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Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the third and fourth years of exposure

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ABSTRACT

Two major components of climate change, increasing atmospheric [CO_2] and increasing temperature, may substantially alter the effects of water availability to plants through effects on the rate of water loss from leaves. We examined the interactive effects of elevated [CO_2] and temperature on seasonal patterns of stomatal conductance (g_s), transpiration (E) and instantaneous transpiration efficiency (ITE) in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. Seedlings were grown in sunlit chambers at either ambient CO_2 (AC) or ambient + 180 μmol mol^{-1} CO_2 (EC), and at ambient temperature (AT) or ambient + 3·5 °C (ET) in a full-factorial design. Needle gas exchange at the target growth conditions was measured approximately monthly over 21 months. Across the study period and across temperature treatments, growth in elevated [CO_2] decreased E by an average of 12% and increased ITE by an average of 46%. The absolute reduction of E associated with elevated [CO_2] significantly increased with seasonal increases in the needle-to-air vapour pressure deficit (D). Across CO_2 treatments, growth in elevated temperature increased E an average of 37%, and did not affect ITE. Combined, growth in elevated [CO_2] and elevated temperature increased E an average of 19% compared with the ACAT treatment. The CO_2 supply and growth temperature did not significantly affect stomatal sensitivity to D or the relationship between g_s and net photosynthetic rates. This study suggests that elevated [CO_2] may not completely ameliorate the effect of elevated temperature on E, and that climate change may substantially alter needle-level water loss and water use efficiency of Douglas-fir seedlings.

Key-words: *Pseudotsuga menziesii*; climate change; Douglas-fir; elevated CO_2; leaf water relations; photosynthesis; seasonal patterns; stomatal conductance; temperature; transpiration.

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INTRODUCTION

Water stress limits carbon uptake for part or most of the year in most terrestrial ecosystems (Webb *et al.* 1983). Two major components of climate change, increasing atmospheric [CO_2] and increasing temperature, may substantially alter the effects of water availability to plants through effects on the rate of water loss from leaves (Morison 1993). Increasing temperature within normal physiological ranges often increases leaf-level transpiration rates (E) by increasing the driving gradient for water loss through stomates (Lambers, Chapin & Pons 1998). In contrast, short-term exposure to elevated [CO_2] reduces E by inducing reductions in stomatal conductance (g_s) in response to increased intercellular [CO_2] (Morison 1987). If this short-term effect is sustained during long-term exposure, increasing atmospheric [CO_2] may moderate the effects of increasing temperatures on leaf-level water loss (Kellomäki & Wang 1996; Koike *et al.* 1996; Tjoelker, Oleksyn & Reich 1998; Wayne, Reekie & Bazzaz 1998).

Although long-term exposure to elevated [CO_2] often reduces g_s (Morison 1985; Ceulemans & Mousseau 1994; Drake, Gonzàlez-Meler & Long 1997; Curtis & Wang 1998), there is considerable variability in the response (Saxe, Ellsworth & Heath 1998; Medlyn *et al.* 2001). For example, trees generally show smaller responses compared to herbaceous plants (Saxe *et al.* 1998; Mooney *et al.* 1999). Further, coniferous trees often show smaller responses compared with deciduous trees (Saxe *et al.* 1998), although this effect may be confounded with effects of plant age (Medlyn *et al.* 2001). Young field-grown conifers often show a significant reduction in g_s associated with long-term exposure to elevated [CO_2] (Surano *et al.* 1986; Hollinger 1987; Tissue, Thomas & Strain 1997), whereas older conifers may show little or no response (Medlyn *et al.* 2001).

In addition, long-term exposure to elevated [CO_2] has been shown to reduce the sensitivity of g_s to the leaf-to-air vapour pressure deficit (D) in some tree species (Hollinger 1987; Heath 1998). If elevated [CO_2] induces a uniform reduction in g_s across D, the effect of elevated [CO_2] on E should increase with increasing evaporative demand (Jarvis, Mansfield & Davies 1999). However, if elevated

[CO_2] reduces the sensitivity of g_s to D, then the effect of elevated [CO_2] on E may actually decline with increasing D. If this occurs, long-term exposure to elevated [CO_2] may have minimal effects on the impacts of predicted increases in temperature on leaf water relations.

Potential effects of climate change on leaf water relations are a particularly important issue for forests that experience extended periods of drought (Franklin *et al.* 1991; Field, Jackson & Mooney 1995). In the Pacific North-west, water availability during the summer is a primary factor limiting growth of many species (Waring & Franklin 1979), including Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), a dominant tree species at lower elevations in this region (Franklin & Dyrness 1988; Hermann & Lavender 1990). Mean annual temperatures in the Columbia River Basin are predicted to increase by 2·6–3·4 °C by 2050 compared to the 1961–90 means, whereas annual precipitation is predicted to fall within the current range (Mote *et al.* 1999).

Increasing temperature generally increases g_s of Douglas-fir seedlings (Livingston & Black 1987; Apple *et al.* 2000), whereas elevated [CO_2] has been shown to significantly reduce (Hollinger 1987) or not affect g_s (Hollinger 1987; Apple *et al.* 2000). As a result, increasing temperature associated with climate change may exacerbate summer water loss by Douglas-fir, whereas the effects of elevated [CO_2] on water loss are unclear. In addition, elevated [CO_2] has been shown to reduce stomatal sensitivity of Douglas-fir to D (Hollinger 1987), suggesting that the mediatory affect of elevated [CO_2] may decrease with seasonal increases in D. Because previous studies have only reported the effects of climate change on stomatal responses of Douglas-fir at one or two points in time, it is unclear to what extent these effects may vary over time.

