The effects of ultraviolet-B radiation on photosynthesis of different aged needles in field-grown loblolly pine

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Summary

We examined the effect of supplemental UV-B radiation (290–320 nm) on photosynthetic characteristics of different aged needles of 3-year-old, field-grown loblolly pine (Pinus taeda L.). Needles in four age classes were examined: I, most recently fully expanded, year 3; II, first flush, year 3; III, final flush, year 2; and IV, oldest needles still present, year 2. Enhanced UV-B radiation caused a statistically significant decrease (6%) in the ratio of variable to maximum fluorescence (F_v/F_m) following dark adaptation only in needles from the youngest age class, suggesting transient damage to photosynthesis. However, no effects of enhanced UV-B radiation on other instantaneous measures of photosynthesis, including maximum photosynthesis, apparent quantum yield and dark respiration, were seen for needles of any age. Foliar nitrogen concentration was unaffected by UV-B treatment. However, the $^{13}C/^{12}C$ carbon isotope ratios ($\delta^{13}C$—a time integrated measure of photosynthetic function) of needles in age classes II and IV were 3% ($P < 0.01$) and 2% ($P < 0.05$) more negative, respectively, in treated plants than in control plants. Exposure to enhanced UV-B radiation caused a 20% decrease in total biomass and a 4% ($P < 0.05$), 25% ($P < 0.01$), and 9% ($P < 0.01$) decrease in needle length of needles in age classes I, II, and IV, respectively. The observed decreases in $\delta^{13}C$, and F_v/F_m of the needles in the youngest needle age class suggest subtle damage to photosynthesis, although overall growth reductions were probably a result of decreased total leaf surface rather than decreased photosynthetic capacity. Needles of age class IV had lower light- and CO_2-saturated maximum photosynthetic rates (39%), lower dark respiration (34%), lower light saturation points (37%), lower foliar nitrogen concentration (28%), and lower $\delta^{13}C$ (14%) values than needles of age class I. Apparent quantum yield and F_v/F_m did not change with needle age. The observed changes in photosynthesis and foliage chemical composition with needle age are consistent with previous studies of coniferous trees and may represent adaptations of older needles to shaded conditions within the canopy.

Keywords: carbon isotope ratio, leaf age, leaf nitrogen, ozone, photosynthesis, Pinus taeda, UV-B radiation.

Introduction

A continuing decline in stratospheric ozone concentrations caused by atmospheric pollutants (Kerr 1988, NASA 1988, Kerr 1991) has stimulated interest in the consequences of increased penetration of ultraviolet-B (UV-B, 290–320 nm) radiation to the surface of the Earth. Recent reports indicate stratospheric ozone losses over the northern mid-latitudes of 4 to 5% per decade (Kerr 1991). This could correspond to a 7 to 10% increase in UV-B radiation flux at Beltsville, MD (39° N latitude) on the summer solstice (based on the model of Green et al. 1980). Many

agronomic species are susceptible to UV-B damage (Tevini and Teramura 1989, Barnes et al. 1990); however, relatively few native plants including trees have been examined. Ultraviolet-B radiation is absorbed by a wide spectrum of macromolecules and may disrupt a number of physiological processes including photosynthesis, which can ultimately result in reduced growth (Sisson 1986, Caldwell et al. 1989, Tevini and Teramura 1989).

Plants with long-lived foliage such as coniferous evergreen trees may be especially vulnerable to UV-B induced damage, because the degree of damage appears to depend on cumulative exposure (e.g., Sisson and Caldwell 1976, 1977, Sisson 1981, Tevini and Iwanzik 1983). Although visible and UV-B radiation are strongly attenuated in roughly equal proportion through evergreen canopies (DeLucia et al. 1991), the longevity of conifer foliage may lead to large cumulative lifetime doses. Depending on the species and environment, conifers may retain foliage for 2 to more than 15 years; and 1-year-old and older foliage can contribute significantly to the annual carbon budget of these species (Larson 1964, Dickmann and Kozlowski 1968, Schulze et al. 1977, Tesky et al. 1984). Schulze et al. (1977) reported that 15% of the total annual CO_2 uptake for *Picea abies* was attributable to current-year foliage and 35% was attributable to 4-year-old and older needles. Cumulative UV damage to older foliage may therefore cause substantial reductions in canopy carbon gain of evergreen trees.

