

Metadata of the chapter that will be visualized in SpringerLink

Book Title	Plant Responses to Drought Stress	
Series Title		
Chapter Title	Water Use Strategies of Plants Under Drought Conditions	
Copyright Year	2013	
Copyright HolderName	Springer-Verlag Berlin Heidelberg	
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Abstract	<p>The growing concerns about water scarcity have focused more attention on water management in agriculture and promotion of water conservation through improved water use efficiency (WUE). Depending on the main purpose of the study, WUE can be estimated at multiple scales, from leaf to whole plant, crop, yield, and ecosystem levels. Drought resistance and WUE are not synonymous and their association is often misunderstood. Effectively, two water use strategies may be employed by woody plants under drought conditions. The prodigal water use behavior is beneficial in conditions where water supply is interrupted for short periods only. The conservative water use is favorable in conditions where a long dry period prevails and is associated with high capacity for drought resistance and slow growth rates. In this chapter, we also examine how human manipulation such as breeding and agricultural management techniques will offer new opportunities to improve plant water use under drought conditions.</p>
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Chapter 6

Water Use Strategies of Plants Under Drought Conditions

Eunice L. V. A. Bacelar, José M. Moutinho-Pereira, Berta M. C. Gonçalves, Cátia V. Q. Brito, José Gomes-Laranjo, Helena M. F. Ferreira and Carlos M. Correia

Abstract The growing concerns about water scarcity have focused more attention on water management in agriculture and promotion of water conservation through improved water use efficiency (WUE). Depending on the main purpose of the study, WUE can be estimated at multiple scales, from leaf to whole plant, crop, yield, and ecosystem levels. Drought resistance and WUE are not synonymous and their association is often misunderstood. Effectively, two water use strategies may be employed by woody plants under drought conditions. The prodigal water use behavior is beneficial in conditions where water supply is interrupted for short periods only. The conservative water use is favorable in conditions where a long dry period prevails and is associated with high capacity for drought resistance and slow growth rates. In this chapter, we also examine how human manipulation such as breeding and agricultural management techniques will offer new opportunities to improve plant water use under drought conditions.

6.1 Introduction

Climate change has emerged in recent years as one of the most critical phenomena affecting our planet. The latest scientific findings reported by the Intergovernmental Panel on Climate Change (IPCC), show changes in some extreme events, particularly daily temperature extremes and heat waves. Precipitation has

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26 generally increased over land north of 30°N from 1900 to 2005, but there is
27 evidence that droughts will intensify over the coming century in southern Europe
28 and the Mediterranean region, central Europe, central North America, Central
29 America and Mexico, northeast Brazil, and southern Africa (IPCC 2011). Given
30 these facts, the question is which are and will be their impacts on plants, animals,
31 and ecosystems.

32 Climate change can have both beneficial and detrimental impacts on plants.
33 Researchers believe that climate change will increase productivity in certain
34 crops and regions, and reduce productivity in others (Karl et al. 2009). Many
35 species show positive responses to elevated carbon dioxide and low levels of
36 warming, but higher levels of warming often negatively affect growth and yields.
37 Nevertheless, it is expected that heat, drought, climate-associated pests and
38 diseases, flooding, and extreme weather events will all contribute to a decline in
39 plant productivity.

40 As a result of increasing populations and unsustainable practices in water usage,
41 severe strains on freshwater resources are being felt around the world, especially in
42 the rapidly developing countries. Water is an important component of the
43 metabolism of all living organisms, facilitating many vital biological reactions by
44 being a solvent, a transport medium, and an evaporative coolant (Bohnert et al.
45 1995). In plants and other photoautotrophs, water plays the additional role of
46 providing the energy necessary to drive photosynthesis. Thus, any limitation in the
47 availability of water has a great influence on plant life. The growing concerns
48 about water scarcity have focused more attention on water management in agri-
49 culture and promotion of water conservation through improved water use effi-
50 ciency (WUE). The way to increase WUE in plants is through human manipulation
51 such as breeding or crop management techniques. Manipulating plant drought
52 resistance mechanisms will offer new opportunities to improve WUE in water-
53 scarce environments.

