

Dynamics of Changing Intercellular CO₂ Concentration (c_i) during Drought and Determination of Minimum Functional c_i

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Nine conifer species with narrow (<5 mm), single-veined leaves were selected for the purpose of examining changes in intercellular CO₂ concentration (c_i) during drought. Due to the leaf morphology of the study plants, the confounding effects of nonhomogenous photosynthesis common to most reticulate-veined angiosperms were largely avoided, giving a clear picture of c_i dynamics under increasing drought. A characteristic biphasic response was observed in all species, with an initial stomatal control phase resulting in a substantial reduction in c_i as stomatal conductance (g_s) decreased. As g_s reached low levels, a strong nonstomatal limitation phase was observed, causing c_i to increase as g_s approached a minimum. This nonstomatal phase was linked to a concomitant rapid decrease in the fluorescence parameter quantum efficiency, indicating the onset of nonreversible photoinhibition. The ratio of internal to atmospheric CO₂ concentration (c_i/c_a) decreased from values of between 0.68 and 0.57 in undroughted plants to a minimum, (c_i/c_a)_{min}, which was well defined in each species, ranging from 0.10 in *Actinostrobus acuminatus* to 0.36 in *Acropyle pancheri*. A high correlation was found to exist between (c_i/c_a)_{min} and leaf water potential measured at (c_i/c_a)_{min}. Species developing high maximum intrinsic water use efficiencies (low [c_i/c_a]min), such as *A. acuminatus*, did so at lower leaf water potentials (−4.5 MPa) than more mesic species (−1.75 MPa for *A. pancheri*). It is concluded that in the absence of patchy stomatal closure, (c_i/c_a)_{min} gives a good representation of the drought tolerance of foliage.

It has long been established that during drought, g_s decreases, reducing water loss from the leaf (Slatyer, 1967). In the absence of other effects, such a decrease in conductance will produce a CO₂ “supply” limitation of photosynthesis (Farquhar and Sharkey, 1982) by causing the c_i to become depleted. Recently, however, the importance of reduced g_s in limiting A during drought has been questioned. Many authors have described “nonstomatal” inhibition of photosynthesis as an important influence during drought, attributing this to impaired chloroplast function in leaves subject to water deficits (Boyer, 1971; Graan and Boyer, 1990; Majumdar et al., 1991; Gunasekera and Berkowitz, 1993).

Nonstomatal inhibition of photosynthesis has been commonly invoked to explain results of gas-exchange studies showing unchanging or increasing c_i in the leaves of droughted plants and a strong depression in the relationship between A and c_i (Ehleringer and Cook, 1984; Wong et

al., 1985; Grieu et al., 1988; VanRensberg and Kruger, 1993; Anderson et al., 1995). The suggestion that during drought nonstomatal effects become important soon after ψ_1 begins to drop is not consistent, however, with data obtained in fluorescence studies, which indicate that leaf photochemistry is resistant to moderate drought stress (Sharkey and Badger, 1982; Genty et al., 1987; Cornic et al., 1989) and that during drought an increased flow of electrons to O₂ reflects decreasing c_i (Cornic and Briantais, 1991). Integrated measures of c_i , such as carbon isotope discrimination in leaves, also suggest that c_i is lower in water-stressed plants (Smith and Osmond, 1987; Hubick et al., 1988; Ehleringer et al., 1992; Donovan and Ehleringer, 1994). This disparity between gas-exchange data and other measures of c_i has been attributed to patchy stomatal closure in response to drought.

Implicit in the calculation of A and g_s from gas-exchange parameters is uniform stomatal aperture on the leaf being measured (Farquhar and Sharkey, 1982). In the case of patchy stomatal closure, the whole A(c_i) relationship is erroneously depressed, leading to the conclusion that photosynthesis is being directly inhibited (Terashima et al., 1988). Heterogeneity in the distribution of photosynthesis has been observed in most species examined (Pospisilova and Santrucek, 1994) and appears to be associated with the reticulate venation typically found in broad-leaved angiosperms. This type of venation tends to subdivide the leaf lamina, especially in the case of heterobaric leaves, in which bundle-sheath extensions from the veins to the epidermis compartmentalize the leaf into small aereoles. Studies of chlorophyll fluorescence (Downton et al., 1988; Cardon et al., 1994; Siebke and Weis, 1995) and radiolabeling (Terashima et al., 1988) have shown that during drought or ABA application, groups of aereoles can be actively photosynthetic while adjacent parts of the leaf are not measurably assimilating. This spatial heterogeneity in leaf photosynthesis has prevented an accurate understanding of the dynamics of changing c_i during drought.

