Plant water relations at elevated CO$_2$ – implications for water-limited environments

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ABSTRACT

Long-term exposure of plants to elevated [CO$_2$] leads to a number of growth and physiological effects, many of which are interpreted in the context of ameliorating the negative impacts of drought. However, despite considerable study, a clear picture in terms of the influence of elevated [CO$_2$] on plant water relations and the role that these effects play in determining the response of plants to elevated [CO$_2$] under water-limited conditions has been slow to emerge. In this paper, four areas of research are examined that represent critical, yet uncertain, themes related to the response of plants to elevated [CO$_2$] and drought. These include (1) fine-root proliferation and implications for whole-plant water uptake; (2) enhanced water-use efficiency and consequences for drought tolerance; (3) reductions in stomatal conductance and impacts on leaf water potential; and (4) solute accumulation, osmotic adjustment and dehydration tolerance of leaves. A survey of the literature indicates that the growth of plants at elevated [CO$_2$] can lead to conditions whereby plants maintain higher (less negative) leaf water potentials. The mechanisms that contribute to this effect are not fully known, although CO$_2$-induced reductions in stomatal conductance, increases in whole-plant hydraulic conductance and osmotic adjustment may be important. Less understood are the interactive effects of elevated [CO$_2$] and drought on fine-root production and water-use efficiency, and the contribution of these processes to plant growth in water-limited environments. Increases in water-use efficiency and reductions in water use can contribute to enhanced soil water content under elevated [CO$_2$]. Herbaceous crops and grasslands are most responsive in this regard. The conservation of soil water at elevated [CO$_2$] in other systems has been less studied, but in terms of maintaining growth or carbon gain during drought, the benefits of CO$_2$-induced improvements in soil water content appear relatively minor. Nonetheless, because even small effects of elevated [CO$_2$] on plant and soil water relations can have important implications for ecosystems, we conclude that this area of research deserves continued investigation. Future studies that focus on cellular mechanisms of plant response to elevated [CO$_2$] and drought are needed, as are whole-plant investigations that emphasize the integration of processes throughout the soil–plant–atmosphere continuum. We suggest that the hydraulic principles that govern water transport provide an integrating framework that would allow CO$_2$-induced changes in stomatal conductance, leaf water potential, root growth and other processes to be uniquely evaluated within the context of whole-plant hydraulic conductance and water transport efficiency.

Key-words: Atmospheric CO$_2$ enrichment; carbon allocation; global change; osmotic adjustment; osmotic potential; stomatal conductance; transpiration; water-use efficiency; water stress.

INTRODUCTION

Exposing plants to long-term CO$_2$ enrichment invariably leads to a range of growth and physiological responses (Mousseau & Saugier 1992; Norby et al. 1999; Pritchard & Rogers 2000). In general, elevated [CO$_2$] increases plant biomass, root mass and total leaf area (Rogers, Runion & Krupa 1994; Curtis & Wang 1998) and alters leaf net photosynthetic rates, stomatal conductance and water-use efficiency (WUE) (Gunderson & Wullschleger 1994; Saxe, Ellsworth & Heath 1998). Although these growth and physiological effects can be considerable, there is evidence that the magnitude of such responses is dependent on the availability of other potentially limiting resources (Chaves & Pereira 1992; Morison 1993; Campbell, Stafford Smith & McKeon 1997). Soil resources, such as nitrogen, and environmental variables, such as light and temperature, are all significant in this respect (Long 1991; Field, Jackson & Mooney 1992; Stitt & Krapp 1999). However, for the vast majority of terrestrial ecosystems, water is considered the primary factor limiting growth and productivity (Schulze et al. 1987) and thus, the interaction between elevated [CO$_2$] and soil water is an important scientific issue with relevance to both the basic plant sciences and global change research.

Many studies have addressed the interactions that arise between elevated [CO$_2$] and drought, and most have focused on one or more components of plant water relations (Morse et al. 1993; Tschaplinski, Norby & Wullschleger 1993; Centritto et al. 1999b; Ellsworth 1999; Tognetti, Raschi & Jones 2000a). A few studies have addressed the potential interaction between elevated [CO$_2$] and drought by direct multifactor manipulations (Johnsen 1993; Centritto, Lee & Jarvis 1999a; De Luis, Irigoyn & Sanchez-Diaz 1999), whereas others have taken a more
observational approach by comparing drought-induced changes in plant water relations at natural CO$_2$ springs (Tognetti et al. 1999a) or inferring CO$_2$–drought interactions by observing seasonal patterns of response (Ellsworth 1999). Regardless of how such studies are conducted, there has long been the temptation to interpret results in the context of the potential ameliorating effects that elevated [CO$_2$] may have on the drought response of plants. Increased allocation of carbon to root growth and osmotic adjustment in plants exposed to elevated [CO$_2$] may, for example, ameliorate the negative impacts of water stress by improving the capacity to extract soil water, whereas CO$_2$-induced reductions in stomatal conductance may ameliorate drought by increasing leaf or whole-plant WUE, thus enabling plants to better exploit water-limited environments. Elevated [CO$_2$] may also influence water relations and plant responses to drought by altering developmental processes, including root and shoot architecture (Berntson & Woodward 1992; Miao, Wayne & Bazzaz 1992) and leaf morphology (Thomas & Harvey 1983). However, despite these interpretations, there are few examples illustrating the effects of elevated [CO$_2$] on plant water relations and, in turn, the significance of these effects to plant and/or ecosystem productivity under water-limited conditions. One notable exception is that of Owensby and colleagues who have successfully used a combination of approaches (measurements of leaf water potential to whole-ecosystem gas exchange) to show that reduced water use in a C$_4$ tallgrass prairie exposed to elevated [CO$_2$] was sufficient to increase above- and below-ground biomass production in years when water stress was frequent (Knapp, Hamerlynck & Owensby 1993; Ham, Owensby & Coyne 1995; Owensby et al. 1997).

