C 4 photosynthesis and water stress

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Background In contrast to C 3 photosynthesis, the response of C 4 photosynthesis to water stress has been less well studied in spite of the significant contribution of C 4 plants to the global carbon budget and food security. The key feature of C 4 photosynthesis is the operation of a CO 2-concentrating mechanism in the leaves, which serves to saturate photosynthesis and suppress photorespiration in normal air. This article reviews the current state of understanding about the response of C 4 photosynthesis to water stress, including the interaction with elevated CO 2 concentration. Major gaps in our knowledge in this area are identified and further required research is suggested.

Scope Evidence indicates that C 4 photosynthesis is highly sensitive to water stress. With declining leaf water status, CO 2 assimilation rate and stomatal conductance decrease rapidly and photosynthesis goes through three successive phases. The initial, mainly stomatal phase, may or may not be detected as a decline in assimilation rates depending on environmental conditions. This is because the CO 2-concentrating mechanism is capable of saturating C 4 photosynthesis under relatively low intercellular CO 2 concentrations. In addition, photorespired CO 2 is likely to be refixed before escaping the bundle sheath. This is followed by a mixed stomatal and non-stomatal phase and, finally, a mainly non-stomatal phase. The main non-stomatal factors include reduced activity of photosynthetic enzymes; inhibition of nitrate assimilation, induction of early senescence, and changes to the leaf anatomy and ultrastructure. Results from the literature about CO 2 enrichment indicate that when C 4 plants experience drought in their natural environment, elevated CO 2 concentration alleviates the effect of water stress on plant productivity indirectly via improved soil moisture and plant water status as a result of decreased stomatal conductance and reduced leaf transpiration.

Conclusions It is suggested that there is a limited capacity for photorespiration or the Mehler reaction to act as significant alternative electron sinks under water stress in C 4 photosynthesis. This may explain why C 4 photosynthesis is equally or even more sensitive to water stress than its C 3 counterpart in spite of the greater capacity and water use efficiency of the C 4 photosynthetic pathway.

Key words: C 3 and C 4 photosynthesis, stomatal and non-stomatal limitation, high CO 2, water stress.

INTRODUCTION

Water stress is one of the most limiting environmental factors to plant productivity worldwide, and can be caused by both soil and atmospheric water deficits. The response of C 3 photosynthesis to water stress has been well studied and reviewed, as indicated by the large number of research (e.g. Sharkey and Seemann, 1988; Ortiz-López et al., 1991; Cornic et al., 1992; Tezara et al., 1999; Cornic and Fresneau, 2002) and review (e.g. Cornic, 2000; Lawlor, 1995, 2002; Lawlor and Cornic, 2002; Flexas et al., 2004) articles published on this topic. In general, C 3 photosynthesis is negatively affected by water stress measured as changes in leaf water potential (Ψ leaf) or relative water content (RWC). In the early phase of water stress, when leaf RWC is still greater than 70 %, the decline in CO 2 assimilation rates (A) is largely the result of reduced intercellular CO 2 concentration (C i) due to decreased stomatal conductance (g s). Under these conditions, maximal photosynthetic capacity and quantum yield remain unaffected when measured under saturating irradiance and carbon dioxide concentration ([CO 2]). In addition, photosynthetic inhibition usually recovers relatively quickly when plants are re-hydrated.

If water stress persists and leaf RWC falls below 70 %, the loss of photosynthetic activity becomes increasingly less responsive to high [CO 2] and A fails to recover to pre-stress values following the removal of water stress. The exact mechanisms underlying this non-stomatal phase, also termed metabolic inhibition, are diverse and less well understood (for more details and reviews on this topic, see Cornic, 2000; Lawlor and Cornic, 2002; Lawlor, 2002; Flexas et al., 2004; and references therein).

In contrast, the response of C 4 photosynthesis to water stress has been less well studied. This is in spite of the fact that C 4 plants make a significant contribution to the global carbon budget, and C 4 crops, such as maize and sorghum, are pivotal to current and future global food security (Lloyd and Farquhar, 1994; Ehleringer et al., 1997; Brown, 1999; Pingali, 2001). Moreover, C 4 plants predominate in hot, arid regions which are prone to frequent drought. This fact is likely to be exacerbated by global climate change in three main ways: (1) global warming and changes in precipitation patterns are likely to expose many ecosystems, including C 4-dominated ones, to increasing soil and atmospheric water stresses (IPCC, 2007); (2) the impact of rising atmospheric [CO 2] on the productivity of C 4 plants is greatly influenced.
The C4 photosynthetic pathway is strongly represented in the grasslands (Lloyd and Farquhar, 1994; Ehleringer et al., 1994; Crimp et al., 2002). Therefore, it is important to understand how water stress influences the primary processes of CO₂ fixation in C₄ plants. In this article, I review the evidence related to the response of C₄ photosynthesis to water stress and attempt to summarize the current state of understanding in this area, including the interaction of elevated [CO₂] with the effects of water stress on C₄ photosynthesis.

