

How Plants Cope with Water Stress in the Field. Photosynthesis and Growth

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Received: 16 August 2001 Returned for revision: 4 December 2001 Accepted: 12 February 2002

Plants are often subjected to periods of soil and atmospheric water deficit during their life cycle. The frequency of such phenomena is likely to increase in the future even outside today's arid/semi-arid regions. Plant responses to water scarcity are complex, involving deleterious and/or adaptive changes, and under field conditions these responses can be synergistically or antagonistically modified by the superimposition of other stresses. This complexity is illustrated using examples of woody and herbaceous species mostly from Mediterranean-type ecosystems, with strategies ranging from drought-avoidance, as in winter/spring annuals or in deep-rooted perennials, to the stress resistance of sclerophylls. Differences among species that can be traced to different capacities for water acquisition, rather than to differences in metabolism at a given water status, are described. Changes in the root : shoot ratio or the temporary accumulation of reserves in the stem are accompanied by alterations in nitrogen and carbon metabolism, the fine regulation of which is still largely unknown. At the leaf level, the dissipation of excitation energy through processes other than photosynthetic C-metabolism is an important defence mechanism under conditions of water stress and is accompanied by down-regulation of photochemistry and, in the longer term, of carbon metabolism.

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Key words: Carbon assimilation, high temperature stress, *Lupinus*, photosynthesis, *Quercus ilex*, *Quercus suber*, stomatal functioning, *Vitis vinifera*, water-stress, xanthophyll cycle.

INTRODUCTION

Periods of soil and/or atmospheric water deficit often occur during a plant's life cycle even outside the arid/semi-arid regions, as reported for temperate deciduous forests (Law *et al.*, 2000; Wilson *et al.*, 2001) or tropical rainforests (Grace, 1999). In the latter, for example, water limitation may prove to be a critical constraint to primary productivity under future scenarios of more arid climates due to global environmental change (Fischer *et al.*, 2001).

Plant responses to water scarcity are complex, involving adaptive changes and/or deleterious effects. Under field conditions these responses can be synergistically or antagonistically modified by the superimposition of other stresses. Plant strategies to cope with drought normally involve a mixture of stress avoidance and tolerance 'strategies' that vary with genotype. This complexity is well illustrated in Mediterranean-type ecosystems where plants with predominant drought-avoidance strategies (e.g. deep-rooted perennials or winter/spring annuals), which die when they run out of water, coexist with drought-tolerant sclerophylls.

Early responses to water stress aid immediate survival, whereas acclimation, calling on new metabolic and structural capabilities mediated by altered gene expression, helps

to improve plant functioning under stress (Bohnert and Sheveleva, 1998). Some of these responses occur at the leaf level in response to stimuli generated in the leaf itself or elsewhere in the plant. They have a negative influence on carbon assimilation and growth. However, it is the integrated response at the whole plant level, including carbon assimilation and the allocation of photoassimilates to different plant parts and reproductive ability, that finally dictates survival and persistence under environmental stress (Pereira and Chaves, 1993).

Some of the differences among species in growth and survival can be traced to different capacities for water acquisition and transport rather than to drastic differences in metabolism at a given water status. Nevertheless, carbon assimilation at the whole plant level always decreases as a consequence of limitations to CO₂ diffusion in the leaf, diversion of carbon allocation to non-photosynthetic organs and defence molecules, or changes in leaf biochemistry that result in the down-regulation of photosynthesis. Acclimatory changes in the root : shoot ratio or the temporary accumulation of reserves in the stem (Rodrigues *et al.*, 1995) under water deficit are accompanied by alterations in carbon and nitrogen metabolism, the fine regulation of which is still largely unknown (Pinheiro *et al.*, 2001). In perennial plants, when leaves have to withstand drought, the dissipation of excitation energy at the chloroplast level through processes other than photosynthetic C-metabolism

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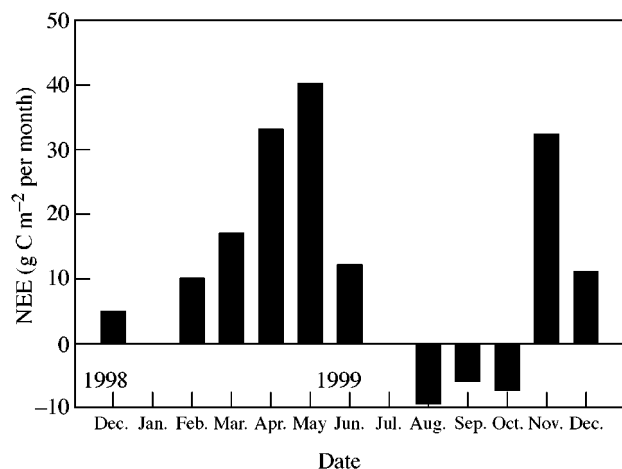


FIG. 1. Net ecosystem exchange (NCE) (in g C m^{-2} per month) by the ecosystem consisting of an evergreen oak woodland ('montado') in Évora, southern Portugal, from December 1998 to December 1999. Measurements were done by the eddy covariance method (M. Rayment *et al.* unpubl. res.).

is an important defence mechanism, which is accompanied by down-regulation of photochemistry and, in the longer term, of photosynthetic capacity and growth.

Here we review different plant strategies to cope with drought, and discuss how regulation of leaf photosynthesis, whole plant carbon assimilation and allocation takes place in response to slowly imposed water deficits under field conditions, presenting examples from our own work with Mediterranean species.

