot. 50:215-219.

Blum, U. T., and D. T. Tingey. 1977. A study of the potential ways in which ozone could reduce root growth and nodulation of soybean. Atmos. Environ. 11:737-739.

Botkin, D. B., W. H. Smith, R. W. Carlson, and T. W. Smith. 1972. Effects of ozone on white pine saplings: Variation in inhibition and recovery of net photosynthesis. Environ. Pollut. 3:273-289.

Bouma, D. 1970. Effects of nitrogen nutrition on leaf expansion and photosynthesis of *Trifolium subterraneum* L. I. Comparison between different levels of nitrogen supply. Ann. Bot. (London) 34:1131-1142.

Brewer, R. F., F. B. Guillemet, and R. K. Creveling. 1961. Influence of N-P-K fertilization on incidence and severity of oxidant injury to mangels and spinach. Soil Sci. 92:298-301.

Brix, H., and L. F. Ebell. 1969. Effect of nitrogen fertilization on growth, leaf area and photosynthesis rate in Douglas-fir. For. Sci. 15:189-196.

Brown, K. A., and T. M. Roberts. 1988. Effects of ozone on foliar leaching in Norway spruce (*Picea abies* L. Karst.): Confounding factors due to NO_x production during ozone generation. Environ. Pollut. 55:55-73.

Cannell, M. G. R. 1986. Dry matter partitioning in tree crops. *In* M. G. R. Cannell, and J. E. Jackson, eds. Attributes of Trees as crop plants. Institute of Terrestrial Ecology, Midlothian, Scotland. pp. 160-193.

Carlson, R. W. 1979. Reduction in photosynthetic rate of *Acer*, *Quercus*, and *Fraxinus* species caused by sulfur dioxide and ozone. Environ. Pollut. 18:159-170.

Castillo, F. J., and H. Greppin. 1988. Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone exposure. Environ. and Exp. Bot. 28:231-238.

Chappelka, A. H., III, and B. I. Chevone. 1986. White ash seedling growth response to ozone and simulated acid rain. Can. J. For. Res. 16:786-790.

Chappelka, A. H., III, and B. I. Chevone. 1988. Growth and physiological responses of yellow-poplar seedlings exposed to ozone and simulated acid rain. Environ. Pollut. 49:1-18.

Chappelka, A. H., III, B. I. Chevone, and T. E. Burk. 1985. Growth response of yellow-poplar seedlings to ozone, sulfur dioxide, and simulated acidic precipitation, alone and in combination. Environ. and Exp. Bot. 25:233-244.

Chevone, B. I, Y. S. Yang, and G. S. Reddick. 1984. Acidic precipitation and ozone effects on growth of loblolly and shortleaf pine seedlings. Phytopathology 74:756.

Chimiklis, P. E., and R. L. Heath. 1975. Ozone-induced loss of intracellular potassium ion from *Chlorella sorokiniana*. Plant Physiol. 56:723-727.

Clarkson, D. T., and J. B. Hanson. 1980. The mineral nutrition of higher plants. Ann. Rev. Plant Physiol. 31:239-298.

Coleman, J. S., C. G. Jones, and W. H. Smith. 1987. The effect of ozone on cottonwood-leaf rust interactions: Independence of abiotic stress, genotype, and leaf ontogeny. Can. J. Bot. 65:949-953.

Coleman, J. S., C. G. Jones, and W. H. Smith. 1988. Interactions between an acute ozone dose, eastern cottonwood, and *Marssonina* leaf spot: Implications for pathogen community dynamics. Can. J. Bot. 66:863-868.

Conroy, J. P., M. Küppers, B. Küppers, J. Virgona, and E. W. R. Barlow. 1988. The influence of CO_2 enrichment, phosphorus deficiency and water stress on the growth, conductance and water use of *Pinus radiata* D. Don. Plant, Cell, Environ. 88:91-98.

Cooley, D. R., and W. J. Manning. 1987. The impact of ozone on assimilate partitioning in plants: A review. Environ. Pollut. 47:95-113.

Cooley, D. R., and W. J. Manning. 1988. Ozone effects on growth and assimilate partitioning in alfalfa, *Medicago sativa* L. Environ. Pollut. 49:19-36.

Coulson, C. L., and R. L. Heath. 1974. Inhibition of photosynthetic capacity of isolated chloroplasts by ozone. Plant Physiol. 53:32-38.

Cowling, D. W., and M. J. Koziol. 1982. Mineral nutrition and plant response to air pollutants. *In* M. H. Unsworth, and D. P. Ormrod, eds. Effects of Gaseous Air Pollutants in Agriculture and Horticulture. Butterworth Scientific, London, U.K. pp. 349-375.