**SIGNIFICANCE AND DISTRIBUTION OF THE C₄ PHOTOSYNTHETIC PATHWAY**

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary CO₂-fixing enzyme in plants, has poor kinetic properties. Compared with other enzymes, Rubisco is a slow catalyst with a low affinity for its substrate CO₂. Most importantly, Rubisco has a low ability to discriminate between molecular CO₂ and O₂ (Jordan and Ogren, 1981; Andrews and Lorimer, 1987). The latter feature is particularly problematic because O₂ is the by-product of the light reactions of photosynthesis (Edwards and Walker, 1983) and is in high concentration in the atmosphere. By reacting RuBP with O₂, Rubisco fixes less CO₂ and initiates a series of reactions, photorespiration, which culminates in the release of CO₂ back to the atmosphere (Edwards and Walker, 1983). Under the current atmospheric [CO₂] and a temperature of 25°C, photorespiration runs at about 20–30% of photosynthesis in C₃ leaves (Sage, 2001, 2004). With increasing temperature, photorespiration increases faster than photosynthesis (Jordan and Ogren, 1984; Sage and Kubien, 2007). The C₄ photosynthetic pathway has evolved as an adaptation to high photorespiratory pressures resulting from various combinations of stresses which include low atmospheric [CO₂], high temperature, aridity and/or salinity (Ehleringer et al., 1991, 1997; Sage, 2001, 2004; Tipple and Pagani, 2007). According to carbon isotope discrimination records, plant fossils and molecular taxonomy studies, it is likely that C₄ plants formed a minor component of the world’s flora for a long time before the recent expansion of C₄ grasslands some 5–10 million years ago (Cerling, 1999; Kellogg, 1999; Sage, 2004). It is estimated that the C₄ photosynthetic pathway has evolved independently some 45 times in three monocot and 16 dicot lineages (Kellogg, 1999; Sage et al., 1999; Sage, 2004).

Although C₄ plants represent a mere 4% of the world’s flora, they contribute about 20% of global primary productivity, mainly because of the high productivity of C₄ grasslands (Lloyd and Farquhar, 1994; Ehleringer et al., 1997). The C₄ photosynthetic pathway is strongly represented in the grass (Poaceae) family, comprising about 50% of total grasses (Hattersley, 1992; Sage et al., 1999). C₄ plants are grouped into three biochemical subtypes [NAD malic enzyme (NAD-ME), NADP malic enzyme (NADP-ME) and phosphoenolpyruvate carboxykinase (PCK)] following the major C₄ acid decarboxylation enzyme in the bundle sheath (Hatch, 1987; Hattersley, 1992). The major C₄ crops, such as maize, sugarcane and sorghum belong to the NADP-ME subtype. At the regional level, the geographic distribution of C₄ grasses is strongly influenced by rainfall level. With decreasing rainfall (from 900 mm to 50 mm per annum), the abundance of NAD-ME grasses increases while that of NADP-ME grasses decreases. The distribution of PCK grasses is weakly correlated with rainfall gradient (Ellis et al., 1980; Hattersley, 1992; Taub, 2000). This distribution suggests that C₄ grasses with different biochemical subtypes may have different water use efficiency (WUE) or drought tolerance. The first attribute has been validated with NAD-ME grasses having a greater whole-plant WUE under water stress than their NADP-ME counterparts (Ghannoum et al., 2002). However, there is no evidence suggesting that the three C₄ biochemical pathways have different sensitivities to water stress. Hence, in the context of the current review, it is possible to discuss the effects of water stress on C₄ photosynthetic metabolism in general.

**THE CO₂-CONCENTRATING MECHANISM IN C₄ LEAVES**

The key feature of C₄ photosynthesis is the operation of a CO₂-concentrating mechanism in the leaves of C₄ plants, which consists of a series of biochemical and structural modifications around the ancestral C₃ photosynthetic pathway (Hatch, 1987). Although there are many ways, biochemically and anatomically, of achieving C₄ photosynthesis, the most common C₄ syndrome in higher plants involves the operation of two photosynthetic cycles (C₃ and C₄) across two photosynthetic cell types (mesophyll and bundle sheath), which are arranged in concentric layers around the vascular bundle (Fig. 1; Hatch, 1987). The first steps of C₄ photosynthesis occur in the mesophyll and involve the hydration of CO₂ into bicarbonate, which reacts with phosphoenolpyruvate (PEP) with the aid of PEP carboxylase (PEPC) to produce oxaloacetate, a C₄ acid, hence the terms C₃ cycle and C₄ photosynthesis. Oxaloacetate is converted into other C₄ acids (malate, aspartate or alanine) which diffuse into the bundle sheath cells where they are decarboxylated, releasing CO₂ for fixation by Rubisco and the rest of the C₃ cycle. The C₃