54 6.2 Water Use Strategies and Drought Resistance 55 Mechanisms

56 Many environmental conditions can lead to water deficit in plants. Periods of little
57 or no rainfall can lead to drought episodes. The period of time without rainfall
58 actually needed to produce a drought episode depends mainly on the water holding
59 capacity of the soil and the rate of evapotranspiration (ET) (Jones 1992; Larcher
60 1995; Kozłowski and Pallardy 1997). Water deficit in plants can also occur in
61 environments in which water is not limiting (Bray et al. 2000). In saline habitats,
62 the presence of high salt concentrations makes it more difficult for plant roots to
63 extract water from the environment. Soil temperatures between 0 and 15 °C reduce
64 soil and plant hydraulic conductance and eventually water uptake through an
65 increase in the viscosity of water. However, the decrease in water viscosity cannot



66 fully explain the decrease in the root water uptake rate (Bloom et al. 2004) and the
67 reduction of root hydraulic conductivity has also biological–metabolic causes
68 (Aroca et al. 2012). It has been suggested that the decrease in root hydraulic
69 conductivity upon exposure to low temperatures could be caused by an inhibition
70 of aquaporin activity (Wan et al. 2001; Murai-Hatano et al. 2008; Ionenko et al.
71 2010; Aroca et al. 2012). Exposure to temperatures below 0 °C, known as freezing
72 temperatures, can lead to cellular dehydration as water leaves the cells and forms
73 ice crystals in the intercellular spaces. Occasionally, well-watered plants will show
74 periodic signs of water stress such as transient loss of turgor during some periods
75 of the day. In this case, wilting indicates that the transpirational water loss has
76 exceeded the rate of water absorption.

77 The ability of plants to survive the consequences of drought is termed drought
78 resistance. It is important not to confound the significance of drought resistance
79 and WUE. In fact, these terms are not synonymous, although this is often the case
80 in the literature (Blum 2005; Morison et al. 2008). Both features are often con-
81 ferred by plant traits that are mutually exclusive (Blum 2005). In some situations,
82 higher drought resistance is linked to identical (Nelson et al. 2007) or even lower
83 (Moutinho-Pereira et al. 2007; Bacelar et al. 2009) WUE, whereas in others
84 enhanced WUE does not necessarily mean an improved drought resistance (Masle
85 et al. 2005). In other cases, however, increased drought resistance can be asso-
86 ciated with higher WUE (Bacelar et al. 2007a; Moutinho-Pereira et al. 2007;
87 Rivero et al. 2007). Differences in the time of the day and season when they are
88 determined and on the genotypes, plant age, and the experimental environmental
89 conditions may explain these discrepancies.

90 Drought adaptation depends on the severity of water deficit and two different
91 water use strategies may be employed by woody plants (Passioura 1982). The
92 prodigal water use behavior is beneficial in conditions where water supply is
93 interrupted for short periods only. In this situation there is little danger of serious
94 desiccation despite rapid water use, and it enables a plant to grow quickly. In
95 this strategy, plants present high stomatal conductance, high C_i/C_a ratio, and low
96 WUE_{leaf} that are positively correlated with high photosynthetic rate (Bacelar
97 et al. 2009). The conservative water use behavior is beneficial in conditions
98 where a long dry period prevails, enabling the plant to use the available water
99 efficiently. Conservative water use strategies are associated with high WUE_{leaf} ,
100 high capacity for drought resistance, and slow growth rates.

101 There is no universal way by which drought resistance can be achieved and in
102 consequence the different components of such capacity have been classified in
103 different ways by different authors. Classically, plant resistance to drought has
104 been divided into escape, avoidance, and tolerance strategies (Levitt 1972;
105 Turner 1986). Nevertheless, these strategies are not mutually exclusive and, in
106 practice, plants may combine a range of response types (Ludlow 1989).

107 **6.2.1 Drought Escape**

108 Plants that escape drought, like desert ephemerals and annual crop and pasture plants,
109 exhibit a high degree of developmental plasticity, being able to complete their life
110 cycle before water deficit occurs. Escape strategies rely on successful reproduction
111 before the onset of severe stress. Improved reproductive success also includes better
112 partitioning of assimilates to developing fruits and seeds. This is associated with the
113 plant's ability to store reserves in some organs (stems and roots) and to mobilize them
114 for fruit production, a response well documented in crop plants, such as cereals
115 (Bruce et al. 2002) and some legumes (Chaves et al. 2002).

116 **6.2.2 Drought Avoidance**

117 Plants that tend to avoid drought generally have tissues that are very sensitive to
118 dehydration, and thus they have to avoid water deficits whenever water shortage
119 occurs (Ludlow 1989). Dehydration avoidance is common to both annuals and
120 perennials and is associated with a variety of adaptive traits. The most effective
121 protection against drought is a deep, extensively branched root system that can
122 absorb water from a large volume of soil (Arndt 2000). Moreover, roots with low
123 hydraulic conductance or few but long roots can permit a slow but sustainable
124 supply of water to the plant (Passioura 1983).