In this study, we examined the effects of elevated [CO_2], increasing temperature associated with climate change, and seasonal changes in D on stomatal regulation of E of Douglas-fir seedlings over a 21 month period. In addition, we examined the effects of climate change on the relationship between leaf-level water loss and carbon uptake by assessing changes in instantaneous transpiration efficiency (ITE). Potential reductions in E associated with increasing atmospheric [CO_2] may increase ITE, and this effect may be magnified because growth in elevated [CO_2] increases net photosynthetic rates in Douglas-fir (Hollinger 1987; Lewis *et al.* 2001). In contrast, because net photosynthetic rates in Douglas-fir are relatively constant across a broad temperature range (Helms 1964; Helms 1965; Brix 1967; Lewis *et al.* 2000; Lewis *et al.* 2001), increasing temperature may reduce ITE by increasing E. Because effects of climate change on leaf water relations may be regulated by reductions in the sensitivity of g_s to D, we examined the effects of climate change on stomatal sensitivity to D. In addition, we examined whether g_s and photosynthesis respond in parallel to climate change. Although elevated [CO_2] increases net photosynthetic rates in Douglas-fir, seasonal down-regulation has been observed (Lewis, Olszyk & Tingey 1999). Because g_s and photosynthesis are often

assumed to respond in parallel to climate change (Medlyn *et al.* 2001), photosynthetic down-regulation may be expected to enhance reductions in g_s associated with elevated [CO_2]. Specifically, we addressed the following questions: (1) do elevated [CO_2] and elevated temperature have counteracting effects on leaf-level water loss and ITE in Douglas-fir seedlings; (2) do changes in stomatal sensitivity to D regulate seasonal patterns in the effects of climate change on leaf water relations; and (3) do g_s and photosynthesis respond in parallel to elevated [CO_2] and elevated temperature?

MATERIALS AND METHODS

Growth conditions

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed lots were collected at five low-elevation seed zones (< 500 m) in the Coast Range, Willamette Valley and the west slopes of the Cascade Mountains around Corvallis, Oregon, USA. Seedlings were grown for 1 year in seed beds and 1 year in nursery beds. In June 1993, 14 seedlings were transplanted as bare-root, 2-year-old stock into each 1 m × 2 m surface area chamber at the US Environmental Protection Agency Environmental Research Laboratory in Corvallis, Oregon. Each chamber consisted of a sun-lit upper compartment (1·2–1·5 m high) where air temperature, [CO_2] and vapour pressure deficit were monitored and controlled, and a lower soil lysimeter (0·9 m deep) filled with a native coarse-textured sandy loam in which soil parameters such as temperature and soil moisture were monitored (Tingey *et al.* 1996).

Ambient [CO_2] and air temperature were monitored at an adjacent meteorological station. The chambers were controlled to continuously track ambient [CO_2] (AC) or ambient + 200 μmol mol^{-1} CO_2 (EC) and ambient air temperature (AT) or ambient + 4 °C (ET; Tingey *et al.* 1996). Target dew point depression in the chambers was based on ambient conditions and controlled to track equivalent vapour pressure deficits across treatments. Actual chamber conditions across the course of the experiment differed slightly from targets: elevated [CO_2] averaged 179 μmol mol^{-1} above ambient, elevated temperature averaged 3·5 °C above ambient, and the vapour pressure deficit of chamber air in the ET treatment averaged 0·10 kPa above ambient (Olszyk *et al.* 1998a).

The experimental design was a full factorial with three replicate chambers in each of the four treatment combinations: ambient CO_2 and ambient temperature (ACAT); ambient CO_2 and elevated temperature (ACET); elevated CO_2 and ambient temperature (ECAT); and elevated CO_2 and elevated temperature (ECET). Treatments were applied 24 h per day beginning in August 1993 and continuing until the end of the study in July 1997. Mid-day (1000–1400 Pacific Standard Time) [CO_2] during the growing season in 1996 typically ranged between 360 and 400 μmol mol^{-1} in the ambient CO_2 treatment.

Seedlings were grown under ambient light, and without

supplemental nutrients. Soil moisture content was controlled to reflect seasonal changes in soil moisture typical for the wet winter – dry summer climate in the Pacific North-west (Griffiths & Caldwell 1990; Griffiths *et al.* 1991). Weekly water additions to the ACAT treatment were calculated based on this pattern of soil moisture content. All treatments received the same weekly water additions.

Physiological measurements

Needle-level transpiration rates, stomatal conductance and net photosynthetic rates were measured using infrared gas analysers built into a leaf cuvette in an open-flow gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA). The ITE was calculated from these measurements as CO_2 uptake per unit H_2O transpired. Needle gas exchange measurements began in November 1995, 27 months after treatments were initiated, and continued to be made approximately monthly until July 1997, when the study was terminated. All measurements were made on intact fully expanded, unshaded needles from the most recent fully expanded needle cohort. In 1996, the average date at which new needles reached full expansion was 28 June, and there were no significant treatment effects on needle development (Olszyk *et al.* 1998b). As a result, needles from the 1995 cohort were used for measurements made during the interval between November 1995 and the beginning of July 1996, whereas the 1996 cohort was used for the remainder of the experiment.

Needles were arranged in the cuvette such that self-shading was minimized and all needles were parallel to the plane of the leaf chamber. The projected surface area of the measured needles was estimated using measurements of needle length and width. All measurements were made using ambient light. Photosynthetic photon flux densities (PPFD) at the upper needle surface ranged between 1200 and 2000 µmol photons $m^{-2} s^{-1}$ for 84% of measurements. No measurements were made at PPFD below 800 *µ*mol photons $m^{-2} s^{-1}$. Irradiances above 800 *µ*mol photons $m^{-2} s^{-1}$ are saturating for photosynthesis in Douglas-fir (Bond *et al.* 1999; Lewis *et al.* 1999, 2000).

The airstream entering the cuvette was maintained at the growth $[CO_2]$ (either 360 or 560 *µ*mol mol^{-1} CO_2) using the LI-6400 computer-controlled CO_2 mixing system. Needle, cuvette and air temperatures were measured with thermocouples linked to the LI-6400 computer. Needle temperature was maintained at the target temperature using a computer-controlled peltier module mounted on the cuvette. Needle and cuvette air temperatures were generally similar during measurements. The needle-to-air vapour pressure deficit (*D*) in the cuvette was maintained at the target *D* by regulating the air flow rate, and by using desiccant to scrub the incoming airstream as necessary.