**FIG. 1.** A simplified, schematic representation of C₃ (left) and C₄ (right) photosynthesis.
product of the decarboxylation reaction returns to the mesophyll, completing the C3 cycle (Fig. 1). The C4 cycle acts like a CO2-concentrating mechanism for two main reasons: (1) PEPC is faster than Rubisco and insensitive to O2; and (2) the bundle sheath cell wall presents a significant gaseous diffusion barrier (Hatch, 1987; Brown and Byrd, 1993). Consequently, the high [CO2] generated by the C4 CO2-concentrating mechanism in the bundle sheath leads to the suppression of apparent photorespiration in air as well as the saturation of C4 photosynthesis at a lower ambient [CO2] than for C3 plants (Fig. 2). In addition, photorespired CO2 is released within the bundle sheath, and either is refixed or contributes to increasing bundle sheath [CO2] ([CO2]BS), which in turn, leads to reducing photorespiration. High [CO2]BS gives rise to the characteristic A/Ci curve of C4 leaves. Relative to C3 photosynthesis, the C4 A/Ci curve is characterized by abrupt saturation at a relatively low Ci (Fig. 2). This constitutes the basis of a number of advantages conferred by the C4, relative to C3, photosynthetic pathway, chief of which is higher WUE (Osmond et al., 1982; Long, 1999).

**C4 PHOTOSYNTHESIS AND WATER STRESS**

C3 and C4 photosynthesis share most of the fundamental photosynthetic processes such as the C3 cycle, light harvesting complexes and electron transport components. Hence, the two photosynthetic pathways may be expected to show, by and large, similar responses to water availability. Nevertheless, significant differences exist between the two photosynthetic types, which could make their response to water stress differ at a number of levels. A cursory examination of the literature reveals that the observed responses of C4 photosynthesis to water stress are as diverse as those reported for C3 photosynthesis. Some studies concluded that inhibition of C4 photosynthesis under water stress is mainly due to stomatal closure, while others concluded that non-stomatal factors play a major role (e.g. Lawlor and Fock, 1978; Becker and Fock, 1986; Loreto et al., 1995; Lal and Edwards, 1996; Saccardy et al., 1996; Maroco et al., 2000; Ghannoum et al., 2003; Marques da Silva and Arrabaca, 2004a; Ripley et al., 2007; Carmo-Silva et al., 2008). These studies used different C4 species subjected to different severities and methods of inducing water stress (e.g. withholding watering, using an osmotic agent or drying of detached leaves) and made photosynthetic measurements using different techniques (e.g. various gas exchange instruments or O2 electrodes) and under different conditions of light intensity and leaf temperatupe. Consequently, the different responses could be attributed to any combination of these factors. Therefore, there is a need to dissect the available evidence in order to draw a more comprehensive picture of the mechanisms underlying the response of C4 photosynthesis to water stress. These mechanisms are summarized in Fig. 3 and, as commonly argued in the literature, are divided into stomatal and non-stomatal factors. The stomatal factors refer to the downstream effects of CO2 limitation on photosynthetic activity. The non-stomatal factors encompass everything else, including the direct effects of reduced leaf and cellular water status on the activity of enzymes involved in the CO2 fixation and electron transport reactions, induction of early senescence, and changes to leaf anatomy and ultrastructure (Fig. 3).

**THE ROLE OF STOMATAL FACTORS IN THE INHIBITION OF C4 PHOTOSYNTHESIS UNDER WATER STRESS**

Similarly to what has been reported in C3 plants, stomatal conductance of C4 plants decreases with declining leaf water status, and this invariably coincides with reduced photosynthetic rates (e.g. Kalapos et al., 1996; Maroco et al., 2000; Ghannoum et al., 2003; Carmo-Silva et al., 2008). The concomitant decline of A and g, particularly under mild water stress (i.e. for leaf RWC >70%), has been interpreted in a causal way in C3 and C4 plants alike, based on four main lines of

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**Fig. 2.** The response of CO2 assimilation rates (A) to intercellular CO2 concentration (Ci) in one C3 (*Panicum laxum*) and two C4 (*Cenchrus ciliaris* and *Panicum coloratum*) grasses. Gas exchange measurements were made at 30 °C and 1200 μmol quanta m–2 s–1. The dotted lines represent the slope of stomatal conductance, g = A/(Ci – Cj), where Cj and Ci are the ambient and intercellular [CO2], respectively. The arrows indicate A at the operational Cj (i.e. Cj at normal air [CO2]; O. Ghannoum, unpubl. res.).

**Fig. 3.** Summary of the main effects of water stress on the photosynthetic parameters of C4 leaves. Stomatal and non-stomatal factors are indicated by dashed and continuous lines, respectively. The ‒ sign indicates an effect in the opposite direction. The term leakiness (θ) is defined as the fraction of CO2 fixed by PEPC which leaks out of the bundle sheath.
Intercellular CO₂ of C₄ plants subjected to water stress