The aim of this study was to measure changes in c_i and A during drought in species with a low potential for nonuniform gas exchange. By using functionally amphiph-

Abbreviations: A, assimilation; c_a , ambient CO₂ concentration; c_i , intercellular CO₂ concentration; (c_i/c_a)_{min}, minimum c_i/c_a attained during drought; F_v/F_m , quantum efficiency; g_s , stomatal conductance; ψ_1 , leaf water potential.

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tomatous species with single-veined leaves < 5 mm in width, the problem of leaf subdivision was avoided and the potential for rapid lateral diffusion of CO₂ throughout the leaf was maximized. Potential patchy effects were also avoided by slowly and evenly inducing drought (Gunasekera and Berkowitz, 1992; Wise et al., 1992). These measurements allowed determination of a minimum attainable c_i/c_a during drought, a parameter that could be compared between species and that reflects the relative drought tolerance of the species examined.

MATERIALS AND METHODS

Plant Material

Cuttings of the conifers *Acropyle pancheri* (Brongn. and Glis) Pilger, *Diselma archerii* Hook.f., *Lagarostrobos franklinii* (Hook.) C.J.Quinn, *Podocarpus lawrencii* Hook.f., and *Microstrobos niphophilus* Garden and Johnson, and seed from *Actinostrobus acuminatus* Parlatore, *Athrotaxis selaginoides* D.Don, *Callitris rhomboidea* R.Br., *Dacrycarpus dacrydioides* (Rich.) de Laubenfels, and two angiosperms, *Eucalyptus tenuiramis* Miq. and *Acacia melanoxylon* R.Br., were propagated in sand in Hobart (Tasmania, Australia). Seedlings and rooted cuttings were transferred to a pine-bark potting mix in large (3 L) pots and grown under ambient glasshouse light and temperature. A minimum of three individuals per species were used in experimental work.

During droughting, water was withheld from plants, causing a gradual decrease in soil water content (due to the large pot size and relatively small plants). Water was withheld until the dark-adapted F_v/F_m was approximately 0.7, at which point plants were at their maximum drought tolerance (see "Results"). This took between 2 and 4 weeks, during which time gas-exchange measurements were taken every 1 to 5 d depending on desiccation rate.

Gas Exchange

Fluxes of CO₂ and water vapor were measured on leaves using an open-flow system. An ADC mk. 3 (Hoddleson, UK) IR gas analyzer was used to measure CO₂ differentials between cuvette and reference air, and a modified Dew-10 dew-point hygrometer (General Eastern, Waterton, MA) was used to monitor water vapor content of pre- and postcuvette air.

Air was supplied from compressed air cylinders, and different partial pressures of CO₂ were produced by removing different proportions of CO₂ from the air during tank fills. In undroughted plants, c_a was varied from 360 to 160 $\mu\text{mol mol}^{-1}$ to give c_i values within the range likely to be found in the field, and photosynthetic CO₂ compensation concentration was measured by recirculating air through the leaf chamber, allowing the leaf to draw down c_a until net CO₂ uptake ceased. Measurements during drought were made with c_a held constant at approximately atmospheric concentration ($360 \pm 15 \mu\text{mol mol}^{-1}$). The dew point of the influx air was maintained at 0°C by passing air over water at 0°C or at around 8°C by passing air through a column of hydrated ferrous sulfate, resulting in leaf to air vapor pressure deficits of 8 to 10 mbar bar^{-1} .

The leaf cuvette was constructed from ultra-high-density polyethylene and glass, and all tubing was stainless steel so as to minimize adsorption of water vapor and CO₂. Gas-flow rates were generally 300 to 400 mL min^{-1} and the volume of the cuvette was 20 mL. At these relatively high flow rates, the boundary layer conductance of the leaves, which were generally >3 mm in width, was at least 1 order of magnitude greater than the maximum leaf conductance.