Cunningham, M., M. B. Adams, R. J. Luxmoore, W. M. Post, and D. L. DeAngelis. 1989. Quick estimate of root length using a video image analyzer. Can. J. For. Res. 19:335-340.

Drew, M. C., and L. R. Saker. 1975. Nutrient supply and the growth of the seminal root system of barley. II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. J. Exp. Bot. 26:79-90.

Düball, S., and A. Wild. 1988. Investigation on the nitrogen metabolism of spruce needles in relation to the occurrence of novel forest decline. J. Plant. Physiol. 132:491-498.

Duchelle, S. F., J. M. Skelly, and B. I. Chevone. 1982. Oxidant effects on forest tree seedling growth in the Appalachian Mountains. Water, Air and Soil Pollut. 18:363-373.

Environmental Protection Agency. 1985. Air quality criteria for ozone and other photochemical oxidants. EPA/600/8-84/020AF-EF. Environmental Criteria and Assessment Office, Research Triangle Park, NC.

Flagler, R. B., R. P. Patterson, A. S. Heagle, and W. W. Heck. 1987. Ozone and soil moisture deficit effects on nitrogen metabolism of soybean. Crop Sci. 27:1177-1184.

Friend, A. L. 1988. Nitrogen stress and fine root growth of Douglas-fir. Ph.D. Disseration, University of Washington, Seattle 115 p.

Greitner, C. S., and W.E. Winner. 1988. Increases in $\delta\ ^{13}C$ values of radish and soybean plants caused by ozone. New Phytol. 108:489-494.

Gross, K. 1987. Gaswechselmessungen an jungen Fichten und Tannen während Begasungen mit Ozon und Schwefeldioxid (allein und in kombination) im Kleinphytotron. Allgemeine Forst und Jagdzeitung 158:31-36.

Grossman, W. D. 1988. Products of photo-oxidation as a decisive factor of the new forest decline? Results and considerations. Ecol. Modelling 41:281-395.

Guerrero, M. G., J. M. Vega, and M. Losada. 1981. The assimilatory nitrate-reducing system and its regulation. Ann Rev. Plant Physiol. 32:169-204.

Halliwell, B. 1984. Chloroplast metabolism. Rev. ed. Oxford University Press, New York.

Harkov, R. S., and E. Brennan. 1980. The influence of soil fertility and water stress on the ozone response of hybrid poplar trees. Phytopathology 70:991-994.

Harkov, R. S., and E. Brennan. 1982. An ecophysiological analysis of woody and herbaceous plants to oxidant injury. J. Environ. Manag. 15:251-261.

Heagle, A. S. 1979. Effects of growth media, fertilizer rate and hour and season of exposure on sensitvity of four soybean cultivars to ozone. Environ. Pollut. 18:313-322.

Heagle, A. S., R. B. Flagler, R. P. Patterson, V. M. Lesser, S. R. Shafer, and W. M. Heck. 1987. Injury and yield response of soybean to chronic doses of ozone and soil moisture deficit. Crop Sci. 27:1016-1024.

Heath, R. L. 1975. Ozone. *In* J. B. Mudd, and T. T. Kozlowski, eds. Responses of Plants to Air Pollution. Academic Press, New York. pp. 23-55.

Heath, R. L. 1980. Initial events in injury to plants by air pollutants. Ann. Rev. Plant Physiol. 31: 395-431.

Heggestad, H. E., E. L. Anderson, T. J. Gish, and E. H. Lee. 1988. Effects of ozone and soil water deficit on roots and shoots of field-grown soybeans. Environ. Pollut. 50:259-278.

Hill, A. C., and N. Littlefield. 1969. Ozone. Effect on apparent photosynthesis, rate of transpiration, and stomatal closure in plants. Environ. Sci. Tech. 3:52-56.

Hofstra, G., A. Ali, R. T. Wukasch, and R. A. Fletcher. 1981. The rapid inhibition of root respiration after exposure of bean (*Phaseolus vulgaris* L.) plant to ozone. Atmos. Environ. 15:483-487.

Hogsett, W. E., M. Plotcher, V. Wildman, D. T. Tingey, and I. P. Bennett. 1985. Growth response of two varieties of slash pine seedlings to chronic ozone exposures. Can. J. Bot. 63:2369-2373.

Ingestad, T. 1979. Nitrogen stress in birch seedlings. II. N, K, P, Ca, and Mg nutrition. Physiol. Plant. 45:149-157.

Ingestad, T. 1979b. Mineral nutrient requirements of *Pinus sylvestris* and *Picea abies* seedlings. Physiol. Plant. 45:373-380.

Isaac, R. A., and W. C. Johnson. 1976. Determination of total nitrogen in plant tissue using a block digestor. J. Assoc. Off. Anal. Chem. 59:98-100.