Decreased C₄ due to reduced stomatal conductance has been taken as a proof of CO₂ limitation for C₄ photosynthesis. The operation of a CO₂-concentrating mechanism during C₄ photosynthesis introduces additional layers of complexity to this otherwise straightforward argument. A closer look at the literature shows that C₄ decreases only during the early phases of water stress as has been reported for maize (Becker and Fock, 1986; Lal and Edwards, 1996; Leakey et al., 2004), sorghum (Williams et al., 2001), sugarcane (Du et al., 1996), amaranthus (Lal and Edwards, 1996) and a non-crop C₄ grass species (Marques da Silva and Arrabaca, 2004a). During the later stages of drought, it is often observed that C₄ increases while A continues its decline (e.g. Becker and Fock, 1986; Du et al., 1996; Kalapos et al., 1996; Lal and Edwards, 1996). In contrast, some studies using various C₄ plants reported no change in C₄ under water stress (e.g. Salindra et al., 1996, Ripley et al., 2007) or for most of the water stress period, with C₄ increasing under severe stress at the end of the drying cycle (Kalapos et al., 1996; Lal and Edwards, 1996).

By raising [CO₂] at the sites of Rubisco, the C₄ CO₂-concentrating mechanism serves to CO₂-saturate A and virtually suppress photorespiration in normal air (Hatch, 1987). This is illustrated in Fig. 4 which uses the C₄ model of von Caemmerer (2000) to simulate the response of some key photosynthetic parameters to C₄ in a mature C₄ leaf measured under optimal light and temperature. The shaded area highlights the range of C₄ measured in well-watered and moderately water-stressed leaves (Fig. 4). The modelling predicts little change in A with C₄ declining down to 50 μmol·m⁻²·s⁻¹ (Fig. 4A). Thus, based on our theoretical understanding, the CO₂-concentrating mechanism endows C₄ photosynthesis with a significant buffering capacity against short-term fluctuations in C₄ down to a certain concentration, such as those usually observed in mildly water-stressed C₄ leaves. This is supported by the results of Lal and Edwards (1996) who found that the initial decline in C₄, up to 50% of control values, had no effect on A during the early phases of water stress in both maize and amaranthus. Hence, it may be concluded that during the early stages of water stress, stomatal closure may not always reduce C₄ enough to cause a detectable decline in A. In addition to inter-species variations amongst C₄ plants, whether or not a decline in C₄ will elicit a reduction in A depends largely on growth and measuring conditions that influence the position of the operational C₄ (i.e. C₄ at normal air [CO₂]; Fig. 2). For example, conditions of high irradiance and nutrition tend to shift the operational C₄ down to the CO₂-responsive part of the A/C₄ curve (Ghannoun et al., 1997; Ghannoun and Conroy, 1998). In contrast, low irradiance tends to shift C₄ to the flat part of the A/C₄ curve, which necessitates a large decline in C₄ before A is affected (e.g. Lal and Edwards, 1996). The interaction between environmental conditions (such as irradiance, nutrition, temperature) and the response of C₄ photosynthesis to water stress has not yet received its due attention.
Super-saturating \([\text{CO}_2]\) was reported to restore \(\text{O}_2\) evolution order to force \(\text{CO}_2\) to diffuse across the whole leaf surface and reasons, some researchers used \(\text{O}_2\) electrodes to measure conductance in response to water stress (Cornic, 2000). For these part of the inhibitory effects of water stress on \(\text{O}_2\) evolution rates (Saccardy et al.). Reduced the sites of \(\text{Rubisco}\). Increased photorespiration (e.g. due to of \(\text{CO}_2\) fixation (\(\text{C}_4\)), and indicates that \(\text{A}\) is \(\text{CO}_2\)-limited. In \(\text{C}_4\) plants, the relationship between \(\text{C}_i\), photorespiration and \(\text{J}/\text{A}\) is more complex (Fig. 4A and B). Photorespiration in \(\text{C}_4\) leaves remains very low under a range of environmental and genetic conditions, and runs at about 3.5–6 % of \(\text{A}\) (Lacuesta et al., 1997; Carmo-Silva et al., 2008). On the one hand, photorespiration may increase – from a very low base – with decreasing \(\text{C}_i\) without any measurable impact on \(\text{A}\) (Fig. 4A). This is because photorespired \(\text{CO}_2\) is most likely refixed within the bundle sheath before escaping to the atmosphere. The modelling results are supported by work on the oxygen sensitivity of \(\text{C}_4\) photosynthesis. In an early study using maize subjected to osmotic stress, Lawlor and Fock (1978) found that \(\text{A}\) changed little in response to increasing \([\text{O}_2]\) from 1.5 % to 21 %. The decline of \(\text{A}\) with \(\Psi_{\text{leaf}}\) was almost indistinguishable between 1.5 % and 21 % \([\text{O}_2]\) (Lawlor and Fock, 1978). In a recent study using three \(\text{C}_4\) grasses subjected to mild and severe water stress, Carmo-Silva et al. (2008) observed no changes in \(\text{A}\) with increasing \([\text{O}_2]\) above an optimum of 10 %, and estimated photorespiration rates were small under all water stress conditions (Carmo-Silva et al., 2008). On the other hand, if water stress were to reduce Rubisco activity independently of \(\text{C}_i\), then both the carboxylation and oxygenation reactions of Rubisco would decrease in equal proportions. Accordingly, photorespiration is predicted to decrease rather increase under water stress (Fig. 3). This is in line with findings by Carmo-Silva et al. (2008). They found that photorespiration increased slightly between well-watered and moderate water-stress conditions, then decreased under severe water stress in two \(\text{C}_4\) grasses (Carmo-Silva et al., 2008). Consequently, while a small \(\text{CO}_2\)-limitation may occur in the early phases of water stress, severe water stress tends to inhibit both photosynthesis and photorespiration in \(\text{C}_4\) plants (Fig. 3).