KEEPING THE WATER BALANCE RIGHT

It is possible to separate the effects of water deficit that occur before a large part of a plant's rooting zone has been depleted of water from the effects of severe dehydration that may occur in late summer in the Mediterranean (Pereira and Chaves, 1993). For example, the ecosystem net carbon uptake by a Mediterranean evergreen oak woodland, dominated by *Quercus ilex* ssp. *rotundifolia* and *Q. suber*, declines from around 12 g C m^{-2} per month in June to values close to zero in July and even to negative values during late summer (Fig. 1). This is the result of a diminished net carbon gain by the foliage as well as of increased autotrophic and heterotrophic respiration. The decreased net carbon gain results from large decreases in the rates of photosynthesis at the leaf level (Fig. 2A), due in part to stomatal closure (Fig. 2B) which restricts water losses, but also due to the down-regulation of photosynthesis when drought, high light and high temperatures co-occur (Fig. 3).

We compared the two evergreen oak species, growing side by side near Évora, Portugal, and found that there were no significant differences in net carbon assimilation rates when there was sufficient soil moisture or in mildly water-stressed plants in early July (Faria *et al.*, 1998). However, by the end of the dry, hot summer (September), midday gas exchange in *Q. ilex* ssp. *rotundifolia* was significantly less depressed than that of *Q. suber* (Faria *et al.*, 1998). This

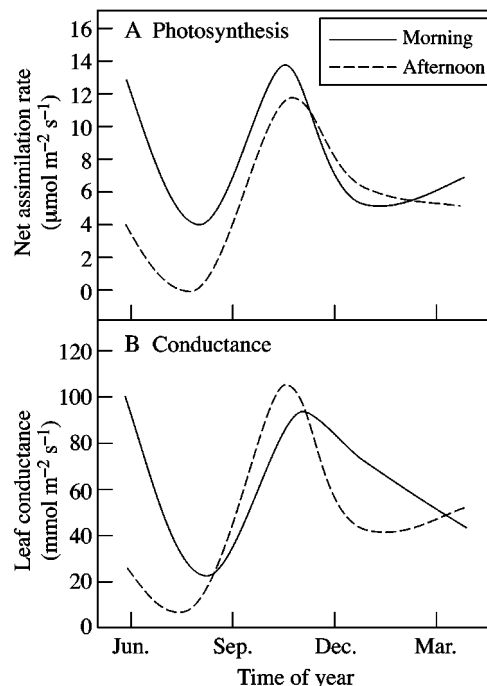


FIG. 2. Seasonal variation in net photosynthetic rate (A) and in leaf conductance (B) of *Quercus suber*, measured in the morning and afternoon, in southern Portugal.

would suggest a less severe water stress in the former. In fact, significantly higher pre-dawn leaf water potentials were observed by the end of summer 1999 in *Q. ilex* ssp. *rotundifolia* (-1.52 MPa) as compared with the -2.38 MPa measured in *Q. suber* (unpubl. res.). We hypothesized that *Q. ilex* roots were able to tap water from deeper soil layers, allowing this species to maintain higher water influx and leaf carbon assimilation rates for a longer period than *Q. suber*.

Herbaceous annuals, such as *Lupinus albus*, also show a promotion of root growth under water deficit. For example, water deficits induced for 15 d at the end of flowering led to a significant increase in fine root length per unit soil volume, even in deeper soil layers (Fig. 8D). In general, shoot growth is more sensitive to water deficit than root growth (Sharp and Davies, 1989). The mechanisms underlying the sustained root growth under water stress include osmotic adjustment (Saab, 1992) and an increase in the loosening capacity of the cell wall (Hsiao and Xu, 2000). The involvement of drought-induced abscisic acid (ABA) and ethylene in shoot and root growth is still under debate (Spollen *et al.*, 2000; Sauter *et al.*, 2001; Sharp and LeNoble, 2002). It seems that an important role of endogenous ABA accumulation in the maintenance of root elongation under drought is the inhibition of ethylene production (Sharp and LeNoble, 2002).

STOMATAL CLOSURE: TRADING WATER SAVINGS FOR CARBON ASSIMILATION

Stomatal control of water losses has been identified as an early event in plant response to water deficit under field

