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Response of three eastern tree species to supplemental UV-B radiation: leaf chemistry and gas exchange

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Abstract

Quantitative changes in foliar chemistry in response to UV-B radiation are frequently reported but less is known about the qualitative changes in putative UV-screening compounds. It has also not been conclusively shown whether qualitative differences in screening compounds or differences in localization patterns influence the sensitivity of plants to damage from UV-B radiation and there is some question as to whether differences in the amounts of soluble screening compounds correlate with physiological sensitivity to UV-B radiation. This study represents the first part of a multiple-year study designed to answer the above questions. In this study we evaluated whether differences in soluble UV-screening compounds were linked with possible effects on gas exchange and photosynthetic carbon assimilation. Branches of mature trees of sweet gum (*Liquidambar styraciflua*), tulip poplar (*Liriodendron tulipifera*) and red maple (*Acer rubrum*) were exposed to supplemental levels of UV-B radiation over three growing seasons. Controls for UV-A were also measured by exposing branches to supplemental UV-A only and additional branches not irradiated were also used for controls. These species demonstrated differing levels of screening compounds with poplar being the most responsive in terms of epidermal accumulation of phenolics. These were separately identified as flavonols, chlorogenic acid and hydroxycinnamates (HCAs). Red maple had the highest levels of constitutive UV-absorbing compounds but these showed little response to supplemental UV-B radiation. Leaf area was marginally influenced by UV exposure level with both UV-A and UV-B tending to reduce leaf area in red maple and poplar and increase it in sweet gum, when averaged over the 3-year period. Assimilation was generally not reduced by UV-B radiation in these species and was enhanced in red maple by both UV-B and UV-A and by UV-A in sweet gum. These findings are consistent with a hypothesis that epidermal attenuation of UV-B would only be reduced in poplar, which accumulated the additional epidermal screening compounds. It is possible that photosynthetic efficiency was enhanced in red maple by the increased absorption of blue light within the mesophyll due to elevated levels of HCAs. Stomatal conductance was generally reduced and this led to an increase in water use efficiency (WUE) in red maple and poplar. Since few detrimental effects of supplemental UV-B were observed, these results suggest that these tree species utilized a range of UV-screening compounds and deposition patterns to achieve UV-B tolerance and further, that subtle responses to UV-B could have ecological significance in the absence of reduced productivity or photosynthetic efficiency.

Keywords: UV-A radiation; UV-B radiation; Phenolics; Photosynthesis; Water use efficiency

1. Introduction

Though representing only a fraction of the total solar electromagnetic spectrum, UV-B has a dispro-

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portionately large photobiological effect, due to its absorption by important macromolecules such as proteins and nucleic acids (Giese, 1964). Therefore, it is not surprising that both plant and animal life are greatly affected by UV-B radiation. Approximately 400 species of plants have been screened for sensitivity to UV-B radiation and of these, about two-thirds were found to be sensitive in some parameter (Sullivan and Rozema, 1999). However a wide range of responses have been found and the interpretation of these studies is hindered by the contrasting experimental conditions employed (e.g. field versus controlled environment studies), interacting environmental factors such as low light levels and water stress. Nonetheless, observed symptoms of visual damage may occur in very sensitive species and include chlorosis or bronzing of leaf tissue and general stunting. Physiological damage has been observed as reductions in photosynthesis in sensitive species (e.g. Sullivan and Teramura, 1989; Ziska et al., 1992; Stewart and Hoddinott, 1993) as a result of damage to photosystem II (Bornman, 1989) or changes in the stomatal limitations to assimilation (Teramura, 1983). Reviews by Caldwell and Flint (1994), Jordan (1996), Sullivan (1997), Caldwell et al. (1998) and Sullivan and Rozema (1999) more thoroughly review the general responses of plants to UV-B radiation. While some plants are sensitive to even present ambient fluences (Bogenrieder and Klein, 1982; Krizek et al., 1997), many plants appear quite tolerant to even high fluences of UV-B (Sullivan and Teramura, 1992; Barnes et al., 1987; Allen et al., 1998). This variation in response to UV-B radiation makes it difficult to generalize about UV-B effects, and perhaps more importantly, infers the importance of UV-B radiation throughout the evolution of land plants.