Ito, O., K. Okano, M. Kuroiwa, and T. Totsuka. 1985. Effects of nitrogen dioxide and ozone alone and in combination on kidney bean plants: Growth, partitioning of assimilates and root activities. J. Exp. Bot. 36:652-662.

Jagels, R. 1986. Acid fog, ozone and low elevation spruce decline. IAWA Bull. 7:299-307.

Jaworski, E. G. 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 46:531-537.

Jensen, K. F. 1981. Ozone fumigation decreased root carbohydrate content and dry weight of green ash seedlings. Environ. Pollut. 26:147-152.

Johnson, D. W., and D. E. Todd. 1988. Nitrogen fertilization of young yellow poplar and loblolly pine plantations at differing frequencies. Soil Sci. Soc. Am. J. 52:1468-1477.

Johnston, J. W., D. S. Shriner, and C. H. Abner. 1986. Design and performance of an exposure system for measuring the response of crops to acid rain and gaseous pollutants in the field. J. Air Pollut. Control Assoc. 36:894-899.

Jones, J. B. 1975. Collaborative study of the elemental analysis of plant tissue by direct reading emmission spectroscopy. J. Assoc. Off. Anal. Chem. 58:764-769.

Keller, T. 1972. Gaseous exchange of forest trees in relation to some edaphic fators. Photosynthetica 6:197-206.

King, D. A. 1987. A model for predicting the influence of moisture stress on crop losses caused by ozone. Ecol. Modelling 35:39-44.

King, D. A., A. S. Heagle, and R. B. Flagler. 1988. Evaluation of an ozone x moisture stress interaction model for soybean. Ecol. Modelling 41:269-279.

Kogelschatz, U., and P. Baessler. 1987. Determination of nitrous oxide and dinitrogen pentoxide concentrations in the output of air-fed ozone generators of high power density. Ozone Science and Engineering 9:195-206.

Kramer, P. J. 1983. Drought tolerance and water use efficiency. *In* P. Kramer, ed. Water Relations of Plants. Academic Press, New York. pp. 390-415.

Kramer, P. J., and T. T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, New York.

Krause, G. H. M. 1988. Ozone-induced nitrate formation in needles and leaves of *Picea abies*, *Fagus sylvatica*, and *Quercus robur*. Environ. Pollut. 52:117-130.

Krause, G. H. M., V. Arndt, C. J. Brandt, J. Bucher, G. Kenk, and E. Matzner. 1986. Forest decline in Europe: Development and possible causes. Water, Air and Soil Pollut. 31:647-668.

Krause, G. H. M., B. Prinz, and K. D. Jung. 1983. Forest effects in West Germany. *In* D. D. Davis, A. A. Millen, and L. Dochinger, eds. Air Pollution and the Productivity of the Forest. Izaak Walton League, Arlington, VA. pp. 297-332.

Kress, L. W., and J. M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. Plant Dis. 66:1149-1152.

Krupa, J. V., and W. J. Manning. 1988. Atmospheric ozone: Formation and effects on vegetation. Environ. Pollut. 50:101-137.

Lajtha, K., and M. Klein. 1988. The effect of varying nitrogen and phosphorus availability on nutrient use by *Larrea tridentata*, a desert evergreen shrub. Oecologia 75:348-353.

Lehnherr, B., A. Grandjean, F. Mächler, and J. Fuhrer. 1987. The effect of ozone in ambient air on ribulosebisphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. J. Plant Physiol. 130:189-200.

Leone, I. A., and E. Brennan. 1970. Ozone toxicity in tomato as modified by phosphorus nutrition. Phytopathology 60:1521-1524.

Lucas, P. W., D. A. Cottam, L. J. Sheppard, and B. J. Francis. 1988. Growth responses and delayed winter hardening in Sitka spruce following summer exposure to ozone. New Phytol. 108:495-504.

Maas, E. V., G. J. Hoffman, S. L. Rawlins, and G. Ogata. 1973. Salinity-ozone interactions on pinto bean: Integrated response to ozone concentration and duration. J. Environ. Qual. 2:400-404.

MacDowall, F. D. H. 1965. Predisposition of tobacco to ozone damage. Can. J. Plant Sci. 45:1-12.

Mackay, C. E., T. Senaratna, B. D. McKersie, and R. A. Fletcher. 1987. Ozone induced injury to cellular membranes in *Triticum aestivum* L. and protection by the triazole S-3307. Plant Cell Physiol. 28:1271-1278.

Mahoney, M. J., B. I. Chevone, J. M. Skelly, and L. D. Moore. 1985. Influence of mycorrhizae on the growth of loblolly pine seedlings exposed to ozone and sulfur dioxide. Pytopathology 75:679-682.