125 Water movement from the roots to the atmosphere is controlled by the con-
126 ductance of the components of the water pathway (Lovisolo and Schubert 1998).
127 Traditionally, stomatal conductance and root conductivity have been considered
128 the main controlling factors of water flow in the plant (Jones 1992). However, the
129 efficiency of water transport depends on constraints to water movement that affect
130 hydraulic conductivity (Tyree and Ewers 1991). It has been reported that water
131 stress affects shoot conductivity by inducing embolism in xylem vessels (Schultz
132 and Matthews 1988; Tyree and Sperry 1989; Tognetti et al. 1996) or by a reduction
133 in the vessel diameter (Lovisolo and Schubert 1998). Adaptation requires a fine
134 balance because features that reduce vulnerability to cavitation, narrow conduits,
135 and many inter-conduit connections, also reduce hydraulic conductivity that
136 generates the high xylem tensions that trigger embolism (Sperry 2003). In general,
137 vessels with narrow diameters are less susceptible to embolism (Lovisolo and
138 Schubert 1998). However, variations in xylem conduit diameter can radically
139 affect the function of the conducting system because of the fourth-power rela-
140 tionship between radius and flow through a capillary tube, as described by the
141 Hagen-Poiseuille law (Fahn et al. 1986). Under water stress conditions, some
142 plants are able to increase or maintain the level of protection against cavitation and
143 turn more efficient the water transport through the xylem, revealing a trade-off
144 between safety (low vulnerability to cavitation) and efficiency (large hydraulic
145 conductivity) (Bacelar et al. 2007a).

146 Leaves growing under water stress usually develop smaller but more densely
147 distributed stomata. This modification makes a leaf able to reduce transpiration by
148 a quicker onset of stomatal regulation (Larcher 1995). Stomata are mainly con-
149 fined to the abaxial surface and are often hidden beneath dense hairs (peltate scales
150 or trichomes) or in depressions (grooves or crypts). In this way the boundary layer
151 resistance is increased and the air outside the stomata becomes moister (Larcher
152 1995). Other morpho-anatomical traits that help to minimize water loss during
153 drought include leaf rolling (Schwabe and Lionakis 1996), dense leaf pubescence
154 (Karabourniotis and Bornman 1999; Liakoura et al. 1999; Bacelar et al. 2004), a
155 thick cuticle and epicuticular wax layer (Leon and Bukovac 1978; Liakoura et al.
156 1999; Richardson and Berlyn 2002; Bacelar et al. 2004), heavily lignified tissue
157 (Richardson and Berlyn 2002), smaller mesophyll cells and less intercellular
158 spaces (Bongi et al. 1987; Mediavilla et al. 2001). Moreover, leaf movements,
159 such as paraheliotropism, can also prevent damage by high temperatures, dehy-
160 dration, and photoinhibition (Ludlow 1989). Another common feature in water
161 stressed plants is the reduction of the canopy leaf area through reduced growth and
162 shedding of older leaves (Bacelar et al. 2007a). This usually begins with the oldest
163 leaves and progresses toward stem tips. Although the loss of leaves results also in a
164 reduction of the photosynthetic surface it consequently reduces water loss and
165 prolongs survival (Kozłowski et al. 1991).

166 Because of their ability to postpone or avoid tissue water deficits, plants that avoid
167 drought could be said to have good short-term survival. However, they have poor
168 long-term survival, because the avoidance mechanisms eventually fail to prevent
169 dehydration of tissues that are relatively sensitive to desiccation (Ludlow 1980).

170 **6.2.3 Drought Tolerance**

171 Drought tolerance is the ultimate drought strategy (Connor 2005) and it refers to
172 adaptations that allow the plant to withstand the stress, with or without a reduction
173 in performance.

174 Osmotic adjustment is generally regarded as an important drought tolerance
175 mechanism. Most of the adjustment can usually be accounted for by increases in
176 concentration of a variety of common solutes including sugars, organic acids, and
177 ions, especially K^+ (Taiz and Zieger 1998). Enzymes extracted from the cytosol of
178 plant cells have been shown to be severely inhibited by high concentrations of
179 ions. These accumulations during osmotic adjustment appears to occur mainly in
180 the vacuoles, that are kept out of contact with enzymes in the cytosol or subcellular
181 organelles (Taiz and Zieger 1998). Because of this compartmentation of ions, other
182 solutes must accumulate in the cytosol to maintain water potential equilibrium
183 within the cell. These other solutes, called compatible solutes or compatible
184 osmolytes, are organic compounds that do not interfere with enzyme functions.
185 These compatible solutes may protect the cell membrane and the metabolic
186 machinery under dehydration (Chaves et al. 2003).

187 The osmotic compounds synthesized in response to water stress include amino
188 acids like proline, aspartic acid, and glutamic acid (Samuel et al. 2000; Hamilton
189 and Heckathorn 2001; Bacelar et al. 2009), methylated quaternary ammonium
190 compounds (Rathinasabapathi et al. 2001), carbohydrates (Vijn and Smeekens
191 1999), polyols (Smirnov 1998) and low-molecular-weight proteins (Ingram and
192 Bartels 1996).

