Effect of irradiance and vapour pressure deficit on stomatal response to CO₂ enrichment of four tree species

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Received 18 March 1997; Accepted 26 August 1997

Abstract

The stomatal response of seedlings grown in 360 or 720 μmol mol⁻¹ to irradiance and leaf-to-air vapour pressure deficit (VPD) at both 360 and 720 μmol mol⁻¹ CO₂ was measured to determine how environmental factors interact with CO₂ enrichment to affect stomatal conductance. Seedlings of four species with different conductances and life histories, Cercis canadensis (L.), Quercus rubra (L.), Populus deltoides (Bartr. ex Marsh.) × P. nigra (L.), and Pinus taeda (L.), were measured in hopes of identifying general responses. Conductance of seedlings grown at 360 and 720 μmol mol⁻¹ CO₂ were similar and responded in the same manner to measurement CO₂ concentration, irradiance and VPD. Conductance was lower for all species when measured at 720 than when measured at 360 μmol mol⁻¹ CO₂ at both VPDs (≈1.5 and ≈2.5 kPa) and all measured irradiances greater than zero (100, 300, 600, >1600 μmol m⁻² s⁻¹). The average decrease in conductance due to measurement in elevated CO₂ concentration was 32% for Cercis, 29% for Quercus, 26% for Populus, and 11% for Pinus. For all species, the absolute decrease in conductance due to measurement in CO₂ enrichment decreased as irradiance decreased or VPD increased. The proportional decrease due to measurement in CO₂ enrichment decreased in three of eight cases: from 0.46 to 0.10 in Populus and from 0.18 to 0.07 in Pinus as irradiance decreased from 1600 to 100 μmol m⁻² s⁻¹ and from 0.35 to 0.24 in Cercis as VPD increased from 1.3 to 2.6 kPa.

Key words: Stomatal conductance, CO₂ enrichment, irradiance, vapour pressure deficit.

Introduction

Stomatal conductance generally decreases when the atmospheric CO₂ concentration is elevated, but the magnitude of stomatal response to CO₂ enrichment is quite variable between and within species (Curtis, 1996; Field et al., 1995; Morison, 1987; Raschke, 1986). Between-species differences in physiological responses to the environment are to be expected, but the large amount of variation reported in stomatal sensitivity to CO₂ concentration within species is somewhat surprising and particularly troublesome for trying to predict the effect of atmospheric CO₂ enrichment on plant water use. Loblolly pine (Pinus taeda L.) is an excellent example of a species for which large differences in stomatal sensitivity to CO₂ concentration have been reported. Responses of loblolly pine to CO₂ enrichment include a decrease in stomatal conductance of as much as 50% depending on irradiance and water status (Tolley and Strain, 1985), a decrease in conductance up to 40% depending on growth CO₂ concentration (Fetcher et al., 1988), a decrease in conductance between 10% and 21% depending on nutrient status (Thomas et al., 1994), a 7% decrease in sap flow (Ellsworth et al., 1995) and no significant effect on stomatal conductance (Ellsworth et al., 1995; Liu and Teskey, 1995; Teskey and Shrestha, 1985).

These large intraspecific differences in stomatal sensitivity to CO₂ concentration suggest factors such as genotype, foliage age, nutrition, measurement environment or growth environment interact with the effect of CO₂ concentration. To predict accurately the effect of increasing atmospheric CO₂ concentration on plant water use, these interactions must be better understood. An excellent way to determine how the stomatal response to CO₂ enrichment will be affected by environmental fluctuations is to grow a set of plants in ambient and a set of plants in enriched CO₂ concentrations and then to measure all plants in both ambient and enriched CO₂ concentrations while manipulating environmental conditions.