Leaf temperature was maintained at $20 \pm 1^\circ\text{C}$ by pumping water from a controlled-temperature water bath through a jacket surrounding the cuvette and was measured by a thermocouple placed in contact with the abaxial surface of the leaf. The light source was a 24-V, 250-W tungsten lamp that provided 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to the leaf surface. Leaf area was measured using a digital camera (EDC-1000, Electrim, Princeton, NJ) to determine the projected area of foliage in the chamber.

Calculation of gas-exchange parameters were made, according to von Caemmerer and Farquhar (1981), with c_i corrected for water vapor efflux from the leaf.

ψ_1

Measurements of ψ_1 were carried out immediately after gas-exchange readings using a Scholander (Commonwealth Scientific and Industrial Research Organization, Hobart, Tasmania, Australia) pressure bomb. Due to the destructive nature of the Scholander technique and the small amount of plant material available, ψ_1 could be determined only once or twice per plant. These measurements were made as c_i approached a minimum, enabling the ψ_1 to be defined at minimum c_i .

Fluorescence

In one species (*Podocarpus lawrencii*), the optimum F_v/F_m was measured to determine nonreversible photochemical inhibition during drought. Measurements were made on every second plant after the completion of gas-exchange readings. This involved dark adapting whole plants for 3 h (to ensure complete oxidation of the plastoquinone pool) before F_v/F_m readings were taken. Fifteen leaves from each plant were measured, using a PSM mk. 2 fluorometer (Biomonitor, Umeå, Sweden).

Stomatal Aperture

Stomatal aperture was measured every 3 d during the drought of a single *Acacia melanoxylon* plant. It was not possible to use *Eucalyptus* or any of the conifers, since the sunken nature of the stomata did not allow accurate impressions to be made of the guard cells. Leaves were detached after measurement of gas exchange and epidermal impressions were made by immediately coating the abaxial surface of the leaf with nail varnish (cellulose acetate). Closed stomata were easily distinguished from open or partially open stomata when impressions were examined under the light microscope, so it was possible to measure the percentage of closed stomata in leaves subject to increasing drought.

RESULTS

Gas Exchange and Fluorescence in *P. lawrencii*

Within the range of c_i produced by varying c_a from compensation to atmospheric concentration, a linear regression ($r^2 = 0.935$) described the A versus c_i response curve for well-watered *P. lawrencii* (Fig. 1). At an ambient CO_2 concentration of $360 \mu\text{mol mol}^{-1}$ (approximately atmospheric), c_i for *P. lawrencii* was $205 \pm 5 \mu\text{mol mol}^{-1}$.

Once water was withheld, g_s and A decreased slowly over the 1st week. Generally, gas exchange was found to drop sharply during the 2nd week of drought, continuing at low values during the 3rd and 4th weeks. Figure 2 illustrates this trend in a single replicate. Accompanying the decrease in gas exchange was a steep decline in c_i , typically from around $205 \mu\text{mol mol}^{-1}$ (c_i/c_a of around 0.6) on the 1st d of water stress, to minimum values between 71 and $88 \mu\text{mol mol}^{-1}$ (Fig. 1). c_i/c_a is expressed in preference to c_i since it accounts for variation in c_a (Fig. 2c). Minimum values of c_i were typically reached after 20 to 28 d without water, at which point ψ_1 was -3.0 MPa and c_i was quite close to the compensation value of $53 \mu\text{mol mol}^{-1} \text{ CO}_2$. Until the point of minimum c_i there was no difference in carboxylation efficiency between droughted and undroughted plants (Fig. 1), indicating that nonstomatal limitation was not significantly increased. Only when A and g_s reached very low levels was there any deviation from the unstressed carboxylation curve, indicating a rapid increase in nonstomatal limitation. This can be seen in Figure 1, where c_i approaches and then exceeds c_a as assimilation approaches 0. This rapid increase in nonstomatal limitation occurred when ψ_1 fell below -3.0 MPa .