193 One of the most studied compatible solutes is the amino acid proline. Accumulation
194 of high proline content in cells has also been associated with prevention of protein
195 denaturation, preservation of enzyme structure and activity (Rajendrakumar et al.
196 1994; Samuel et al. 2000), and protection of membranes from damage by reactive
197 oxygen species (ROS) produced under drought and high light conditions (Hamilton
198 and Heckathorn 2001).

199 Betaines are quaternary ammonium compounds in which the nitrogen atom is fully
200 methylated (Chen and Murata 2002). The most common betaines in plants include
201 glycine betaine (GB, the most widely studied betaine), as well as proline betaine, β -
202 alanine betaine, choline-O-sulfate, and 3-dimethylsulfoniopropionate (Rhodes and
203 Hanson 1993; McNeil et al. 1999). GB is widely distributed in higher plants and is
204 synthesized in many species at elevated rates in response to environmental stresses,
205 appearing to be a critical determinant of stress tolerance (Rhodes and Hanson 1993). In
206 addition, studies in vitro have shown that GB is effective as a compatible solute in
207 stabilizing the quaternary structures of enzymes and complex proteins, as well as in
208 maintaining the highly ordered state of membranes (Papageorgiou and Murata 1995).

209 In several species, polyols accumulate in leaves in response to water stress
210 (Noiraud et al. 2000). The most common polyols in plants include acyclic forms,
211 mannitol, glycerol, sorbitol, and cyclic forms (cyclitols), ononitol, and pinitol
212 (Ashraf and Harris 2004). In addition to their role in osmoregulation, polyols also
213 function as oxygen radical scavengers. For example, mannitol was found in vitro
214 to act as a scavenger of hydroxyl radical (Halliwell and Gutteridge 1999), thereby
215 protecting the proteins from oxidative damage in drought stressed plants (Moran
216 et al. 1994). Recent studies support that species like olive tree cope with drought
217 and salinity by coordinating mannitol transport with intracellular metabolism
218 (Conde et al. 2011).

219 In general, soluble sugar content tends to be maintained in the leaves of
220 droughted plants, in spite of lower rates of carbon assimilation (Chaves et al.
221 2003). This is achieved at the expenses of starch, which drastically declines
222 (Chaves 1991). This response favors osmoregulation, enhances desiccation toler-
223 ance and allows metabolic activity to be regained more quickly in young leaves
224 (Chaves et al. 2003).

225 During drought periods, plants undergo many physiological changes and induce a
226 large number of genes for adaptation (Ingram and Bartels 1996). A typical change in
227 gene expression is the induction of genes involved in the synthesis of low-molecular-
228 weight proteins, e.g., dehydrins and late embryogenesis abundant (LEA) proteins
229 (Ingram and Bartels 1996). The functions of LEA proteins are multiple as they are
230 involved not only in seed maturation and desiccation but also in vegetative organs,
231 where they can stabilize enzyme complexes and membrane structures and promote

ion sequestration (Close 1996; Garay-Arroyo et al. 2000). Dehydrins are involved in a range of responses to abiotic stress that share a dehydration component. However, the mechanisms by which dehydrin synthesis is enhanced and functions in vivo remain poorly understood (Cellier et al. 2000). There is increasing evidence that another class of proteins, the small heat-shock proteins (sHsps), may play a role in cellular protection during dehydration (Alamillo et al. 1995; Alpert and Oliver 2002). Strong evidence supports that sHsps function as molecular chaperones (Hendrick and Hartl 1995; Hong and Vierling 2000) that bind to partially folded or denatured substrate proteins and thereby prevent irreversible aggregation or promote correct substrate folding (Sun et al. 2002). They are also described as being developmentally regulated, being abundant in dry mature seeds, and disappearing during germination (Wehmeyer et al. 1996).

Membrane-associated carriers or transporters are probably involved in differentially distributing osmolytes within the cell and may also participate in regulating the transport and distribution of these solutes throughout the plant (Bray et al. 2000). Aquaporins have been implicated as the major facilitators for the movement of water across membranes (Chrispeels and Agre 1994; Bray et al. 2000). In addition, the capacity of aquaporins to transport small neutral solutes and/or gases has raised the intriguing possibility that aquaporins may work as membrane channels with multiple functions (Tyerman et al. 2002). Aquaporins may facilitate water movement in drought stressed tissues and promote the rapid recovery of turgor on watering (Bray et al. 2000).