A few studies have taken this approach. For two of three species, Hollinger (1987) found stomatal conduct-
ance was lower when measured in CO₂ enrichment, and that seedlings grown and measured in CO₂ enrichment exhibited a smaller per cent change in stomatal conductance in response to higher leaf-to-air vapour pressure deficit (VPD) than seedlings grown and measured in ambient CO₂ concentration. In three out of four species tested, Bunce (1993) found that the absolute and relative effect of increasing VPD on stomatal conductance decreased when plants were grown and measured in CO₂ enrichment. In two of the species, Bunce found that growth in CO₂ enrichment may have altered the response of stomatal conductance to CO₂ concentration. Berryman et al. (1994) found that in Eucalyptus tetrodonta (F. Muell) seedlings, the absolute difference in stomatal conductance due to measurement in ambient and elevated CO₂ concentrations decreased when leaf-to-air VPD increased or irradiance decreased. Needless to say, there is great uncertainty as to the importance of CO₂ x environment interactions and their effect on stomatal conductance.

In these experiments, seedlings of four species were grown in 360 μmol mol⁻¹ (ambient) or 720 μmol mol⁻¹ (2 x ambient) CO₂ concentrations under controlled conditions in growth chambers. Species were chosen that represent a range of conductance, life histories and taxonomic groups in the hope of identifying general relationships. Stomatal response to irradiance and, in a separate experiment, leaf-to-air VPD, of all seedlings was measured at both 360 and 720 μmol mol⁻¹ CO₂ concentration thus testing the effect of growth CO₂ concentration, measurement CO₂ concentration, either irradiance or VPD, and all interactions that may contribute to the conflicting results concerning the effect of CO₂ enrichment on stomatal conductance.

It was hypothesized that measurement CO₂ concentration x irradiance and measurement CO₂ concentration x VPD interactions occur that would cause the absolute decrease in stomatal conductance due to measurement in CO₂ enrichment to diminish as irradiance decreased or VPD increased. It was also hypothesized that as irradiance decreased or VPD increased, the proportional decrease due to measurement in CO₂ enrichment would also decrease. If both hypotheses are correct and the absolute and proportional effect of CO₂ enrichment on stomatal conductance both decrease as environmental conditions become less optimal, the effect of CO₂ enrichment on stomatal conductance is magnified under optimal conditions and approaches zero under suboptimal conditions.

Materials and methods

Between 29 April and 2 May 1996, one-year-old bare root seedlings of Cercis canadensis (L.), Quercus rubra (L.) Pinus taeda (L.), and the Populus hybrid, Robusta (Populus deltoides Bartr. ex Marsh. x P. nigra L.), obtained from commercial sources, were planted in 5 1 pots filled with Fafard 3B potting mix (Conrad Fafard Inc., Agawam, MA, USA). Seedlings were maintained in a greenhouse under ambient conditions for several days after planting and then moved into growth chambers at or before bud burst. The four growth chambers used in the experiment were 2.5 m wide x 1.3 m deep x 2 m tall and controlled temperature at 23°C, relative humidity at 60%, and carbon dioxide at a concentration of either 360 or 720 mmol mol⁻¹ (Environmental Growth Chambers, Chagrin Falls, OH, USA). Irradiance at the top of the seedlings was approximately 700 μmol m⁻² s⁻¹ and was supplied for a 13 h photoperiod by a combination of incandescent and high output fluorescent bulbs so that all wavelengths within the visible spectrum were present. Seedlings were placed in two chambers, one of which was maintained at 360 and the other at 720 μmol mol⁻¹ CO₂. Seedlings were rotated between chambers once per week to balance any chamber effects. Chamber CO₂ concentrations were adjusted when seedlings were rotated to maintain the appropriate treatments. Seedlings were watered as needed and fertilized every 5 d with a solution containing 0.06 g l⁻¹ N, 0.12 g l⁻¹ P, 0.08 g l⁻¹ K, 0.0002 g l⁻¹ B, 0.0004 g l⁻¹ Cu, 0.0008 g l⁻¹ Fe, 0.0004 g l⁻¹ Mn, 0.0004 g l⁻¹ Zn, and 0.000004 g l⁻¹ Mo supplied as Stern’s Miracle-Gro (Stern’s Miracle-Gro Products Inc., Port Washington, NY, USA). In addition, supplemental iron was supplied to the Pinus at a rate of 0.0004 g l⁻¹ chelated Fe (Miller Iron Chelate DP, Miller Chemical, Hanover, PA, USA).