Because significant interactions do occur between elevated [CO$_2$] and drought, and because many effects of plant water relations have important implications for ecosystems in water-limited environments, we feel that this area of research deserves continued investigation. Therefore, in this paper, we examine four topics of plant water relations research that represent critical, yet uncertain, themes related to the response of plants to elevated [CO$_2$] under water-limited conditions. These topics span a range of scales from cellular to whole plant and encompass a diversity of processes from CO$_2$-induced increases in solute accumulation and osmotic adjustment of leaves, to the enhanced production of fine roots in plants grown at elevated [CO$_2$] and implications for whole-plant water use. Our primary focus is on the response of woody perennials to elevated [CO$_2$] and drought, but where appropriate, we also consider how these responses relate to other studies with herbaceous crops and grassland species. The interactions that arise from a combination of atmospheric CO$_2$ and drought are complex and we note that a clear picture has been slow to emerge from the many studies conducted in the area of plant water relations. We discuss what might be contributing to this unfortunate lack of scientific certainty and make recommendations whereby additional insights might be derived from future investigations.

**FINE-ROOT PROLIFERATION AND IMPLICATIONS FOR WHOLE-PLANT WATER UPTAKE**

*Increases in fine root production under elevated CO$_2$ represent yet another mechanism whereby forests might adjust to future climates. If greater rooting density and/or depth is attained by forests in the future they may be able to tap new water sources and maintain normal function under altered atmospheric conditions.*

(Hanson & Weltzin 2000)

In his review, Morison (1993) observed that greater carbon allocation to roots as a mechanism to improve plant water status at elevated [CO$_2$] was an attractive teleonomic argument, but cautioned that such an effect lacked consistent experimental evidence. From a historical perspective, there have been many observations that root systems become larger when plants are grown in CO$_2$-enriched atmospheres, dating back to some of the earliest experiments with potted plants in growth chambers. For example, wheat (*Triticum aestivum* L.) plants grown in deep pots in growth cabinets with different levels of soil moisture displayed increases in root weight in response to elevated [CO$_2$] under dry conditions only (Gifford 1979). Moreover, growth of white oak (*Quercus alba* L.) seedlings in nutrient-poor soil increased in elevated [CO$_2$], with the dry mass of tap roots increasing 93% and that of fine roots increasing 111% (Norby, O’Neill & Luxmoore 1986). Responses such as these were recognized almost immediately to be a potentially important determinant of how plants respond to drought under field conditions, especially if increased growth at elevated [CO$_2$] allowed roots to gain access to deeper layers of soils and associated soil water.

If plants grow larger overall at elevated compared to ambient [CO$_2$], root mass can logically be expected to increase as well. To consider whether there is a specific CO$_2$-induced stimulation of root growth, i.e. whether dry matter distribution is affected by elevated [CO$_2$], many researchers have considered the response of the root-to-shoot ratio (R/S). Stulen & den Hertog (1993) pointed out that many experimental problems exist with this statistic, but nevertheless concluded that although R/S is not influenced by elevated [CO$_2$] when water and nutrients are non-limiting, it is increased by elevated [CO$_2$] when soil resources are limiting, as was the case with the wheat and white oak examples cited earlier. A thorough compilation of the literature, however, does not support a general effect of elevated [CO$_2$] on R/S (Norby 1994). When differences in R/S are noted between CO$_2$ treatments, they often are associated with an effect of [CO$_2$] on plant ontogeny. That is, plants grow faster in elevated [CO$_2$], and R/S changes during plant development. When viewed allometrically, few effects of elevated [CO$_2$] on dry matter allocation have been observed (Norby 1994; Tissue, Thomas & Strain 1997).

The capacity of the root system to take up water depends not on root mass, but on rooting volume (or rooting depth) and fine-root area and activity. The size of root
systems of white oak seedlings was significantly stimulated by elevated [CO$_2$] in a growth chamber experiment (Norby et al. 1986), although there was no significant difference in whole-plant water use between treatments over a 40 week period. In a subsequent experiment with the same species (Norby & O’Neill 1989), root growth was not increased in elevated [CO$_2$] and water use was lower than in seedlings grown in ambient air. In both cases, whole-plant WUE increased significantly in elevated [CO$_2$], but in neither of these experiments were the plants subjected to drought. Thus, the importance of the increased WUE to drought tolerance or avoidance cannot be evaluated. Increases in the size of root systems were observed with CO$_2$ enrichment in five studies of intact native grasslands, but no significant increases occurred in seven others (Arnone et al. 2000). King, Thomas & Strain (1997) observed increased root surface area in Pinus taeda (lobolly pine) and P. ponderosa (Ponderosa pine) seedlings in elevated [CO$_2$], which was entirely attributable to whole-plant growth, not a redistribution of biomass. Increased fine root production and seasonal increases in fine root standing crop have also been observed in forest stands exposed to elevated [CO$_2$] (Norby & Jackson 2000; Matamala & Schlesinger 2000). However, in none of these cases has the increased fine root production been associated with increased water uptake. Indeed, water uptake is more likely to be reduced because of elevated CO$_2$ effects on canopy transpiration (Wullschleger & Norby 2001).

Atmospheric CO$_2$ enrichment can change the distribution of roots in the soil, which might also have implications for water uptake. Fine roots at the soil surface may be beneficial in capturing water after a dry soil is rewetted (Kosola & Eissenstat 1994). Several experiments suggest that crop plants in a higher CO$_2$ atmosphere will have larger root systems that are more highly branched, especially at shallow depths, and this should increase the capacity for resource acquisition, but at lower efficiency (Pritchard & Rogers 2000). CO$_2$ enrichment of a grassland community led to an upward shift in root length density with a greater proportion of roots in the top 6 cm soil layer (Arnone et al. 2000). This shift was perhaps related to increased soil moisture and its effect on nutrient availability. In a sandy, nutrient-poor, oak-palmetto system, CO$_2$ enrichment stimulated root production both near the surface, where nutrient availability was greatest, and at greater depth, where water was most available (Day et al. 1996).