Figure 3a shows the response of c_i/c_a to decreasing g_s during drought. The curve shown in Figure 3a represents

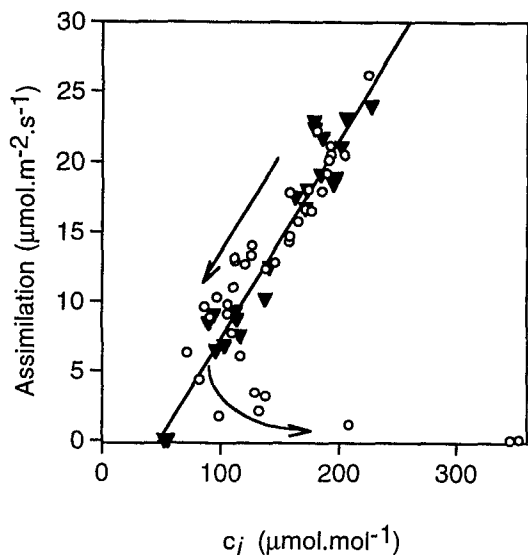


Figure 1. The relationship between A and c_i in undroughted *P. lawrencii* (itrif), with c_a ranging from compensation to atmospheric concentration, and droughted plants (O), with c_a constant at $360 \mu\text{mol mol}^{-1} \text{ CO}_2$. A linear regression ($r^2 = 0.935$) was used to fit the data from undroughted plants. Arrows show the progression of A and c_i during droughting.

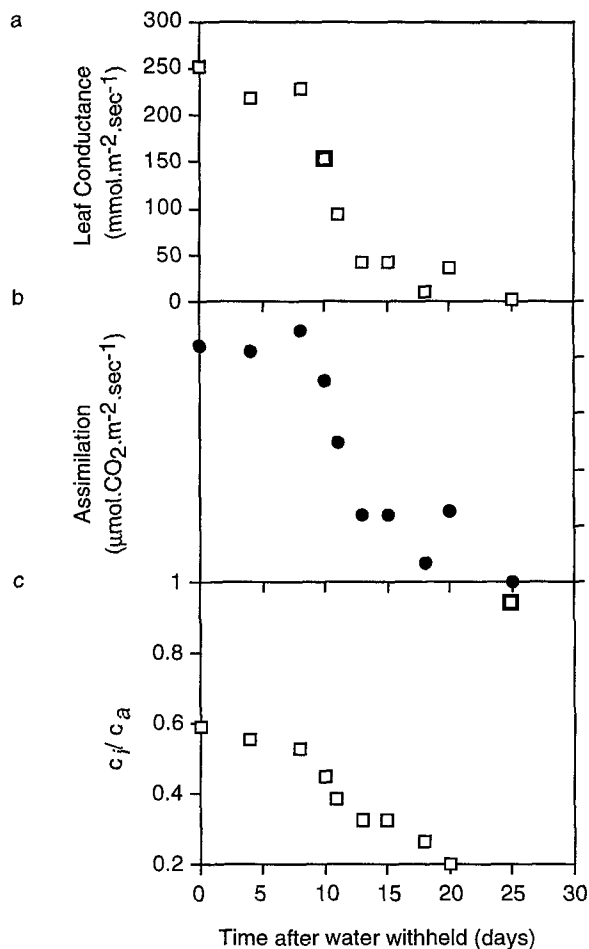


Figure 2. Decreasing g_s (a), A (b), and c_i/c_a (c) in a single replicate of *P. lawrencii* during droughting. Minimum g_s in plants was generally reached 25 to 30 d after water was withheld.

the expected response if assimilation was limited only by g_s , i.e. nonstomatal factors were insignificant. Points falling at increasing distances above this curve reflect an increasing component of nonstomatal limitation. During the initial stomatal limitation phase between conductances of 320 and $45 \text{ mmol m}^{-2} \text{ s}^{-1}$, a curvilinear relationship was observed between c_i/c_a and g_s . The lowest value of c_i/c_a occurred at a g_s of $45 \text{ mmol m}^{-2} \text{ s}^{-1}$, with c_i/c_a (and hence nonstomatal limitation) increasing rapidly at conductances below this level. Photosynthesis in plants that were allowed to reach this phase of rapidly increasing c_i/c_a did not completely recover after rewating, and several individuals sustained leaf damage.

The average F_v/F_m of *P. lawrencii* leaves from unstressed plants was 0.78 ± 0.008 , which falls within the normal range for unstressed plants (Björkman and Demmig, 1987). During droughting, F_v/F_m decreased only slightly as g_s dropped from 320 to $45 \text{ mmol m}^{-2} \text{ s}^{-1}$. Below this g_s however, F_v/F_m was found to fall rapidly to values below 0.70 (Fig. 3b). In all plants where the average F_v/F_m fell below 0.7 , leaves became necrotic or were shed after rewating.