Although forests may account for over two-thirds of global NPP, compared to about 11% for agricultural land (Barnes et al., 1998), only limited studies on the effects of UV-B on growth or productivity of trees have been published to date. In some early studies, Basioumy and Biggs (1975) demonstrated visual stunting of 1-month-old peach seedlings under supplemental UV-B in a greenhouse while Semeniuk (1978) found no visual symptoms in other greenhouse studies on six ornamental species. Bogenrieder and Klein (1982), however, demonstrated in field studies that biomass accumulation in young seedlings of four tree species was increased when ambient UV-B was excluded. Two additional studies (Kossuth and Biggs, 1981; Sullivan and Teramura, 1988) screened a total of 15 coniferous species and found that roughly half (7 of 15) exhibited reduced growth under elevated UV-B fluences in growth chambers or greenhouses.

Kaufmanm (1978) conducted the first outdoor UV-B radiation supplementation study of trees in the field and irradiated seedlings of *Picea engelmannii* and *Pinus contorta* in the field for one season. Although he failed to detect any detrimental effects during that year, he found visible symptoms and increased mortality during the subsequent growing season following irradiation. This raised the question of whether UV-B might be cumulative in trees, especially evergreens. Therefore, Sullivan and Teramura (1992) grew loblolly pine for 3 years in the field under supplemental UV-B irradiation. They found that the growth reductions increased over the study period in plants from two of four seed sources studies and suggested that the effects of UV-B may be cumulative in some tree species. Further studies on tree species have shown variable effects of exposure to supplemental or reduction of ambient levels of UV-B. In sensitive species, the responses may include alterations in leaf chemistry, gas exchange and photosynthetic leaf area (e.g. Sullivan and Teramura, 1989, 1992; Naidu et al., 1993; Sullivan, 1994; Dillenburg et al., 1994; Sullivan et al., 1996; Sullivan and Rozema, 1999; Searles et al., 2001). In many cases, substantial damage to photosynthesis has not been demonstrated in trees, but the loss of photosynthetic area may contribute to reduced biomass over extended periods of time. In loblolly pine in particular, the lack of apparent damage to the photosynthetic system may be related to minimal penetration of UV-B through the epidermis (Sullivan et al., 1996). While epidermal transmittance was about 10 to 20% in sweet gum, it was only ca. 1% in loblolly pine. The absence of evidence of consistent photosynthetic damage in these species, and the high degree of epidermal absorption of UV-B (Sullivan et al., 1996), suggest that growth reductions are manifested at the epidermal level. One hypothesis is that reduced leaf size in loblolly pine may be mediated by changes in cell wall biochemistry that leads to cell wall thickening and inhibits cell wall expansion (Dale, 1988; Liu and McClure, 1995; Liu et al., 1995). This could lead to a reduction in epidermal cell size and possibly to reduced needle length

(Sullivan, 1994; Sullivan et al., 1996). Present ambient levels of UV radiation have been shown to modify leaf morphology and gas exchange in several tree species (Schumaker et al., 1997; Nagel et al., 1998).

This wide range of response differences suggests that some plants have well-developed UV-protection mechanisms in place (i.e. Beggs et al., 1986), while others may lack them to some extent. Some responses to UV-B, such as the accumulation of UV-screening compounds, have generally been considered as adaptive. The accumulation of phenolics and flavonoids in particular has commonly been reported in response to UV-B radiation (e.g. Robberecht and Caldwell, 1978, 1983; Caldwell et al., 1983; Sullivan and Teramura, 1989; Tevini et al., 1991). In fact Searles et al. (2001) found in a recent meta analysis of a large part of the UV-B database, that one of the primary response to UV-B radiation across the studies they included was that of an increase in UV-absorbing compounds. Flavonoids and other phenolics, especially HCAs (Sheahan, 1996), absorb strongly in the UV-B range. The accumulation of these compounds in the epidermis has been shown to reduce UV-B radiation transmittance and hypothesized to protect sensitive targets (Beggs et al., 1986; Caldwell et al., 1983 and many others). However, it has not been clearly shown whether qualitative differences in phenolic chemistry or in chemical deposition patterns within the leaf structure are linked with tolerance to UV-B radiation. In fact very few studies have specifically identified or quantified specific UV-absorbing compounds, described localization patterns of those putative protective compounds or attempted to ascertain whether specific chemical types or deposition patterns may provide enhanced UV-tolerance or whether all types were equally effective. For this reason, we initiated multiple-year field studies on seedlings and mature trees in order to describe their chemical composition and deposition patterns and assess whether these species differences altered the penetration of UV-B into the mesophyll or the response to UV-B radiation. In this manuscript we discuss photosynthetic and gas exchange responses of these species to supplemental levels of UV-B and UV-A radiation and whether such responses may be associated with quantitative and qualitative differences in UV-screening compounds.