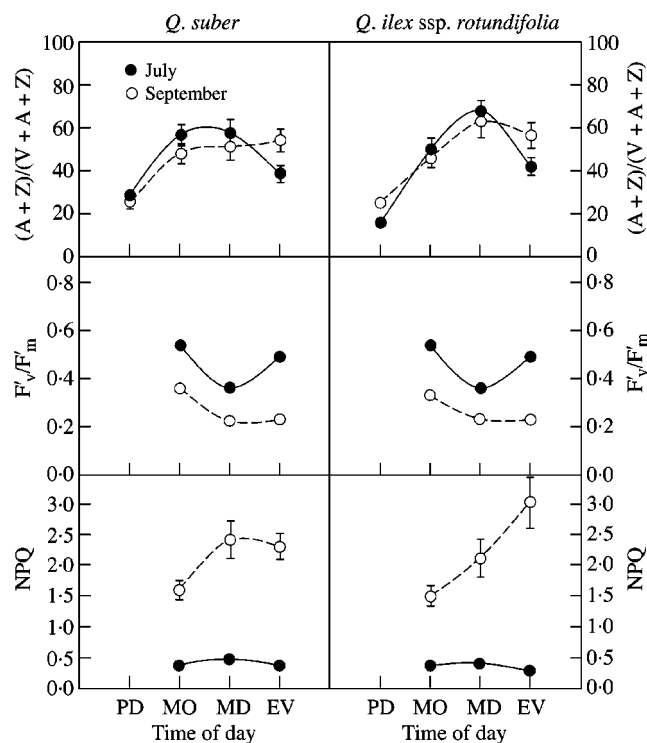


FIG. 3. Diurnal time course of the ratio of xanthophylls ($A + Z$)/($V + A + Z$), PSII efficiency (F_v'/F_m') and non-photochemical quenching of chlorophyll a fluorescence (NPQ) in sun leaves of *Quercus suber* and *Quercus ilex* ssp. *rotundifolia* in early July (closed symbols) and September (open symbols). The data are means $\pm$ s.e. of 12 leaves from three different trees with comparable exposure. A, antheraxanthin; Z, zeaxanthin; V, violaxanthin; PD, pre-dawn; MO, morning; MD, midday; EV, evening. (Adapted from Faria *et al.*, 1998.)

conditions, leading to a limitation of carbon uptake by the leaves (Chaves, 1991; Cornic and Massacci, 1996). Stomata close in response to either a decline in leaf turgor and/or water potential (e.g. Ludlow, 1980) or to a low-humidity atmosphere (Schulze *et al.*, 1986; Maroco *et al.*, 1997). Various experiments have shown that stomatal responses are often more closely linked to soil moisture content than to leaf water status. This suggests that stomata are responding to chemical signals (e.g. ABA) produced by dehydrating roots, whilst leaf water status is kept constant (Gowing *et al.*, 1990; Davies and Zang, 1991). Although most evidence for this kind of response has been obtained under controlled conditions on small plants grown in containers (Davies and Zang, 1991; Jackson *et al.*, 1995), experiments with field-grown plants, such as maize (Tardieu *et al.*, 1991), grapevine (Correia *et al.*, 1995; Stoll *et al.*, 2000) and clover (Socias *et al.*, 1997), also support this hypothesis. Much is known about the role of ABA in closing stomata, as well as its production in dehydrating roots and its circulation in the plant. However, there is still limited knowledge about the exact relationship between water deficit and ABA long-distance signalling and the nature of interactions between ABA and other chemical signals, such as cytokinins and ethylene (Sauter *et al.*, 2001). In mature trees, where long-distance transport of the chemical signal from the roots to

the shoots would be required, the evidence is even less clear (Jackson *et al.*, 1995). Changes in plant hydraulic conductivity have been invoked as playing a major role in short-term stomatal regulation of woody plants (e.g. Saliendra *et al.*, 1995). The interactions between root chemical signalling and changes in plant hydraulic conductivity during drought remain obscure and need further attention (Jackson *et al.*, 2000).

As drought progresses, stomatal closure occurs for increasingly longer periods of the day in field-grown plants, beginning in mid-morning (Tenhunen *et al.*, 1987). This depression in gas exchange simultaneously reduces daily carbon assimilation and water loss at the time of highest evaporative demand in the atmosphere, and leads to a near optimization of carbon assimilation in relation to water supply (Cowan, 1981; Jones, 1992). The causes for this depression in net carbon uptake are still not fully understood and seem to involve mechanisms at both the stomatal (Downton *et al.*, 1988) and chloroplastic level (Correia *et al.*, 1990).

We could not explain the decline in leaf photosynthesis during the day in field-grown plants (such as *Vitis vinifera* L. or *Quercus suber* L.) as being entirely the result of increased light, temperature or leaf-to-air vapour pressure deficit (Correia *et al.*, 1995; Faria *et al.*, 1996). In fact, even when leaves were maintained at near optimal conditions of these parameters a decline in stomatal conductance (g_s) and leaf net carbon assimilation (A) was observed in the afternoon. One hypothesis to explain this depression is the increase in ABA concentration in the transpiration stream throughout the day (Gowing *et al.*, 1993). However, we found no increase in xylem ABA concentration or in the rate of delivery of this compound by the transpiration stream after the morning peak in g_s in field-grown grapevine (Correia *et al.*, 1995). In the absence of diurnal changes in xylem ABA concentration, the midday decline in stomatal conductance may be due to an increased sensitivity to xylem-carried ABA, induced by low leaf water potentials (Tardieu *et al.*, 1993), by increasing xylem sap alkalinity (Schurr and Schulze, 1995; Wilkinson *et al.*, 1998) or by calcium concentration (Schurr *et al.*, 1992). A clear time dependency in stomatal responsiveness to air humidity and leaf water status was also found (Franks *et al.*, 1997; Mencuccini *et al.*, 2000), suggesting that some of the diurnal changes in stomatal function may result from metabolic processes with a circadian rhythm.