Mahoney, M. J., J. M. Skelly, B. I. Chevone, and L. D. Moore. 1984. Response of yellow poplar (*Liriodendron tulipifera* L.) seedling shoot growth to low concentrations of O_3, SO_2, and NO_2. Can J. For. Res. 14:150-153.

McCool, P. M., and J. A. Menge. 1983. Influence of ozone on carbon partitioning in tomato: Potential role of carbon flow in regulation of the mycorrhizal symbiosis under conditions of stress. New Phytol. 94:241-247.

McEvers, J. A., T. L. Bowers, and N. T. Edwards. 1988. Air Pollution effects field research facility: I. Ozone flow control and monitoring system. ORNL/TM-10758. Oak Ridge National Laboratory, Oak Ridge, TN.

McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical review. J. Air Pollut. Control Assoc. 35:512-534.

McLaughlin, S. B., M. B. Adams, N. T. Edwards, P. J. Hanson, P. A. Layton, E. G. O'Neill, and W. K. Roy. 1988. Comparative sensitivity, mechanisms, and whole plant physiological implications of responses of loblolly pine genotypes to ozone and acid deposition. ORNL/TM-10777. Oak Ridge National Laboratory, Oak Ridge, TN.

McLaughlin, S. B., and R. K. McConathy. 1983. Effects of SO_2 and O_3 on allocation of ^{14}C-labelled photosynthate in *Phaseolus vulgaris*. Plant Physiol. 73:630-635.

McLaughlin, S. B, R. K. McConathy, D. Duvik, and L. K. Mann. 1982. Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. For. Sci. 28:60-70.

Meagher, J. F., N. T. Lee, R. J. Valente, and W. J. Parkhurst. 1987. Rural ozone in the Southeastern United States. Atmos. Environ. 21:605-615.

Mehlhorn, H., and A. R. Wellburn. 1987. Stress ethylene formation determines plant sensitivity to ozone. Nature 327:417-418.

Mehlmann, M. A., and C. Borek. 1987. Toxicity and biochemical mechanisms of ozone. Environ. Research 42:36-53.

Miller, P. R. 1983. Ozone effects in the San Bernardino National Forest. *In* D. D. Davis, A. A. Millen, L. S. Dochinger, eds. Air Pollution and the Producivity of the Forest. Izaak Walton League, Arlington, VA. pp. 161-197.

Mooi, J. 1980. Influence of ozone on the growth of two poplar cultivars. Plant Dis. 64:772-773.

Moorby, J., and R. T. Besford. 1983. Mineral nutrition and growth. *In* A. Läuchli, and R. L. Bieleski, eds. Encyclopedia of Plant Physiology. new series vol 15b. Springer-Verlag, Berlin. pp. 481-527.

Mudd, J. B. 1982. Effects of oxidants on metabolic function. *In* M. H. Unsworth, and D. P. Ormrod, eds. Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworth Scientific, London. pp. 189-203.

Nakamura, H., and H. Saka. 1978. Photochemical oxidants injury in rice plants. III. Effect of ozone on physiological activities in rice plants. Jpn. J. Crop Sci. 47:704-714.

Natr, L. 1972. Influence of mineral nutrients on photosynthesis of higher plants. Photosynthetica 6:80-99.

Nobel, P. S. 1983. Biophysical Plant Physiology and Ecology. W. H. Freeman and Company, New York.

Noland, T. L., and T. T. Kozlowski. 1979. Influence of potassium nutrition on susceptibility of silver maple to ozone. Can. J. For. Res. 9:501-503.

Oaks, A., and B. Hirel. 1985. Nitrogen metabolism in roots. Ann Rev. Plant Physiol. 36:345-65.

Okano, K., O. Ito, G. Takeba, A. Shimizu, and T. Totsuka. 1984. Alteration of ^{13}C assimilate partitioning in plants of *Phaseolus vulgaris* exposed to ozone. New Phytol. 9:155-163.

Omielan, J. A., and E. J. Pell. 1988. The role of photosynthetic activity in the response of isolated *Glycine max* mesophyll cells to ozone. Can. J. Bot. 66:745-749.

Patton, R. L., and M. O. Garroway. 1986. Ozone-induced necrosis and increased peroxidase activity in hybrid poplar leaves. Environ. Exp. Bot. 26:137-142.

Pell, E. J., and N. S. Pearson. 1983. Ozone-induced reduction in quantity of ribulose-1,5-bisphosphate carboxylase in alfalfa foliage. Plant Physiol. 73:185-187.

Peterson, D. L., M. J. Arbaugh, V. A. Wakefield, and P. R. Miller. 1987. Evidence of growth reduction in ozone-injured Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) in Sequoia and Kings Canyon National Parks. J. Air Pollut. Control Assoc. 37:906-912.