To relate the size of the root system more closely to water balance, it is reasonable to consider the ratio of fine-root mass (length or area would be better) to leaf area (FR/LA); that is, the balance between the supply organ and the demand organ. The FR/LA of yellow-poplar (Liriodendron tulipifera L.) seedlings increased in elevated [CO$_2$], and this response was interpreted as a compensatory response that increased whole-plant WUE through morphological adjustments rather than through stomatal adjustments (Norby & O’Neill 1991). In six CO$_2$ enrichment experiments with deciduous trees in the field, FR/LA increased in elevated [CO$_2$] (Norby et al. 1999). However, Tingey, Phillips & Johnson (2000) concluded that there was no consistent effect of elevated [CO$_2$] on FR/LA in conifers.

Although it is reasonable to speculate that an increase in FR/LA increases the capacity of a plant to maintain a favourable water balance, there are no field observations that directly support this hypothesis. Why is this? In pot studies, effects of elevated [CO$_2$] on water use can often be explained simply by differences in the amount of leaf area. In field experiments as well, effects of elevated [CO$_2$] on water balance will be manifested primarily through effects on leaf area and canopy conductance. Describing a CO$_2$ effect on water uptake related to root deployment requires separating out numerous confounding influences and systemic feedbacks. When soil moisture is adequate to meet transpirational losses, CO$_2$ effects on root volume are presumably irrelevant, so detection of this mechanism would be possible only under specific conditions. Reduced canopy transpiration in high [CO$_2$] could reduce root uptake of water regardless of a prior effect on root volume, and this could result in increased soil moisture (Hungate et al. 1997) and secondary effects on root growth. The response of fine-root production to changes in soil moisture varies considerably in different experiments and may be confounded by concurrent changes in N availability (Joslin, Wolfe & Hanson 2000). Any interactions between CO$_2$ and water via root activity might be swamped by interactions between CO$_2$ and N or some other soil resource.

Much of the focus of investigations of root system responses to CO$_2$ enrichment has been toward long-term changes that might affect carbon flux to soil or plant-soil N dynamics. Short-term changes may be more important in consideration of water balance. An acceleration of root growth in elevated [CO$_2$] could result in a seedling becoming established more rapidly and avoiding water deficits. Polley et al. (1996) observed this in an experiment with Prosopis glandulosa (honey mesquite) seedlings. Low water availability restricts the establishment of this invasive shrub in some grasslands. Root growth in their experiment was stimulated by CO$_2$ enrichment, a response that Polley et al. (1996) observed as an increase in the ratios of lateral root to total root mass and lateral root mass to leaf area. Seedling survival during drought was significantly higher, and the authors attributed this to a combination of reduced water use per leaf and an increase in potential water uptake per leaf following from the higher ratio of lateral root to leaf area. Additional observations by Tischler et al. (cited in Polley et al. 1996) showed that CO$_2$ enrichment increased rooting depth of mesquite seedlings, and that the rapid establishment of deep roots effectively uncoupled the shrub from competition for water with more shallow-rooting grasses. Similarly, Tolley & Strain (1984) speculated that since CO$_2$ enrichment increased the R/S ratio of sweetgum (Liquidambar styraciflua L.) more than lobolly pine, sweetgum would become more competitive and better able to become established in drier areas currently dominated by pine seedlings. Jifon, Friend & Berrang (1995) studied the competitive interactions of these two species under elevated [CO$_2$] and concluded that resource-rich soil con-
ditions enhanced the CO$_2$-induced stimulation of soil colonization by sweetgum roots in sweetgum-pine mixtures.

In conclusion, the premise that increased root growth of plants in elevated [CO$_2$] will increase water uptake, improve water balance, or help to avoid water deficits may be well founded, but it has not yet been well supported with experimental data. The extent to which such mechanisms operate in nature and their biological significance must be evaluated in the context of other effects of elevated [CO$_2$], interacting environmental influences, and internal and system-level feedbacks. As a result, observations that support the importance of root proliferation to whole-plant water uptake are likely to remain anecdotal.

ENHANCED WATER-USE EFFICIENCY AND CONSEQUENCES FOR DROUGHT TOLERANCE

It is sometimes assumed that because increases in atmospheric CO$_2$ concentrations usually enhance water use efficiency per unit leaf area, there will be a tendency for plants to show greater drought tolerance... in the future. (Beerling et al. 1996)

Many studies have shown an increase in instantaneous transpiration efficiency (ITE) for leaves exposed to atmo-
spheric CO$_2$ enrichment, primarily as a result of reduced stomatal conductance, enhanced photosynthesis, or both factors in combination (Eamus 1991; Drake, González-Meler & Long 1997; Saxe et al. 1998). Increases in ITE due to elevated [CO$_2$] range from 25 to 229% (average 93% over 13 studies) and represent probably the most consistent and responsive measure in CO$_2$ effects research (Saxe et al. 1998). However, leaf-level measurements of ITE are hard to interpret in the context of plant water relations, and few mechanistic insights about the response of whole plants to elevated [CO$_2$] are conveyed by this simple metric (Jarvis, Mansfield & Davies 1999). Total leaf area per plant, shoot and root hydraulic conductance, above- and below-ground allocation of biomass and the duration of leaf area development can all be important in determining water use and, hence, the drought tolerance of a species, yet these properties are not reflected in ITE. A more meaningful, albeit less reported, measurement of plant response to elevated [CO$_2$] is WUE or the amount of total biomass produced per unit water used (Eamus 1991). Although conceptually similar to ITE, the term WUE conveys a more integrated measure of plant response to elevated [CO$_2$].