MATCHING BIOCHEMICAL CAPACITY FOR CARBON ASSIMILATION WITH CO_2 AVAILABILITY

Changes in cell carbon metabolism are also likely to occur early in the dehydration process as shown by Tezara *et al.* (1999) and Lawlor (2002), although some of them are possibly mediated by low CO_2 availability due to stomatal closure (Sage *et al.*, 1990; Meyer and Genty, 1999). As Cornic (2000) states, some of the metabolic changes that occur as a result of drought are themselves a consequence of the resistance of the photosynthetic apparatus to dehydration, as seems to be the case for the reversible decrease in

nitrate reductase and sucrose phosphate synthase activities (Vassey *et al.*, 1991). These changes can contribute to the maintenance of osmotic pressure within photosynthetic cells by increasing the nitrate concentration and decreasing carbohydrate export. Direct inhibition of shoot growth by water deficit also contributes to solute accumulation and, eventually, to osmotic adjustment (Osório *et al.*, 1998).

When water stress is imposed slowly, as is generally the case under field conditions, a reduction in the biochemical capacity for C assimilation and utilization may occur along with restrictions in gaseous diffusion. For example, in grapevines growing in the field, CO₂ assimilation was limited to a significant extent due to stomatal closure as summer drought progressed, but there was also a proportional reduction in the activity of various enzymes of the Calvin cycle (Fig. 4, Maroco *et al.*, 2002). By mid-summer, Rubisco maximum carboxylation capacity, RuBP regeneration and Triose-P utilization were significantly attenuated at 'veraison' (the stage corresponding to the change in berry colour), when rain-fed vines had a pre-dawn water potential (ψ_{PD}) of -0.97 ± 0.01 MPa as compared with -0.13 ± 0.01 MPa of well-watered plants. The tight co-regulation between mesophyll photosynthesis and stomatal aperture observed in this experiment and by others (Correia *et al.*, 1990; Gunasekera and Berkowitz, 1993; Ort *et al.*, 1994; Tourneux and Peltier, 1995) may reflect a down-regulation of the photosynthetic apparatus by the low carbon availability.

Ort *et al.* (1994) argued that, although light-saturated photosynthesis in field-grown sunflower subjected to soil water deficit was strongly dependent on leaf conductance, an underlying dependence on intercellular CO₂ concentrations (C_i) was also apparent. They showed that there was a decrease (of approx. 25 %) in the rate of net photosynthesis following a 5 min treatment at low C_i (close to the CO₂ compensation point). According to these authors, the response of photosynthesis to C_i indicates that the biochemical demand for CO₂ was down-regulated in response to declining CO₂ availability, associated with drought-induced stomatal closure. This type of down-regulation observed in the photosynthetic demand for CO₂ demonstrates how quickly these adjustments can occur at the chloroplast level.

In summary, under field conditions when mild water deficit develops slowly, one of the first events to take place in plants is presumably stomatal closure in response to the migration of chemical compounds synthesized in dehydrating roots (including ABA). The decline in intercellular CO₂ following stomatal closure apparently induces, in the long-term, a down-regulation of photosynthetic machinery to match the available carbon substrate.

COPING WITH MULTIPLE STRESSES AT THE LEAF LEVEL

Under the Mediterranean-type climate an evergreen habit may be advantageous because it allows plants to take advantage of every environmentally favourable opportunity for carbon uptake and growth (Larcher, 2000). However, long-lived leaves have to survive periods when conditions

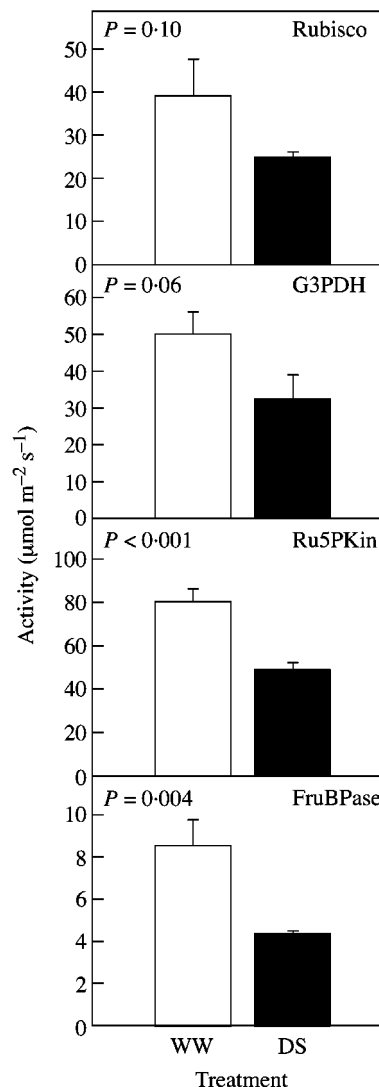


FIG. 4. *In vitro* activities of key enzymes of C metabolism; Rubisco, G3PDH, Ru5PKin and FruBPase in well-watered (open bars) and drought-stressed grapevine (closed bars) in the middle of the summer in Évora, Portugal. Values are means $\pm$ s.e. (From Maroco *et al.*, 2002).

are hostile. This requires various protective measures, ranging from anatomical/morphological characteristics, such as sclerophylly to resist extreme climatic events and herbivory (Turner, 1994), a dense trichome layer as in *Oleae europaeae* for increased reflectance (Larcher, 2000), or steep leaf angles as in 'macchia' shrubs (Werner *et al.*, 1999), to biochemical mechanisms targeted at dissipating excess radiant energy (e.g. the xanthophyll cycle) (Demmig-Adams and Adams, 1996; García-Plazaola *et al.*, 1997).