Changes in the elastic properties of plant cell walls can also contribute to turgor maintenance under drought (Patakas and Noitsakis 1999). An inelastic cell wall should act to increase the uptake of water from drying soil because more negative water potentials would result from a relatively small decrease in water content (Schulte 1993). On the other hand, a more flexible cell should allow shrinkage during water loss, thus maintaining high turgor for a given change in water content (Patakas and Noitsakis 1999). In a study with olive tree (Bacelar et al. 2009) it was observed that cultivars that employ a prodigal water use strategy revealed high tissue elasticity, whereas conservative water use cultivars revealed high tissue rigidity.

An extreme desiccation tolerance is found in the resurrection plants as well as in some ferns, non-vascular plants, algae, and lichens (Chaves et al. 2003). The leaves of resurrection plants, like *Craterostigma plantagineum*, can equilibrate with air close to 0–2 % (v/v) relative humidity and are still able to fully recover their physiological activity upon rehydration (Bartels et al. 1990). When water is withheld from mature individuals of *C. plantagineum*, changes rapidly occur at the messenger RNA and protein levels, eventually leading to the tolerant state (Ingram and Bartels 1996).

The disadvantage of tolerance of water deficits is that the continued carbon fixation is inevitably accompanied by water loss. Thus, plant water status must ultimately fall, what can result in some cases in death (Arndt 2000).

When the use of absorbed light in either photosynthesis or photorespiration and the thermal dissipation are not enough to cope with excess energy, the production of highly reactive molecules is exacerbated (Chaves et al. 2003). The excess of

277 reducing power determines a redirection of photon energy into processes that favor
278 the production of ROS, mainly in the photosynthetic (Asada 1999) and mito-
279 chondrial electron transport chains (Møller 2001). To mitigate the oxidative
280 damage initiated by ROS, plants have developed a complex antioxidative defence
281 system that includes enzymatic and non-enzymatic antioxidants. These compounds
282 and enzymes are not distributed uniformly, so defence systems vary among spec-
283 ific subcellular compartments. The degree to which the activities of antioxidant
284 enzymes and the amount of antioxidants increase under drought stress are extre-
285 mely variable among several plant species and even between cultivars of the same
286 species (Bacelar et al. 2006; Bacelar et al. 2007b).

287 6.3 Estimation of WUE at Multiple Scales

288 WUE could be defined in many ways, depending on the scale of measurement and
289 the main purpose of the studies. For plant physiologists, the basic unit of pro-
290 duction could be moles of carbon gained in photosynthesis (A) in relation to water
291 used in transpiration (E) or to stomatal conductance (g_s), which permits the cal-
292 culation of instantaneous WUE_{leaf} (A/E) or intrinsic WUE_{leaf} (A/g_s), also found as
293 WUE_i in the literature. For farmers and agronomists, the unit of production is
294 much more likely to be the accumulated plant biomass or the yield of harvested
295 product achieved from the water made available to the plants through precipitation
296 or irrigation. With this in mind, in this chapter we present the concepts of whole
297 plant WUE (WUE_{WP}), crop WUE (WUE_C), and yield WUE (WUE_Y). Finally,
298 mainly for forestry and environmental issues the concept of ecosystem WUE
299 (WUE_E) is important, defined as the net carbon uptake per amount of water lost
300 from the ecosystem.

301 Intrinsic WUE_{leaf} presents substantial intra and interspecific variation and is
302 also dependent on environmental conditions, mainly drought and co-occurring
303 stresses such as elevated temperature, high vapor pressure deficit, and elevated
304 photosynthetic photon flux density. For instance, A/g_s was shown to range between
305 around 20 and 80 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ during a typical summer day, with low
306 values generally in the afternoon, when comparing three Portuguese grapevine
307 cultivars (Moutinho-Pereira et al. 2007). On the other hand, in Touriga Nacional
308 grapevine cultivar WUE_{leaf} decreased during the day in the more stressful con-
309 ditions of Port wine country, while it increased more than 100 % in other sites of
310 the Douro Valley, to values higher than 100 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ (Moutinho-
311 Pereira et al. 2004). Meanwhile, Bacelar et al. (2007a) found in olive tree intrinsic
312 WUE_{leaf} values in the range of 80–130 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$, being the higher
313 values under regulated deficit irrigation (RDI), at midday and during the afternoon,
314 whereas in droughted trees it decreased from 110 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ in the
315 morning to 80 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ at midday. Meanwhile, in full irrigated trees,
316 WUE_{leaf} increased from 80 to 90 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ between these periods. Thus,
317 although A/g_s typically increases under water stress conditions (Bota et al. 2001;

318 Flexas et al. 2002), under some environmental conditions this may not occur, such
319 as in some regions of the inland Iberian Peninsula or other regions with Medi-
320 terranean weather conditions. Thus, these results support the possibility of
321 improving WUE_{leaf} by genetic engineering. WUE_{leaf} could be ameliorated by the
322 increment of net carbon assimilation at leaf level (i.e., the difference between
323 carbon gain in photosynthesis and carbon loss in respiration), or by the reduction
324 in stomatal conductance or transpiration rate.