Pinkerton, J. E., and A. S. Lefohn. 1987. The characterization of ozone data for sites located in forested areas of the Eastern United States. J. Air Pollut. Control. Assoc. 37:1005-1010.

Pye, J. M. 1988. Impact of ozone on the growth and yield of trees: A review. J. Environ. Qual. 17:347-360.

Radin, J. W., L. L. Parker, and C. R. Sell. 1978. Partitioning of sugar between growth and nitrate reduction in cotton roots. Plant Physiol. 62:550-553.

Reich, P. B. 1987. Quantifying plant response to ozone: A unifying theory. Tree Physiol. 3:63-91.

Reich, P. B., and R. G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.

Reich, P. B., and J. P. Lassoie. 1984. Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant, Cell, Environ. 7:661-668.

Reich, P. B., and J. P. Lassoie. 1985. Influence of low concentrations of ozone on growth, biomass partitioning and leaf senescence in young hybrid poplar plants. Environ. Pollut. 39:39-51.

Reich, P. B., A. W. Schoettle, H. F. Stroo, J. Troiana, and R. G. Amundson. 1985. Effects of ozone, sulfur dioxide, and acidic rain on mycorrhizal infection in northern red oak seedlings. Can. J. Bot. 63:2049-2055.

Reich, P. B., A. W. Schoettle, H. F. Stroo, J. Troiana, and R. G. Amundson. 1987. Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils. I. Net photosynthesis and growth. Can. J. Bot. 65:977-987.

Richards, B. L., J. T. Middleton, and W. B. Hewitt. 1958. Air pollution with relation to agronomic crops: V. Oxidant stipple of grape. Agron. J. 50:559-561.

Rowland, A. J., M. C. Drew, and A. R. Wellburn. 1987. Foliar entry and incorporation of atmospheric nitrogen dioxide into barley plants of different nitrogen status. New Phytol. 107:357-371.

Rowland-Bamford, A. J., and M. C. Drew. 1988. The influence of plant nitrogen status on NO_2 uptake, NO_2 assimilation on the gas exchange characteristics of barley plants exposed to atmospheric NO_2. J. Exp. Bot. 39:1287-1297.

Rufty, T. W., Jr., C. D. Raper, Jr., and S. C. Huber. 1984. Alterations in internal partitioning of carbon in soybean plants in response to nitrogen stress. Can. J. Bot. 62:501-508.

Ryle, G. J. A., and J. D. Hesketh. 1969. Carbon dioxide uptake in nitrogen deficient plants. Crop Sci. 9:451-454.

Schere, K. L. 1988. Modeling ozone concentrations. Environ. Sci. Technol. 22:488-495.

Shafer, S. R., A. S. Heagle, and D. M. Camberato. 1987. Effects of chronic doses of ozone on field-grown loblolly pine: seedling responses in the first year. J. Air Pollut. Control Assoc. 37:1179-1184.

Shriner, D. S., W. W. Cure, A. S. Heagle, W. W. Heck, D. W. Johnson, R. J. Olson, and J. M. Skelly. 1982. An analysis of potential agriculture and forestry impacts of long-range transport air pollutants. ORNL Report No. 5910, Oak Ridge, TN.

Simmons, G. L., J. M. Kelly. 1989. Effects of acidic precipitation, O_3, and soil Mg status on throughfall, soil, and loblolly pine seedling nutrient concentrations. Water, Air and Soil Pollut. 42:(in press).

Skärby, L., E. Troeng, and C. Å. Bostrom. 1987. Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration in Scots pine. For. Sci. 33:801-808.

Skeffington, R. A., and T. M. Roberts. 1985. The effects of ozone and acid mist on Scots pine saplings. Oecologia 65:201-206.

Srivastava, H. S., and D. P. Ormrod. 1984. Effects of nitrogen dioxide and nitrate nutrition on growth and nitrate assimilation in bean leaves. Plant Physiol. 76:418-423.

Strasser, R. J., B. Schwarz, and J. B. Bucher. 1987. Simultane Messung der Chlorophyll Fluoreszenz-Kinetik bei verschiedenen Wellenlängen als rasches Verfahren zur Frühdiagnose von Immissionsbelastungen an Waldbäumen: Ozonwirkungen auf Buchen und Pappeln. Eur. J. For. Path. 17:149-157.

Tamm, O. C. 1979. Nutrient cycling and productivity of forest ecosystems. *In* Impact of Intensive Harvesting on Forest Nutrient Cycling. Northeast Forest Exp. Stn., Broomall, PA.