In contrast to the aforementioned works, Lal and Edwards (1996) reported increased \(\text{J}/\text{A}\) in maize and amaranthus exposed to water stress, and concluded that \(\text{A}\) was \(\text{CO}_2\)-limited in these two \(\text{C}_4\) species under water stress (Lal and Edwards, 1996). In this study, the ambient \([\text{CO}_2]\) used for the low \([\text{CO}_2]\) comparison (Fig. 4 in Lal and Edwards, 1996) was much lower than the \(\text{C}_i\) observed in the water-stressed leaves (fig. 1 in Lal and Edwards, 1996). Hence, the two situations, water stress and low \(\text{C}_i\), were not comparable in their study. In the modelling example presented in Fig. 4, \(\text{J}/\text{A}\) showed a biphasic response to \(\text{C}_i\) (Fig. 4B). Below a \(\text{C}_i\) of \(\sim 0.2\ \text{µbar}\), \(\text{J}/\text{A}\) increases with decreasing \(\text{C}_i\) due to increasing photorespiration (Fig. 4A and B). This is comparable to the low \([\text{CO}_2]\) and moderate water stress treatments in Lal and Edwards (1996) and Carmo-Silva et al. (2008), respectively. Above a \(\text{C}_i\) of \(\sim 20\ \text{µbar}\), \(\text{J}/\text{A}\) increases with increasing \(\text{C}_i\), which may be due to increased leakiness (\(\Psi\), the fraction of \(\text{CO}_2\) fixed by \(\text{PEPC}\) which leaks out of the bundle sheath). It should be noted that, although leakiness is predicted to increase with \(\text{C}_i\) (Fig. 4B), this was not confirmed experimentally (Henderson et al., 1992). Nevertheless, there is some evidence in the literature suggesting that leakiness increases under water stress (Bowman et al., 1989; Saliendra et al., 1996; Williams et al., 2001). Increased \(\text{J}/\text{A}\) as a result of increased leakiness could explain the water stress results of Lal and Edwards (1996). Conclusive testing of this proposition requires the use of sophisticated techniques such as on-line measurement of carbon and oxygen isotopes discrimination by mass spectrometry or tube diode laser.

**Photorespiration in \(\text{C}_4\) plants subjected to water stress**

In \(\text{C}_3\) plants, low \(\text{C}_i\) causes a decrease in \(\text{A}\) and an increase in the rate of photorespiration due to a decreased \([\text{CO}_2] : [\text{O}_2]\) ratio at the sites of Rubisco. Increased photorespiration (e.g., due to reduced \(g\) under drought) causes an increase in the electron cost of \(\text{CO}_2\) fixation (\(\text{J}/\text{A}\), the ratio of electron transport to \(\text{CO}_2\) assimilation rates), and indicates that \(\text{A}\) is \(\text{CO}_2\)-limited. In \(\text{C}_4\) plants, the relationship between \(\text{C}_i\), photorespiration and \(\text{J}/\text{A}\) is more complex (Fig. 4A and B). Photorespiration in \(\text{C}_4\) leaves remains very low under a range of environmental and genetic conditions, and runs at about 3.5–6 % of \(\text{A}\) (Lacuesta et al., 1997; Carmo-Silva et al., 2008). On the one hand, photorespiration may increase – from a very low base – with decreasing \(\text{C}_i\).

**Recovery of photosynthetic rates following re-hydration of \(\text{C}_4\) plants subjected to water stress**

In addition to the aforementioned arguments, there remains one related to the recovery of \(\text{A}\) following re-hydration. Some
studies reported that when plants which have been deprived of water for 3–10 days were re-hydrated, photosynthetic rates, measured in normal air, returned to near control values (i.e. well-watered plants) relatively quickly (Lal and Edwards, 1996; Saccardy et al., 1996; Foyer et al., 1998). This has been interpreted as proof that the photosynthetic capacity remains intact under water stress. However, most of these studies measured the recovery of photosynthetic rates using the C₄ crop maize, which has been exposed to relatively mild stress such as withholding watering for several days (Lal and Edwards, 1996; Saccardy et al., 1996; Foyer et al., 1998). In a study using sorghum, recovery of A was only partial in response to re-hydration (Loreto et al., 1995). Hence, it is important to undertake these measurements using C₄ species other than maize, exposed to different degrees of water stress. In these future studies, it is also important to distinguish whether the recovery of A occurs at the level of the same stressed leaf or the plant.

