Effect of Elevated Atmospheric CO₂ and Temperature on Leaf Optical Properties and Chlorophyll Content in *Acer saccharum* (Marsh.)

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Running Title: _Effects of CO₂ and Temperature on Leaf Optics_
Elevated atmospheric CO₂ pressure and numerous causes of plant stress often result in decreased leaf chlorophyll contents and thus would be expected to alter leaf optical properties. Hypotheses that elevated carbon dioxide pressure and air temperature would alter leaf optical properties were tested for sugar maple (*Acer saccharum* Marsh.) in the middle of its fourth growing season under treatment. The saplings had been growing since 1994 in open-top chambers at Oak Ridge, Tennessee under the following treatments: 1) ambient CO₂ pressure and air temperature (control); 2) CO₂ pressure approximately 30 Pa above ambient; 3) air temperatures 3 °C above ambient, and 4) elevated CO₂ and air temperature. Spectral reflectance, transmittance and absorptance in the visible spectrum (400 - 720 nm) did not change significantly (*p*=0.05) in response to any treatment compared with control values. Although reflectance, transmittance and absorptance at 700 nm correlated strongly with leaf chlorophyll content, chlorophyll content was not altered significantly by the treatments. The lack of treatment effects on pigmentation explained the non-significant change in optical properties in the visible spectrum. Optical properties in the near-infrared (721-850 nm) were similarly unresponsive to treatment with the exception of an increased absorptance in leaves that developed under elevated air temperature alone. This response could not be explained by the data, but might have resulted from effects of air temperature on leaf internal structure. Results indicated no significant potential for detecting leaf optical responses to elevated CO₂ or temperature by the remote sensing of reflected radiation in the 400-850 nm spectrum.

**Key Words:** *Acer saccharum*, sugar maple, leaf, reflectance, transmittance, absorptance, chlorophyll, spectroradiometry, remote sensing.
INTRODUCTION

The reflection, transmission and absorption of light by leaves can be influenced significantly by growth environment. For example, unfavorable growth conditions tend to result in a reduced capacity for chlorophyll production (Hendry et al., 1987), yielding decreased absorption and increased reflection and transmission in the visible spectrum. In particular, leaf optical properties at wavelengths near 700 nm have correlated strongly with leaf chlorophyll content and physiological responses to the environment in numerous species (for review see Gitelson and Merzlyak, 1996; Lichtenthaler, Gitelson and Lang, 1996; Carter, Cibula and Miller, 1996; Carter, 1998).

Although CO₂ enrichment is not considered broadly to cause plant stress, it alters leaf nutrition and development in ways that at least partially resemble stress responses. Recent meta-analyses indicate that elevated CO₂ pressures generally result in decreased leaf N concentrations (Cotrufo, Ineson and Scott, 1998; Curtis and Wang, 1998). Leaf pigment content may decline also by various amounts (Delucia, Sasek and Strain, 1985; Nederhoff and Buitelaar, 1992; Wullschleger, Norby and Hendrix, 1992; Sicher, 1997; 1998) and the ratio of chlorophyll a to chlorophyll b may decrease (Cave, Tolley and Strain, 1981; Delucia et al., 1985). However, foliar N levels do not always change under elevated CO₂ (Rey and Jarvis, 1997) and this effect appears less significant when N concentrations are expressed on a leaf area rather than mass basis (Norby et al., 1999). Pigmentation may be affected only slightly in some cases (Rey and Jarvis, 1998), and the occurrence of reduced leaf chlorophyll contents can be species-dependent under identical experimental conditions (Holbrook et al., 1993). Leaf dry mass per unit area is often increased by CO₂ enrichment, indicating a change in leaf anatomy such as increased cell size, cell number, or number of cell layers (Saxe et al., 1998). When
grown under elevated CO\textsubscript{2} in glasshouses, leaves of some species have developed structural abnormalities (Tripp et al., 1991; Nederhoff, DeKoning and Rijstdijk, 1992).

Given these influences on leaf pigmentation and structure, significant influences of elevated CO\textsubscript{2} pressures on leaf optical properties would be expected. Elevated CO\textsubscript{2} appeared to ameliorate leaf damage and reduce effects on leaf optical properties caused by O\textsubscript{3} (Carter, Rebbeck and Percy, 1995), but to our knowledge specific effects of CO\textsubscript{2} on leaf optical properties throughout the visible to near-infrared spectrum have not been published. Such information could provide a basis for larger-scale remote sensing of free-air CO\textsubscript{2} enrichment sites (e.g., Pinter et al., 1992).