2. Materials and methods

The field site was located at Beltsville, MD, 5 km north of the University of Maryland on a farm owned by the U.S. Department of Agriculture. Branches of mature trees (ca. 75 years old) of sweet gum (*Liquidambar styraciflua*), tulip poplar (*Liriodendron tulipifera*) and red maple (*Acer rubrum*) were irradiated with supplemental levels of UV-A and UV-B radiation. Branches from each species were selected for uniformity of exposure (southern exposure) and approximate branch age of 20+ years. Supplemental UV radiation was supplied to the terminus of each branch by filtered Q-panel UVB-313 lamps following the general procedures outlined by Sullivan and Teramura (1988, 1989). The incident spectral irradiance was measured with an Optronic Model 754 Spectroradiometer. The Spectroradiometer was calibrated before each use against a N.I.S.T. traceable 200 W tungsten halogen lamp (OL 752-10) and wavelength alignment checked against mercury vapor emission lines (OL 752-150). The absolute spectral irradiance was weighted with the generalized plant action spectrum (Caldwell, 1971) and normalized at 300 nm to obtain daily or instantaneous biologically effective dose ($UV\text{-}B_{BE}$). Lamps were filtered with either polyester films which absorb almost all radiation below 316 nm (+UV-A) or with cellulose diacetate, which transmits UV-B down to about 293 nm (+UV-A and UV-B). The distance between the lamps and the branches was 1 m and filters were changed each week. Supplemental exposures were initiated just prior to leaf-out in the spring and continued for the entire growing season from 1999 through 2001 and were provided for a maximum of 6 h daily centered around solar noon using a square-wave irradiation system. Separate branches that were not irradiated were used as controls for supplemental irradiation. Since UV-A levels were similar between the polyester filtered and acetate-filtered lamps, a net effect of UV-B can be inferred from the difference between the responses under these two conditions. The levels of supplemental UV-B radiation were calculated using the empirical models of Green et al. (1980) and later derivations of this model (Bjorn and Murphy, 1985) to estimate anticipated UV-B irradiances associated with a 15% ozone depletion and adjusted seasonally and on a daily basis during extreme cloudy days. Maximum daily

supplemental UV-B was $4\,kJ\,m^{-2}$ and total UV-B exposure ranged from essentially 0 to $10\,kJ\,m^{-2}$.

The primary measurements described in this study were those of leaf area and leaf level gas exchange measured during the growing season of 2001. Photosynthetic parameters measured in the field were instantaneous photosynthesis at saturating light and ambient CO_2 concentrations, functional responses of photosynthesis to light and internal carbon dioxide concentration (C_i) and chlorophyll florescence. Gas exchange analyses utilized a Li-Cor 6400 (Lincoln, NE) portable photosynthesis system and were restricted to the hours of 09:00–14:00 to restrict the range of ambient temperature and vapor pressure deficit. Photosynthetic response to C_i was measured at a light level of $2000\,\mu mol\,m^{-2}\,s^{-1}$ supplied by a Li-Cor 6400-02B red-blue LED light source, a leaf temperature of 25 °C, and a leaf-to-air vapor pressure deficit of $2.0\,kPa\,Pa^{-1}$. Steady-state photosynthetic rates were measured initially at an ambient CO_2 concentration of $370\,\mu mol\,mol^{-1}$, then CO_2 concentration was changed to $350\,\mu mol\,mol^{-1}$ where photosynthesis was recorded again, followed by successive measurements at CO_2 concentrations of 250, 150, 50, 350, 500, 800, 1000 and $1200\,\mu mol\,mol^{-1}$. Lower CO_2 concentrations were measured first to prevent stomatal closure effects. Photosynthetic response to light was measured at 25 °C temperature and $2.0\,kPa\,Pa^{-1}$. Steady-state photosynthetic rates were sequentially measured at light levels of 2000, 1500, 1000, 500, 200, 100, 50 and $0\,\mu mol\,m^{-2}\,s^{-1}$. Fluorescence measurements were conducted with a pulse modulated fluorometer (PAM 2000 Waltz, Inc.) Photosynthetic pigments were determined according to Knudson et al. (1977) using 100% ethanol and absorbance of these pigments was measured with a double beam spectrophotometer (Shamadzu Model UV-1601).