Loblolly pine (*Pinus taeda* L.) produces two to three flushes of needles during one growing season and maintains needles for 2 years. Thus, at any time there are typically four to six foliage age cohorts present. During the early stages of growth, loblolly pine is highly susceptible to UV-B damage. Kossuth and Biggs (1981) reported significant reductions in biomass of loblolly pine seedlings in response to supplemental UV-B radiation and reductions were greater under higher doses of UV-B. Sullivan and Teramura (1988, 1989) also observed significant reductions in biomass of loblolly pine seedlings exposed to supplemental UV-B radiation (simulating 16 to 40% decreases in stratospheric ozone), as well as reductions in photosynthetic capacity. These experiments, however, were of relatively short duration and the possibility of cumulative damage to long-lived foliage is still in question.

The objective of this study was to evaluate the effects of supplemental UV-B radiation on photosynthetic characteristics of needles of various ages in field-grown loblolly pine seedlings. We predicted that, as a result of a greater cumulative dose of UV-B radiation, older needles (matured during the previous year) would exhibit greater UV-induced inhibition of photosynthesis than current-year foliage. In addition to instantaneous measurement of photosynthetic function, foliage nitrogen and carbon isotope composition, as well as growth, were measured to detect potentially subtle cumulative effects of long-term exposure to supplemental UV-B radiation under field conditions.

Materials and methods

Loblolly pine (*Pinus taeda* L.) seeds were stratified for 30 days at 3 °C and then

germinated in "cone-tainers" (Cone-tainer nursery, Wilsonville, Oregon) under greenhouse conditions. Six weeks after germination, seedlings were transplanted to 8-liter pots in a mix of milled pine bark, peat, and sand (1/1/1, v/v). Pots were then transferred to the experimental treatment at a field site located 5 km north of the University of Maryland where they were buried in the ground. After the first year, trees were transplanted to 20-liter pots and grown for two additional years. Seedlings were spaced at sufficient distances to prevent shading from direct sunlight and supplemental UV irradiation. Total daily photosynthetic photon flux density (PPFD, 400–700 nm) received by the plants under the UV lamps was 90% of that above the lamps. Supplemental UV-B irradiation, provided by filtered UVB-313 lamps (Q-Panel, Cleveland, OH) was administered for 6 h each day centered around solar noon as described by Teramura et al. (1990). Plants were covered with thermal blankets during winter.

Lamps used to provide the UV-B treatment were filtered with pre-solarized (8 h) 0.13-mm thick cellulose diacetate (transmits wavelengths above 290 nm). Control lamps were filtered with 0.13-mm thick Polyester (transmits wavelengths above 320 nm). Ultraviolet-B doses were calculated by weighting with the generalized plant action spectrum (Caldwell 1971), normalized to 300 nm, and are referred to as biologically effective doses (UV-B_{BE}). Polyester-filtered lamps provided a weighted daily supplemental UV-B irradiance of zero; therefore, plants under these lamps received only ambient levels of UV-B (8.5 kJ m^{-2} UV-B_{BE} on the summer solstice). Cellulose diacetate filtered lamps supplied a weighted daily supplemental irradiance of 5.0 kJ m^{-2} UV-B_{BE}, giving a total of 13.5 kJ m^{-2} UV-B_{BE} to treatment plants. The supplemental dose of UV-B irradiation simulated that which would be received at Beltsville, MD (39° N) with a 25% reduction in stratospheric ozone during a cloudless day on the summer solstice.

The UV-B dose was checked weekly and maintained by varying the lamp height above the plants and changing the cellulose diacetate filters. Spectral irradiance under the lamp banks was measured with a spectroradiometer equipped with a double monochromator with dual holographic gratings (Model 742, Optronics, Orlando, FL). The spectroradiometer was calibrated using a 1000-W tungsten halogen quartz lamp traceable to the National Institute of Standards and Technology, and wavelength alignment was checked against known mercury emission lines. Detailed information about growth and treatment conditions has been reported previously (Sullivan and Teramura 1992).

Five plants from each treatment were randomly chosen for measurement toward the end of the third growing season. Needle ages were determined by counting bud scars. Needles were divided into four age classes: I, most recently fully expanded, year 3; II, first flush, year 3; III, final flush, year 2; and IV, oldest needles still present, year 2. Lengths of 20 needles were measured for each age class on each seedling. Additional measurements were made on age classes I, II, and IV only.