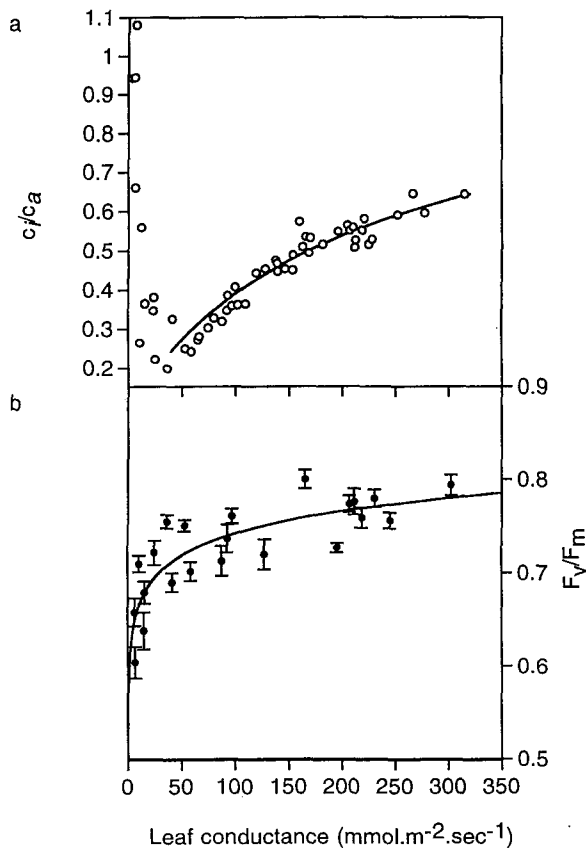


Figure 3. Changing ratios of c_i/c_a (a) and F_v/F_m (b) as g_s declined during drought in *P. lawrencii*. Each value of F_v/F_m represents the average of 15 leaves. A log function has been used to fit the F_v/F_m data ($r^2 = 0.696$). c_i/c_a also decreased logarithmically before reaching a minimum value, c_i/c_a min, increasing markedly thereafter. A line representing the theoretical relationship between conductance and c_i/c_a under conditions in which g_s was the primary factor controlling decreasing photosynthesis is shown using the regression in Figure 1. This has been extended only to the g_s at which c_i/c_a was a minimum.

Other Conifers

The response described above for *P. lawrencii* was characteristic of all the conifers measured. In all species c_i/c_a decreased during drought to $(c_i/c_a)_{min}$ at low g_s . Continued droughting beyond this point resulted in a pronounced increase in c_i/c_a as direct (nonstomatal) limitation of photosynthesis became significant. The minimum c_i/c_a for each species was averaged from at least three replicates (Table I).

A large degree of interspecific variation was observed in $(c_i/c_a)_{min}$, with a maximum value of 0.36 in *A. pancheri* ranging to a minimum of 0.10 in *A. acuminatus*. Minimum c_i/c_a was strongly correlated ($r^2 = 0.976$) with the ψ_1 measured at $(c_i/c_a)_{min}$ (Fig. 4).

Broad-Leaved Angiosperms

In contrast to the conifers, both *A. melanoxylon* and *E. tenuiramis* showed only minor decreases in c_i/c_a during the drought period. There was, however, a similar rapid increase in c_i/c_a as g_s approached 0. As in Figure 3a, the curves in Figures 5 and 6 represent the expected response if decreasing A was a pure function of g_s (insignificant nonstomatal component). The early divergence from this curve as g_s decreased would typically be interpreted as indicating an increase in nonstomatal limitation during the early stages of drought.

Increasing numbers of closed stomata were observed in stomatal impressions from *A. melanoxylon* leaves as a single plant was subject to drought (Fig. 5). Although average stomatal aperture was found to decrease as plants were droughted, closed stomata were clearly distinguished from partially open stomata, and a high proportion of stomata were found to close early during drought, while leaf conductance was still relatively high.