Small and thick leaves of evergreens are well adapted to the high light, high temperature environments that prevail in most arid regions. Such leaf anatomy enables the greatest carbon gain over transpiration losses under a prolonged hot and dry season (Givnish, 1979). The better light interception and higher water use efficiency permitted by increased thickness may be counteracted by the tendency to become hotter than ambient air when stomata close and to restrict

TABLE 1. Proportion of the VAZ (violaxanthin + antheraxanthin + zeaxanthin) pool compounds per unit of total chlorophyll in sun leaves of *Quercus suber* and *Q. ilex* ssp. *rotundifolia*, in early July and September

	<i>Quercus suber</i>		<i>Quercus ilex</i>	
	Early July	September	Early July	September
V + A + Z [mmol mol(Chl) ⁻¹]	75.0 ± 6.9	132 ± 20	64.6 ± 7.3	104 ± 16

Means ± s.e. of at least 12 replicates. Differences between species were not significant using Student–Newman–Keuls test. (Adapted from Faria *et al.*, 1998.)

latent heat exchange under drought. However, leaf temperature does not rise much above air temperature because the small size of the leaves allows for increased heat dissipation through convection/conduction. Even so, leaf temperatures 4–8 °C higher than air temperatures have been reported for *Q. ilex* during the summer (Larcher, 2000).

A decline in photosynthesis was observed by the end of the dry season (September in Portugal) as compared with early summer (July), in both *Q. ilex* and *Q. suber* (Faria *et al.*, 1996, 1998). This decline was associated with a decrease in quantum yield of photosystem II (PSII) (F'_v/F'_m) that was most marked during the warmest part of the day when carbon assimilation was limited by the decrease in stomatal conductance, and which may be viewed as an important protective mechanism under drought in these evergreen trees. This down-regulation of photosynthesis resulted from the thermal dissipation of excessive excitation energy in the chloroplasts, as shown by the increase of non-photochemical quenching (NPQ) in September compared with July (Fig. 3). In the latter month, more than 60–70 % of the photon energy absorbed by the leaves at midday was dissipated thermally. Martinez-Ferri *et al.* (2000) reported similar values for various Mediterranean tree species. This ability to dissipate energy is associated with an increase in the concentration of de-epoxidized xanthophyll cycle components, antheraxanthin (A) and zeaxanthin (Z), at the expense of violaxanthin, occurring during the day, as reported by Demmig-Adams and Adams (1996) and García-Plazaola *et al.* (1997).

In the case of *Q. ilex* and *Q. suber*, down-regulation of photosynthesis was also associated with the increased capacity of the xanthophyll cycle pool (Table 1) and the accompanying decrease in leaf chlorophyll concentration (Fig. 5), which was observed from July to September. A smaller pool and reduced efficiency of PSII open centres, driven by lower protein and chlorophyll contents, was also observed in rain-fed grapevines after a prolonged period of drought (Maroco *et al.*, 2002). However, no permanent damage to PSII centres was observed under these conditions, as indicated by PSII quantum yield values of dark-adapted leaves which remained close to the optimal value of 0.8. In grapevines, the lower light use efficiency under drought was accompanied by the down-regulation of C metabolism, understood to be an adjustment of the

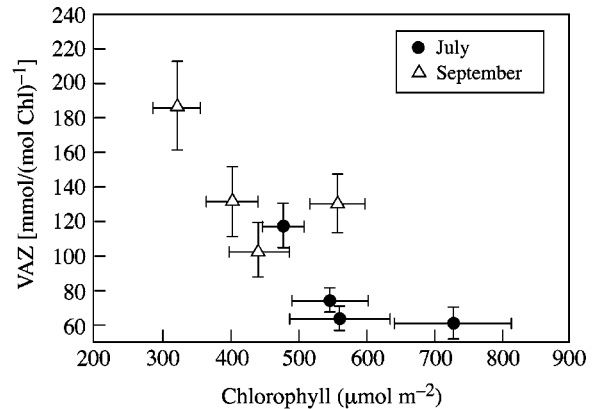


FIG. 5. Relationship between the amount of VAZ (violaxanthin, antheraxanthin and zeaxanthin) pool compounds and total chlorophyll in sun leaves of *Quercus suber*, *Quercus ilex* ssp. *rotundifolia*, *Olea europaea* and *Eucalyptus globulus* trees in early July (closed symbols) and September (open symbols) ($r^2 = 0.67$). Data are means ± s.e. for six leaves from three different trees with comparable exposure. (Adapted from Faria *et al.*, 1998.)

photosynthetic machinery to a reduction in available resources (water, nutrients, carbon).

When it co-occurs with high light and temperature, water stress exerts some of its effect through oxidative damage, which may be associated with an increase in the Melher reaction (Biehler *et al.*, 1996; Haupt-Herting and Fock, 2002). Antioxidants, as scavengers of reactive oxygen species (Foyer *et al.*, 1994; Smirnov, 1998), play a role in the protection of the photosynthetic machinery against excitation energy not dissipated via PSII or other processes such as non-radiative decay or photorespiration, which may increase during drought (Wingler *et al.*, 1999). High concentrations of antioxidant systems were observed during the summer in Mediterranean woody species, either enzymatic (superoxide dismutase, ascorbate peroxidase and glutathione reductase), as in the case of *Quercus suber* leaves (Faria *et al.*, 1996), or non-enzymatic (α -tocopherol or diterpenes), as in *Rosmarinus officinalis* (Munné-Bosch *et al.*, 1999).