Tissue samples for photosynthetic measurements were taken from different trees and different age classes at varying times of day (between 0900 and 1400 h, EST) over a 10-day period. Five to 10 fascicles were taken for each sample and a "disk"

was created by using a small amount of tape to fasten needles together edge-to-edge with adaxial side toward the lamp. Photosynthetic measurements were based on one-sided leaf area which was determined by measuring the length and width of each needle segment.

Light- and CO_2-saturated (10% hydrated CO_2 in air) net photosynthesis, dark respiration, and light compensation point were measured as oxygen evolution with a leaf-disk oxygen electrode (LD2, Hansatech Ltd., Norfolk, England) according to Delieu and Walker (1981). Apparent quantum yield was calculated as the slope of net photosynthesis versus irradiance for irradiances greater than 30 and less than 120 μmol m^{-2} s^{-1} (PPFD). A CO_2 concentration of 10% is required to saturate (> 90% of A_{max}) net photosynthesis of excised needles from well-watered loblolly pine (Day et al. 1991). Various irradiances were provided by passing light from a fixed output metal halogen lamp (LS2, Hansatech) through combinations of neutral-density filters (Melles-Griot, Irvine, CA). Irradiance was measured with a quantum sensor (LI-185B, Li-Cor, Lincoln, NE). Temperature in the chamber was maintained at 25 °C by a circulating refrigerated water bath. An infrared filter consisting of 500 ml of 3% $CuSO_4$ was placed between the light source and the chamber to facilitate temperature control.

After an initial induction period of repeated cycles of light (ca. 1000 μmol m^{-2} s^{-1}) and dark, leaf tissue was dark adapted for 15 min during which time dark respiration was measured. After dark adaptation the ratio of variable to maximum fluorescence (F_v/F_m) was measured during exposure to a 240 μmol flash of actinic light (white light passed through a blue-green filter—Schott BG-18—via a camera shutter). The induction kinetics of chlorophyll fluorescence were measured with a filtered (Wratten 88A) probe attached to a transient recorder (TR1, Hansatech) operating at 3.4 kHz. The signal was played back at a slow speed to a chart recorder and the F_v/F_m ratio was calculated from the trace. Initial fluorescence (F_o) was taken as the point of initial non-linearity in the fluorescence emission after shutter opening.

Foliage samples and additional needles from the same tree and cohort were oven-dried at 70 °C to a constant mass, and dried tissue was ground to 40 mesh in a Wiley mill. Nitrogen concentration was determined with an ammonium analyzer (model 360, Wescan Inst., Santa Clara, CA) following Kjeldahl digestion (Lowther 1980). The $^{13}C/^{12}C$ carbon isotope ratio was measured on combusted samples with a mass spectrometer at the Duke University Phytotron. Values for $\delta^{13}C$ are expressed relative to the PDB standard (Craig 1957).

Twelve plants were harvested after the third growing season and partitioned into roots, stems, and needles. Biomass was measured after drying at 70 °C for two weeks.

Statistical analysis was performed on the biomass data with the Statistical Analysis System (SAS 1985) incorporating the General Linear Model Procedures. Significantly different means were compared using t-tests. Other data were analyzed by means of Statistix computer software (version 3.0, Analytical Software, St. Paul, MN) using a general analysis of variance to determine age and age × treatment effects. The effects of enhanced UV-B treatment within each age group were

determined using t-tests. Significant differences are reported at the $P < 0.05$ level.

Results and discussion

Ultraviolet-B effects

Light- and CO_2-saturated rates of net photosynthesis measured as oxygen evolution (A_{max}), dark respiration (R_d), apparent quantum yield (ϕ), and the light compensation point (Γ) were unaffected by supplemental UV-B irradiation (Figure 1); and there were no statistically significant age × treatment interactions. These measurements of instantaneous photosynthetic performance did, however, show highly significant ($P < 0.01$) reductions with needle age. Measurement of the fluorescence induction kinetics following dark adaptation revealed a small but statistically significant ($P < 0.05$) decrease in the ratio of variable to maximum fluorescence (F_v/F_m) for the youngest foliage, needle age class I, under supplemental UV-B radiation. The F_v/F_m values for needles from age classes I, II, and IV were 0.760, 0.730, and 0.722, respectively, for control plants; and were 0.716, 0.712, and 0.714, respectively, for treatment plants.