The stomatal conductance-irradiance relationship was measured at both CO₂ concentrations (360 and 720 μmol mol⁻¹) for all seedlings. Seedlings were continuously exposed to their growth CO₂ concentrations (360 or 720 μmol mol⁻¹) from bud burst to when they were measured: 6–10 weeks for Populus, 10–14 weeks for Cercis, 16–19 weeks for Quercus, and 19–22 weeks for Pinus. Stomatal conductance was measured in the chambers using a LiCor LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln NE, USA) at the target irradiances of >1600, 600, 300, 100, and 0 μmol m⁻² s⁻¹ PPFD. Supplemental light was provided by a heat shielded 50 W quartz halogen bulb (OEX/CG, General Electric Co., Nela Park, OH, USA) 20 cm above the cuvette. Irradiance in the cuvette was manipulated by placing screens between the light source and the cuvette. Actual measured mean irradiances were 1597, 601, 302, 99, and 0 μmol m⁻² s⁻¹ for Populus, 1612, 609, 303, 98, and 0 μmol m⁻² s⁻¹ for Cercis, 1609, 601, 300, 101, and 0 μmol m⁻² s⁻¹ for Quercus, and 2129, 645, 313, 102, and 0 μmol m⁻² s⁻¹ for Pinus. Leaf temperature during measurements was maintained at 26°C and cuvette CO₂ concentration was maintained at either 360 or 720 μmol mol⁻¹ by the LI-6400 system. A similar VPD was maintained at all irradiances by adjusting the flow rate and the proportion of dry air entering the cuvette. Mean VPD at the different irradiances ranged from 1.10–1.35 kPa for Populus, 1.39–1.58 kPa for Cercis, 1.49–1.58 kPa for Quercus, and 1.41–1.50 kPa for Pinus. A curve at each CO₂ concentration was measured on consecutive days on the same leaf or needles. The order of measurement CO₂ concentration was randomized. Measurements were not taken until the leaves had fully equilibrated to the new irradiance. Equilibration time ranged between 45 min for Populus and 10 min for Quercus. Ten Populus seedlings (n = 5) and twelve Quercus, Cercis and Pinus seedlings (n = 6) were measured.

In a separate experiment, stomatal conductances of the same seedlings continuously grown at either 360 or 720 μmol m⁻² s⁻¹ CO₂ were measured at a low and high leaf-to-air VPD at both
CO₂ concentrations. For each species, the VPD response was measured after the light response: approximately 9–10 weeks after bud burst for Populus, 12–14 weeks after bud burst for Cercis, 18–19 weeks after bud burst for Quercus, and 21–22 weeks after bud burst for Pinus. As in the light experiment, the reciprocal CO₂ measurements were conducted on the same foliage over two consecutive days with the order of measurement CO₂ randomized. Mean measurement VPDs were restricted by the conductance of the species and were 1.1 (0.075 s.d.) and 2.5 kPa (0.072 s.d.) for Populus, 1.3 (0.045 s.d.) and 2.6 kPa (0.033 s.d.) for Cercis, 1.5 (0.063 s.d.) and 2.6 kPa (0.094 s.d.) for Quercus, and 1.4 (0.039 s.d.) and 2.5 kPa (0.026 s.d.) for Pinus. Leaf temperature was controlled at 26°C and mean irradiance was 2053 μmol m⁻² s⁻¹ for Pinus, 1506 μmol m⁻² s⁻¹ for Populus, 1459 μmol m⁻² s⁻¹ for Quercus, and 1590 μmol m⁻² s⁻¹ for Cercis. For all species, 12 plants, six grown in 360 and six grown in 720 μmol mol⁻¹ CO₂ were measured.