325 The carbon isotope discrimination ($\Delta^{13}C$), a measure of the $^{13}C/^{12}C$ ratio in
326 plant material relative to the value of the same ratio in the air on which plants feed,
327 can be used as a long-term indicator of WUE_{leaf} . Negative correlations between
328 $\Delta^{13}C$ and water use efficiency have been demonstrated in many species, as pre-
329 dicted by theory (reviewed in Farquhar et al. 1989). These observations point to
330 $\Delta^{13}C$ as a potential candidate for use in breeding for greater agronomic water use
331 efficiency (Condon et al. 2004). In addition, the measurements of the O isotope
332 enrichment of plant organic material ($\Delta^{18}O$) can provide complementary infor-
333 mation to that inferred from $\Delta^{13}C$ in analyses of plant water use efficiency. Spe-
334 cifically, $\Delta^{18}O$ could provide information about the ratio of ambient to
335 intercellular vapour pressures, and thus about the leaf to air vapor pressure dif-
336 ference, during photosynthesis (Cernusak et al. 2008).

337 Whole plant WUE represents the whole plant carbon and biomass acquisition per
338 amount of transpired water. Thus, higher WUE_{WP} could be obtained by the drop of
339 plant water losses, the decrease of growth respiration and, mainly, the decrease of
340 maintenance respiration, that uses a substantial proportion of the total carbon
341 assimilated (Amthor 2000), or the increase of photosynthesis by either increasing
342 total leaf surface or increasing leaf photosynthetic capacity. Water losses depend on
343 leaf transpiration rate, which is a function of leaf conductance (including cuticular
344 and especially stomatal conductances) to water vapor, and on dimension and
345 structure of canopy, such as leaf area index and leaf orientation. Leaf conductance
346 depends on well-identified morpho-anatomical and physiological traits that may be
347 targeted for genetic manipulation. Meanwhile, the dimension and structure of the
348 canopy could be regulated by agronomic management techniques.

349 Closely related with WUE_{WP} is WUE_C , although in this case the water con-
350 sumed by the crop includes the transpired water plus the water lost by physical
351 processes, such as soil evaporation and runoff. Thus, in addition to the aspects
352 reported before to WUE_{WP} , higher WUE_C could be achieved by the implemen-
353 tation of agronomic practices that reduce the amount of water lost without being
354 used by the plants.

355 WUE_Y is a variant of WUE_{WP} that represents the carbon and biomass allocated
356 to yield components (mainly fruits and seeds, but also to other plant organs,
357 depending on plant species and the aim of the production). Therefore, higher
358 partitioning to yield organs could increase WUE_Y .

359 It is important to emphasize that improving WUE_{leaf} may not necessarily result
360 in improving WUE_{WP} , WUE_C , and WUE_Y , because of the interference of canopy
361 and ambient processes (Flexas et al. 2010). The absence of a significant association
362 between A/g_s and WUE_{WP} was found by our group in olive tree (Bacelar et al.

363 2007a) reflecting the difference in timescale of both processes (from seconds to
364 months) and non-accounted energy expenses in growth and maintenance in the
365 long-term water use (Maroco et al. 2000). The study of Bacelar et al. (2007a) also
366 shows that WUE_{WP} was enhanced by 64 % in Cobrançosa cultivar under drought
367 conditions, while in the rest of the genotypes it was either unaffected ('Verdeal
368 Transmontana') or considerably decreased ('Madural'). Thus, for production on
369 relatively dry sites, cultivars that are characterized by high values of WUE_{WP} , such
370 as 'Cobrançosa', appear to be the most promising.

371 For the determination of WUE_E , two different methods based on Bowen ratio
372 and eddy covariance systems could be used to evaluate the H_2O and CO_2 fluxes
373 (Emmerich 2007). One method uses the ratio of net ecosystem exchange (NEE)
374 of carbon dioxide to ET by the ecosystem for the growing season or some other
375 time period (Tubiello et al. 1990). A more instantaneous evaluation method of
376 WUE_E is the regression of daily daytime NEE versus ET with the slope value of
377 the regression line, a measure of WUE_E (Baldocchi et al. 2001). Ecosystem
378 differences in CO_2 and H_2O fluxes have important management implications
379 including primary productivity, carbon sequestration, and rangeland health
380 (Emmerich 2007).