Soluble phenolic pigments were analyzed spectrophotometrically. Leaf disks (diameter = 1.35 cm) from the leaves used for gas exchange analysis were placed in 20-ml high-density polyethylene (HDPE) scintillation vials covered with 10 ml of slightly acidified aqueous methanol (MeOH:H_2O:AcOOH, 50:50:1, v:v:v), tightly capped and held in the dark at room temperature with gentle agitation (50 rpm) for 48–72 h. During extraction, vials were sealed with polyethylene lined caps, since it was found that foil lined caps would occasionally corrode and contaminate the extracts leading to the non-uniform formation of metal-flavonol complexes and bathochromic shifting of the absorbance spectra (e.g. Markham, 1982). To insure that photosynthetic pigments were not present in the phenolic extracts, the extract absorbance was determined from 260 to 760 nm at 1-nm intervals with a Shimadzu UV-1601 Spectrophotometer dual beam spectrophotometer. For presentation, data are only shown for absorbance at 300 nm in this manuscript but additional qualitative and quantitative analyses made using HPLC with NMR for compound identification may be found in Sullivan et al., 2002 and in subsequent publications.

The study design was a random complete block study with three species and light treatment levels (control, supplemental UV-A and supplemental UV-A and B). Due to logistics and construction costs there was only one tree for each species so the leaf samples were treated as the experimental unit. Replication at the tree level is being attempted for future studies and this is most desirable. However, each tree will have unique growth and shading characteristics and therefore cannot be truly replicated in a real sense. On the other hand the treatments were grouped as closely as possible to standardize canopy height, exposure, etc. Analysis consisted of one-way ANOVA for light treatment (UV) effects. Means separation was done by the Student–Newman–Keuls multiple range test at $\alpha = 0.05$ (SAS, 1985).

3. Results and discussion

Absorption profiles of extracts from these species suggested contrasting quantities of UV-absorbing compounds and qualitative differences in the chemical species (Sullivan et al., 2002). However, when soluble extracts were measured at 300 nm, a commonly used technique in UV-B radiation studies, only poplar showed a significant increase in response to UV-B. In this study a trend toward increases in red maple was found but it was not statistically significant at the 0.05 level (Fig. 1). However, Sullivan et al. (2002) found using absorption spectra from 260 to 440 nm that the concentration of absorbing compounds in maple increased at wavelengths below 300 and that levels of absorbing compounds were much higher in red maple than in the other two species. This suggests

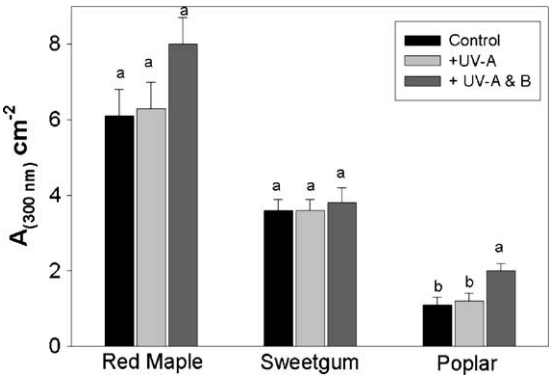

Fig. 1. Relative absorbance of methanol extracts for three tree species exposed to either ambient conditions (control) or ambient + supplemental UV-A (+UV-A) or ambient + supplemental UV-A and B (+UV-A and B). Values are the mean ± S.E. of eight samples measured at 300 nm. Statistical comparisons between species were not made and different letters over a bar within a species signify statistically different means at $P < 0.05$ according to Student–Newman–Keuls multiple range test.

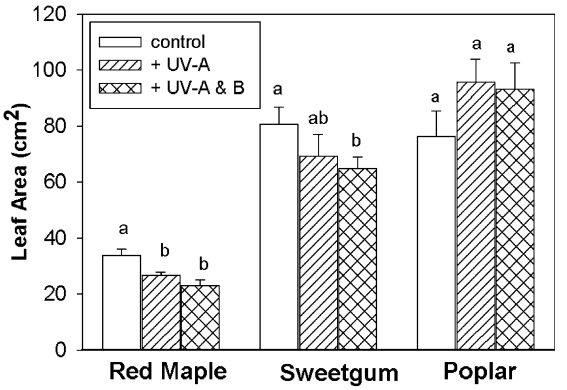

Fig. 2. The effects of UV-B radiation on leaf area (as measured by single leaf size) for three tree species exposed to either ambient conditions (control) or ambient + supplemental UV-A (+UV-A) or ambient + supplemental UV-A and B (+UV-A and B). Values are the mean ± S.E. of 15 mature leaves collected following leaf maturation each year for 3 years. Since leaf size did not vary significantly between years, the data were pooled for analysis. Different letters over a bar within a species signify statistically different means at $P < 0.05$ according to Student–Newman–Keuls multiple range test.

that while simple measures of extracts at 300 nm may be adequate in some species, such as soybean, it may not be so in other species.