For a given measurement period, the target needle temperature and *D* for the ambient temperature treatment reflected average ambient conditions between 1000 and 1400 h. Target needle temperatures for the ET treatment were 4·0 °C higher than for the AT treatment. The target *D*
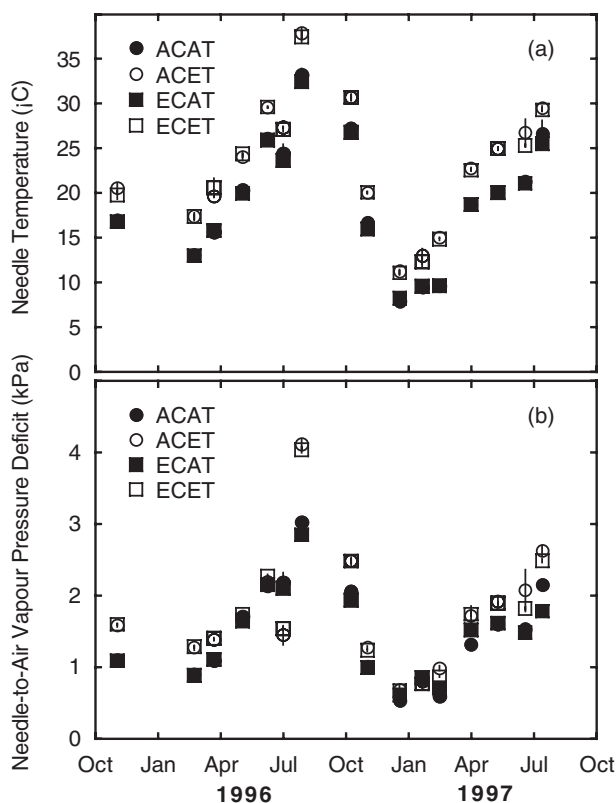

Figure 1. Mean needle temperatures (a) and needle-to-air water vapour pressure deficit (*D*; b) during measurements at each measurement period in each treatment. Needle temperatures in the elevated temperature treatment were 4·0 °C higher on average than needle temperatures in the ambient temperature treatment, and *D* was 0·3 kPa higher on average than *D* in the ambient temperature treatment. ACAT = ambient CO_2 and ambient temperature; ACET = ambient CO_2 and elevated temperature; ECAT = elevated CO_2 and ambient temperature; and ECET = elevated CO_2 and elevated temperature. *n* = 3 between November 1995 and July 1996; for the remainder of the experiment, *n* = 2 for the ACAT and ECAT treatments, *n* = 3 for the ACET and ECET treatments.

was the same for all treatments. Mean (± SE) needle temperatures and *D* at each measurement period in all treatments are shown in Fig. 1. The actual cuvette conditions deviated somewhat from the actual chamber conditions because the cuvette conditions were regulated to match the target chamber conditions, which themselves varied somewhat from actual chamber conditions (see above). Furthermore, although the average measurement $[CO_2]$ and needle temperatures matched the target conditions, the actual *D* were 0·3 kPa higher on average in the ET treatment compared with the AT treatment.

Prior to each measurement, needles were equilibrated in the cuvette at saturating PPFD, the growth $[CO_2]$, the measurement temperature and *D*. Needles were considered equilibrated if the gas exchange parameters were stable for 1 min. In general, the equilibration period lasted approximately 5 min. For a given measurement day, measurements were initiated at approximately 0900 h Pacific Standard

Time, and typically were completed by 1200 h Pacific Standard Time.

Stomatal sensitivity to vapour pressure deficit

The relationship between stomatal conductance and the needle-to-air vapour pressure deficit was examined using the following linear model (Jarvis 1976; Medlyn *et al*. 2001):

$$g_s = g_{smax}(1 - D/D_0) \qquad (1)$$

where g_s is stomatal conductance, g_{smax} is the maximum stomatal conductance under optimal environmental conditions, D is the needle-to-air vapour pressure deficit, and D_0 is the vapour pressure deficit at which g_s becomes zero. The effect of elevated $[CO_2]$ on stomatal sensitivity to D was assessed by examining the effect of elevated $[CO_2]$ on D_0.

Relationship between stomatal conductance and net photosynthetic rates

The relationship between stomatal conductance and net photosynthetic rates was examined using the Ball, Woodrow & Berry (1987) model:

$$g_s = g_0 + g_1 A h_s / C_a \qquad (2)$$

where A is the net photosynthetic rate at the growth $[CO_2]$, h_s is the relative humidity at the leaf surface, C_a is the treatment $[CO_2]$ (360 or 560 μmol mol^{-1}) and g_0 and g_1 are the parameters to be determined. Treatment effects on the relationship between g_s and A were assessed by examining changes in g_0 and g_1. We used the Ball–Berry model rather than the Leuning (1995) model because the Ball–Berry model has fewer parameters to fit.

Statistical analyses

Treatment effects on seasonal patterns in needle gas exchange parameters were analysed using repeated measures analysis of variance with growth $[CO_2]$ and temperature as the between-subjects factors and measurement period as the within-subjects factor. Analyses were performed using the multivariate general linear model function (MGLH) in SYSTAT (SPSS, Inc., Chicago, IL, USA). In general, needle gas exchange measurements were performed on one seedling in each chamber per measurement period. Individual branches were not repeatedly sampled over time, and across the study period measurements were made on several seedlings from each chamber. As the chamber was the experimental unit, measurements on multiple branches and seedlings from a chamber at a given measurement period were combined and the mean value used in the analyses. Data from the 1995 and 1996 needle cohorts were analysed separately. One chamber each in the ACAT treatment and the ECAT treatment were excluded from the 1996 needle cohort analyses because of extensive insect damage to seedlings in these chambers.

RESULTS

Across temperature treatments, the elevated $[CO_2]$ (EC) treatment was associated with a significant reduction in E and g_s of the 1995 needle cohort, but g_s of the 1996 cohort did not significantly vary between CO_2 treatments (Fig. 2, Table 1). The 1995 cohort was measured between November 1995 and July 1996, whereas the 1996 cohort was measured between August 1996 and July 1997. For both years, the EC treatment was associated with significant increases in net photosynthetic rates and ITE.