To determine how differences between stomatal conductance measured at 360 and 720 μmol mol⁻¹ CO₂ translated to differences in water use, whole plant water use was gravimetrically measured at both CO₂ concentrations on consecutive days for all plants. Plants were watered beyond saturation at night and then weighed at 08.00 and 17.00 h the following day. Immediately following the weighing at 17.00 h, CO₂ concentration was adjusted to the reciprocal and the protocol was repeated with the seedlings in the same chamber and chamber position. The difference in weight minus a constant value representing evaporation (averaged from several pots without seedlings) was considered to be the amount of water transpired by the plant. The sample size was 17 for Populus, 10 for Cercis, 12 for Quercus, and 13 for Pinus.

Stomatal conductance was measured by counting the number of stomata using a light microscope at 400 × magnification for Populus, Cercis and Quercus or using a dissecting microscope at 40 × magnification for Pinus. Ten fields per leaf were averaged. A fan was placed near the microscope to prevent condensation of water on the lens.

For all experiments, species were analysed separately. The light experiment was analysed with a multivariate repeated-measures analysis of variance (SAS Institute Inc., 1987) with two repeated measures factors, light and measurement CO₂ concentration, and the between subject factor, growth CO₂ concentration. Stomatal density data were analysed with an ANOVA to determine whether the effect of growth CO₂ concentration was significant.

Results

Light experiment

For all species, there were no differences in stomatal conductance between seedlings grown in 360 and seedlings grown in 720 μmol mol⁻¹ CO₂ for the effect of CO₂ concentration during measurement. The effect of light during measurement or their interactions (no growth CO₂ concentration effect or interactions involving growth CO₂ concentration). Therefore, discussion of results concentrates on the effect of measurement CO₂ concentration. Data from seedlings of both growth concentrations were pooled for presentation in figures and tables.

For all four species, stomatal conductance increased (P<0.05) with increasing irradiance (light effect) and was greater (P<0.05) when measured at 360 than when measured at 720 μmol mol⁻¹ CO₂ (measurement CO₂ effect) (Fig. 1). In addition, the absolute decrease in stomatal conductance due to measurement in CO₂ enrichment increased (P<0.05) with increasing irradiance (light × measurement CO₂ interaction) (Fig. 1). For all species, the linear and quadratic components of the light effect and the light × measurement CO₂ interaction were significant (P<0.05). When the effect of measurement CO₂ concentration was examined separately at each irradiance, conductance of Cercis, Quercus and Pinus was lower (P<0.05) when measured at 720 than when measured at 360 μmol mol⁻¹ CO₂ at all measured irradiances above 0 μmol m⁻² s⁻¹ PPFD and conductance of Populus was lower (P<0.05) at all measured light intensities except 0 and 100 μmol m⁻² s⁻¹ PPFD (Fig. 1).

Given the significant linear and quadratic components of the measurement CO₂ × light interactions, the proportional decrease in stomatal conductance due to measurement in CO₂ enrichment was calculated to determine how irradiance was influencing the relative effect of measurement CO₂ concentration. For all species, the proportional decrease at 0 μmol m⁻² s⁻¹ PPFD was approximately 0 and less than the proportional decreases calculated at the other irradiances (Table 1). For Cercis and Quercus, the proportional decrease due to measurement in CO₂ enrichment was similar at 100, 300, 600, and 1600 μmol m⁻² s⁻¹ PPFD (Table 1). For Populus and Pinus however, the proportional decrease due to measurement in CO₂ enrichment increased as irradiance increased (Table 1).

VPD experiment

As in the light experiment, there were no differences in the stomatal response between seedlings grown in 360
Fig. 1. Mean light response curves for stomatal conductance of *Cercis canadensis*, *Quercus rubra*, *Populus deltoides* × *P. nigra*, and *Pinus taeda* measured at 360 and 720 μmol mol⁻¹ CO₂ concentration. Growth CO₂ concentration did not influence the response to light or measurement CO₂ so measurements from seedlings grown in 360 and 720 μmol mol⁻¹ CO₂ were pooled. An asterisk indicates a significant difference due to measurement CO₂ concentration at a particular irradiance. Vertical bars represent standard errors.