Tanaka, K., H. Saji, and N. Kondo. 1988. Immunological properties of spinach glutathione reductase and inductive biosynthesis of the enzyme with ozone. Plant Cell Physiol. 29:637-642.

Taylor, G. E. Jr., and R. J. Norby. 1985. The significance of elevated levels of ozone on natural ecosystems of North America. *In* S. D. Lee, ed. International Specialty Conference on Evaluation of the Scientific Basis for Ozone/Oxidants Standards. Air Pollution Control Association, Pittsburgh, PA. pp. 152-175.

Taylor, O. C. 1974. Air pollutant effects influenced by plant-environmental interaction. *In* M. Dugger, ed. Air Pollution Effects on Plant Growth. American Chemical Society, Washington, D.C. pp. 1-7.

Temple, P. J., R. W. Benoit, R. W. Lennox, C. A. Reagan, and O. C. Taylor. 1988. Combined effects of ozone and water stress on alfalfa growth and yield. J. Environ. Qual. 17:108-113.

Teskey, R. O., J. A. Fites, L. J. Samuelson, and B. C. Bongarten. 1986. Stomatal and nonstomatal limitations to net photosynthesis in *Pinus taeda* L. under different environmental conditions. Tree Physiol. 2:131-142.

Timmer, V. R. 1985. Response of a hybrid polar clone to soil acidification and liming. Can. J. Soil Sci. 65:727-735.

Timmer, V. R., and E. L. Stone. 1978. Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Soil Sci. Am. J. 42:125-130.

Tingey, D. T. 1974. Ozone induced alterations in the metabolite pools and enzyme activities of plants. *In* M. Dugger, ed. Air Pollution Effects on Plant Growth. American Chemical Society. pp. 40-57.

Tingey, D. T., R. C. Fites, and C. Wickliff. 1973. Ozone alteration of nitrate reduction in soybean. Physiol. Plant. 29:33-38.

Tingey, D. T., and W. Hogsett. 1985. Water stress reduces ozone injury via a stomatal mechanism. Plant Physiol. 77:944-947.

Tingey, D. T., K. D. Rodecap, E. H. Lee, T. J. Moser, and W. E. Hogsett. 1986. Ozone alters the concentrations of nutrients in bean tissue. Angew. Botanik. 60:481-493.

Tingey, D. T., and G. E. Taylor, Jr. 1982. Variation in plant response to ozone: A conceptual model of physiological events. *In* M. H. Unsworth, and D. P. Ormrod, eds. Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworth Scientific, London, U.K. pp. 113-138.

Tingey, D.T., R. G. Wilhour, and F. Standley. 1976. The effect of chronic ozone exposures on the metabolite content of ponderosa pine seedlings. For. Sci. 22:234-241.

Trumble, J. T., J. D. Hare, R. C. Musselman, and P. M. McCool. 1987. Ozone-induced changes in host-plant suitability: Interactions of *Keiferia lycopersicella* and *Lycopersicon esculentum*. J. Chem. Ecol. 13:203-217.

Tseng, E. C., J. R. Seiler, and B. I. Chevone. 1988. Effects of ozone and water stress on greenhouse-grown Frasier fir seedling growth and physiology. Environ. Exp. Bot. 28:37-41.

Vessey, J. K., and D. B. Layzell. 1987. Regulation of assimilate partitioning in soybean: Initail effects following change in nitrate supply. Plant Physiol. 83:341-348.

Weetman, G. F., and D. Algar. 1974. Jack pine fertilization and nutrition studies: Three year results. Can J. For. Res. 4:381-398.

Wells, C. G. 1970. Nitrogen and potassium fertilization of loblolly pine on a South Carolina piedmont soil. For. Sci. 16:172-176.

Wilkinson, T. G., and R. L. Barnes. 1973. Effects of ozone in $^{14}CO_2$ fixation patterns in pine. Can. J. Bot. 51:1573-1578.

Yandow, T. S., and R. M. Klein. 1986. Nitrate reductase of primary roots of red spruce seedlings. Plant Physiol. 81:723-725.

Yang, Y. S., J. M. Skelly, B. I. Chevone, and J. B. Birch. 1983. Effects of long term ozone exposure on photosynthesis and dark respiration of eastern white pine. Environ. Sci. Technol. 17:371-373.

Zech, W., Th. Suttner, and E. Popp. 1985. Elemental analyses and physiological responses of forest trees in SO_2-polluted areas of NE-Bavaria. Water, Air and Soil Pollut. 25:175-182.