The objective of this paper was to test the hypothesis that leaf reflectance, transmittance and absorptance in sugar maple (Acer saccharum Marsh.) would change significantly in response to a 30 Pa increase above ambient CO\textsubscript{2} pressure and a 3 °C elevation in air temperature. Given the reported occurrences of decreased chlorophyll contents under elevated CO\textsubscript{2} and the tendency of increased temperature to induce drought, we hypothesized that wavelength-dependent optical responses to these variables would be similar to those reported earlier for a variety of causes of plant stress (Carter, 1993; Carter et al., 1995).

MATERIALS AND METHODS

Research site and experimental design

The elevated CO\textsubscript{2} and air temperature ($T_{\text{air}}$) treatments were implemented in open-top chambers that had been modified for temperature control (Norby et al., 1997) on the Oak Ridge National Laboratory Environmental Research Park in Tennessee, USA. Five, one year old, bare-rooted sugar maple seedlings were planted directly into the soil within each
of 12 chambers on 19 April 1994. Additional sugar maple and red maple (*Acer rubrum* L.) seedlings that were planted into the chambers in 1995 were not used in this study. The elevated $T_{air}$ and CO$_2$ exposures began on 11 May and 12 July, 1994, respectively. Four treatments were assigned to the 12 chambers in a randomized, complete block design: 1) near-ambient CO$_2$ and $T_{air}$ (control); 2) CO$_2$ partial pressure of 65 Pa maintained day and night throughout the growing season; 3) $T_{air}$ approximately 3 °C above ambient, maintained continuously all year; and 4) elevated CO$_2$ and $T_{air}$ in combination. The performance of these chambers and chamber influences on other microclimatic variables have been described previously (Norby et al., 1997). The chambers were covered with a black polypropylene mesh that transmitted 27 % of solar irradiance to create a more realistic light environment for these shade-tolerant species. No fertilizer or irrigation was added to the soil during the four-year experiment.

*Leaf Optical Properties*

Leaf reflectance and transmittance were measured throughout the 400-850 nm spectrum using a spectroradiometer coupled to an integrating sphere via a fiber optic cable (models LI1800UW and LI1800-12S, LI-COR, Inc., Lincoln, NE, USA) and methods described earlier in detail (Daughtry, Ranson and Biehl, 1989). Data were acquired on 3 June, 1997, when the trees were midway through their fourth growing season of exposure to the treatments. The trees were approximately 3 m tall with a dense canopy. Three upper canopy leaves within each chamber were excised, sealed in plastic bags and placed immediately on water ice in the dark to avoid pigmentation changes and minimize water loss during the brief (1 min) transport to the field laboratory. In the laboratory, a leaf was selected for measurement and condensed moisture was blotted
from its surfaces. The leaf then was placed against the sample port of the integrating sphere such that the adaxial surface was irradiated with the beam from a tungsten halogen lamp. Radiance reflected from the 1.65 cm² leaf area exposed to the sphere interior was transmitted to the spectroradiometer through the fiber optic. Similar measurements were made for the radiance reflected from a white reference (BaSO₄) while the adaxial leaf surface faced the sphere interior, and for the intensity of stray light caused by imperfect collimation of the lamp beam. Spectral reflectance was computed by subtracting stray light radiance from the raw leaf and reference radiances, then dividing leaf reflected radiance by reference reflected radiance. The resulting quantity was multiplied by 100 to yield units of % reflectance. Leaf transmittance was measured by illuminating the adaxial leaf surface such that light passed through the leaf into the integrating sphere. Radiance from the white reference was measured while the abaxial surface faced the sphere interior. Transmitted radiance was multiplied by 100 and divided by reference radiance to yield % transmittance. Leaf absorptance was computed as 100 - (reflectance + transmittance). True spectral bandwidth produced by the 0.5 mm slitwidth of the monochromator was 4 nm. Data were recorded at 1 nm intervals throughout the 400-850 nm range.

Leaf Chlorophyll Content

After leaf optical properties were measured, chlorophyll contents of the same leaves were determined to provide at least a partial explanation for possible differences in optical properties among treatments. For this analysis, the leaves were frozen on dry ice and shipped overnight to another laboratory. The leaves were allowed to thaw at room temperature and five circular disks, each 0.32 cm² in surface area, were punched from
the leaf portion for which optical properties were measured. The disks were placed immediately into 8 ml of buffered 80% aqueous acetone and pigments allowed to extract in the dark at 4 °C for 24 h. Absorbances of the clear extract at 646.6 and 663.6 nm were recorded and concentrations of chlorophylls a, b and \(a+b\) computed after Porra, Thompson and Kriedemann (1989). Chlorophyll concentration of the extract and the total disk surface area of 1.6 cm\(^2\) were used to compute chlorophyll contents per unit leaf area.