A sampling of recent literature indicates that whole-plant WUE, like that observed for ITE, increases considerably with elevated [CO$_2$] (Table 1). Calculated increases in WUE range from no effect in the case of a well-watered species to as high as 50% in the case of a drought-stressed species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Conditions</th>
<th>Change in WUE (%)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Arrhenatherum elatius</td>
<td>GH, Watered</td>
<td>+30</td>
<td>Arp et al. 1998</td>
</tr>
<tr>
<td></td>
<td>GH, Stressed</td>
<td>+45</td>
<td></td>
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<td>Calluna vulgaris</td>
<td>GH, Watered</td>
<td>+18</td>
<td>Arp et al. 1998</td>
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<tr>
<td></td>
<td>GH, Stressed</td>
<td>+38</td>
<td></td>
</tr>
<tr>
<td>Erica tetralix</td>
<td>GH, Watered</td>
<td>+31</td>
<td>Arp et al. 1998</td>
</tr>
<tr>
<td></td>
<td>GH, Stressed</td>
<td>+33</td>
<td></td>
</tr>
<tr>
<td>Grassland – Sandstone</td>
<td>OTC, High water</td>
<td>+27</td>
<td>Field et al. 1997</td>
</tr>
<tr>
<td></td>
<td>OTC, Med water</td>
<td>+43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OTC, Low water</td>
<td>+51</td>
<td></td>
</tr>
<tr>
<td>Grassland – Serpentine</td>
<td>OTC, High water</td>
<td>−1</td>
<td>Field et al. 1997</td>
</tr>
<tr>
<td></td>
<td>OTC, Med water</td>
<td>+22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OTC, Low water</td>
<td>+16</td>
<td></td>
</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td>FACE</td>
<td>+28</td>
<td>Wullschleger &amp; Norby 2001</td>
</tr>
<tr>
<td>Medicago sativa</td>
<td>GC, Watered</td>
<td>+180</td>
<td>De Luis et al. 1999</td>
</tr>
<tr>
<td></td>
<td>GC, Stressed</td>
<td>+150</td>
<td></td>
</tr>
<tr>
<td>Molinia caerulea</td>
<td>GH, Watered</td>
<td>+29</td>
<td>Arp et al. 1998</td>
</tr>
<tr>
<td></td>
<td>GH, Stressed</td>
<td>+36</td>
<td></td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>SPAR, Flooded</td>
<td>+41</td>
<td>Baker et al. 1997b</td>
</tr>
<tr>
<td></td>
<td>SPAR, Reflooded</td>
<td>+39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SPAR, Droughted</td>
<td>+125</td>
<td></td>
</tr>
<tr>
<td>Prunus avium</td>
<td>OTC, Watered</td>
<td>+47</td>
<td>Centritto et al. 1999a</td>
</tr>
<tr>
<td></td>
<td>OTC, Stressed</td>
<td>+52</td>
<td></td>
</tr>
<tr>
<td>Quercus robur</td>
<td>OTC, Watered</td>
<td>+47</td>
<td>Picon et al. 1996</td>
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<td></td>
<td>OTC, Stressed</td>
<td>+18</td>
<td></td>
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<tr>
<td>Rumex obtusifolius</td>
<td>GH, Watered</td>
<td>+39</td>
<td>Arp et al. 1998</td>
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<td></td>
<td>GH, Stressed</td>
<td>+50</td>
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<tr>
<td>Tallgrass prairie</td>
<td>OTC</td>
<td>+36</td>
<td>Owensby et al. 1997</td>
</tr>
<tr>
<td>Vaccinium myrtillus</td>
<td>GH, Watered</td>
<td>+36</td>
<td>Arp et al. 1998</td>
</tr>
<tr>
<td></td>
<td>GH, Stressed</td>
<td>+47</td>
<td></td>
</tr>
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Table 1. Whole-plant, stand or ecosystem water-use efficiency (WUE) expressed as a percentage change with atmospheric CO$_2$ enrichment. Plants were exposed to elevated [CO$_2$] either in growth chambers (GC), soil–plant–atmosphere-research (SPAR) chambers, greenhouse (GH), open-top chambers (OTC) or free-air CO$_2$ enrichment (FACE) facilities.
serpentine grassland (Field et al. 1997) to 180% increase in WUE for a study in which potted alfalfa (Medicago sativa L.) was exposed to elevated [CO₂] and a 15 d drought (De Luis et al. 1999). The majority of values, however, fall within the range of 30–50% and, in general, the impact of elevated [CO₂] on WUE appears greater in plants and ecosystems under drought conditions (Field et al. 1997; Arp et al. 1998). The period of time over which the studies summarized in Table 1 assessed WUE varied from daily to seasonal. In one of the longer studies to date, Wullschleger & Norby (2001) reported that for 12-year-old sweetgum trees exposed to free-air CO₂ enrichment (FACE) for a 7 month growing season was 1.90 kg of woody biomass produced per kilogram water used compared to 1.48 kg for trees in the ambient CO₂ treatment. This 28% increase in stand-level WUE was the combined result of a 15% increase in dry matter increment (Norby et al. 2001) and a 12% decrease in stand water use (as measured by sap flow techniques).

Although our discussion has thus far dealt with CO₂-induced effects on ITE and WUE, it should be emphasized that the total amount of water used by plants, stands and ecosystems exposed to elevated [CO₂] and drought is also of much interest. An increase in WUE per se does not ensure that water use on an individual plant basis will be less or, in turn, that soil moisture will be different between CO₂ treatments. Centritto et al. (1999a) observed in droughted cherry (Prunus avium L.) seedlings that although whole-plant WUE increased 56–103% with elevated CO₂ (Fig. 1b), there were no effects of CO₂ on water use (Fig. 1a). Picon, Guehl & Aussenc (1996) observed an increase in WUE for well-watered (47%) and droughted (18%) Quercus robur L. seedlings exposed to atmospheric CO₂ enrichment, but noted that whole-plant water use was similar between the two CO₂ treatments. It should be emphasized that in both of these studies, a lack of effect on total water use occurred because leaf area was higher in plants grown at elevated [CO₂], which emphasizes the overriding importance of plant and canopy development in determining responses observed in many CO₂ enrichment studies with young trees.