APPENDIXES

APPENDIX A: SOIL ANALYSIS

Table A1. Mean (± SD) nutrient concentrations of six random soil samples taken during planting. Six random soil samples were obtained from the soil supply during transplanting and analyzed by the Soil Testing and Plant Analysis laboratory of the University of Georgia Athens, Georgia. The samples were extracted using the Mehlich 1 procedure, an acid extraction method using 0.05 N HCl and 0.025 N H_2SO_4, for determination of P, K, Ca, Mg, Zn, and Mn concentration. Extractable NH_4 and NO_3 were obtained using a 2 M KCl solution.

Element	Concentration (μg/g)
P	6.8 ± 0.42
K	71.1 ± 31.0
Ca	168.2 ± 15.0
Mg	17.8 ± 0.88
Zn	1.1 ± 0.20
Mn	58.3 ± 2.44
NH_4	11.9 ± 2.80
NO_3	84.4 ± 6.18
Soluble N	96.3 ± 7.83

Table A2. Mean (± SD) composition of six replicate soil samples taken during planting.

Soil component	Percentage (dry weight)
organic matter	2.8 ± 0.17
sand	50.8 ± 1.29
silt	31.2 ± 1.21
clay	17.9 ± 1.03

APPENDIX B: STATISTICAL MODEL

Table B1. Statistical model of sources of variance used to test main effects and interactions of response variables. Analysis of variance was used to test the significance of the various main effects and interactions on the response variables using the model shown. Each species was tested separately using a 5 percent significance level ($\alpha = 0.05$). Type III sums of squares were used in the analysis and means separation tests using the F-ratios shown.

Source of variation	Degrees of freedom	F ratio
Whole plot		$\frac{\text{MS O}}{\text{MS C(O)}}$
Ozone [O]	2	
Chamber(Ozone)[1] [C(O)]	6	$\frac{\text{MS C(O)}}{\text{MS N*C(O)}}$
Subplot		
Nitrogen [N]	2	$\frac{\text{MS N}}{\text{MS N*C(O)}}$
Whole x Subplot		
Ozone x Nitrogen [O*N]	4	$\frac{\text{MS O*N}}{\text{MS N*C(O)}}$
Nitrogen x Chamber(Ozone)[1] [N*C(O)]	12	
Error	27	$\frac{\text{MS N*C(O)}}{\text{MS Error}}$
Total	53	

[1]Random source of variation.

APPENDIX C: OZONE GENERATION AND CONCOMITANT NITRIC ACID VAPOR DEPOSITION TO LOBLOLLY PINE SEEDLINGS

Introduction

Reactive trace nitrogen gases, such as dinitrogen pentoxide (N_2O_5) and its hydration product nitric acid vapor (HNO_3) are formed as a consequence of ozone generation using air-fed electric discharge ozone generators (Kogelschatz and Baessler, 1987). Concurrent deposition of trace nitrogen gases to plant and soil surfaces exposed to elevated ozone levels could result in inadvertent N fertilization. In experiments with Norway spruce fumigated with purified and unpurified ozone, Brown and Roberts (1988) found rapid accumulation of nitrate in the needles exposed to the unpurified ozone, attributed to deposition of trace nitrogen gases.

Recent analysis of the air stream entering the open-top chambers used in this study has revealed mean HNO_3 concentrations estimated at 2.0, 4.1 and 16.3 $\mu g/m^3$ in the charcoal-filtered, ambient, and ambient + 60 nL/L ozone treatment chambers, respectively (Grizzard et al., 1988, unpublished data). They concluded that the total N deposition amounted to only 1 to 6 percent of the total seasonal N demand of loblolly pine in the ambient and elevated ozone chambers, respectively. Assuming that a substantial portion of the HNO_3 is deposited to needle and bark surfaces and subsequently removed via throughfall, they concluded that HNO_3 formation and deposition to plant foliage was probably physiologically insignificant.

Deposition of trace nitrogen gases to N-deficient seedlings, however, may be physiologically significant. For example, atmospheric NO_2 has been shown to be a significant N source for N-deficient plants (Srivasta and Ormrod, 1984; Rowland et al., 1987; Rowland-Bamford and Drew, 1988). In this study, current-year needles of loblolly pine seedlings exposed to elevated ozone levels,

exhibited an 18 percent increase in N concentration relative to the subambient (charcoal-filtered) ozone treatment (Table 11). This increase in N concentration may be attributable to HNO_3 vapor deposition concurrent with the elevated ozone exposure. The higher needle N concentration and the higher fine root nitrate reductase activity (Fig. 7) in seedlings exposed to the elevated ozone levels relative to the ambient and subambient treatments observed in this study led to this hypothesis. Needle-deposited HNO_3 could potentially be assimilated by the enzyme, nitrate reductase, whereas soil deposited HNO_3 vapor (or nitrate washed from the plant surfaces) entering the soil solution could stimulate fine root nitrate reductase activity (NRA). The objective of the following N deposition calculations was to estimate the potential contribution of HNO_3 vapor deposition to the total seasonal N uptake in loblolly pine seedlings.