**Data Analysis**

Treatment effects on reflectance, transmittance, and absorptance at each 1 nm wavelength interval, as well as effects on leaf chlorophyll content and the chlorophyll \(a/b\) ratio were determined by analysis of variance (ANOVA) (SAS 6.0, SAS Institute, Cary, NC, USA). Values for the three leaves sampled per chamber were averaged and the mean value per chamber was used in the statistical analyses. The statistical model incorporated one degree of freedom each for CO\(_2\), \(T_{air}\) and the CO\(_2\) \(\times\) \(T_{air}\) interaction, two degrees of freedom for block effects and six error degrees of freedom. Dunnett's test (Steel and Torrie, 1960) determined significant differences between treatment and control means. Regression analyses determined the wavelengths at which reflectance, transmittance or absorptance correlated most strongly with leaf chlorophyll content (REG procedure, SAS) as well as best-fit regression equations at those wavelengths (TableCurve 2D 4.0, SPSS, Inc., Chicago, IL, USA).
RESULTS AND DISCUSSION

Mean reflectance, transmittance and absorptance for leaves under the control treatment were typical of healthy green leaves (Fig. 1). Reflectances and transmittances were minimal in the blue and red spectra, with peaks in the green spectrum and maxima in the near-infrared. Absorptance was maximum in the visible spectrum and minimal in the near-infrared. Mean spectra for the remaining treatments generally differed little from the controls. Neither reflectance nor transmittance changed significantly ($p=0.05$) in response to elevated CO$_2$, $T_{air}$ or CO$_2$ + $T_{air}$ (Figs. 2, 3). Reflectance and transmittance tended to increase maximally near 700 nm under elevated CO$_2$ or $T_{air}$, a response that is typical for leaves subjected to stress conditions that induce chlorosis (Carter, 1993; Carter et al., 1995). Elevated $T_{air}$ tended to decrease reflectance and transmittance in the near-infrared. The combination of elevated CO$_2$ + $T_{air}$ tended to reduce reflectance and transmittance differences with the control.

Both elevated CO$_2$ and elevated $T_{air}$ tended to decrease absorptance at wavelengths near 700 nm, similar to absorptance changes induced by elevated O$_3$ (Carter et al., 1995). However, the only statistically significant change in optical properties was increased near-infrared absorptance under elevated $T_{air}$ (Fig. 4). As with reflectance and transmittance, elevated CO$_2$ and $T_{air}$ in combination tended to minimize differences with the control. Block effects were non-significant in all cases.

Although reflectance, transmittance and absorptance at 700 nm correlated strongly with leaf chlorophyll contents when data for all leaves were combined ($n=36$) (Fig. 5), there were no statistically significant effects of any treatment on chlorophyll content or the chlorophyll $a/b$ ratio (Table 1). This explains the lack of significant optical responses to treatment in the visible spectrum. The breakdown of chlorophyll is accelerated under high
light intensities (Hendry et al., 1987). Thus, significant treatment effects on chlorophyll contents and optical properties in the visible spectrum might have occurred had the chambers not been partially shaded. As with the optical parameters, block effects were non-significant for the chlorophylls and the chlorophyll \(a/b\) ratio. Chlorophyll concentrations also did not differ among treatments in 1996 (D. Tissue, pers. comm.). However, during an unusually hot, dry period of the second summer (1995), leaves exposed to elevated \(T_{air}\) were visibly chlorotic and chlorophyll concentrations were significantly depressed (Norby et al., 1998). This apparent stress reaction was ameliorated when CO\(_2\) was elevated along with \(T_{air}\).

The significant increase in near-infrared absorptance under elevated \(T_{air}\) cannot be explained by the data. Two weeks prior to the measurement of optical properties, leaves in the elevated \(T_{air}\) chambers had an 8.6% greater leaf dry mass per area (\(p=0.11\)) (D. Tissue, pers. comm.), perhaps indicating an increased leaf thickness. A greater near-infrared absorptance, primarily by leaf water, would be expected for thicker versus thinner leaves as a result of increased internal scattering and the concomitant increase in absorption pathlength (Gausman et al., 1970; Sinclair, Schreiber and Hoffer, 1973). Nevertheless, a recent study of leaf optical properties in 26 species that represented broad ranges in leaf thickness and dry mass per unit area (Knapp and Carter, 1998) indicated that such a small change in mass per unit area would not significantly alter near-infrared absorptance. Alternatively, absorptance could have been affected if elevated \(T_{air}\) induced changes in cell size, shape and amount of intercellular air space (Gausman, Allen and Cardenas, 1969).
CONCLUSIONS