The 6% decrease in F_v/F_m for needles of age class I suggests that there may have been transient damage to the photosynthetic machinery during early needle expansion but that the needles recovered rapidly as they aged. The F_v/F_m ratio is a measure
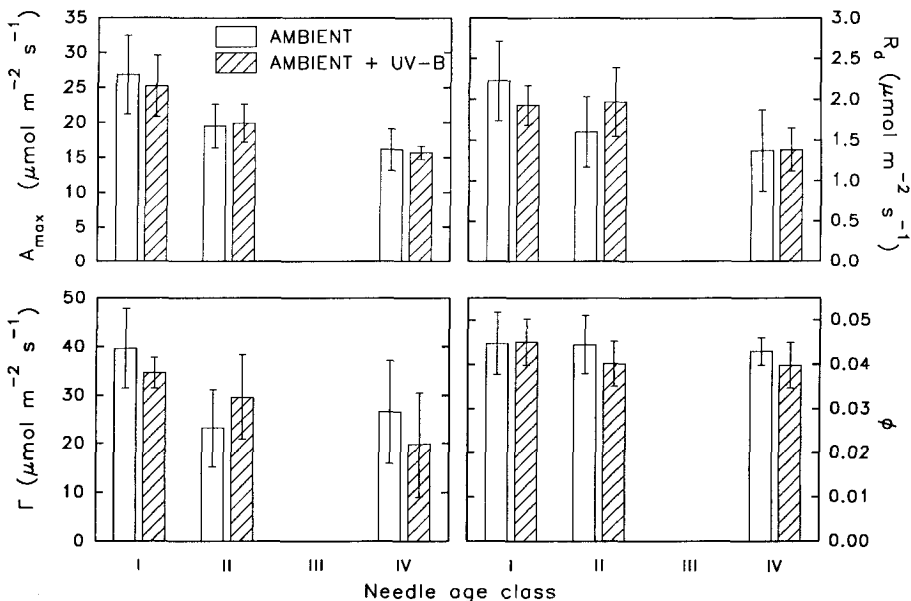

Figure 1. Light- and CO_2-saturated net photosynthesis (A_{max}), dark respiration (R_d), light compensation point (Γ) measured as oxygen evolution, and apparent quantum yield (ϕ) calculated as the slope of net photosynthesis versus irradiance for irradiances > 30 and < 120 μmol m^{-2} s^{-1} (PPFD) of different aged *Pinus taeda* needles treated with ambient (open bars) or enhanced (striped bars) UV-B radiation. Bars represent averages of four or five plants, and error bars are plus or minus one standard deviation.

of the quantum efficiency of photosystem II (PSII, Butler and Kitajima 1975). Under many situations, the quantum efficiency of PSII is correlated with the apparent quantum yield of oxygen evolution. In this experiment, however, apparent quantum yield was not affected by enhanced UV-B (Figure 1). The emission of chlorophyll fluorescence from intact leaves typically occurs from near the leaf surface. In *Medicago sativa*, 90% of the 688-nm fluorescence originates from the outer 47 μm of the palisade layer (Bornman et al. 1991); and Tevini and Iwanzik (1983) observed that UV damage to photosynthesis, as measured by chlorophyll fluorescence, did not completely penetrate radish cotyledons. In contrast to fluorescence, gas-exchange measurements of apparent quantum yield may integrate photosynthetic function throughout more of the leaf. Thus, it is possible that changes in F_v/F_m represent selective damage to the photosynthetic machinery of the surface cell layers. Alternatively, the lack of a correlation between F_v/F_m and φ may indicate that the quantum efficiency of PSII was not limiting gas exchange.

Few studies have examined the effects of supplemental UV-B exposure on the photosynthetic function of leaves through time. In studies where fully expanded leaves were exposed to UV-B for several days, photosynthesis became progressively inhibited with increasing duration of UV-B exposure (Sisson and Caldwell 1976, Sisson 1981). However, for leaves that were allowed to expand under supplemental UV-B radiation, initial decreases in photosynthesis were not compounded through time, i.e., older leaves did not show greater decreases in photosynthesis than younger leaves (Sisson and Caldwell 1977, Sisson 1981), suggesting that initial UV-induced damage may stimulate protective responses that lessen further damage caused by cumulative dose. Also, herbaceous plants (*Rumex patientia* L. and *Cucurbita pepo* L. in the studies cited above) have relatively short-lived leaves that preclude the large cumulative doses that may occur in the long-lived foliage of conifers.