Table 1. Summary of levels of statistical significance (*P*) from univariate comparisons (repeated measures analysis of variance) on transpiration rates, mean light-saturated net photosynthetic (P$_s$) rates, conductance and instantaneous transpiration efficiency (ITE) of the 1995 and 1996 needle cohorts with CO_2 supply and temperature as the between-subjects factors and measurement period as the within-subjects factor. Results from multivariate and single degree of freedom polynomial contrasts were similar. Measurements on the 1995 needle cohort were made between November 1995 and July 1996, whereas the 1996 needle cohort was measured between August 1996 and July 1997

Factor	Transpiration rate			Net P$_s$ rate		Conductance		ITE	
	1995	1996	1996[a]	1995	1996	1995	1996	1995	1996
Between subjects									
CO$_2$ (C)	**0·031**	**0·021**	**0·042**	**0·007**	**0·048**	0·056	0·109	**0·001**	**0·032**
Temperature (T)	**< 0·001**	**< 0·001**	**0·003**	**0·001**	**0·011**	**0·019**	0·163	0·862	0·290
Temperature × CO$_2$	0·682	0·107	0·871	0·517	0·481	0·310	0·195	0·587	0·657
Within subjects									
Period (Pd)	**< 0·001**	**< 0·001**	**< 0·001**	**0·046**	**< 0·001**	**0·038**	**< 0·001**	**< 0·001**	**< 0·001**
Pd × C	0·653	0·817	0·725	0·307	0·388	0·607	0·893	0·745	0·429
Pd × T	0·818	**0·082**	0·137	0·201	0·214	**0·016**	**0·053**	0·168	0·647
Pd × C × T	0·909	**0·003**	0·896	0·974	0·982	0·942	0·738	0·870	0·834

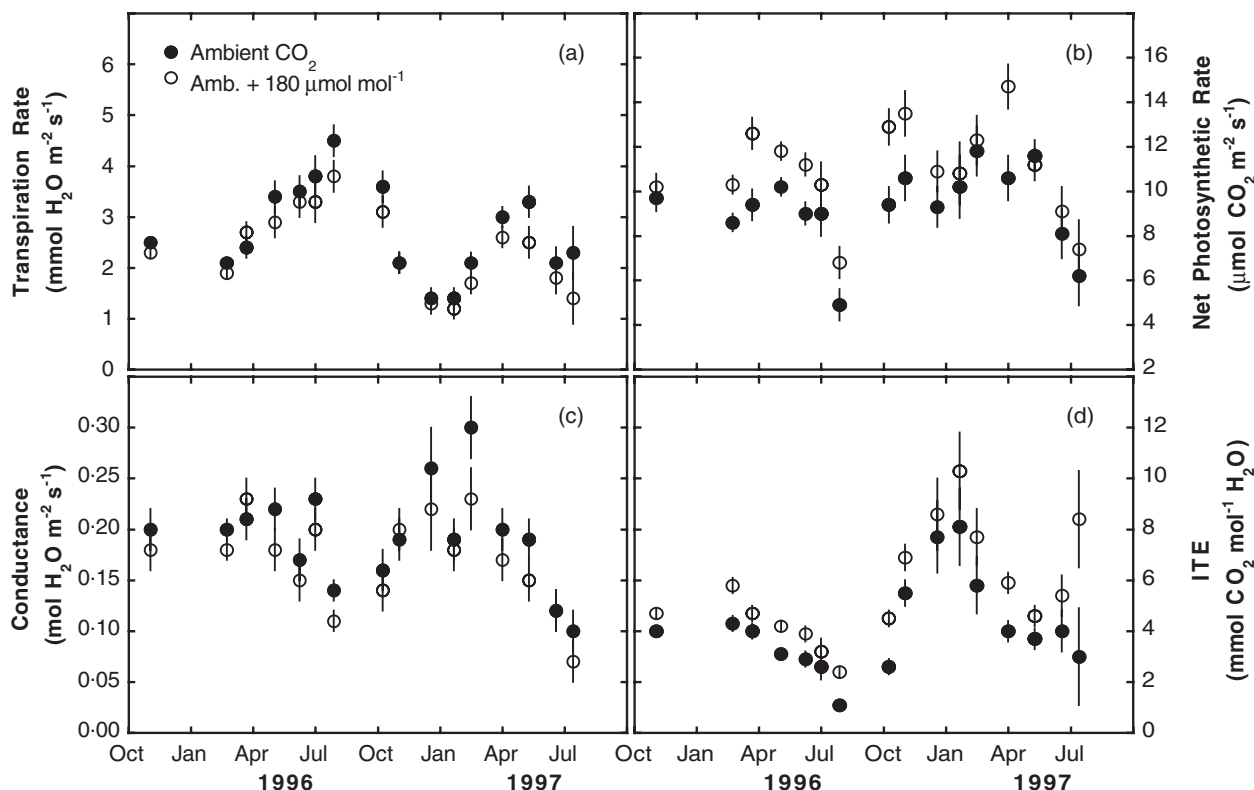[a]Data from August 1996 excluded (see Results).

Figure 2. Effect of CO_2 supply on monthly patterns of transpiration rates (a), net photosynthetic rates (b), conductance (c), and instantaneous transpiration efficiency (ITE; d) for the 1995 and 1996 needle cohorts. Data are combined across temperature treatments. The ambient CO_2 treatment tracked ambient $[CO_2]$ at an adjacent meteorological station; actual $[CO_2]$ in the elevated CO_2 treatment was ~180 μmol mol^{-1} higher on average across the study period. Measurements between November 1995 and July 1996 were made on the 1995 needle cohort, whereas the 1996 needle cohort was measured between August 1996 and July 1997. $n = 6$ for both treatments for the 1995 cohort; for the 1996 cohort, $n = 5$ for both treatments.

There was a significant interaction between CO_2 supply, temperature treatment and measurement period on E of the 1996 cohort (Table 1). This interaction reflected a significant interaction between CO_2 supply and temperature treatment during the August 1996 measurement period. At this measurement period, elevated $[CO_2]$ was associated with significantly lower E in the ambient temperature treatment (means ± SE: ACAT = 5·0 ± 0·4; ECAT = 1·9 ± 0·5), but E did not significantly vary between CO_2 treatments in the elevated temperature treatment (ACET = 4·0 ± 0·4; ECET = 5·6 ± 0·4). Excluding the August 1996 data, there were no significant interactions between CO_2 treatment, temperature treatment or measurement period on E of the 1996 cohort (Table 1), and the EC treatment was associated with a significant reduction in E of the 1996 cohort, paralleling the results for the 1995 cohort (Fig. 2).

Across CO_2 treatments, the elevated temperature (ET) treatment was associated with a significant increase in E of the 1995 cohort (Fig. 3, Table 1). Excluding August 1996 (see above), the ET treatment was similarly associated with a significant increase in E of the 1996 cohort. The ET treatment was also associated with a significant increase in net

photosynthetic rates across the study period, although ITE did not significantly vary between temperature treatments. Across both years, there were significant interactions between growth temperature and measurement period on g_s. Stomatal conductance was higher in the ET treatment at 11 of 16 measurement periods, but either did not vary between temperature treatments or was lower in the ET treatment during the other five periods. There were no other significant interactions between CO_2 treatment, temperature treatment or measurement period on any parameter.