Equivalent rates of water use for plants exposed to ambient and elevated [CO₂] suggest that there are important trade-offs between increases in leaf area and reductions in stomatal conductance, such that in many cases there are few, if any, effects of elevated [CO₂] on whole-plant water use. One question that needs to be asked in this regard is how much of an increase in leaf area per plant (or LAI for a stand or ecosystem) is required to balance a CO₂-induced reduction in stomatal conductance? The answer to this question is dependent on the initial leaf area (closed-canopy versus isolated plants) and the extent to which canopy transpiration is under stomatal control. For example, in canopies with high LAI, leaf boundary layer and aerodynamic conductance may exert a stronger control on water vapour exchange than stomatal conductance, so that any change in stomatal conductance induced by elevated [CO₂] may only marginally affect transpiration and hence, plant and stand water use. Niklaus, Spinnler & Körner (1998) reported that ecosystem-level controls of the water balance (soil moisture, leaf area) can, in responsive systems like grasslands, far outweigh the physiological effects of elevated [CO₂] observed at the leaf level.

In comparison with studies that report limited or no effect of elevated [CO₂] on whole-plant water use, some studies have shown that water use in highly responsive plants and ecosystems may be considerably lower at elevated, compared to ambient, [CO₂] (Field et al. 1997; Owensby et al. 1997; Niklaus et al. 1998). Field et al. (1997) estimated that water savings in serpentine grasslands exposed to elevated [CO₂] reached maximum values of 0.3–0.4 mm d⁻¹ in each of three water treatments, whereas in sandstone grasslands a marked 2.15 mm d⁻¹ decrease in evapotranspiration (ET) due to CO₂ enrichment was observed. Integrated over the season, these CO₂-induced reductions in ET led to cumulative savings of water under elevated [CO₂] that ranged from 23 to 87 mm, depending on watering regime. One important consequence of these effects of elevated [CO₂] on water use should be a conservation of soil moisture and, thus, soil water content in plots exposed to elevated [CO₂] should be generally higher than under ambient conditions. In the sandstone grasslands, elevated [CO₂] led to increased soil moisture during at least

Figure 1. Total water loss (a) and plant water-use efficiency (b) of droughted cherry seedlings grown in ambient (cross-hatched) and elevated (open) [CO₂], during the first-growing season drought cycle. Data are the means of three plants per treatment ±1 SE. Letters (a, b) indicate significant differences at P < 0.05. Modified from Centritto et al. (1999a).
part of the growing season (Field et al. 1997). Niklaus et al. (1998) also observed such a response in calcareous grasslands and concluded that non-significant responses of water loss (i.e. transpiration) to elevated CO2 as measured by gas exchange accumulated over time and resulted in significantly higher soil moisture in CO2-enriched plots (Fig. 2).

Few studies have quantitatively evaluated the significance of these soil moisture effects on plant growth and/or physiology during drought. Higher soil water contents in elevated, compared to ambient, CO2 treatments might convey a margin of drought tolerance to plants and ecosystems exposed to elevated [CO2] and we calculate, using a WUE expression of 15\(\text{g biomass produced per kilogram water consumed}\), sufficient to explain a 20% increase in above-ground biomass due to conservation of soil water alone. Other studies have indicated that reduced water use by plants at elevated [CO2] was sufficient to extend the photosynthetically active period when water became limiting in the ecosystem (Owensby et al. 1997), but this particular study did not quantify the magnitude of the response. Wullschleger & Norby (2001) reported a 20 mm difference in water use during the month of May for a sweetgum stand exposed to elevated [CO2] and we calculate, using a WUE of 1·90 \(\text{g biomass produced per kilogram water consumed}\), that this would lead to an additional 38 g biomass produced per square metre ground area if indeed this conserved water were available for growth. Such an increase in the production of biomass due to water conservation alone represents 4·1% of the annual biomass increment for trees in the elevated CO2 treatment.

Our analysis suggests that although ITE and WUE are often reported to increase in response to elevated [CO2], it is difficult to separate CO2-induced effects on photosynthesis and growth from those on leaf transpiration and whole-plant water. As a result, these metrics convey only limited mechanistic information on the response of plants to atmospheric CO2 enrichment. More important is whether plants and ecosystems exposed to elevated [CO2] conserve soil water compared to ambient [CO2]. Unfortunately, few studies actually quantify the magnitude of water conserved at elevated [CO2] and still fewer consider how this conservation of soil water may potentially impact plant growth and physiology in water-limited environments. For those studies that do characterize the significance of reduced water use at elevated [CO2] in terms of maintaining growth or carbon gain during drought, the benefits of CO2-induced improvements in soil water content appear minor. Nonetheless, even small changes in soil water content can be important to nitrogen mineralization, organic matter decomposition and the survival of young plants in areas where periodic droughts are frequent. Thus, CO2 enrichment studies that link plant and soil-based processes should be encouraged.

**REDUCTIONS IN STOMATAL CONDUCTANCE AND IMPACTS ON LEAF WATER POTENTIAL**

Elevated [CO2], by causing a decline in stomatal conductance, might reduce transpiration rate, leading to an increase in plant water potential and a delay in the onset of drought... Moreover, it is reasonable to suppose that less negative plant water potentials in drying soils enable plants to remain turgid and functional for longer. (Centritto et al. 1999b)

A common expectation from many, albeit not all, studies that address the physiological response of plants to elevated [CO2] is that stomatal conductance will be reduced. In herbaceous species, these reductions can approach 27–40% (Morison 1985; Field et al. 1995), whereas in some coniferous species the response may be considerably less (Teskey 1995; Tissue et al. 1997; Ellsworth 1999). Nonetheless, this response has long fuelled the debate as to whether CO2-induced reductions in stomatal conductance will be of sufficient magnitude to impact leaf water potentials, particularly during periods of water-deficit stress. Moreover, if significant effects on plant water relations are observed, what then will be the consequences of this to leaf, plant and ecosystem function?