Changes in F_v/F_m in *E. tenuiramis* during drought were similar to those seen in *Podocarpus* (Fig. 3b), with no significant decrease until g_s fell below about $70 \text{ mmol m}^{-2} \text{ s}^{-1}$, at which point a significant depression in quantum efficiency was found to occur.

The ψ_1 immediately prior to the rapid increase in c_i/c_a was -2.9 and -3.20 MPa in *A. melanoxylon* and *E. tenuiramis*, respectively.

Table I. Mean c_i/c_a at approximately atmospheric $[\text{CO}_2]$ for the nine species of conifers used

$(c_i/c_a)_{min}$ refers to the mean minimum c_i/c_a attained during drought. ψ_1 at $(c_i/c_a)_{min}$ represents the lowest value of ψ_1 measured at $(c_i/c_a)_{min}$.

Species	Habitat ^a	c_i/c_a at $c_a = 360 \mu\text{mol mol}^{-1}$	$(c_i/c_a)_{min}$	ψ_1 at $(c_i/c_a)_{min}$
				MPa
<i>A. acuminatus</i>	Dry sandy heathland	0.63 ± 0.0016	0.10	-4.50
<i>C. rhomboidea</i>	Open woodland	0.68 ± 0.0017	0.21	-3.20
<i>P. lawrencii</i>	Wet forest-alpine forest	0.59 ± 0.012	0.21	-3.00
<i>D. archerii</i>	Wet montane-alpine forest	0.57 ± 0.014	0.25	-2.90
<i>M. niphophilus</i>	Wet alpine forest	0.60 ± 0.010	0.26	-2.70
<i>A. selaginoides</i>	Cool-temperate rainforest	0.66 ± 0.0075	0.29	-2.10
<i>L. franklinii</i>	Cool-temperate rainforest	0.65 ± 0.0068	0.30	-2.30
<i>D. dacrydioides</i>	Cool-temperate rainforest	0.68 ± 0.0057	0.33	-1.90
<i>A. pancheri</i>	Tropical rainforest	0.67 ± 0.020	0.36	-1.75

^a Habitat descriptions are from Enright and Hill (1995).

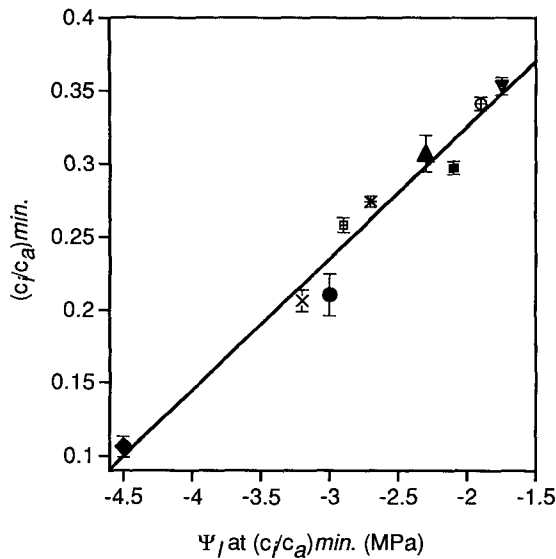


Figure 4. The relationship between c_i/c_a min and ψ_l at c_i/c_a min in the nine conifer species examined: *A. pancheri*, \blacktriangledown ; *A. selaginoides*, \blacksquare ; *A. acuminatus*, \blacklozenge ; *C. rhomboidea*, \times ; *D. dacrydioides*, \oplus ; *D. archerii*, \boxplus ; *L. franklinii*, \blacktriangle ; *P. lawrencii*, \bullet ; *M. nipphophilus*, $*$. A highly significant linear regression is shown ($r^2 = 0.976$). Each point represents the average c_i/c_a min from at least three individuals, and ψ_l at c_i/c_a min represents the water potential reading that corresponded most closely with c_i/c_a min.