The combined effects of elevated $[CO_2]$ and elevated temperature on E, g_s, net photosynthetic rates and ITE are shown in Fig. 4. Across both cohorts, elevated $[CO_2]$ and elevated temperature significantly increased E, net photosynthetic rates and ITE, but did not significantly affect g_s, compared with seedlings in the ACAT treatment.

Treatment effects on g_s paralleled the effects on net photosynthetic rates, based on results from fitting the data to the Ball *et al.* (1987) model. Neither $[CO_2]$ supply nor temperature treatment significantly affected model parameters, and there were no significant treatment interactions
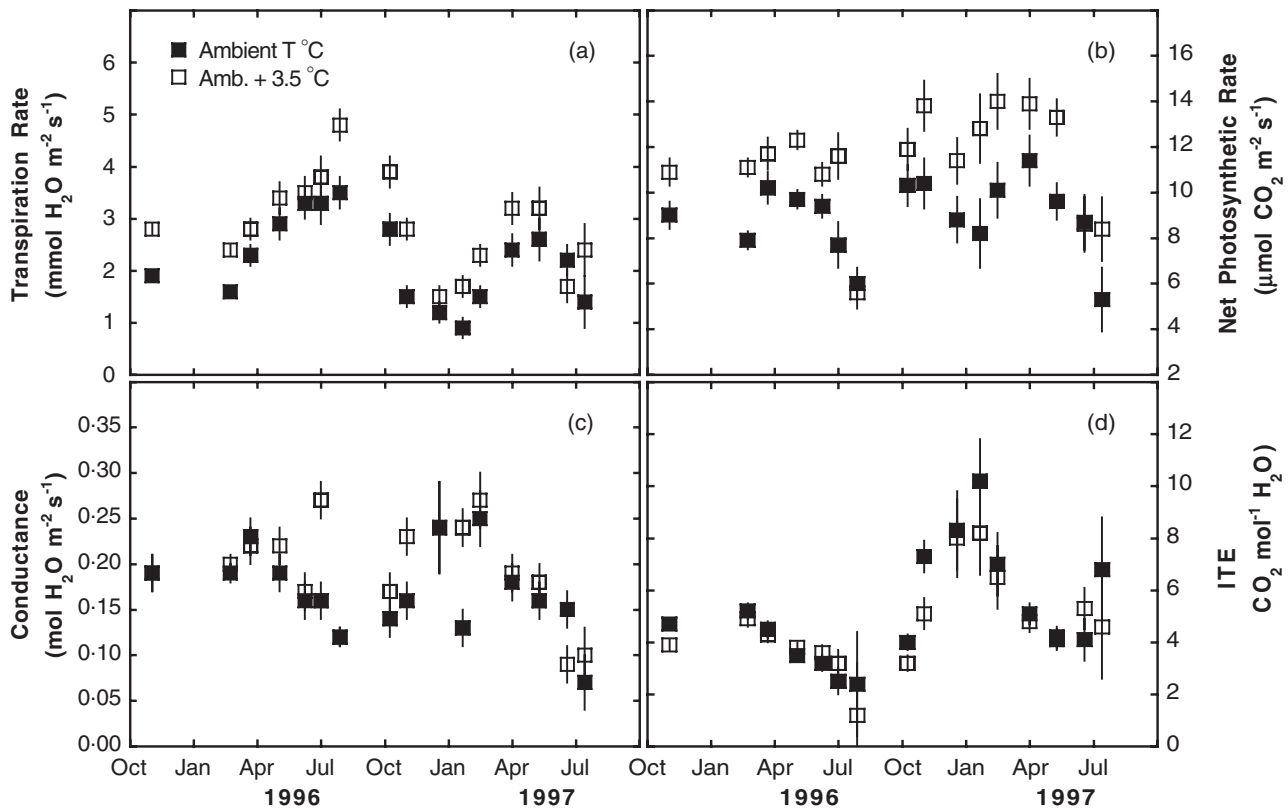
Figure 3. Effect of growth temperature on monthly patterns of transpiration rates (a), net photosynthetic rates (b), conductance (c), and instantaneous transpiration efficiency (ITE; d) for the 1995 and 1996 needle cohorts. Data are combined across CO_2 treatments. The ambient temperature treatment tracked the ambient air temperature at an adjacent meteorological station. Across the study period, actual air temperatures in the elevated temperature treatment were 3·5 °C higher on average compared to the ambient temperature treatment. Measurements between November 1995 and July 1996 were made on the 1995 needle cohort, whereas the 1996 needle cohort was measured between August 1996 and July 1997. $n = 6$ for both treatments for the 1995 cohort; for the 1996 cohort, $n = 4$ for the Ambient treatment, $n = 6$ for the Amb. + 3·5 °C treatment.

($P > 0·196$ in all cases, data not shown). Sensitivity of g_s to D, assessed by examining changes in D_0, did not significantly vary between CO_2 or temperature treatments ($P > 0·360$ in all cases), and the absolute or relative responses of g_s to elevated [CO_2] or temperature did not significantly vary with D (Fig. 5). As a result, CO_2 supply and temperature treatment did not affect seasonal patterns in g_s (Figs 2c & 3c) or E (Figs 2a & 3a), which generally reflected seasonal changes in D (Fig. 1b). Seasonal changes in D also accounted for 31% of the variability in the absolute response of E to elevated [CO_2] ($P = 0·014$; adjusted $r^2 = 0·312$; Fig. 6). As D increased, the absolute reduction in E associated with elevated [CO_2] increased. This occurred because E increased with increasing D, but the relative effect of [CO_2] on E did not significantly vary with D ($P = 0·215$). There were no significant effects of D on the absolute or relative responses of E or g_s to elevated temperature ($P \geq 0·170$ in all cases). Similarly, across measurement periods, temperature treatment did not significantly affect the mean relative responses of E and g_s to elevated [CO_2] ($P \geq 0·120$ in both cases).