Attempts to address the influence of elevated [CO2] on plant water relations have now spanned almost 25 years (Cure & Acock 1986; Lawlor & Mitchell 1991). Early studies, conducted mostly with agricultural crops, showed marked responses of leaf water potential to elevated [CO2]...
(Rogers et al. 1984; Bhattacharya et al. 1990; Prior et al. 1991; Allen et al. 1994) and significant interactions between atmospheric CO2 and drought have been observed (Bhattacharya et al. 1990; Prior et al. 1991). Leaf water potentials in soybean (*Glycine max* L.) exposed to elevated [CO2] during a 4 d drought were more than 0.5 MPa higher (less negative) than those from plants exposed to ambient [CO2], with the greatest effects observed during the latter stages of an imposed water stress period (Rogers et al. 1984). At the time these studies were conducted, a typical conclusion was that elevated [CO2] ameliorated, mitigated or compensated for the negative impact of drought on plant growth. Indeed, Rogers et al. (1984) concluded that even though greater growth and leaf area production were observed at high atmospheric [CO2], lower rates of water use in soybean delayed the onset of severe water stress under conditions of low soil moisture. More recently, however, quantifying the long-term effects of elevated [CO2] and drought on plant water relations has been recognized as problematic given complications that arise from increases in whole-plant leaf area, effects of elevated [CO2] on plant ontogeny (Centritto et al. 1999a), difficulties associated with maintaining similar soil moisture levels among plants with different rates of soil water extraction (De Luis et al. 1999), species differences (Ferris & Taylor 1995) and day-to-day variation in radiation, vapour pressure deficit and precipitation.

Notwithstanding these difficulties, a number of studies have indicated that elevated [CO2] can lead to conditions whereby plants maintain higher (less negative) leaf water potentials. A survey of the literature published in the past decade (1990–2000) shows that there have been repeated attempts to examine the impact of elevated [CO2] on one or more aspects of plant water relations, including measures of leaf water and/or turgor potential (Table 2). Many of these

| Table 2. Literature survey on the influence of elevated [CO2] either alone or in combination with drought on leaf water potential and turgor potential. Plants were grown either in growth chambers (GC), soil-plant-atmosphere-research (SPAR) chambers, greenhouse (GH), open-top chambers (OTC), free-air CO2 enrichment (FACE) facilities or natural CO2 springs |
|-----------------|-----------------|-----------------|-----------------|
| Species         | Conditions       | Water potential | Turgor potential | Reference |
| Abutilon theophrasti | GH              | +               |                | Bunce & Ziska 1998 |
| Amaranthus hypochondriacus | GH              | +               |                | Bunce & Ziska 1998 |
| Andropogon gerardii          | OTC             | +               |                | Owensby et al. 1993 |
| Anthyllis vulneraria          | GC              | o               | +              | Ferris & Taylor 1994 |
| Avena barbata                | OTC             | +               |                | Jackson et al. 1994 |
| Erica arborea                | CO2 Spring      | +               | +              | Tognetti et al. 2000a |
| Glycine max                  | OTC, Watered    | o               |                | Prior et al. 1991 |
|                             | OTC, Stressed   | +               |                | Allen et al. 1994 |
|                             | SPAR, Watered   | +               | o              | Allen et al. 1998 |
|                             | SPAR, Stressed  | +               | +              | Field et al. 1997 |
|                             | GC              | +               |                | Allen et al. 1998 |
| Grassland spp.               | OTC             | +               |                | Field et al. 1997 |
| Ipomoea batatas              | OTC, Watered    | o               |                | Bhattacharya et al. 1990 |
|                             | OTC, Stressed   | +               |                | Tognetti et al. 2000a |
| Juniperus communis           | CO2 Spring      | +               | +              | Tognetti et al. 2000b |
| Lotus corniculatus           | GH              | o               | o              | Ferris & Taylor 1994 |
| Medicago sativa              | GC, Watered     | o               |                | De Luis et al. 1999 |
|                             | GC, Stressed    | –               |                | |
| Myrtus communis              | CO2 Spring      | +               | +              | Tognetti et al. 2000a |
| Panicum coloratum            | GC              | +               |                | Senewereea, Ghannoum & Conroy 1998 |
| Picea mariana                | GC, Watered     | o               |                | Johnsen 1993 |
|                             | GC, Stressed    | +               |                | |
| Pinus taeda                  | FACE            | o               | o              | Ellsworth 1999 |
| Plantago media               | GH              | o               | o              | Ferris & Taylor 1994 |
| Prosopis glandulosa          | GH              | +               |                | Polley et al. 1996 |
| Prunus avium                 | OTC, Watered    | o               |                | Centritto et al. 1999b |
|                             | OTC, Stressed   | –               |                | |
| Quercus ilex                 | CO2 Spring      | +               |                | Tognetti et al. 1998 |
| Quercus pubescens            | CO2 Spring      | +               |                | Tognetti et al. 1999a |
| Quercus robur                | GC              | +               |                | Schulte et al. 1998 |
| Sanguisorba minor            | GC              | o               | o              | Ferris & Taylor 1994 |
| Zea mays                     | GH              | –               |                | Bunce & Ziska 1998 |
studies were conducted under field conditions using open-top chambers (OTC), free-air CO2 enrichment (FACE) technology or natural CO2 springs, with a few explicitly considering the potential interaction of elevated [CO2] and drought. A brief examination of Table 2 shows that plant growth at elevated [CO2] can, as suggested by earlier investigations, lead to higher leaf water and turgor potentials. Tognetti et al. (2000b) recently conducted a comprehensive examination of plant water relations for three Mediterranean shrubs (Erica arborea L., Myrtus communis L. and Juniperus communis L.) growing at a natural CO2-emitting vent near Laiatico, Italy. It was observed that differences in leaf conductance between CO2 vent and control sites were, over an annual cycle, significant for all three species, with plants at the CO2 vent showing lower leaf conductances than plants at the control site. These changes were accompanied by small, but highly significant effects on leaf water potential (Fig. 3). In the plants growing near the CO2 vent, both pre-dawn and midday water potentials were higher (less negative) than those at the control site, and differences were most pronounced between July and September when drought was severe (soil water content approaching 12%).