CONTRIBUTION OF NON-STOMATAL FACTORS TO THE INHIBITION OF C₄ PHOTOSYNTHESIS UNDER WATER STRESS

As for stomatal factors, arguments related to non-stomatal inhibition of A are very similar to those advanced for C₃ photosynthesis subjected to water stress (Lawlor, 2002). They include reduced activity of photosynthetic enzymes, decreased ATP concentration, inhibition of nitrate assimilation, induction of early senescence, and changes to the leaf anatomy and ultrastructure amongst others (Fig. 3 and Table 1). These metabolic factors have been reviewed recently by Lawlor (2002), Flexas and Medrano (2002) and Flexas et al. (2004). Therefore, in this review, my discussion is limited to evidence from the C₄ literature for the operation of such factors under water stress. In particular, I focus on the main point of difference with C₃ photosynthesis, which is the differential impact of water stress on the activity of C₃ and C₄ cycle enzymes.

Impact of water stress on the activity of C₃ and C₄ cycle enzymes

A number of studies have reported significant changes in the activity of photosynthetic enzymes in C₃ plants subjected to water stress (Table 1). For Rubisco, most studies reported decreased activity under water stress, while a couple of studies found no change (Table 1). In contrast, the response of the key C₄ cycle enzymes appears to be less consistent, with some studies reporting a decrease in activity, while others report no change or even increased activity under water stress (Table 1). This makes it difficult to draw firm conclusions about the role of these enzymes in water stress-induced photosynthetic inhibition in C₄ plants. This is further complicated by the fact that the literature offers only patchy data on a limited number of C₄ species. Nevertheless, a number of observations can be made regarding this aspect. In particular, there seems to be a more consistent inhibition of the activity of C₃ (e.g. Rubisco) than C₄ (e.g. PEPC) cycle enzymes in response to water stress (Table 1). In other words, the available, albeit limited, data suggest that water stress may lead to a decrease in the activity ratio of C₃/C₄ cycle enzymes in C₄ plants. This argument is supported by studies which reported increased leakiness in water-stressed C₄ plants (Bowman et al., 1989; Saliendra et al., 1996). Increased

### Table 1. A literature survey of the effects of water stress on the activity of selected enzymes in a number of C₄ species

<table>
<thead>
<tr>
<th>Species</th>
<th>Assay basis</th>
<th>Stress indicator</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zea mays</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 20%</td>
<td>Becker and Fock (1986)</td>
</tr>
<tr>
<td>Saccharum sp</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 30%</td>
<td>Bekker and Fock (1986)</td>
</tr>
<tr>
<td>Amaranthus cruentus</td>
<td>Leaf area</td>
<td>RWC</td>
<td>slightly</td>
<td>Lal and Edwards (1996)</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 50%</td>
<td>Lal and Edwards (1996)</td>
</tr>
<tr>
<td>Setaria sphacelata</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 30%</td>
<td>Saccardy et al. (1996)</td>
</tr>
<tr>
<td>Cynodon dactylon</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 50%</td>
<td>Lal and Edwards (1996)</td>
</tr>
<tr>
<td>Paspalum dilatatum</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 30%</td>
<td>Saliendra et al. (1996)</td>
</tr>
<tr>
<td>Zoysia japonica</td>
<td>Leaf area</td>
<td>RWC</td>
<td>by 18%</td>
<td>Marques da Silva and Arrabaca (2004)</td>
</tr>
<tr>
<td>Scenedesmus obliquus</td>
<td>Chlorophyll</td>
<td>RWC</td>
<td>by 50%</td>
<td>Marques da Silva and Arrabaca (2004)</td>
</tr>
</tbody>
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leakiness may be caused by a number of factors, one of which is reduced activity of C₃ relative to C₄ cycle enzymes (von Caemmerer and Furbank, 1999). In particular, if the carboxylation activity decreases more than the decarboxylation activity, CO₂ consumption will fall in the bundle sheath, leading to an increase in [CO₂]ᵦᵣᵦₑ. A greater [CO₂]ᵦᵦₑ leads to a greater [CO₂] gradient across the bundle sheath cell walls, and hence a greater leakage of CO₂. In the study by Saliendra et al. (1996), increased leakiness was related to a decrease in Rubisco/PEPC activity ratio as a result of no change in Rubisco activity and a slight increase in PEPC activity. Bowman et al. (1989) concluded that a decrease in the C₃/C₄ activity ratio was the likely factor behind increased leakiness based on two main reasons. First, the changes in leakiness in response to water stress underwent diurnal fluctuations. This indicated that increased leakiness was caused by biochemical rather than anatomical factors (e.g. changes in the properties of bundle sheath cell wall and membranes). Secondly, there was a linear relationship between changes in leakiness and photosynthetic inhibition in response to water stress. This indicates that activities of C₃ cycle enzymes are more sensitive to water stress, assuming that this cycle is limiting C₄ photosynthesis (Bowman et al., 1989). The differential response of C₃ and C₄ cycle enzymes to water stress and their eventual impacts on leakiness in C₄ plants is an important aspect which awaits further work.