Emissions of biogenic isoprenoid compounds from Mediterranean woody species were also reported to increase thermotolerance during summer stress (Loreto *et al.*, 1998; Logan *et al.*, 2000).

In addition to the escape strategy already mentioned, herbaceous plants in Mediterranean-type climates show some leaf tissue tolerance to dehydration allowing rapid recovery of the photosynthetic apparatus following short spells of drought. A remarkable resistance to dehydration of the photosynthetic apparatus was observed, e.g. in lupins, especially in the younger leaves (Quick *et al.*, 1992; Pereira and Chaves, 1993). Upon rehydration, younger leaves of white lupin, (*Lupinus albus*; a Mediterranean winter annual) showed higher Rubisco content, as well as higher soluble sugar (glucose) accumulation, compared with older leaves (David *et al.*, 1998). Soluble sugars may act as osmoprotectants as well as being sources of carbon for maintenance and re-growth during recovery.

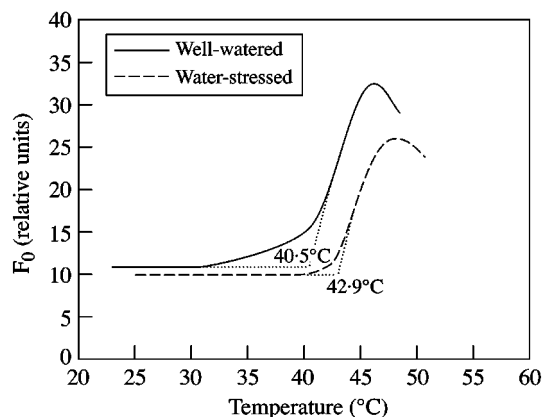


FIG. 6. Response of basal chlorophyll *a* fluorescence (F_0) to leaf temperature in well-watered and water-stressed *Lupinus albus* L.

The effects of water deficit on photosynthetic capacity in lupins were shown to be dependent on leaf temperature and incident light (Chaves *et al.*, 1992). The data indicated that at optimal or sub-optimal temperatures for photosynthesis (25 and 15 °C, respectively), photosynthetic capacity only decreased at leaf relative water contents (RWC) around 60 %. This confirms previous reports for other species showing that photosynthetic machinery is highly resilient to water deficit (see Chaves, 1991; Cornic and Massacci, 1996; Cornic and Fresneau, 2002; Lawlor, 2002, for a review of stomatal vs. non-stomatal limitation of photosynthesis). However, when the temperature rose above the optimum (35 °C), photosynthetic capacity was affected at a higher leaf water status (RWC = 80 %).

On the other hand, a study of the heat-induced response of leaf chlorophyll fluorescence (Fig. 6) indicated that critical temperatures for photosynthesis (T_c , i.e. the temperature at which tissue necrosis and a sharp increase in F_0 occurred) increased in water-stressed white lupin by approx. 2.5 °C compared with well-watered plants. Havaux (1992) obtained similar data in various Solanaceae, showing that photosynthesis was significantly less inhibited by temperatures above 38–40 °C in dehydrated plants compared with well-watered plants. Therefore, it seems that when temperatures are close to critical values, water deficit may have a protective role against heat stress. The nature of the protection of PSII against extreme heat stress in water-stressed plants is not yet clear, one hypothesis being that membrane stability increases in dehydrated tissues.

It is well known that temperature also affects the stomatal aperture of leaves (Jones, 1992). In *L. albus*, stomata were more open at higher temperatures (25 °C) than at lower temperatures (15 °C), in either well-watered or water-stressed conditions (Correia *et al.*, 1999). This response may increase leaf cooling under heat stress, which may be critical to survival and acclimation in these heat-sensitive species.

Lupins are also able to get rid of excessive energy by thermal dissipation, associated with an increase in the concentration of the xanthophyll pigments, zeaxanthin and antheraxanthin, at the expense of violaxanthin, as was

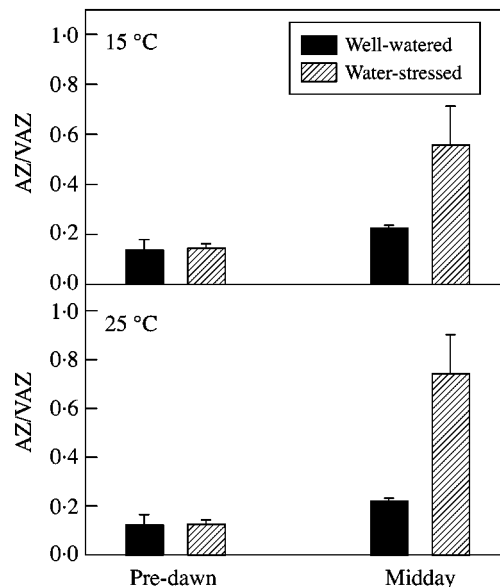


FIG. 7. Ratio of concentrations of the xanthophyll pigments antheraxanthin + zeaxanthin (AZ) to the total xanthophyll pool (violaxanthin + antheraxanthin + zeaxanthin, VAZ), measured pre-dawn and at midday in well-watered and water-stressed *Lupinus albus* L. grown at 15/10 °C and 25/20 °C (day/night).

observed at midday, especially in water-starved plants (Fig. 7). The thermal regime during growth also influenced the total pool of xanthophylls as a proportion of the chlorophyll present, the values being higher in plants grown at a lower temperature regime (15 °C day/10 °C night) than in plants grown at higher temperatures (25 °C day/20 °C night) (Table 2). The high proportion of xanthophylls in plants in the lower temperature regime may serve as a protection mechanism in leaves where the chlorophyll concentration is almost double that of plants grown at higher temperatures (Table 2).