DISCUSSION

The combination of narrow, single-vein leaf morphology and the drought regime used in this study should have minimized patchy stomatal closure and photosynthetic heterogeneity, adding a high degree of confidence to c_i calculations presented here. In contrast to previous studies

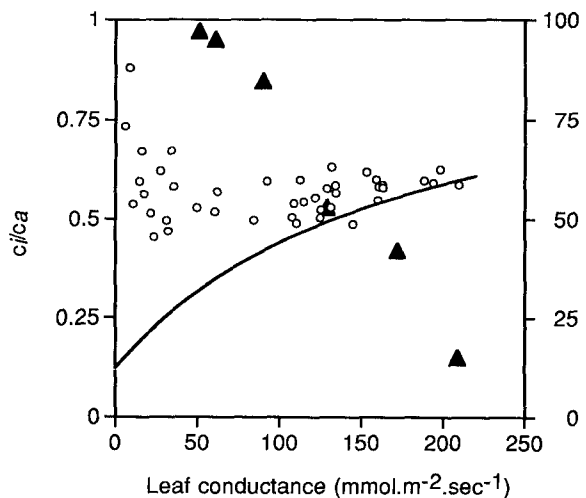


Figure 5. Response of c_i/c_a to decreasing g_s during drought in *A. melanoxylon* (\blacktriangle). The curve shown represents the theoretical response if decreasing g_s explained the decline in photosynthesis (cf. Fig. 3a). Also shown is the percentage of stomata seen to be completely closed at six times during drought intensification in a single *A. melanoxylon* plant (\circ).

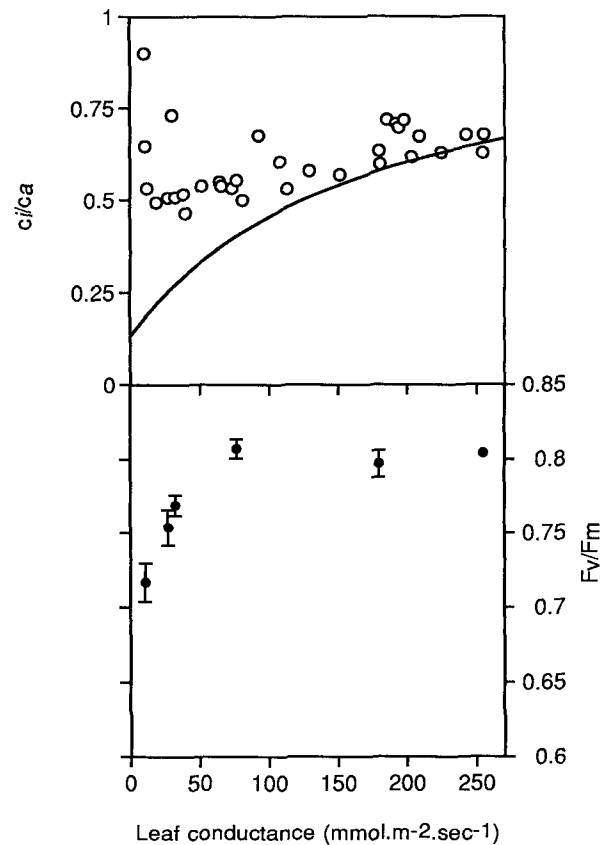


Figure 6. Drought response of c_i/c_a and g_s in *E. tenuiramis*; again the curve represents the theoretical response if decreasing photosynthesis was stomatally driven. Also shown are F_v/F_m data for a single replicate during drought.

(Wong et al., 1985), large variations in c_i were found to occur during drought, with an initial draw-down of c_i to between 25 and 55% of unstressed levels, followed by a rapid increase as ψ_l fell below a critical level.

During the initial "draw-down" phase, there was no evidence of nonstomatal limitation in any of the conifers studied. This is illustrated for *P. lawrencii* in Figure 3a, where the data show no systematic deviation from the theoretical curve produced if photosynthesis were responding only to increasing stomatal limitation of gas exchange (insignificant, nonstomatal component). Nonstomatal factors did, however, become important in plants droughted beyond $(c_i/c_a)_{min}$, producing a sharp increase in c_i/c_a as g_s approached 0 (Fig. 3a). The fluorescence data for *P. lawrencii* also illustrate these two phases in drought response. Initially, F_v/F_m was found to slowly decline in parallel with c_i/c_a , presumably as a result of increased nonphotochemical energy dissipation, since Calvin cycle activity was inhibited by limiting CO_2 concentration in the chloroplast (Cornic et al., 1989; Cornic and Briantais, 1991). As g_s approached 0 and c_i/c_a began to increase, there was a concomitant steep drop in F_v/F_m (Fig. 3b). This indicates a rapid loss of fluorescence from PSII for some reason other than CO_2 limitation and may represent direct inhibition of