The absorbing compounds were localized in the epidermis in poplar, much like in pine species (e.g. Laakso et al., 2000; Schnitzler et al., 1996, 1997) but primarily in the mesophyll in red maple and to a lesser extent in sweet gum (Sullivan et al., 2002). The predominant absorbing compounds were hydroxycinnamates (HCAs) in red maple and sweet gum and glycosylated flavonols, hydroxycinnamates and chlorogenic acid in poplar. Since epidermal concentrations of putative UV-protection compounds increased only in poplar, this suggests that epidermal attenuation of UV-B would likewise only be altered by previous exposure to UV-B in poplar. Sullivan et al. (1996) found that exposure to UV-B radiation did not affect epidermal attenuation of UV-B at 300 nm in sweet gum. However, we are not aware of studies that have measured this parameter in red maple or tulip poplar. The question then arises as to whether epidermal accumulation of phenolics is a prerequisite for UV-B tolerance? In this study, leaf area was reduced by UV-B in both red maple and sweet gum and by UV-A in red maple (Fig. 2), suggesting that increased epidermal attenuation, hypothesized to occur only in poplar, may have prevented UV (A or B) mediated reductions in leaf area in that species.

Soluble phenolic extract concentration does not always increase in response to UV-B radiation (e.g. Barnes et al., 1987; Dillenburg et al., 1994; Sullivan et al., 1994) and simple correlations may not exist between the apparent concentrations of soluble UV-absorbing compounds and UV-sensitivity. For example, Barnes et al. (1987) suggested that some species adapted to high ambient UV-B fluxes were inherently tolerant to UV-B radiation because the tolerance could not be attributed to changes in soluble phenolics. The accumulation of bound phenolics that would not be detected in measurement of only soluble extracts may also contribute to UV-B screening and UV tolerance. This has been shown to be the case in some conifer species where soluble extracts were not found to increase in response to UV-B radiation (e.g. Laakso et al., 2000; Schnitzler et al., 1996, 1997). It is also possible that compound localization may be important in providing protection to a particular target (e.g. chloroplasts) by screening compounds within the mesophyll as well as in the epidermis.

Supplemental UV-B radiation did not negatively impact the functional response of CO_2 assimilation to either light or internal CO_2 concentrations (Figs. 3 and 4). In fact light saturated assimilation (A) (Fig. 3A)