Methods

Total seasonal HNO_3 vapor deposition was calculated based on the average seasonal needle surface area for each loblolly pine seedling. Needle surface area was estimated using the following regression equation of needle area as a function of needle dry weight:

$$\text{Area} = 195.28 \cdot \text{DW}, \; R^2 = 0.73,$$

where DW = total plant needle dry weight (g) and Area = needle surface area (cm^2). The regression equation was generated using needle surface area calculations for 27 loblolly pine seedlings. At final harvest, current and previous year's needles were removed from each plant and photocopied for area determination using a video method (Cunningham et al., 1989), and subsequently oven-dried (65° C) for dry weight determination. Mean seasonal needle surface

area for each plant was then estimated as average of the final harvest needle area and initial leaf area, calculated from previous-year needle dry weight.

The total N deposition to the foliage of each loblolly pine was calculated as:

$$N_D = [HNO_3] \cdot V_s \cdot LA \cdot t,$$

where N_D is the seasonal N deposition (mg), $[HNO_3]$ is the ozone treatment nitric acid vapor concentration, V_s is the surface conductance (range 0.25-0.42 $cm \cdot s^{-1}$), LA is the mean seasonal leaf area (cm^2), and t is the length of the season (126 d). The surface conductance values of 0.25 and 0.42 $cm \cdot s^{-1}$ were used in calculating a range of N deposition values for each plant, according to recent estimates (P. J. Hanson, personal communication). The HNO_3 concentrations were 2.0, 4.1, and 16.3 $\mu g/m^3$ in the charcoal-filtered, ambient, and elevated ozone treatments, respectively (Grizzard, et al., unpublished data). Whole-plant nitrogen uptake was calculated as final harvest plant N content (the sum of the tissue contents (concentration multiplied by tissue dry weight)) minus mean plant N content at the initial harvest of six randomly selected seedlings.

Results and Discussion

Ozone fumigation resulted in the estimated maximum deposition of 9 to 15 mg of N per plant (Table C1). Over the entire growing season, HNO_3 vapor deposition could have resulted in a potential maximum contribution of 13 to 22 percent of the loblolly pine seedlings' total N uptake of approximately 60 mg. This represents a three-fold increase above seedlings exposed to the ambient ozone treatment, which may have received 5 to 7 percent of the total N uptake. Seedlings grown in charcoal-filtered chambers were estimated to receive between 1 and 2 mg of N per plant, only 2 to 3 percent of the seasonal N uptake.

The significant increase in potential N deposition to foliage of plants exposed to the elevated ozone treatment could account for the observed 18 percent increase in N concentration (9.0 mg/g) in loblolly pine needles relative to the charcoal-filtered treatment (7.6 mg/g). Needle NRA may be stimulated if significant amounts of nitrate were to enter the needle tissue. The significant ozone x nitrogen interaction for current needle NRA (Fig. 8) complicates the analysis of the main effect of ozone. However, needle NRA, assayed after 13 weeks of exposure, was significantly higher in the most N-deficient (low N treatment) seedlings in response to the elevated ozone level as compared to the ambient level. Needle NRA was also statistically the same across all soil N levels in plants grown at the high ozone level, however, the pattern was distinctly different from the seedlings exposed to the ambient and subambient ozone levels.

The deposition ranges calculated are maximum potential deposition to needle surfaces and do not take into account soil surface deposition or the washing of plant surface-deposited N to the soil. A subsequent analysis of throughfall chemistry of similar potted loblolly pine seedlings at the same research site has shown significantly higher nitrate and ammonium-nitrogen concentrations in the elevated ozone treatment (Simmons and Kelly, 1989). These increased nitrate concentrations may indicate that a significant amount of the surface-deposited HNO_3 is subsequently washed from the seedlings in the throughfall, therefore, unavailable for foliar assimilation. Presumably, this nitrate could then be available for uptake and assimilation in the roots of these potted seedlings. This may explain the significant increase in fine root nitrate reductase activity in seedlings grown under the elevated ozone exposure level. Clearly, the introduction of HNO_3 vapor into the open-top chambers receiving elevated ozone levels warrants consideration as a significant N source for N-deficient seedlings.

Table C1. Mean seasonal N deposition to loblolly pine seedlings grown under three ozone levels (n = 54).

Ozone level	Needle surface area (cm^2)	N-deposition (mg) V_s=0.25	N-deposition (mg) V_s=0.42	Seasonal N uptake (mg)	Deposition % of uptake
Filtered	993	1.2	2.0	59	2 to 3
Ambient	1025	2.6	4.3	57	5 to 7
Elevated	928	9.2	15.4	69	13 to 22

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