AVOIDANCE AND RESISTANCE TRAITS SECURING THE NEXT GENERATION

Water deficit can cause reproductive failure. To avoid this some Mediterranean annuals exhibit a phenological drought avoidance, meaning that they flower and produce seed before water supplies are exhausted. Others can resist drought-spells by accumulating reserves in different organs, normally stems and roots, prior to drought; the reserves are then remobilized during the reproductive phase. This is a well-known adaptive response to water deficit which has been documented in cereals (Austin, 1977; Palta, 1994; Gebbing, 1999), and was also observed in the Mediterranean native *Lupinus albus* (Rodrigues *et al.*, 1995). Three ecotypes of *L. albus* responded to 15 d of water shortage during flowering by losing 50 % of the total leaf canopy and increasing stem dry weight by 55 %, whilst maintaining total seed production (Fig. 8).

The maintenance of seed production in water-stressed lupin is due to their ability to temporarily accumulate assimilates in the shoot which are later diverted to the pods

during the seed filling stage. ^{13}C -labelling was used to study carbon partitioning in two lupin species, *L. albus* and *L. mutabilis*, during a 20-d water-stress period initiated 10 d after anthesis (pre-dawn water potential decreased to approx. -0.65 MPa by the end of the drought period). Sampling was carried out at three dates: (1) immediately after labelling, just before imposition of water stress; (2) at the end of the stress period, 30 d after anthesis; and (3) at the end of the growing cycle, 60 d after anthesis. Results showed that 10 d after $^{13}\text{CO}_2$ -labelling, a significant increase in $\delta^{13}\text{C}$ occurred in all plant tissues. The largest relative increase was observed in leaves, followed by stems,

pod coats, roots and seeds (Fig. 9). At the end of the drying cycle, $\delta^{13}\text{C}$ decreased significantly in leaves, stems and roots, whereas in pod coats and seeds $\delta^{13}\text{C}$ increased significantly. Moreover, the increase in $\delta^{13}\text{C}$ in pod coats and seeds of water-stressed plants was higher than that in the same organs of well-watered plants. At harvest, 60 d after anthesis, the retention of ^{13}C label was still high in seeds and pod coats of water-stressed plants (with pod coats acting as an intermediate compartment in relation to the seeds), intermediate in stems and was not detectable in leaves and roots.

The increased ability of lupins to divert photoassimilates to pods when subjected to water deficit confirms earlier data by Withers and Forde (1979) showing that the sink capacity of seeds and pods is stimulated by water stress. It is also apparent that a large photosynthetic accumulation prior to flowering is an important factor for plant production and survival during a drought event that does not disrupt the flowering process. Differences among genotypes in the ability to store and utilize stem reserves, as well as in photosynthetic capacity, are likely to be exploited in crop breeding for arid and semi-arid regions.

In plants subjected to drought, biochemical changes in stems and the processes regulating storage of reserves are still not well understood. A recent study by Pinheiro *et al.* (2001) showed that water deficit in *L. albus* brings about tissue-specific responses that are dependent on the intensity of the stress. The stem (specifically the stele) responds to intensification of the stress with striking increases in the concentration of sugars, N and S, the induction of thaumatin-like-protein (TL) and increased activity of the enzymes chitinase (ChT) and peroxidase. These proteins are typically related to adverse conditions, including pathogen attack (Riccardi *et al.*, 1998; Tabaeizadeh, 1998). The activity of invertase (INV_A) increased under mild stress and dramatically decreased with severe water deficit. The particular response of INV_A may be related to the central role played by this enzyme in the modulation of plant responses to abiotic and biotic stresses (Kingston-Smith *et al.*, 1999; Roitsch, 1999).

It is recognized that sucrose and other sugars regulate the expression of many genes involved in photosynthesis, respiration, N and secondary metabolism, as well as defence processes, thus integrating cellular responses to stress (Koch, 1996; Jang and Sheen, 1997). The large alterations