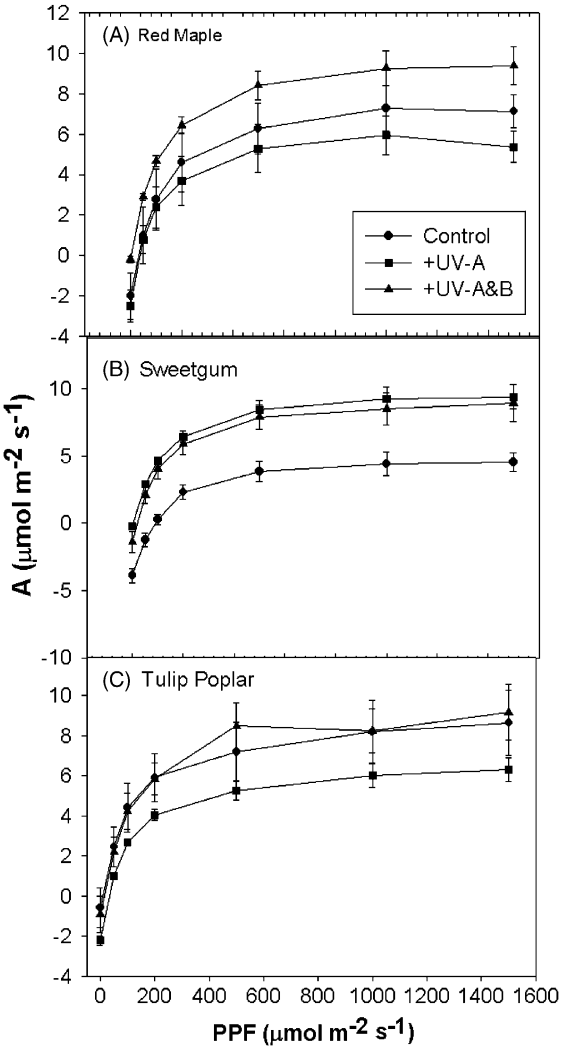

Fig. 3. Response of CO_2 assimilation to light levels for three tree species exposed to either ambient conditions (control) or ambient + supplemental UV-A (+UV-A) or ambient + supplemental UV-A and B (+UV-A and B). Values are the mean ± S.E. of five samples measured with a Li-Cor 6400 portable gas exchange system.

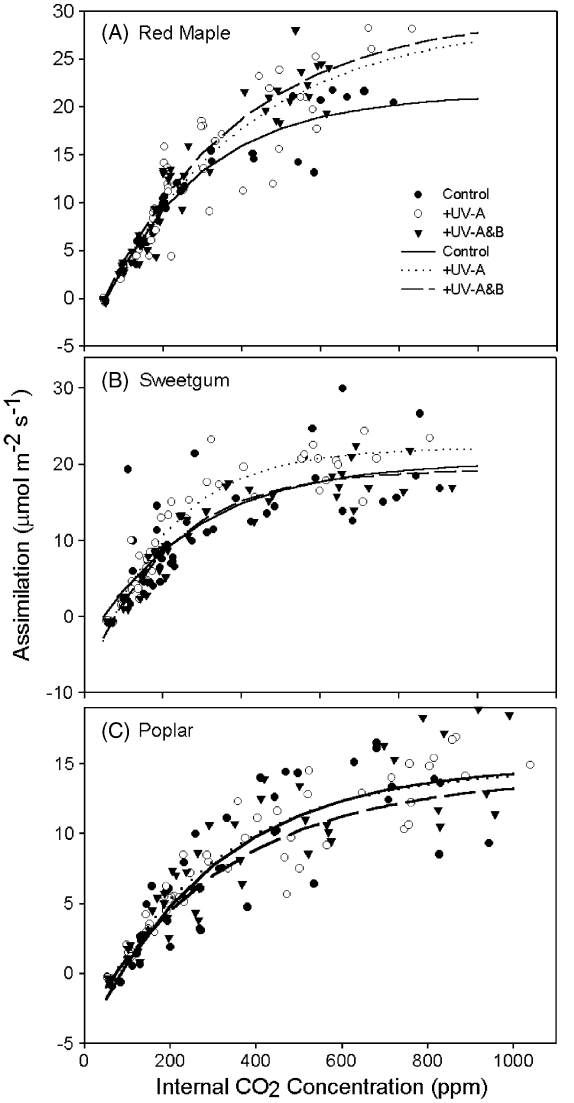

Fig. 4. Response of CO_2 assimilation to changes in internal CO_2 levels (C_i) for three tree species exposed to either ambient conditions (control) or ambient + supplemental UV-A (+UV-A) or ambient + supplemental UV-A and B (+UV-A and B). Curves are non-linear regression fits of all data from each treatment and inter-species comparisons were not attempted. No significant differences were observed for the treatments in poplar and sweet gum and A_{max} was increased by both UV-A and UV-B in red maple.

and photosynthetic capacity (A_{max}) (Fig. 4A) were enhanced by UV-B in red maple, in which UV-A also enhanced the CO_2-saturated assimilation rate (A_{max}). Light saturated assimilation was also enhanced by both UV-A and UV-B in sweet gum (Fig. 3B). The absence of effects of UV levels on assimilation in tulip poplar is consistent with the hypothesis of adequate epidermal screening in this species and are consistent with other studies in which photosynthetic carbon assimilation has not been damaged by UV-B (e.g. Naidu et al., 1993; Allen et al., 1998).