381 6.4 Selection and Breeding Programs to Improve WUE

382 Breeding for drought resistance has long been part of the breeding practice in most
383 crops that have been grown under dryland conditions (Blum 2011). Plant breeders
384 use a step-wise selection procedure to identify the best performing genotypes in
385 early generations (Bänzinger et al. 2000; Atlin 2003). First, many genotypes are
386 evaluated with few replicates and at few sites. Later, the most successful genotypes
387 or their descendants are evaluated with more replicates and at more sites. Finally, the
388 breeder reduces both the number of genotypes and the variation among genotypes,
389 mainly by eliminating the poor-performing fraction. According to Bänzinger et al.
390 (2000), the key to breeding for drought tolerance is to manage stress. This is done by
391 conducting experiments partly or entirely in the dry season and managing the stress
392 through irrigation.

393 In the past, breeding efforts to improve drought tolerance have been hindered
394 by its quantitative genetic basis and our poor understanding of the physiological
395 basis of yield in water-limited conditions (Blum 1998; Passioura 2002). Breeders
396 and physiologists addressed the question of how physiological approaches in
397 plant breeding could have greater impact, namely through (Jackson et al. 1996;
398 Reynolds et al. 2001):

- 399 • Focusing physiological work on a suitable range of germplasm;
- 400 • working with larger populations to enable extrapolation of findings to breeding
- 401 processes;

- 402 • identifying traits for use as indirect selection criteria, in addition to those already
- 403 used in core breeding programs;
- 404 • identifying traits for use as selection criteria in introgression programs;
- 405 • conducting selection trials in more representative environments;
- 406 • developing tools that could be quickly and easily applied to large numbers of
- 407 segregating lines.

409 According to Tuberosa and Salvi (2006), drought is the most devastating stress
410 and the most recalcitrant to breeder's efforts of all the abiotic stresses that curtail
411 crop productivity. Moreover, breeding for specific physiological traits that are
412 expected to impart a yield advantage in dry environments has been notoriously
413 difficult and unsuccessful (Richards et al. 2001). In fact, breeding for drought
414 tolerance is further complicated by the fact that several types of environmental
415 stresses can challenge plants simultaneously. High temperatures, high irradiance,
416 scarcity of water, and nutrient deficiencies are commonly encountered under
417 normal growing conditions but may not be amenable to management through
418 traditional farm practices (Mittler 2006; Fleury et al. 2010). In general, it is cru-
419 cially important, according to Bacon (2004), to select genotypes able to optimize
420 water harvest and WUE. Flexas et al. (2010) suggested the enhancement of WUE
421 by biotechnology and genetic engineering in grapevine, mainly by: (1) increasing
422 CO₂ diffusion to the sites of carboxylation without increasing stomatal conduc-
423 tance, thanks to increase in mesophyll conductance to CO₂, which partly depends
424 on aquaporins; and (2) improving the carboxylation efficiency of Rubisco that
425 could be achieved by replacing Rubisco from grapevine with Rubiscos from other
426 C₃ species with higher specificity for CO₂.

427 On the other hand, indiscriminant selection for higher WUE with the
428 assumption that it equates with improved drought resistance or improved yield
429 under stress might bring about serious negative consequences (Blum 2005). If low
430 water use is the breeder's target it is highly probable that selection for this end can
431 be achieved by directly selecting simple traits, such as reduced leaf area, without
432 measuring WUE. Therefore, Blum (2009) recommended that plant breeders tar-
433 geting water-limited environments should consider skipping the use and reference
434 to WUE and consider plant constitutive and adaptive traits which drive the
435 effective use of water (EUW) and the resultant dehydration avoidance as major
436 traits for yield improvement in drought prone environments.

437 At the molecular level, pathways and gene networks between abiotic stresses
438 overlap (Shinozaki and Yamaguchi-Shinozaki 2007). Moreover, some biochemical
439 mechanisms may have opposing effects under different stresses; therefore tackling
440 tolerance to one stress may lead to sensitivity to another (Fleury et al. 2010).
441 Although the reductionist approach of studying isolated stress has considerably
442 increased our knowledge of tolerance mechanisms, interaction between multiple
443 stresses and stress combinations should be studied so as to make progress relevant
444 to field conditions (Fleury et al. 2010). In addition to these confounding envi-
445 ronmental factors, a drought research program should also consider plant phe-
446 nology (Fukai and Cooper 1995; Fleury et al. 2010). For example, breeding for a

447 shortened crop life cycle has been a very successful strategy in C_3 cereals in
448 Mediterranean conditions (Araus et al. 2002). However, in well-developed agri-
449 cultural regions, crop flowering time has already been optimized by breeders so
450 that the plant's phenology matches its environment (Passioura 2007).