DISCUSSION

Growth in ambient + 180 μmol mol^{-1} CO_2 decreased transpiration rates of Douglas-fir needles an average of 12% across the third and fourth years of exposure compared to seedlings grown in ambient [CO_2]. Stomatal conductance was reduced 8% on average by growth in elevated [CO_2] across the third year of exposure. Across the fourth year, g_s was 14% lower on average in the elevated compared to the ambient CO_2 treatment, but this difference was not statistically significant in part due to smaller sample sizes and larger variability compared with the third year. Elevated [CO_2] did not affect the relationship between g_s and net photosynthetic rates. Although elevated [CO_2] was associated with a significant increase in net photosynthetic rates, photosynthetic acclimation in these seedlings reduced the relative response to elevated [CO_2] (Lewis *et al.* 1999). The results of the present study suggest that g_s and photosynthesis acclimated in parallel to elevated [CO_2], consistent with the findings of other studies (Medlyn *et al.* 2001). In addition, stomatal responses to CO_2 supply were not
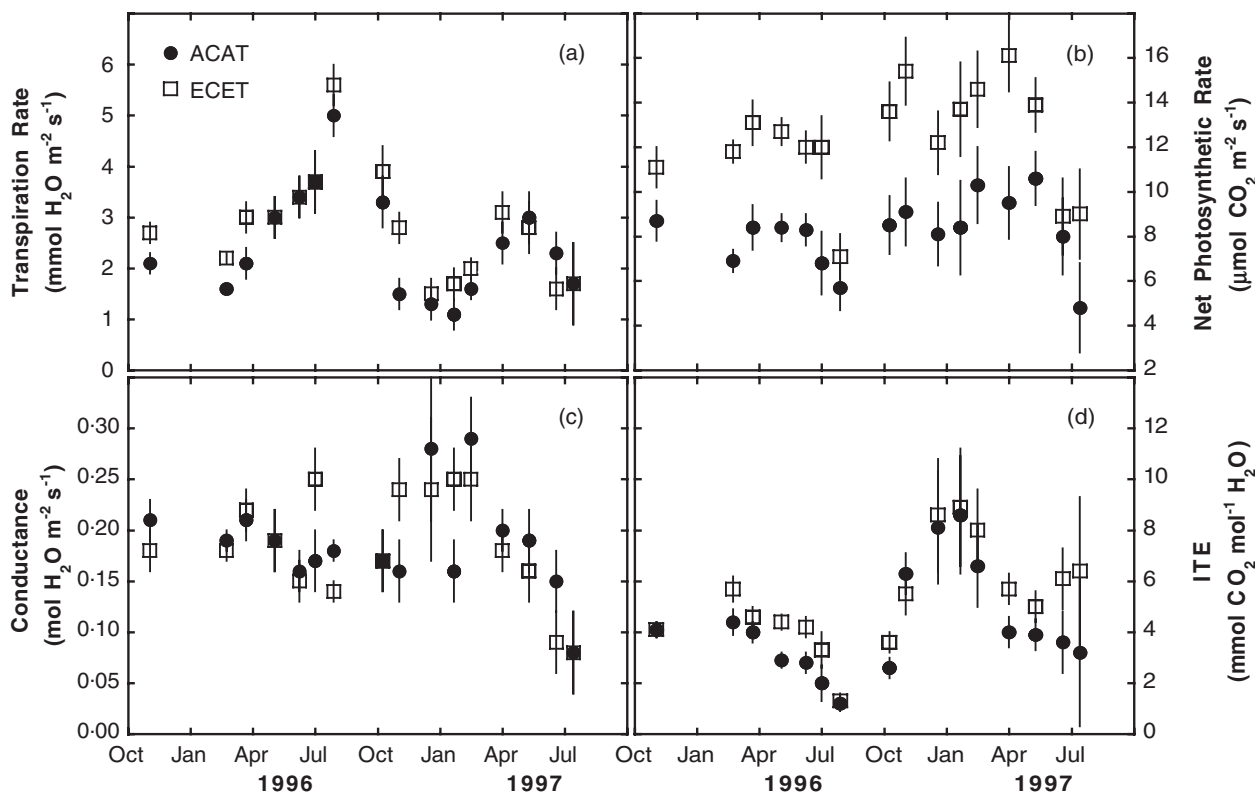
Figure 4. Combined effects of elevated $[CO_2]$ and elevated growth temperature on monthly patterns of transpiration rates (a), net photosynthetic rates (b), conductance (c), and instantaneous transpiration efficiency (ITE; d) for the 1995 and 1996 needle cohorts. Measurements between November 1995 and July 1996 were made on the 1995 needle cohort, whereas the 1996 needle cohort was measured between August 1996 and July 1997. $n = 3$ for both treatments for the 1995 cohort; for the 1996 cohort, $n = 2$ for the ACAT treatment, $n = 3$ for the ECET treatment.

affected by changes in stomatal sensitivity to *D*. Similarly, the CO_2 supply did not significantly affect either the number or density of stomates in these seedlings (Apple *et al*. 2000).

The relatively small effect of elevated $[CO_2]$ on g_s was consistent across temperature treatments, and is consistent with other studies on conifers grown in elevated $[CO_2]$, which generally show reductions of 15% or less (Saxe *et al*. 1998; Norby *et al*. 1999; Medlyn *et al*. 2001). For example, g_s in Douglas-fir seedlings did not significantly vary between ambient or elevated $[CO_2]$ after 120 d of exposure (Hollinger 1987), paralleling the results of the 1997 hydrologic year in this study. Because conifers often show smaller responses to elevated $[CO_2]$ compared to herbaceous plants and deciduous trees (Saxe *et al*. 1998; Medlyn *et al*. 2001), predictions based on these other groups may overestimate the long-term effects of elevated $[CO_2]$ on g_s and *E* in Douglas-fir and other coniferous forests (Ellsworth 1999; Norby *et al*. 1999).