Two aspects of the chemical composition of foliage, foliar nitrogen concentration and the $\delta^{13}C$ isotope ratio provide time-integrated measures of the photosynthetic function of leaves. For leaves of diverse morphology and from widely varying environments, foliar nitrogen concentration is highly correlated with photosynthetic capacity (Field and Mooney 1986); and the carbon isotope ratio of foliage provides a measure of the intercellular CO_2 concentration integrated over the life of the leaf (Farquhar et al. 1982, 1989). Foliar nitrogen concentration decreased with needle age ($P < 0.01$); however, enhanced UV-B had no effect on foliar N concentration and there were no age × treatment interactions. Significant differences ($P < 0.01$) in $\delta^{13}C$ were observed with both needle age and treatment, although the interaction term was not statistically significant (Figure 2).

The $\delta^{13}C$ values in needles of age classes II and IV exposed to supplemental UV-B radiation were 3 and 2%, respectively, more negative than in the controls. The relationship between $\delta^{13}C$ and intracellular CO_2 concentrations (C_i) over the life span of a leaf for plants with C_3 photosynthesis is illustrated by rearrangement of the equation of Farquhar et al. (1982):
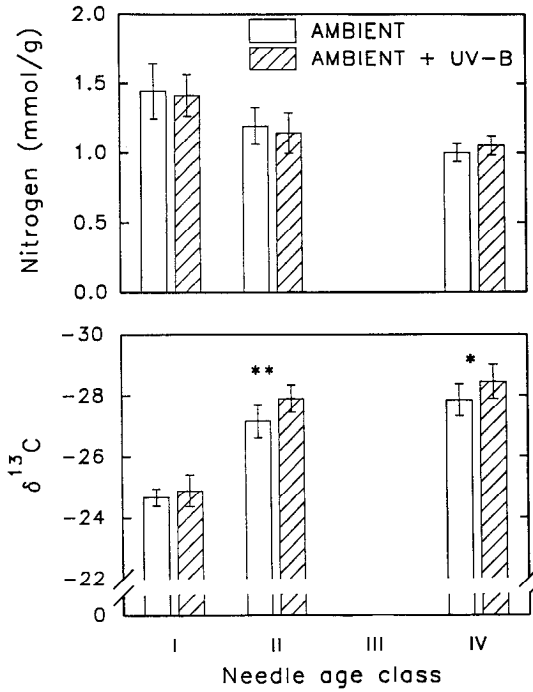
$$C_i = C_a (\delta_{env} - \delta^{13}C - a)/(b - a) ,$$

Figure 2. Foliar nitrogen concentration and carbon isotope ratio ($\delta^{13}C$) of different aged *Pinus taeda* needles treated with ambient (open bars) or enhanced (striped bars) UV-B radiation. Bars represent averages of five plants, and error bars are plus or minus one standard deviation. Asterisks represent significant differences between treatments at $P < 0.05$ (*) and $P < 0.01$ (**).

where C_a is the ambient CO_2 concentration (assumed to be 340 $\mu l \ l^{-1}$), δ_{env} is the isotopic composition of atmospheric CO_2 (−7.8%), $\delta^{13}C$ is the $^{13}C/^{12}C$ ratio in foliage relative to the PDB standard (Craig 1957), *a* is the fractionation caused by diffusion (4.4%), and *b* is the fractionation due to carbon fixation by ribulose bisphosphate carboxylase (27%). Current values for variables used in the model are from O'Leary (1988). Based on this equation, integrated C_i values for needles in age classes I, II, and IV of control plants were 188, 225, and 235 $\mu l \ l^{-1}$, respectively; and for needles of treated plants the values were 191, 236, and 244 $\mu l \ l^{-1}$, respectively.