Table 1. Mean proportional decrease in stomatal conductance due to measurement in CO₂ enrichment ([1 - (measurement at 720 μmol mol⁻¹ CO₂/measurement in 360 μmol mol⁻¹ CO₂)] for seedlings of *Cercis canadensis*, *Quercus rubra*, *Populus deltoides* × *P. nigra* and *Pinus taeda* at five target irradiances (actual irradiances are listed in Materials and methods). Growth CO₂ concentration did not influence the stomatal response to CO₂ concentration or light so measurements of seedlings grown in 360 and 720 μmol mol⁻¹ CO₂ were pooled. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th>Irradiance (μmol m⁻² s⁻¹)</th>
<th>Cercis</th>
<th>Quercus</th>
<th>Populus</th>
<th>Pinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03 (0.047)</td>
<td>0.13 (0.080)</td>
<td>-0.11 (0.133)</td>
<td>-0.02 (0.038)</td>
</tr>
<tr>
<td>100</td>
<td>0.38 (0.044)</td>
<td>0.27 (0.045)</td>
<td>0.10 (0.140)</td>
<td>0.07 (0.028)</td>
</tr>
<tr>
<td>300</td>
<td>0.42 (0.059)</td>
<td>0.31 (0.035)</td>
<td>0.36 (0.057)</td>
<td>0.11 (0.030)</td>
</tr>
<tr>
<td>600</td>
<td>0.37 (0.051)</td>
<td>0.32 (0.036)</td>
<td>0.37 (0.045)</td>
<td>0.18 (0.026)</td>
</tr>
<tr>
<td>1600</td>
<td>0.38 (0.047)</td>
<td>0.29 (0.042)</td>
<td>0.46 (0.039)</td>
<td>0.18 (0.025)</td>
</tr>
</tbody>
</table>

and those grown in 720 μmol mol⁻¹ CO₂ when measured at same CO₂ concentrations and VPDs (no growth CO₂ concentration effect or interactions involving growth CO₂ concentration) so results of seedlings from both growth concentrations were pooled for presentation in figures and tables. For all four species, stomatal conductance was lower at the higher VPD regardless of measurement CO₂ concentration (VPD effect), stomatal conductance of seedlings measured at 720 μmol mol⁻¹ CO₂ was lower than that measured at 360 μmol mol⁻¹ CO₂ regardless of VPD (measurement CO₂ effect) and the absolute difference in stomatal conductance between plants measured at 720 and 360 μmol mol⁻¹ was smaller at the higher VPD than at the lower VPD (VPD × measurement CO₂ interaction) (Fig. 2).

When data from the low and high VPDs were analysed separately, stomatal conductance at both VPDs was lower (P<0.05) when measured at 720 μmol mol⁻¹ CO₂ than
when measured at 360 μmol mol⁻¹ CO₂ concentration (Fig. 2). Given the significant VPD x measurement CO₂ interactions, the proportional decrease in stomatal conductance due to measurement in CO₂ enrichment was calculated at each VPD to determine how the relative effect of measurement CO₂ concentration changed as VPD changed. Only Cercis showed a large reduction in the proportional decrease due to CO₂ enrichment as VPD increased (Table 2).

**Whole plant water use and stomatal density**

In all four species, whole plant water use was lower (P<0.05) during exposure to 720 μmol mol⁻¹ CO₂ than during exposure to 360 μmol mol⁻¹ CO₂ (Table 3). For Cercis, Quercus, and Populus, neither the effect of growth CO₂ concentration or the growth CO₂ x measurement CO₂ interaction influenced this response. For Pinus, however, the growth CO₂ x measurement CO₂ interaction was significant because whole plant water use of seedlings grown in 720 μmol mol⁻¹ CO₂ was more sensitive to measurement CO₂ concentration than seedlings grown in 360 μmol mol⁻¹ CO₂ (P<0.05) (Table 3). Stomatal density was not statistically different between seedlings grown in 360 and 720 μmol mol⁻¹ CO₂ concentration for any of the species. Stomatal densities for seedlings grown in 360 and 720 μmol mol⁻¹ CO₂ concentration for Cercis were 225 and 229 mm⁻², for Quercus were 436 and 417 mm⁻², for Populus were 140 and 136 mm⁻² (abaxial), and 90