Figure 3. Seasonal course of midday shoot water potential measured in the sun crown of (a) Erica arborea (b) Myrtus communis and (c) Juniperus communis plants at a CO2 spring site (closed symbols) and at the control site (open symbols) from October 1996 to September 1997. Modified from Tognetti et al. (2000b).

Tognetti et al. (2000b) interpreted these data to indicate that plants growing near the CO2 vent were either conserving soil water due to direct effects of elevated [CO2] on leaf conductance or that they had improved access to soil water due to a deeper root system. All three species maintained higher turgor potentials under elevated [CO2] during a mid-season drought (Tognetti et al. 2000a).

Not all studies have shown a clear distinction between CO2 treatments in terms of plant water relations (Table 2). Tognetti et al. (1998), in an analysis of Quercus ilex at a natural CO2 spring in central Italy, observed that elevated [CO2] decreased leaf conductance, sap flux density and whole-plant hydraulic resistance, but exerted no effect on leaf water potential. A similar lack of response in leaf water potential to elevated [CO2] was observed by Ferris & Taylor (1994) and Ellsworth (1999), albeit in the latter study with loblolly pine, a significant response might not have been expected given that no effect of atmospheric [CO2] on stomatal conductance was observed. Although it is not surprising that some studies report no effect of elevated [CO2] on leaf water potential, it is interesting that a few recent studies have shown that leaf water potentials may actually be lower (more negative) in plants grown at elevated [CO2] (Centritto et al. 1999b; De Luis et al. 1999). The mechanisms that contribute to these effects are not known. Increases in whole-plant leaf area beyond that which can be compensated for by CO2-induced reductions in stomatal conductance may lead to greater rates of soil drying and associated plant water stress. There is also evidence that during drought, stomatal conductance in some species may be higher (not lower) in elevated [CO2] (Heath & Kerstiens 1997). This creates a condition whereby soils at elevated [CO2] become drier and plants are subjected to a higher severity of drought. Heath & Kerstien (1997) observed in beech (Fagus sylvatica L.) that stomatal conductance during drought was higher at elevated compared to ambient [CO2] and that this resulted in a substantially greater rate of soil drying for plants exposed to atmospheric CO2 enrichment. The explanation for this response was that whole-plant water relations at elevated [CO2] were improved, either through more effective uptake of water by the roots or through increased xylem conductivity at elevated [CO2], which would improve the supply of water to the leaves during periods of high evaporative demand. The consequence of fine-root proliferation to plant water relations in plants exposed to elevated [CO2] and drought has already been discussed. On the other hand, hydraulic conductance of intact plants grown at ambient and elevated [CO2] has been determined, but unfortunately the response has been highly variable. Increases, decreases and no change in leaf-specific hydraulic conductance with elevated [CO2] have been observed (Bunce 1996; Bunce & Ziska 1998; Tognetti et al. 1998; Tognetti et al. 1999b). Regardless of the mechanisms involved, rising [CO2] cannot be assumed to improve the water economy of all plant species, and in some cases there...
may be deleterious effects (Beerling et al. 1996; Robinson, Heath & Mansfield 1998).

Finally, studies that report positive effects of elevated [CO$_2$] on leaf water and turgor potentials have not adequately addressed the question as to the growth and/or physiological benefits derived from these responses. Tognetti et al. (2000a) proposed that since Mediterranean shrubs maintained higher turgor potentials under elevated [CO$_2$], the growth of these species in water-limited environments should be impacted less than plants exposed to drought under ambient [CO$_2$]. However, the causal relationships among effects on leaf water potential, turgor potential and maintenance of growth at elevated [CO$_2$] have seldom been quantified. Ferris & Taylor (1994) tried to demonstrate cause-and-effect relationships among leaf water potential, turgor potential, cell wall elasticity and rate of leaf and leaflet extension in four chalk grassland species exposed to CO$_2$ enrichment, but were unable to identify any single relationship to explain their observations.

**SOLUTE ACCUMULATION, OSMOTIC ADJUSTMENT AND DEHYDRATION TOLERANCE**

In addition to effects of elevated CO$_2$ on plant-water relations that are mediated by stomata, other response mechanisms can be considered. For instance… Elevated CO$_2$ may also directly alter leaf dehydration tolerance in cases where CO$_2$-induced excess carbohydrates serve as osmotica. (Ellsworth 1999)