The circumstances that led to enhanced photosynthesis under elevated UV are not clear. However, some indication of the mechanisms responsible for this may

be gleaned from analysis of fluorescence data, photosynthetic pigments and water use efficiency (WUE). While there was a general trend ($P = 0.08$) of progressive increases in total chlorophyll under supplemental UV-A and UV-A and B (data not shown) in red maple, there was in fact a reduction in chlorophyll in sweet gum; yet both species showed some degree of enhancement in photosynthetic carbon assimilation. Dillenburg et al. (1994) found no effects of UV-B on chlorophyll levels in sweet gum seedlings or on carbon assimilation. In this study on foliage from mature trees the chlorophyll levels were higher than that of the seedling study (Dillenburg et al., 1994) so while they reported a high correlation between carbon assimilation and chlorophyll concentrations in seedlings, other factors may be more limiting to assimilation in these mature trees. Predawn potential photochemical efficiency, as assessed by the ratio of variable to maximal fluorescence (F_v/F_m), was in fact increased by UV-B in sweet gum (Table 1). In red maple, the increased rates of assimilation may have been due to the combination of slightly increased chlorophyll levels and an increase in nonphotochemical quenching (Q_N) and enhanced electron transport rates (Table 1). Photochemical quenching (Q_P) was not altered by UV exposure in either red maple or poplar but was slightly reduced by UV-A + B in sweet gum (Table 1). It is possible that the very high levels of HCAs accumulating around the chloroplast in red maple contributed to assimilation by enhanced blue light absorption or blue light fluorescence induced by UV-B absorbance of these molecules. This mechanism of photosynthetic enhancement by UV-B radiation has been suggested by Mantha et al. (2001) and they have recently suggested that this UV-induced fluorescence could contribute to about a 1% increase in assimilation in some species (T.A. Day, personal communication). This has not been conclusively shown in any tree species that we are aware of but if present could represent an important role for ambient levels of UV-B in plants. Carbon assimilation was not affected by UV-B in poplar and this is consistent with the conceptual model of epidermal screening by UV-absorbing compounds.

At light levels greater than $400\,\mu mol\,m^{-2}\,s^{-1}$, stomatal conductance was reduced by UV-B in red maple and this led to an increase in instantaneous water use efficiency under supplemental UV radiation (Fig. 5). UV-A reduced WUE in sweet gum at these light levels primarily due to increased stomatal conductance, while in poplar UV-A also increased conductance but in this species WUE was not affected by UV-A since assimilation was slightly higher in the UV-A compared to the control plants. In this case C_i (229 and 235 ppm for control and +UV-A, respectively) and WUE were similar between these treatments. Neither WUE or conductance were significantly altered by UV-B in sweet gum, but WUE was enhanced by UV-B in poplar, compared to control leaves (no supplemental irradiance).

Overall UV-B radiation did not appear to damage the photosynthetic apparatus in these plants. Marginal reductions in leaf area were observed in red maple and sweet gum over the 3-year period but these apparently were apparently photomorphogenic rather than

Table 1
Fluorescence parameters measured on three species grown under either ambient conditions or ambient plus either supplemental UV-A or UV-A + B radiation

Species	UV treatment	F_v/F_m (predawn)	F_v/F_m midday	Electron transport rate	Q_P	Q_N
Maple	Control	0.84 ± 0.05 a	0.78 ± 0.01 a	63.6 ± 5 b	0.26 ± 0.01 b	0.85 ± 0.03 b
	+UV-A	0.84 ± 0.04 a	0.78 ± 0.01 a	69.5 ± 7 b	0.23 ± 0.05 b	0.96 ± 0.004 a
	+UV-A + B	0.83 ± 0.05 a	0.75 ± 0.01 b	123.3 ± 7 a	0.44 ± 0.05 b	0.92 ± 0.02 a
Sweet gum	Control	0.86 ± 0.05 b	0.74 ± 0.01 b	64.4 ± 4 a	0.31 ± 0.03 a	0.77 ± 0.04 a
	+UV-A	0.86 ± 0.06 b	0.78 ± 0.01 a	47.0 ± 6 b	0.21 ± 0.02 b	0.64 ± 0.06 a
	+UV-A + B	0.89 ± 0.06 a	0.74 ± 0.01 b	40.1 ± 4 b	0.17 ± 0.02 b	0.69 ± 0.03 a
Tulip poplar	Control	0.82 ± 0.06 a	0.64 ± 0.02 a	21.2 ± 6 a	1.00 ± 0.01 a	0.06 ± 0.03 b
	+UV-A	0.82 ± 0.05 a	0.67 ± 0.02 a	19.8 ± 4 a	0.79 ± 0.14 a	0.73 ± 0.004 a
	+UV-A + B	0.82 ± 0.07 a	0.68 ± 0.02 a	23.8 ± 5 a	0.69 ± 0.19 a	0.47 ± 0.02 ab

Each value is the mean (±S.E.) of at least five measurements. Values within a species followed by the same letter are not significant at $P < 0.05$.