Other non-stomatal factors

For C₄ plants, there is good evidence indicating that nitrate assimilation and nitrate uptake are strongly reduced under water stress (Table 1; Becker and Fock, 1986; Foyer et al., 1998). This may explain the reported decreases in chlorophyll and protein content in a number of C₄ species subjected to water stress (Du et al., 1996; Foyer et al., 1998; Marques da Silva and Arrabaca, 2004b; Carmo-Silva et al., 2007). The decrease in chlorophyll and protein contents under water stress may also be due to generalized protein degradation as a result of induced senescence as suggested by increased contents of amino acids (Becker and Fock, 1986). The induction of senescence under water stress – its timing and the factors which trigger it – is poorly understood.

Using light microscopy, Lal and Edwards (1996) observed ultra-structural distortions (e.g. changes in chloroplast position, distortion of intercellular spaces) in leaves of C₄ species under water stress. Such changes may have significant impacts on CO₂ diffusion inside the leaf as well as light penetration (Flexas et al., 2004). However, due to the lack of data, it is not possible to make much of this aspect at this stage. It is hoped that with the proliferation of more sophisticated microscopic and imaging techniques, especially those which allow the observation of live tissue, there will be more studies published on the effects of water stress on the anatomy of C₃ and C₄ leaves alike.

THE ROLE OF ALTERNATIVE ELECTRON SINKS IN C₄ PHOTOSYNTHESIS EXPOSED TO WATER STRESS

Photospiration results in the release of CO₂ and NH₃ into the atmosphere, and the consumption of ATP and other reducing equivalents. Consequently, photospiration may act as an alternative electron sink in C₃ plants exposed to water stress. By doing so, photospiration can reduce the over-reduction of the photosynthetic electron transport chain (Osmond and Grace, 1995), and allow photosynthesis to recover more quickly after the removal of water stress. For C₃ plants, there is some evidence showing that photosynthetic electron transport increases under mild to moderate water stress, thus maintaining electron flow (e.g. Cornic and Fresneau, 2002; Haupt-Herting and Fock, 2002). Such data is lacking for C₄ plants. However, and as discussed earlier, photospiration remains very low in C₄ plants under a wide range of physiological conditions. Therefore, unlike the case for C₃ photosynthesis, the scope for photospiration acting as a protective electron sink is minimal during C₄ photosynthesis exposed to water stress.

Another photosynthetic, alternative electron sink is the Mehler reaction, which involves the direct reduction of molecular O₂ to superoxide radicals at photosystem I. Most studies involving C₃ plants exposed to moderate water stress indicate that the contribution of the Mehler reaction to total photosynthetic electron flow decreases or remains unchanged (Cornic and Fresneau, 2002; Haupt-Herting and Fock, 2002). For example, in tomato, the percentage of photosynthetic electrons dissipated by the Mehler reaction decreased from 13 % to 6 % in control and water-stressed leaves, respectively, while the proportion of photospiration increased from 23 % to 40 % under water stress (Haupt-Herting and Fock, 2002).

Direct measurements of O₂ exchange in leaves of well-watered C₄ grasses showed that O₂ uptake in the light depends on [CO₂] and light intensity (Siebke et al., 2003). It was estimated that O₂ uptake associated with the Mehler reaction represents about 18 % of total light-dependent O₂ uptake in C₄ leaves (Siebke et al., 2003). This is slightly greater than the rate of Mehler reaction measured in control C₃ leaves, indicating that the Mehler reaction has a slightly greater capacity in C₄ than C₃ leaves. However, it is likely that the Mehler reaction is insensitive – or even slightly suppressed – by water stress in C₄ as in C₃ leaves. Although there are no comprehensive measurements of O₂ exchange in C₄ plants exposed to water stress, a couple of indirect lines of evidence support this conclusion. First, in a study where maize was exposed to mild drought, it was observed that the activities of ascorbate peroxidase and glutathione reductase – enzymes involved in hydrogen peroxide detoxification – were unaffected by drought (Brown et al., 1995). Secondly, in a review, Badger et al. (2000) argued that in higher plants excess light dissipation occurs mainly via non-radiative energy dissipation. Excess electron dissipation by Mehler O₂ uptake is significant mainly in photosynthetic organisms lacking well-developed non-photochemical quenching mechanisms, such as cyanobacteria (Badger et al., 2000). The limited capacity of the Mehler reaction to act as a significant electron sink has been demonstrated by a study using tobacco with a genetically altered amount of Rubisco. Reducing the capacity for both photosynthesis and photospiration in the transgenic, relative to the wild-type, plants did not lead to enhanced electron transport to free O₂ (Rusuksa et al., 2000). Consequently, the limited capacity for the Mehler reaction or photospiration to act as a significant alternative electron sinks may account for the
strong correlation between CO₂ assimilation and electron transport rates observed in C₄ leaves under a wide range of environmental conditions (e.g. Oberhuber and Edwards, 1993).