Intercellular CO_2 concentration is regulated by stomatal conductance (and boundary layer conductance) and the photosynthetic rate. The observed increases in C_i seen in plants subjected to enhanced UV-B could, therefore, be a result of increased stomatal conductance, or decreased photosynthetic rate. We observed no significant reductions in A_{max} when measured with the oxygen electrode at saturating CO_2 concentrations; however, Sullivan and Teramura (1989) reported reduced photosynthesis in loblolly pine exposed to enhanced UV-B when measured at ambient CO_2 with an infrared gas analysis system (IRGA). Their finding is consistent with the suggestion that stomatal closure is the primary cause of the observed decreases in photosynthesis in response to enhanced UV-B (Tevini and Teramura 1989). The

increases in C_i observed in the present study might be the result of slight decreases in photosynthesis caused by supplemental UV-B radiation. Thus, although enhanced UV-B radiation had no significant effect on instantaneous measurements of photosynthetic performance; based on the $\delta^{13}C$ values, we conclude that the UV-B treatment may have caused small, but perhaps chronic, inhibition of photosynthesis.

Loblolly pine plants grown in the presence of supplemental UV-B radiation had significantly ($P < 0.05$) less root (24%), stem (16%), needle (18%), and total biomass (20%) than plants grown in ambient UV-B (Table 1). Root/shoot ratio, plant height, and the number of lateral branches were not affected by supplemental UV-B. Average needle length in the four needle age classes was reduced by exposure to supplemental UV-B (Figure 3), and the decrease was statistically significant for needles in age classes I (4%, $P < 0.05$), II (25%, $P < 0.01$), and IV (9%, $P < 0.01$).

Previous studies have reported that growth of loblolly pine is inhibited by UV-B radiation during the early growth stages under greenhouse conditions. Sullivan and Teramura (1988) grew loblolly pine seedlings for 22 weeks in a greenhouse in the

Table 1. Root, stem, needle, and total dry mass; root-to-shoot ratio (RSR); plant height; and number of lateral branches of 3-year-old, field-grown *Pinus taeda* grown in ambient or supplemental UV-B radiation. Values are the means of 12 plants. Total daily UV-B dose is based on the generalized plant action spectrum (Caldwell 1971) normalized to 300 nm. Asterisks represent significant differences between treatments at $P < 0.05$.

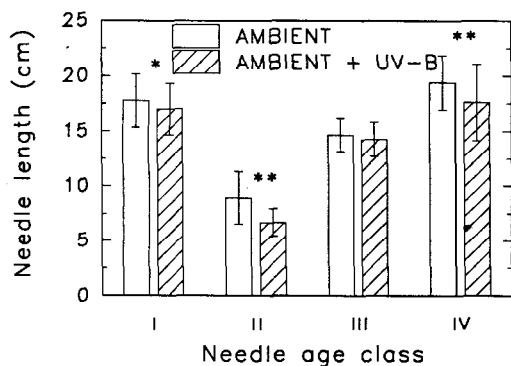| Treatment	Total UV-B dose (kJ m^{-2} UV-B$_{BE}$)	Dry weight (g)				RSR	Height (cm)	No. of lateral branches
		Root	Stem	Needle	Total			
Ambient	8.5	295	286	250	831	0.55	98	20.3
Ambient + UV-B	13.5	225*	239*	205*	668*	0.51	99	20.8

Figure 3. Needle length of different aged *Pinus taeda* needles treated with ambient (open bars) or enhanced (striped bars) UV-B radiation. Bars represent averages of 20 needles from each of five plants, and error bars are plus or minus one standard deviation. Asterisks represent significant differences between treatments at $P < 0.05$ (*) and $P < 0.01$ (**).

presence of 19.1 kJ m^{-2} UV-B$_{BE}$ (simulating a 40% ozone reduction) and saw a 40% reduction in total biomass. Under similar UV-B doses, Kossuth and Biggs (1981) observed a 27% reduction in total biomass in greenhouse-grown loblolly pine seedlings after 11 weeks. When 1-year-old seedlings were irradiated with UV-B for 28 weeks under greenhouse conditions, slight but significant reductions in growth and photosynthetic performance were only observed at a low photon fluence rate (11.5 kJ UV-B$_{BE}$) (Sullivan and Teramura 1989). The 20% reduction in total biomass in response to enhanced UV-B in the current study indicates that loblolly pine is also susceptible to UV-B induced reductions in biomass when grown under field conditions and at later growth stages (cf. Sullivan and Teramura 1992).