The relatively uniform effect of elevated $[CO_2]$ on g_s across the study period and the lack of $[CO_2]$ effect on stomatal sensitivity to *D* resulted in significant seasonal variability in the absolute response of *E* to elevated $[CO_2]$. In general, the absolute reduction in *E* associated with

elevated $[CO_2]$ increased with seasonal increases in *D* (Fig. 6). This response to $[CO_2]$ is consistent with other studies that have shown that elevated $[CO_2]$ has a larger effect on *E* under conditions where *E* is high compared with the conditions where *E* is low (Sage 1994; Kellomäki & Wang 1996; Tissue *et al*. 1997; Will & Teskey 1997; Bunce 2000; Medlyn *et al*. 2001). However, high temperatures or *D* may induce stomatal closure in Douglas-fir (Leverenz 1981; Livingston & Black 1987), minimizing the effects of elevated $[CO_2]$ on *E*. As a result, and because elevated $[CO_2]$ did not significantly affect the *D* at which stomates closed, elevated $[CO_2]$ may not affect *E* in Douglas-fir during the period of the year when water is most limiting. In addition, increasing temperature associated with climate change may result in more days each year where temperature or *D* is high enough to induce stomatal closure. Furthermore, if the increase in temperature associated with climate change varies across the year (e.g. the increase in temperature is greater in winter in comparison with summer), then the effect on needle-level water loss should similarly vary across the year.

In contrast to the relatively small effect of elevated $[CO_2]$ on transpiration rates, growth in ambient +3·5 °C increased *E* by an average of 37% across both years. Increasing tem-

perature increased E due to changes in both g_s and D. Although there was substantial variation in the response of g_s to temperature treatment, g_s was increased 17% on average across both years by growth in the elevated temperature treatment. Additionally, although water vapour in the air was regulated during this study to minimize temperature treatment effects on D, increasing air temperature increased the D by 0·3 kPa, increasing the driving gradient for water loss from leaves to the atmosphere (Lambers *et al.* 1998). Differences in D between temperature treatments may also account for treatment differences in g_s, but temperature may directly affect g_s in Douglas-fir (Living-
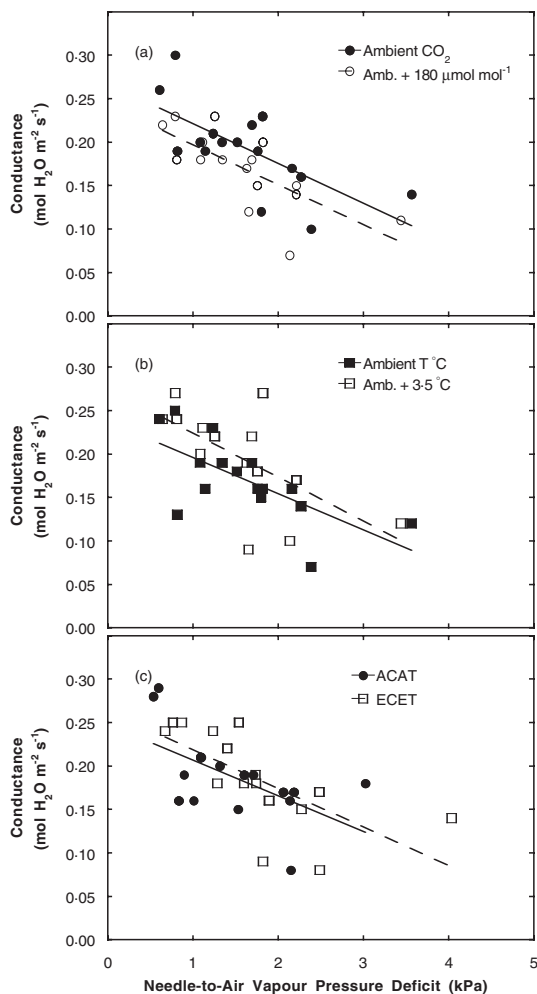
Figure 5. Stomatal sensitivity to D across [CO_2] (a) and temperature treatments (b), and between the ACAT and ECET treatments (c). Equations for the relationships between g_s and D are: $g_s = 0·267 − 0·046 * D$ (ambient [CO_2]); $g_s = 0·243 − 0·046 * D$ (elevated [CO_2]); $g_s = 0·238 − 0·042 \times D$ (ambient temperature); $g_s = 0·275 − 0·051 \times D$ (elevated temperature); $g_s = 0·248 − 0·041 \times D$ (ACAT); and, $g_s = 0·263 − 0·044 \times D$ (ECET). The sensitivity of g_s to D did not significantly vary between CO_2 or temperature treatments, and there were no significant effects of D on the absolute or relative responses of g_s to elevated [CO_2] or temperature ($P \geq 0·498$ in all cases).
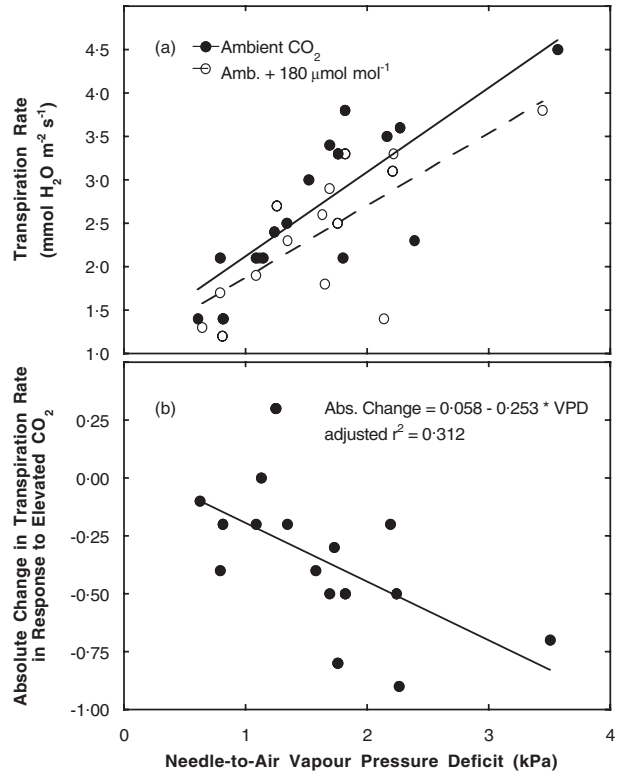

Figure 6. The effect of elevated [CO_2] on the relationship between transpiration rates and D (a), and the effect of D on the absolute response of transpiration rates to elevated [CO_2] (b). Equations for the relationship between transpiration rate and D are: $E = 1·142 + 0·971 \times D$ (ambient [CO_2]); and, $E = 1·038 + 0·831 \times D$ (elevated [CO_2]).

ston & Black 1987). As the effects of elevated temperature on g_s and D were relatively uniform across seasons, differences in E between temperature treatments were also relatively uniform across seasons. The consistent increase in E associated with growth in elevated temperature suggests that even relatively small increases in temperature due to climate change may substantially increase the needle-level water loss from Douglas-fir seedlings.