**Fig. 2.** Mean vapour pressure deficit response curves for stomatal conductance of Cercis canadensis, Quercus rubra, Populus deltoides x P. nigra, and Pinus taeda measured at 360 and 720 μmol mol⁻¹ CO₂ concentration. Growth CO₂ concentration did not influence the response to VPD or measurement CO₂ so measurements from seedlings grown in 360 and 720 μmol mol⁻¹ CO₂ were pooled. An asterisk indicates a significant difference due to measurement CO₂ concentration at a particular vapour pressure deficit. Vertical bars represent standard errors.

<table>
<thead>
<tr>
<th>VPD (kPa)</th>
<th>Cercis</th>
<th>Quercus</th>
<th>Populus</th>
<th>Pinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>~1.5</td>
<td>0.35 (0.039)</td>
<td>0.35 (0.019)</td>
<td>0.33 (0.048)</td>
<td>0.16 (0.027)</td>
</tr>
<tr>
<td>~2.5</td>
<td>0.24 (0.035)</td>
<td>0.34 (0.020)</td>
<td>0.30 (0.091)</td>
<td>0.10 (0.034)</td>
</tr>
</tbody>
</table>

**Table 2.** Mean proportional decrease in stomatal conductance due to measurement in CO₂ enrichment [1 - (measurement at 720 μmol mol⁻¹ CO₂/measuremen in 360 μmol mol⁻¹ CO₂)] for seedlings of Cercis canadensis, Quercus rubra, Populus deltoides × P. nigra and Pinus taeda at two vapour pressure deficits (actual VPDs are listed in Materials and methods). Growth CO₂ concentration did not influence the stomatal response to CO₂ concentration or VPD so measurements of seedlings grown in 360 and 720 μmol mol⁻¹ CO₂ were pooled. Standard errors are in parentheses.
and 90 mm$^{-2}$ (adaxial), and for Pinus were 91 and 91 mm$^{-2}$.

### Discussion

For all species in our study, the absolute difference between stomatal conductance measured in ambient and twice ambient CO$_2$ concentration was smaller at lower irradiance or higher VPD. In addition, for three of the eight experiments (Pinus-light, Populus-light, Cercis-$\text{VPD}$), the proportional decrease due to measurement in CO$_2$ enrichment decreased as irradiance decreased or VPD increased. Thus, the absolute response, and in some cases, the relative response of stomatal conductance to CO$_2$ enrichment was not constant. This changing response makes experimental outcomes depend on environmental conditions during measurement, and predicting the effects of CO$_2$ enrichment on stomatal conductance and water use difficult.

Although the proportional decrease of stomatal conductance due to measurement in CO$_2$ enrichment decreased as environmental conditions became less optimal in three out of eight experiments, the proportional effect of CO$_2$ enrichment probably always becomes negligible as stomata close in response to stress. Interestingly, the effect of CO$_2$ concentration on the stomatal conductance of Cercis, the species most commonly found in the high humidity understory environment, became proportionately smaller at the higher VPD, indicating that stress may have been beginning to override the CO$_2$ effect. Similarly, the effect of CO$_2$ concentration on stomatal conductance of Pinus and Populus, the two shade-intolerant species, became proportionately smaller as irradiance decreased.

In Com melina communis L. (Morison and Jarvis, 1983) and Eucalyptus tetrodonta (Berryman et al., 1994) the absolute difference in stomatal conductance due to measurement CO$_2$ concentration was greater under high than under low irradiance, but the proportional effect due to CO$_2$ concentration did not change as irradiance changed. In contrast, for Eucalyptus pauciflora Sieb. ex Spreng (Wong et al., 1978) and five herbaceous species (Sharkey and Raschke, 1981), little change in the absolute difference in stomatal conductance due to measurement CO$_2$ concentration occurred as irradiance increased. However, the internal CO$_2$ concentrations used by Wong et al. (1978) and Sharkey and Raschke (1981) corresponded to current ambient CO$_2$ concentration and below. Quite possibly, the difference in stomatal conductance due to CO$_2$ concentration could have been larger under high irradiance if conductance had been measured in ambient and enriched CO$_2$ concentrations.