For loblolly pine there was no age × UV-B treatment interaction on needle length, suggesting that cumulative dose may not be critical in determining the magnitude of growth inhibition. Decreases in needle length may be a result of subtle UV-B effects on young expanding foliage, which is less resistant to UV-B penetration than mature foliage (DeLucia et al. 1992). Ultraviolet-B is known to inhibit cell division directly in expanding leaves (Dickson and Caldwell 1978), and may also cause indirect inhibition of growth through its action on plant hormones (Kulandaivelu et al. 1989). Thus, the decreases in needle length might be due to the direct effects of UV-B on cell division and may not necessarily depend on changes in photosynthetic capacity. If the effect of UV-B on needle length was the result of inhibition of cell division, the conditions under which the needles were expanding and not their total cumulative UV-B dose would be most important in determining their final size. Therefore, the various degrees to which different needle age classes were affected may have resulted from seasonal variability in UV-B and other concurrent stresses such as temperature which were present during needle expansion. Age classes II and IV which consist of needles that expanded early in the growing season showed the most significant decreases in needle length (Figure 3).

Early senescence of older foliage may have contributed to the decrease in total needle biomass observed for UV-treated plants (Table 1), although this was not specifically measured in this study. Sisson and Caldwell (1977) reported early leaf senescence in UV-B treated *Rumex patientia*, and Teramura and Sullivan (1987) reported a decrease in leaf area in UV-B treated soybeans during the reproductive stage of growth. Because of the dependence of evergreen conifers on older foliage, premature needle senescence would dramatically alter the canopy carbon gain and nutrient relations. The potential effect of enhanced UV-B radiation on needle longevity in conifers remains, however, unanswered.

Most studies on the potential effects of enhanced UV-B radiation on plant growth and photosynthesis have been conducted in growth chambers or greenhouses that typically have relatively low levels of visible radiation. Previous studies of UV-B effects on loblolly pine were conducted at 200 μmol m^{-2} s^{-1} (Kossuth and Biggs 1981) or 18 mol m^{-2} d^{-1} (Sullivan and Teramura 1988, 1989). Ultraviolet-B damage to photosynthesis is most pronounced under low light conditions (Teramura et al. 1980, Mirecki and Teramura 1984). In the current study, the subtle effect of enhanced UV-B on the photosynthetic performance of loblolly pine, indicated by alterations in

the carbon isotope composition of foliage, suggests that direct UV-inhibition of photosynthesis may be partially masked by other abiotic stresses and high rates of repair under high-light field conditions. Exposure to enhanced UV-B did, however, cause significant reductions in total biomass (Table 1). Presumably, the observed reductions in needle length and needle biomass under the treatment conditions indicate a reduction in photosynthetic leaf area. Under field conditions, reduced leaf area may have contributed more strongly to UV-induced growth reductions than subtle decreases in photosynthesis.

Leaf age effects

Foliar nitrogen concentration, A_{max}, and R_d decreased significantly ($P < 0.01$) with needle age (Figures 1 and 2). However, apparent quantum yield was unaffected, and thus reduced R_d caused a significant decrease ($P < 0.01$) in the light compensation point of older foliage (Figure 1). The response of photosynthetic variables to needle age is consistent with previous studies of evergreen conifers which indicate a decline of 30–50% per year in A_{max} (Chabot and Hicks 1982, Freeland 1952). Concomitant decreases in stomatal conductance (Teskey et al. 1984) and light compensation point (Kajimoto 1990) with needle age have also been reported. For loblolly pine, however, the decrease in $\delta^{13}C$ (Figure 2) indicates that C_i increased with needle age. In herbaceous species, C_i often remains constant with leaf age because of parallel decreases in stomatal conductance and net photosynthesis (Field 1987). The increase in C_i and decrease in A_{max} in older loblolly pine needles suggests that stomatal conductance did not decrease in proportion to photosynthesis with age. Decreases in stomatal conductance and photosynthesis with increasing leaf age have been reported for *Abies amabilis* (Teskey et al. 1984) and *Picea sitchensis* (Watts et al. 1976). However, actual intercellular CO_2 concentrations were not reported in these studies; and it is possible that the reported decreases in stomatal conductance were not sufficient to maintain constant C_i.

Reduced rates of dark respiration and the reduction in the photosynthetic-light compensation point may increase daily carbon gain of older needles in shaded portions of the canopy where light levels are seldom saturating. Moreover, reallocation of foliar N from shaded needles to expanding sun needles may optimize canopy carbon gain with respect to nitrogen (Field 1981, Mooney et al. 1981, Field and Mooney 1983). Thus, age related changes in photosynthetic performance may be adaptive in so far as canopy carbon gain is maximized and nitrogen costs are minimized.

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