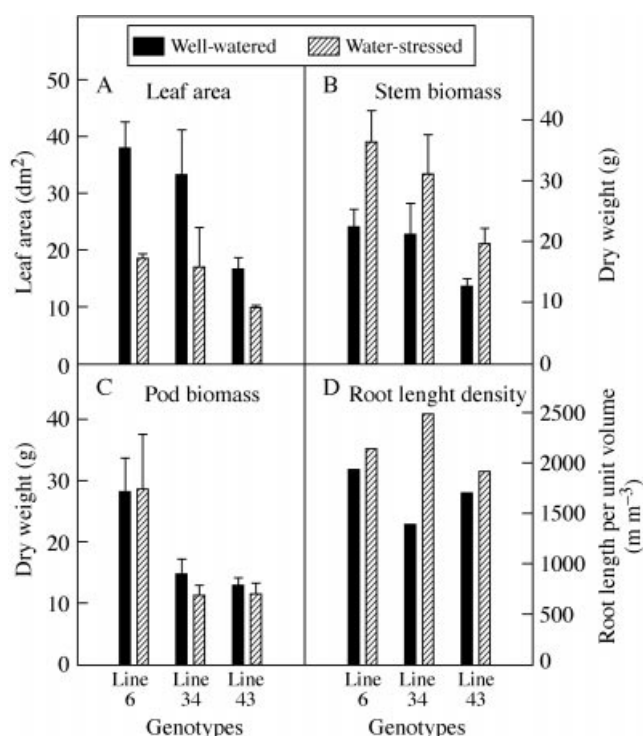


FIG. 8. Leaf area, stem and pod dry weight, and root length per unit soil volume per plant in well-watered and water-stressed *Lupinus albus* L. lines (lines 6, 34 and 43). The drought period of 15 d was imposed at the end of flowering. By the end of the drying period well-watered and water-stressed plants exhibited ψ_{pd} values around -0.1 MPa and -0.6 MPa, respectively. Each value represents the mean ($\pm$ s.e. except root length) of four plants. (Adapted from Rodrigues *et al.*, 1995.)

TABLE 2. Amount and activity of Rubisco per unit leaf area, proportion of the VAZ cycle compounds to total chlorophyll and amount of chlorophyll a + b (Chl) per unit leaf area in well-watered and water-stressed *Lupinus albus* L. grown at 15/10 °C and 25/20 °C

	Rubisco (g m ⁻²)	Initial act. Rubisco ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Total act. Rubisco ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	VAZ (mmol mol ⁻¹ Chl)	Chlorophyll ($\mu\text{mol m}^{-2}$)
15/10 °C					
Well-watered	1.91 $\pm$ 0.14	102.9 $\pm$ 4.5	148.0 $\pm$ 11.7	153.9	575.7 $\pm$ 36.2
Water-stressed	2.05 $\pm$ 0.24	103.9 $\pm$ 6.1	123.8 $\pm$ 5.8	163.2	788.5 $\pm$ 82.2
25/20 °C					
Well-watered	1.18 $\pm$ 0.32	85.8 $\pm$ 4.6	115.0 $\pm$ 9.9	127.0	327.2 $\pm$ 48.5
Water-stressed	1.15 $\pm$ 0.09	79.7 $\pm$ 4.9	100.1 $\pm$ 7.2	149.6	285.0 $\pm$ 53.6

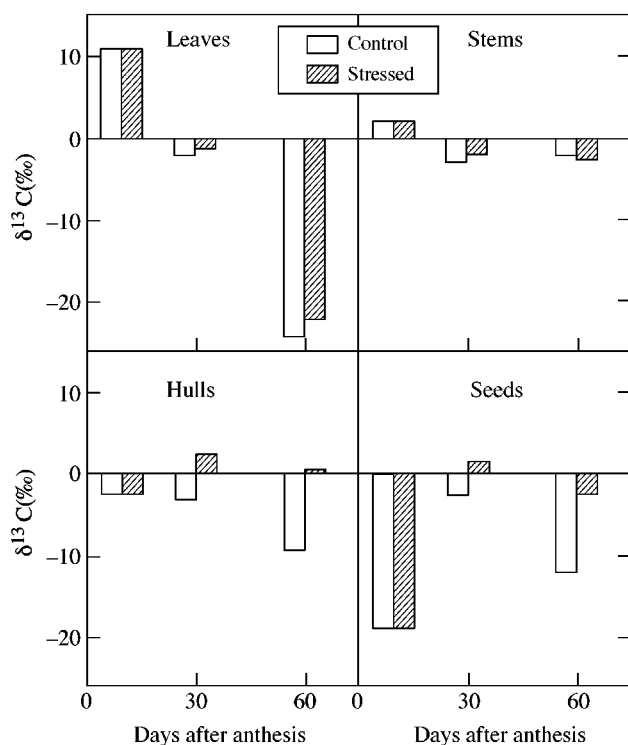


FIG. 9. Discrimination of ^{13}C in the different tissues of well-watered (open bars) and water-stressed (hatched bars) *Lupinus albus* plants at three different times: 10, 30 and 60 d after anthesis. Water was withheld 10 d after anthesis. Mean values of four replicates.

that were observed in sugar metabolism of *L. albus* preceded the accumulation of N and S in the stem and also the increase in stem ChT, TL and peroxidase. Changes in peroxidase, TL and ChT under water deficit could be related to changes in cell wall properties, which are potentially important for plant survival under a variety of environmental stresses and pest attack and disease.

In conclusion, water stress strongly affects photosynthesis, growth and survival of plant species growing in semi-arid climates, such as the Mediterranean. In the field, water deficits do not act alone, but are normally associated with high temperature and high light stresses. Therefore, plant responses to drought during summer also involve adjustments to the stresses associated with drought. While trees and shrubs have developed a 'strategy' of stress tolerance and avoidance, herbs and annuals rely mostly on rapid growth to escape 'summer' stresses as well as on fast responses of the photosynthetic and C metabolism machinery to early signs of stress, including storage of reserves in the stem or roots. When water deficits develop slowly, as in the field, one of the first events to take place in plants is stomatal closure in response to the migration of chemical compounds synthesized in dehydrating roots (including ABA). The decline in intercellular CO_2 following stomatal closure and the lower light use efficiency under drought may induce, in the long-term, a down-regulation of the photosynthetic machinery to match the available carbon substrate.

ACKNOWLEDGEMENTS

We thank Dr Mark Rayment and Professor Paul Jarvis (Institute of Ecology and Resource Management, University of Edinburgh) for providing data used in Fig. 1.

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