Hypothetically, elevated [CO$_2$] could increase drought tolerance of plants if increased rates of net carbon assimilation lead to an increased availability of substrate for osmotic adjustment, thereby lowering osmotic potential at full turgor ($\pi_o$). Elevated [CO$_2$] could therefore have a significant effect on drought tolerance by increasing carbohydrate accumulation and lowering $\pi_o$ when soil moisture availability is high, as well as by enhancing osmotic adjustment during drought. Morse et al. (1993) reported a decline in $\pi_o$ of 0·11 MPa in gray birch (Betula populifolia Marsh.) with elevated [CO$_2$] under mesic conditions. Furthermore, for this same species, a 0·14 MPa osmotic adjustment to xeric conditions under ambient CO$_2$ increased to a 0·2 MPa adjustment under elevated [CO$_2$]. Ferris & Taylor (1994) reported that three of the four chalk grassland herbs that they studied, including salad burnet (Sanguisorba minor Scop.), kidney vetch (Anthyllis vulneraria L.) and hoary plantain (Plantago media L.), had lower foliar osmotic potentials (by 0·3 MPa) adjust-ment at elevated [CO$_2$], which when soil moisture availability is 0·14 MPa in roots of plants exposed to drought was not affected by CO$_2$ enrichment. Although Picon, Ferhi & Guehl (1997) did not determine osmotic potential at full turgor, they reported that a drought-induced increase in hexose concentrations was more pronounced under elevated [CO$_2$]. Again, the lack of sink activity under drought probably led to the modest increase. They noted that only plants from the elevated CO$_2$ treatment had a fourth growth flush, indicating high sink activity. When growth stimulation was evident, carbohydrate accumulation was not observed, and there were only slight increases in sucrose and starch concentrations. Therefore, $\pi_o$ may decrease in response to elevated [CO$_2$] if sink demand for soluble carbohydrates is low.

In contrast, elevated [CO$_2$] could have no effect or even decrease dehydration tolerance if growth promotion depletes the concentrations of organic solutes, such as soluble carbohydrates, phenolic compounds and organic acids, which can be particularly low in rapidly growing seedlings (Tschaplinski & Blake 1989). Leaf $\pi_o$ and osmotic adjustment were not affected by elevated [CO$_2$] in sugar maple (Acer saccharum L.), American sycamore (Platanus occidentalis L.) and sweet gum, where, despite an approximate 50% increase in net carbon assimilation rate (Tschaplinski et al. 1995b), there was no evidence of higher soluble carbohydrate concentrations in leaves or roots (Tschaplinski, Stewart & Norby 1995a). The rapid growth response of seedlings to elevated [CO$_2$] may depress solute concentrations in leaves, increasing $\pi_o$ and lowering drought tolerance. This tendency was exhibited by loblolly pine seedlings grown in elevated [CO$_2$], where $\pi_o$ of needles increased by 0·08 MPa in well-watered seedlings and by 0·16 MPa in stressed seedlings, suggesting that drought tolerance of shoots may have been somewhat less at elevated [CO$_2$] (Tschaplinski et al. 1993). This was offset, however, by an increase in the soluble carbohydrate concentrations in roots of seedlings grown under elevated [CO$_2$], which can potentially facilitate continued water uptake under drought. In well-watered seedlings, elevated [CO$_2$] increased the concentration of soluble carbohydrates in roots by 68% (Tschaplinski et al. 1993). Water stress reduced the soluble carbohydrate concentrations in roots of seedlings growing in ambient [CO$_2$] to 26% that of the well-watered controls. Elevated [CO$_2$] mitigated the water stress-induced decline in soluble carbohydrate concentrations in roots, perhaps due to carbohydrate loading when the seedlings were growing under conditions of higher water supply, rather than to osmotic adjustment to water stress. There were no indications of osmotic adjustment to water stress or that elevated [CO$_2$] enhanced osmotic adjustment in loblolly pine.

A number of recent studies have also confirmed that the effects of elevated [CO$_2$] on $\pi_o$ and osmotic adjustment are minimal. Elevated [CO$_2$] did not affect $\pi_o$ of honey mesquite (Polley et al. 1996, 1999), a species that is known to osmotically adjust both diurnally and seasonally (Nilsen et al. 1983). Similarly, Picon-Cochard & Guehl (1999) reported that although elevated [CO$_2$] increased sucrose
CONCLUSIONS

The effects of elevated \([\text{CO}_2]\) on growth, gas exchange and morphology and, in turn, the influence of these processes on plant water relations are understandably critical issues in predicting the productivity of plants and ecosystems in water-limited environments. Predicting the response of terrestrial ecosystems to the interactive effects of elevated \([\text{CO}_2]\) and drought will require that we understand processes that operate across a range of scales, including plants, leaves, cells and molecules (Saxe et al. 1998). However, as we have shown in this paper, at many (if not all) scales, the implications of elevated \([\text{CO}_2]\) for WUE, water use and whole-plant water relations become difficult to quantify and interpret.

One factor that currently hinders our analysis is that cause and effect relationships are only rarely established between growth, gas exchange, anatomy and plant water relations in the many \([\text{CO}_2]\) enrichment studies conducted to date. Instead, there is heavy reliance on inference. For example, many investigators point to the observation that elevated \([\text{CO}_2]\) often increases R:S ratio or fine-root proliferation, and then conclude that these effects must be beneficial to plants under water-limited conditions. Our intuition supports this conclusion, but actual data fall short, unfortunately, in supporting such causal associations. The same concern can be raised in terms of the presumed benefits associated with \([\text{CO}_2]\)-induced effects on stomatal conductance, transpiration, WUE and leaf water potential. It is rarely shown that these leaf-level impacts of elevated \([\text{CO}_2]\) have implications to either the carbon or water balance of leaves, whole plants or ecosystems. Grasslands and some herbaceous crops apparently represent exceptions to this rule.

We conclude that studies addressing the water relations of plants exposed to elevated \([\text{CO}_2]\) and, in turn, the importance of observed responses to plants in water-limited environments, would benefit from a conceptual framework with which to guide research, identify the need for new measurements and to evaluate the relationships among multiple processes that ultimately contribute to plant growth and development. We suggest that the hydraulic principles that govern water transport provide such a framework, one that allows integration of leaf-level processes including stomatal conductance and water potential with the hydraulic architecture and transport efficiency of plants. Viewed from this perspective, water uptake is controlled by soil and root hydraulic properties, the transport characteristics of stems including xylem anatomy and sapwood area, soil and leaf water potential and stomatal conductance (Sperry 2000). However, rather than being independent measures of response, they are interrelated and collectively provide insights into how elevated \([\text{CO}_2]\) and drought, through effects on water relations, might potentially impact plant growth and development in water-limited environments.

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