Morison and Gifford (1983), Hollinger (1987), Bunce (1993) and Berryman et al. (1994) all found that the absolute difference in stomatal conductance between plants measured under ambient and elevated CO$_2$ concentrations decreased with increasing VPD. Although Berryman et al. (1994) and Morison and Gifford (1983) did not find any large changes in the proportional effect of CO$_2$ concentration at different VPDs, Bunce (1993) found a decrease in the proportional effect of CO$_2$ enrichment as VPD increased for three of four species measured.

Environment $\times$ CO$_2$ concentration interactions do not appear to be the sole cause of the large range in reported stomatal response of some species to CO$_2$ enrichment. When the proportional effect due to measurement CO$_2$ concentration changed with changing irradiance or VPD, three times out of eight, the changes were not very large. For instance, the proportional decrease due to measurement in CO$_2$ enrichment changed from 0.46 to 0.34 for Populus and 0.18 to 0.11 for Pinus when irradiance decreased from $>1600$ to 300 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD and changed from 0.35 to 0.24 for Cercis when VPD increased from 1.3 to 2.6 kPa.

Even though some of the differences in the reported effects of CO$_2$ concentration on stomatal conductance may be due to differences in irradiance and VPD during measurement, other factors must also contribute to the variation. For instance, other studies have found that foliage age (Curtis and Teeri, 1992), growth environment (Talbott et al., 1996), CO$_2$ concentration during growth (Berryman et al., 1994; Conroy et al., 1988), genotype, nutrition (Conroy et al., 1988; Thomas et al., 1994), and time of day (Surano et al., 1986) may all affect the magnitude of the stomatal response to CO$_2$ concentration. Another factor that may affect the reported stomatal response to CO$_2$ concentration is equilibration time because measurements made without allowing adequate equilibration will include potentially large experimental errors. In the present study, 10–45 min was necessary for complete equilibration of stomatal conductance to changes in irradiance or VPD whereas photosynthesis reached steady state in less than five minutes. Others have also reported large differences between the time required for photosynthesis and stomatal conductance to equilibrate to changing environmental conditions (Whitehead and Teskey, 1995; Barradas and Jones, 1996; Ögren and Sundin, 1996).

### Table 3. Mean proportional decrease in whole plant water use due to measurement in CO$_2$ enrichment [$1 - \text{(measurement at 720} \mu\text{mol mol}^{-1} \text{CO}_2]\text{measurement in 360} \mu\text{mol mol}^{-1} \text{CO}_2]$ for seedlings of Cercis canadensis, Quercus rubra, Populus deltoides x P. nigra and Pinus taeda grown at two CO$_2$ concentrations: standard errors are in parentheses

<table>
<thead>
<tr>
<th>Growth CO$_2$ ($\mu$mol mol$^{-1}$)</th>
<th>Cercis</th>
<th>Quercus</th>
<th>Populus</th>
<th>Pinus</th>
</tr>
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<tbody>
<tr>
<td>360</td>
<td>0.41 (0.023)</td>
<td>0.30 (0.041)</td>
<td>0.19 (0.016)</td>
<td>0.10 (0.016)</td>
</tr>
<tr>
<td>720</td>
<td>0.37 (0.018)</td>
<td>0.36 (0.023)</td>
<td>0.19 (0.026)</td>
<td>0.16 (0.013)</td>
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</table>