photochemistry, Calvin cycle activity, or both. The most likely explanation for this rapid onset of photoinhibition is that plants droughted beyond $(c_i/c_a)_{min}$ sustained damage to the photosynthetic apparatus. This is supported by the fact that these plants did not recover predrought photosynthetic rates, and after 2 to 4 d of continued drought they also showed visual signs of leaf damage. ψ_1 measured at $(c_i/c_a)_{min}$ therefore represents the minimum ψ_1 prior to the onset of irreversible photoinhibition. Values of ψ_1 at $(c_i/c_a)_{min}$ ranged widely from -1.75 to -4.5 MPa, reflecting the wide range of habitats from which different species were collected. In comparison, initial cell damage in rapidly desiccated *Helianthus* leaves was found to occur at a ψ_1 of -1.5 MPa, with the majority of mesophyll cells damaged at -2.6 MPa (Fellows and Boyer, 1978). The leaves of the conifer species used here were clearly more robust than those of *Helianthus*, a fact that is hardly surprising considering the perennial, woody nature of these species.

c_i/c_a is inversely proportional to the "intrinsic" water use efficiency and, hence, $(c_i/c_a)_{min}^{-1}$ represents the maximum water use efficiency attainable during drought. Qualitative comparison of $(c_i/c_a)_{min}$ with habitat indicates that species developing low $(c_i/c_a)_{min}$ were from drier habitats (*A. acuminatus* grows in dry heathland), and species with higher $(c_i/c_a)_{min}$ were from rainforest (*A. pancheri* grows in tropical cloud forest) (Table I). These qualitative observations are strongly supported by the highly significant correlation between $(c_i/c_a)_{min}$ and ψ_1 at $(c_i/c_a)_{min}$ (Fig. 4), indicating that $(c_i/c_a)_{min}$ provides a good index of drought tolerance (of the foliage at least) in plants not susceptible to patchy photosynthesis.

The dynamic response described for the narrow-leaved conifers was not found to occur in the broad-leaved angiosperm species used here. Both species exhibited relatively constant c_i/c_a during drought, as described in many other studies (Wong et al., 1985; Anderson et al., 1995). This type of response has classically been interpreted as showing a gradual increase in nonstomatal limitation of photosynthesis during drought. The data presented here do not, however, show any evidence for nonstomatal inhibition in *E. tenuiramis* until leaf conductance had declined to around 25% of the level found in undroughted plants (Fig. 6), indicating that the leaves had responded to drought well before nonreversible photoinhibition became significant.

The fact that both species are relatively drought tolerant, with *A. melanoxylon* foliage found to withstand ψ_1 of around -3.5 MPa (Brodribb and Hill, 1993) and *E. tenuiramis* capable of surviving soil water potentials in the field of around -2.9 MPa without leaf damage (M. Battaglia and N. Davidson, personal communication), also suggests that it is unlikely that direct inhibition of photosynthesis should have occurred soon after water was withheld. Patchy stomatal closure provides a better explanation for the responses illustrated in Figures 5 and 6, and this is confirmed for *A. melanoxylon*, in which the number of stomata found to be fully closed increased rapidly with the onset of drought (Fig 5). This type of preemptive stomatal closure may be explained by fluxes of ABA that are released from the roots and translocated in the xylem (Khalil and Grace,

1993; Jackson et al., 1995), since it has been shown that initial translocation of ABA occurs at ψ_1 values equivalent to only mild drought (Correia and Pereira, 1994).

At some stage during drought it is inevitable that direct inhibition of photosynthesis will occur either by physical effects, such as turgor loss or cell damage, or by loss of chemical function in the chloroplasts. The important question is whether nonstomatal inhibition occurs early during drought, depressing photosynthesis while significant rates of gas exchange are taking place, or whether this effect occurs only in highly stressed plants as g_s approaches a minimum. All narrow-leaved conifers in this study exhibited the latter response to drought, resulting in substantial reductions in c_i/c_a prior to any expression of nonstomatal inhibition. This result supports ^{13}C discrimination and fluorescence data, indicating a dynamic response of c_i/c_a to drought and a minimal nonstomatal influence on assimilation until plants are subject to a severe water stress.

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