Increasing temperature associated with climate change may also offset the beneficial effects of elevated [CO_2] on needle water loss. In this study, the relative effect of elevated temperature on E was uniform across [CO_2] treatments, and was larger than the effect of elevated [CO_2]. As a result, in comparison with ambient conditions, the combined effects of elevated [CO_2] and elevated temperature resulted in a 19% increase on average in E across the 21 month study period. The counteracting effects of elevated temperature and elevated [CO_2] on needle water loss are consistent with general patterns observed in other studies (Kellomäki & Wang 1996; Koike *et al.* 1996; Tjoelker *et al.* 1998; Wayne *et al.* 1998). The extent to which increasing temperature associated with climate change offsets the effect of increasing atmospheric [CO_2] on E is likely to reflect the relative magnitude of the increases in [CO_2] and

temperature, direct effects of changes in g_s on needle temperature, effects of diurnal and seasonal patterns of temperature and D, as well as the potential organism-based differences discussed above. These sources of variability may also account for some of the variability observed between studies on the interactive effects of temperature and $[CO_2]$ on E (Morison & Lawlor 1999).

Differences between studies in effects of elevated $[CO_2]$ on needle water loss may also reflect the differential responses of leaf temperature to changes in g_s (Morison & Lawlor 1999). Decreasing g_s associated with elevated $[CO_2]$ may lead to increased leaf temperature, increasing the driving gradient for water loss from the leaf (Jarvis *et al*. 1999). As a result, elevated $[CO_2]$ may have little or no effect on E when this effect is factored in. In this study, the effect of elevated $[CO_2]$ on E in Douglas-fir may be overestimated because needle temperatures were manipulated during gas exchange measurements to minimize differences in needle temperatures between CO_2 treatments. However, it is unlikely that needle temperatures varied substantially between $[CO_2]$ treatments *in situ*, because the effect of elevated $[CO_2]$ on g_s was small and because needle morphology minimizes temperature increases above ambient air temperature. Similarly, increasing $[CO_2]$ is likely to have relatively small effects on needle temperature in other conifers because of the relatively small responses of g_s to elevated $[CO_2]$ in most conifers and because of similar needle morphologies. The effects of increasing atmospheric $[CO_2]$ on the inter-relationships between g_s, E and regulation of leaf temperature by water loss have been examined for crop plants (Idso *et al*. 1993), but not for trees (Norby *et al*. 1999).

Potential effects of climate change on whole-plant water use due to changes in needle-level transpiration may be mediated by a suite of factors, including climate change effects on morphology and water availability (Field *et al*. 1995). In this study, the elevated temperature decreased total needle biomass (Olszyk *et al*. 1998b), which would tend to reduce effects of increasing temperature on total plant water use compared with needle-level responses. Elevated $[CO_2]$ did not significantly affect either area per individual needle (Apple *et al*. 2000), or total needle area per plant (Olszyk *et al*. unpublished). Hydraulic architecture may also change in response to increasing atmospheric $[CO_2]$ (Atkinson & Taylor 1996; Heath, Kerstiens & Tyree 1997; Pataki, Oren & Tissue 1998) and temperature (Maherali & DeLucia 2000). These changes may influence plant-level water use responses to climate change, and may either offset or enhance leaf-level responses (Pataki *et al*. 1998; Maherali & DeLucia 2000). Crown architecture may affect the link between needle-level and plant-level responses through effects on light availability within the crown, through boundary layer effects, and effects on rainfall penetration to the forest floor (Pataki *et al*. 1998; Norby *et al*. 1999). In this study, g_s and E probably represent maximum values for a given measurement period because all needles were grown and measured under light-saturating conditions. In addition, water was added directly to the soil

surface, so the canopy structure did not affect water availability. Although the amount and timing of water additions were uniform across treatments in this study, climate change may alter the temporal patterns of water availability through effects on root production or the timing and extent of rainfall (Field *et al*. 1995). These changes may also affect the extent to which changes in needle-level water loss affects plant water relations.

The contrasting effects of elevated $[CO_2]$ and elevated temperature on E resulted in differing effects on ITE despite similar effects on net photosynthetic rates. Reductions in E associated with elevated $[CO_2]$, coupled with increases in net photosynthetic rates, increased ITE an average of 46% compared to the ambient CO_2 treatment across the 21 month study period. Increases in leaf-level ITE with increasing $[CO_2]$ have been observed in many studies (e.g. Eamus 1991; Ceulemans & Mousseau 1994; Teskey 1995). For example, Hollinger (1987) found that growth in elevated $[CO_2]$ for 120 d increased ITE by an average of 37% in Douglas-fir. In contrast to the effects of elevated $[CO_2]$ on ITE, elevated temperature did not significantly affect ITE because increased E associated with growth in elevated temperature offset the stimulatory effect of elevated temperature on net photosynthetic rates. As the growth in elevated temperature did not significantly alter ITE, the combined effects of elevated temperature and elevated $[CO_2]$ paralleled the direct effects of elevated $[CO_2]$, and suggest that climate change may increase ITE in Douglas-fir at the needle level. However, as discussed above, increased E associated with the combined effects of increasing temperature and $[CO_2]$ may result in increased water loss from leaves, so that although water use efficiency may increase, water loss may also increase.

In summary, the combined effects of growth in elevated $[CO_2]$ and elevated temperature increased E by an average of 19% compared with growth in ambient conditions, and had essentially no effect on g_s. These results are significant because they suggest that increases in temperature associated with climate change may more than offset potential benefits of elevated $[CO_2]$ on needle-level water loss from Douglas-fir. As a result, increasing temperature associated with climate change may increase the length and severity of the summer drought period in the Pacific North-west. In addition, the interactive effects of elevated $[CO_2]$ and seasonal changes in temperature on E suggest that climate change may substantially alter seasonal patterns in needle-level water loss from Douglas-fir seedlings. However, summer and winter temperature extremes may moderate the effects of climate change on needle-level water use by inducing stomatal closure. Also, the effects of climate change will reflect changes in D, which may be smaller or larger than the 0·3 kPa increase, on average, in the elevated temperature treatment in this study. At the plant level, the effect of climate change on water loss will reflect the interactions between needle-level responses and potential changes in plant morphology and water availability, as well as the relative magnitudes of changes in $[CO_2]$ and temperature.

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