and 90 mm$^{-2}$ (adaxial), and for Pinus were 91 and 91 mm$^{-2}$.
A doubling of $\text{CO}_2$ concentration during measurement decreased the stomatal conductance of the four species that were measured. Mean decreases were 32% for \textit{Cercis}, 29% for \textit{Quercus}, 26% for \textit{Populus}, and 11% for \textit{Pinus}. In 23 out of 25 studies conducted primarily on herbaceous species, stomatal conductance decreased approximately 40% when measured at twice ambient $\text{CO}_2$ concentration (Morison, 1987). The reported effect of $\text{CO}_2$ enrichment on tree species tends to be smaller. For instance, stomatal conductance decreased by 23% on average when data from 29 studies of 23 tree species were compiled (Field et al., 1995). In the present study, the results of the whole plant water use experiment verified not only the large measured decrease in stomatal conductance due to $\text{CO}_2$ enrichment, but that decreases in stomatal conductance translate to decreases in whole plant water use.

Stomatal conductance of seedlings grown in ambient and elevated $\text{CO}_2$ concentrations responded the same to measurement $\text{CO}_2$ concentration, \textit{VPD}, and irradiance and had similar stomatal densities. Therefore, the stomatal conductance and stomatal density of leaves that developed under $\text{CO}_2$ enrichment did not acclimate to growth in twice ambient $\text{CO}_2$ concentration even though leaves were continuously exposed to $\text{CO}_2$ enrichment from bud burst. Rather, stomata of all seedlings reduced water loss at higher $\text{CO}_2$ concentrations via short-term and reversible changes in aperture. The only exception was whole plant water use of \textit{Pinus taeda} seedlings. In this case, seedlings grown in 720 $\mu\text{mol mol}^{-1}$ $\text{CO}_2$ displayed a greater decrease in water use in response to $\text{CO}_2$ enrichment than those grown in 360 $\mu\text{mol mol}^{-1}$ $\text{CO}_2$. The \textit{Pinus} seedlings grown in 720 $\mu\text{mol mol}^{-1}$ $\text{CO}_2$ were larger and supported new foliage growth while those grown in 360 $\mu\text{mol mol}^{-1}$ were smaller and at an earlier phenological stage. If sensitivity of stomatal conductance to $\text{CO}_2$ concentration varies with plant size or foliage age than these size and phenological differences could have caused this difference in whole plant water use. Alternatively, the size differences between the seedlings grown in ambient and twice ambient $\text{CO}_2$ may have led to differences in canopy or boundary layer conductance which could have affected transpiration and water use even though stomatal conductance was not affected. Nevertheless, stomatal conductance measurements on fully expanded needles of similar phenological age did not show a difference due to growth $\text{CO}_2$ concentration.

In a review of the literature, Woodward and Kelly (1995) found that 60% of controlled experiments measuring stomatal density have shown a decrease in response to growth in $\text{CO}_2$ enrichment. Previous reports on the effect of growth in elevated $\text{CO}_2$ concentration on stomatal sensitivity to $\text{CO}_2$ concentration during measurement vary, with growth in $\text{CO}_2$ enrichment sometimes increasing, sometimes decreasing or sometimes not affecting the stomatal response (Eamus, 1991). No difference was found in stomatal response or stomatal density between seedlings grown in 360 and 720 $\mu\text{mol mol}^{-1}$ $\text{CO}_2$ in the four different species which were tested.

Although growth in $\text{CO}_2$ enrichment does not necessarily affect the stomatal response to measurement $\text{CO}_2$ concentration (no effect in this study), $\text{CO}_2$ enrichment usually decreases the stomatal conductance of trees. Even though an interactive combination of several internal and external factors appears to control the magnitude of stomatal response to $\text{CO}_2$ concentration, irradiance and \textit{VPD} do affect the response. Accordingly, the effect of irradiance and \textit{VPD} should be considered when examining intraspecific differences in stomatal sensitivity to $\text{CO}_2$ concentration, when performing future experiments and when modelling the effect of $\text{CO}_2$ enrichment on plant water use.

**Acknowledgement**

We would like to thank William Jennings (Jay) Brown for his help planting the seedlings and his assistance keeping them healthy and vigorous.

**References**


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