INTERACTIVE EFFECTS OF ELEVATED [CO₂] AND WATER STRESS ON C₄ PHOTOSYNTHESIS

Due to unprecedented rates of fossil fuel burning and deforestation since the start of the industrial revolution, atmospheric [CO₂] has been rising rapidly (IPCC, 2007). Understanding the effects of rising [CO₂] on C₄ plants is crucial given their significant contribution to the global carbon budget and food security. C₄ plants were not expected to respond to high [CO₂] because C₄ photosynthesis is mostly CO₂-saturated under current atmospheric [CO₂] due to the operation of the CO₂-concentrating mechanism. However, as more research was done on this topic, it became increasingly evident that C₄ plants can accumulate more biomass at elevated [CO₂], particularly if exposed to some form of water stress during growth. These findings were made consistently in controlled-environment and field studies alike (e.g. Samarakoon and Gifford, 1996; Seneweera et al., 1998, 2001; Wand et al., 1999; Wall et al., 2001; LeCain et al., 2003; Leakey et al., 2004, 2006). Ghannoum et al. (2000, 2006) reviewed the mechanism underlying the response of C₄ plants to high [CO₂]. They concluded that elevated [CO₂] enhances biomass production in C₄ plants predominantly via the indirect effects on stomatal conductance. By reducing leaf and hence canopy transpiration, high [CO₂] leads to soil water conservation (Samarakoon and Gifford, 1996; Seneweera et al., 1998, 2001; Wall et al., 2001; LeCain et al., 2003; Leakey et al., 2006). In general, evidence from the literature about CO₂ enrichment argues against a substantial role for stomatal limitation in the observed decline of C₄ photosynthesis under water stress. In particular, high [CO₂] does not directly alleviate the adverse effects of water stress on C₄ photosynthesis (Ghannoum et al., 2003). The latter conclusion was supported by results from free air [CO₂] enrichment (FACE) studies with the C₄ grass sorghum and maize (Wall et al., 2001; Leakey et al., 2004, 2006) and open-top chamber experiments with the C₄ grass Bouteloua gracilis (LeCain et al., 2003). These experiments tested the interaction between elevated [CO₂] and water stress on C₄ photosynthesis in the field and, in the case of FACE, under natural growing conditions. A key advantage of these studies is that plants experience water stress at rates and severities normally experienced by field-grown plants, thus avoiding the need to get into discussions of whether drought occurred in a ‘realistic’ fashion in pots (or detached leaves). The other main advantage is that field studies allow for the impacts of soil feedbacks to be assessed. It is worth noting that some pot studies have attempted to measure changes in soil moisture with C₄ plants, and reported similar results (e.g. Samarakoon and Gifford, 1996; Seneweera et al., 1998, 2001).

The FACE study undertaken in the North American Corn Belt with maize is particularly illuminating for the following main reasons: plants were grown in the field under rain-fed conditions; plants experienced both wet and dry seasons; and comprehensive diurnal and seasonal gas exchange and fluorescence analyses were carried out under growth conditions (Leakey et al., 2004, 2006). When the crop experienced a wet year due to above-average rainfall, A and all other measured photosynthetic parameters were not stimulated by high [CO₂] at any stage of the day or season (Leakey et al., 2004). The failure of high [CO₂] to affect A during the course of the day is particularly interesting because it reveals that the diurnal drifts in A – particularly those brought about by fluctuations in leaf-to-air vapour pressure deficit – in this C₄ crop are not primarily stomatal in nature. Importantly, during the dry year, A was stimulated by elevated [CO₂] only intermittently during the course of the season. This stimulation was associated with improved soil water content as a result of the consistent reductions in g at high [CO₂] in maize (Leakey et al., 2004). In an open top chamber study using a C₄ grass, Wall et al. (2001) reported a similar pattern of responses. Consequently, these studies indicate that when C₄ plants experience drought in their natural environment, elevated [CO₂] alleviates the effect of water stress almost entirely via the indirect effect of reduced stomatal conductance and subsequent improved soil moisture.

CONCLUSIONS

It is well-established that the physiological advantages, conferred by the higher photosynthetic efficiency of C₄, relative to C₃, photosynthesis under high light and temperature, are crucial for the ecological dominance of C₄ plants in open, hot and arid environments (Osmond et al., 1982; Long, 1999). In particular, the presence of a CO₂-concentrating mechanism in C₄ leaves endows them with higher WUE than their C₃ counterparts when compared under standard conditions (Osmond et al., 1982; Long, 1999). However, it remains questionable whether the higher WUE of C₄, compared with C₃, plants leads to greater tolerance to water stress. In this review, I argue that, although the C₄ CO₂-concentrating mechanism offers C₄ photosynthesis a greater buffering capacity against CO₂ shortages brought about by partial stomatal closure under water stress, the biochemistry of C₄ photosynthesis is as – or even more – sensitive than that of C₃ photosynthesis. The reasons are not clear. However, a greater sensitivity of the C₃, relative to the C₄, cycle emerges as a probable site of metabolic limitation under water stress.

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LITERATURE CITED


photoreduction (Mehler reaction) and Rubisco oxygenation. 