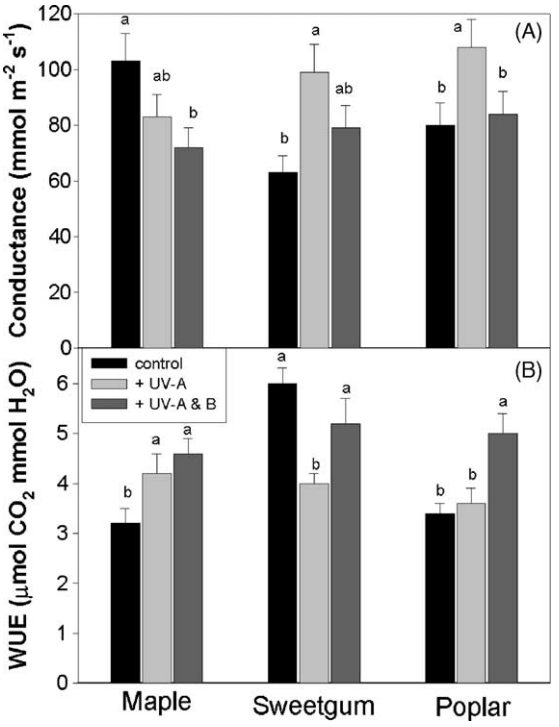

Fig. 5. Responses of stomatal conductance and instantaneous water use efficiency (WUE) at saturating light intensities for three tree species exposed to either ambient conditions (control) or ambient+supplemental UV-A (+UV-A) or ambient+supplemental UV-A and B (+UV-A and B). Values are the mean ± S.E. of five samples measured with a Li-Cor 6400 portable gas exchange system. Statistical comparisons between species were not made and different letters over a bar within a species signify statistically different means at $P < 0.05$ according to Student–Newman–Keouls multiple range test.

damage responses, at least as far as photosynthesis is concerned. To the contrary, this slight reduction in leaf area and decreases in stomatal conductance in red maple may have further contributed to possible improvements in water relations and could have enhanced drought tolerance in red maple and possibly poplar. Other researchers have observed that the response to UV-B could potentially improve drought tolerance. For example, greenhouse studies have shown that UV-B radiation can lead to stomatal closure and reduction in stomatal density in some species (Nogues et al., 1998). It is not known whether either UV-A or UV-B alter stomatal density in these species but such changes could account for the observed responses in conductance and water use efficiency.

UV-B may also alter water use efficiency in manners other than affecting gas exchange. From an anatomical or developmental perspective, Sullivan et al. (1996) observed epidermal thickening in response to UV-B radiation but did not measure actual cell size or cell wall thickness that could have resulted from UV-B induced changes in cell chemistry. In further studies, Laakso et al. (2000) found that lignin, wall-bound phenolics and tannins increased in loblolly pine and Scots pine under supplemental UV-B radiation and that this was accompanied by a thickening of outer epidermal cell walls and increases in surface waxes in these species. Other researchers have also observed changes in the epidermis and cuticle of tree species in response to UV-B radiation and have suggested that this may help protect those plants from water stress (e.g. Manetas et al., 1997; Petropoulou et al., 1995). In addition, Nogues et al. (1999) reported that UV-B radiation affects stomatal conductance through direct effects on stomatal behavior with minimal "damage" to carbon assimilation. These changes may lead to increases in the stomatal limitation to photosynthesis (Allen et al., 1998; Nogues et al., 1998, 1999; Middleton and Teramura, 1993, 1994) and to increases in instantaneous water use efficiency, which could lead to improved drought tolerance. However, many of these studies have been conducted indoors and with very high levels of UV-B radiation. Therefore, field studies designed to specifically test whether UV-B in fact does precondition plants to reduce water stress or increase water use efficiency are needed.

4. Conclusions

These results suggest that there may be no single UV-absorbing compound or specific localization pattern that leads to UV tolerance. In fact plants appear to have evolved a number of means to achieve the same end. Partitioning the response to protect potential targets of damage such as DNA or photosystem II within the mesophyll while utilizing UV-B as a photomorphogenic signal for such factors as leaf size, stomatal development or drought tolerance could have been a potential evolutionary development that provides some selective advantage for "younger" plant species. Clearly further studies are needed before anything definitive could be postulated in this area. As studies

on the response of plants to UV-B radiation continue to mature beyond the "damage" state it is likely that we will find that these shorter wavelengths of the ambient solar spectrum (UV-A and UV-B) may have more important roles that was imagined.

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