The only statistically significant ($p=0.05$) change in the optical properties of sugar maple leaves grown under elevated CO$_2$ and $T_{air}$ was increased absorptance in the near-infrared spectrum in response to elevated $T_{air}$. This response could not be explained by the data, but may have been a result of changes in leaf internal anatomy under the greater temperature. The lack of statistically significant responses in the visible spectrum was explained by the absence of significant treatment effects on leaf chlorophyll contents. Little potential was indicated for detecting leaf optical responses to elevated CO$_2$ or temperature by the remote sensing of reflected radiation in the 400-850 nm spectrum.

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf chlorophyll content (μmol m⁻²)</th>
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<tr>
<td></td>
<td>Chl a</td>
<td>Chl b</td>
<td>Chl a+b</td>
<td>Chl a / Chl b</td>
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<td>Control</td>
<td>273±37</td>
<td>72±12</td>
<td>345±49</td>
<td>3.8±0.2</td>
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<td>Elevated CO₂</td>
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<td>60±15</td>
<td>304±64</td>
<td>4.1±0.2</td>
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<tr>
<td>Elevated T (_{air})</td>
<td>210±64</td>
<td>54±19</td>
<td>264±83</td>
<td>4.1±0.2</td>
</tr>
<tr>
<td>Elevated CO₂ + T (_{air})</td>
<td>255±36</td>
<td>66±12</td>
<td>321±47</td>
<td>3.9±0.2</td>
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Means (n=3 chambers per treatment) were computed for the same leaves used in reflectance and transmittance measurements. Mean values for each chamber (n=3 leaves per chamber) were used in an analysis of variance (ANOVA) to determine significant effects of treatment on leaf chlorophyll content. There were no statistically significant differences (p=0.05).
Figure Legends

Fig. 1. Mean spectral reflectance, transmittance and absorptance for sugar maple leaves grown under control (thicker curves) and elevated $T_{\text{air}}$ (thinner curves) treatments. Curves representing the elevated CO$_2$ and elevated CO$_2$ + $T_{\text{air}}$ treatments are not shown because they differed less from the controls than did values for the $T_{\text{air}}$ treatment. Means at 1 nm wavelength intervals were based on three leaves per chamber and three chambers per treatment ($n=9$ leaves).

Fig. 2. Differences at 1 nm wavelength intervals between the mean reflectance of sugar maple leaves grown under control and elevated CO$_2$ and $T_{\text{air}}$ treatments. Means were based on three leaves per chamber and three chambers per treatment ($n=9$ leaves). Wavelengths (nm) at difference maxima are noted in the figure. None of the differences were statistically significant at $p=0.05$.

Fig. 3. Differences at 1 nm wavelength intervals between the mean transmittance of sugar maple leaves grown under control and elevated CO$_2$ and $T_{\text{air}}$ treatments. Means were based on three leaves per chamber and three chambers per treatment ($n=9$ leaves). Wavelengths (nm) at difference maxima are noted in the figure. None of the differences were statistically significant at $p=0.05$. 
Fig. 4. Differences at 1 nm wavelength intervals between the mean absorptance of sugar maple leaves grown under control and elevated CO$_2$ and $T_{air}$ treatments. Means were based on three leaves per chamber and three chambers per treatment ($n=9$ leaves). Wavelengths (nm) at difference maxima are noted in the figure. Darkened regions indicate differences that were statistically significant at $p=0.05$ according to Dunnett's test.

Fig. 5. Relationships of total chlorophyll (a+b) content with reflectance, transmittance and absorptance at 700 nm wavelength for all sugar maple leaves combined ($n=36$).
Fig. 3
Fig. 4

Absorptance Difference (%)

Elevated CO$_2$

Elevated $T_{air}$

Elevated CO$_2$ and $T_{air}$

Wavelength (nm)
Fig. 5

Reflectance (%)

$y = a + bx^c$

$r^2 = 0.81$

Transmittance (%)

$y = a + b(\ln x)$

$r^2 = 0.76$

Absorptance (%)

$y = (a + b/\ln x)^2$

$r^2 = 0.82$

Chlorophyll $a+b$ (μmol m$^{-2}$)
Effect of Elevated Atmospheric CO2 and Temperature on Leaf Optical Properties and Chlorophyll Content in Acer saccharum (Marsh.)

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