451 Pang et al. (2011) found evidences for the development of new perennial
452 legume forages for a wide range of drought prone agricultural regions in breeding/
453 selection programs. The inter- and intraspecific variability of responses to drought
454 stress in the plants examined by those authors suggests a wide range of strategies
455 in perennial herbaceous legumes to cope with drying conditions. For example, leaf
456 osmotic adjustment was observed in *Bituminaria bituminosa* var. *albomarginata*
457 accessions 6 and 10, both accessions of *Cullen australasicum* and *Kennedia*
458 *prostrata* and leaf water potential was maintained at a high level in *B. bituminosa*
459 var. *albomarginata* accessions 6 and 10 and *Macroptilium atropurpureum*.

460 Campos et al. (2004) refereed that the association between genotype and
461 phenotype must be better understood and quantified to improve the ability to
462 predict phenotypic performance based on genetic information. Tinker (2002)
463 specifies that genomics and high throughput laboratory-based analysis of DNA are
464 indispensable to comprehending gene-phenotype associations. This will be criti-
465 cally important for drought tolerance, where performance is regulated by many
466 loci and subject to multiple genotype x environment and gene x gene interactions
467 (epistasis) (Campos et al. 2004). Also, crop simulation models can assist invest-
468 gation of the physiological and genetic basis of genotype variation (Shorter et al.
469 1991) and help selection decisions in plant breeding (Cooper et al. 1993).

470 6.5 Improving WUE by Agricultural Management 471 Techniques

472 It is widely accepted that water is the most universally limiting factor in agri-
473 cultural production systems (Centritto 2005). Natural factors related with the
474 environment and genetic characteristics are basically responsible by the definition
475 of the plant water requirements and, in the last circumstance, by their WUE.
476 However, the farmer's decision, through the crop managements that ensure a better
477 equilibrium between plants and environment, cannot be minimized. This role is
478 much crucial as the local aridity index is higher. Improving of WUE_C is essential
479 for agricultural sustainability under higher potential ET- associated soil moisture
480 deficits exacerbated by the global climatic change (Flexas et al. 2010). To achieve
481 higher WUE_C the increment/validation of scientific monitoring tools is necessary,
482 such as infrared thermometry (Grant et al. 2007), trunk diameter sensors (Conejero
483 et al. 2007), passive chlorophyll fluorescence (Flexas et al. 2002), hyperspectral
484 reflectance indices (Rodríguez-Pérez et al. 2007), or sap flow meters (Fernández
485 et al. 2008). The impact of agricultural management tools on WUE_C has been
486 studied by many researchers. From the social and economic points of view, this

487 subject is of crucial importance for many world regions where the lack of food is a
488 direct consequence of the insufficient and unreliable rainfall and changing rainfall
489 patterns (FAO 2010). This section intends to refer some management techniques,
490 either at soil or plant level, that can optimize crop water use efficiency and crop
491 yield and its quality attributes.

492 *6.5.1 At Soil Level*

493 In those arid and semi-arid areas where the highest crop water requirements coincide
494 with periods without rain, some soil properties such as texture, effective depth, and
495 organic matter content are important to ensure both a high soil water-holding
496 capacity and a good root development. The soil volume that can be explored by the
497 roots is a key factor that affects the plant–water relations and mineral nutrition and
498 therefore its vigor and yield (Keller 2005). Generally, plants are less affected by
499 severe drought when the root system is deeper. This situation should be enhanced by
500 greater soil depth and adequate fertility (Rodrigues et al. 2011).

501 Soil texture affects the erodibility and the availability of water, air, and mineral
502 nutrients (Northcote 1988). In general, sandy soils show poor water availability
503 while more argillaceous textures lead to soils more compact and oxygen-poor,
504 impairing the development of the root system. In an increasingly mechanized
505 agriculture, this negative aspect is exacerbated by excessive traffic of heavy farm
506 machinery.

507 Soil management has great influence on crop–water relations. Cover crops keep
508 the soil protected against erosion, reduce soil compaction, increase water infiltration,
509 introduce organic carbon, and may prevent nitrate leaching. In periods of greater
510 water scarcity, however, this practice affects crops in a manner comparable to deficit
511 irrigation, because the covers use water and reduce nutrient availability for the crops
512 (Lipecki and Berbeć 1997; Rodrigues et al. 2011). Conversely, the critical situation
513 of water resources in most arid regions makes the soil surface management one of the
514 most critical aspects of the agriculture practices applied to rainfed crops (Rodrigues
515 et al. 2005). Thus, it is desirable that cover vegetation dries out naturally (using
516 species that employ a drought escape strategy) or artificially before the drought
517 period, creating a mulch of dead material on the ground. Therefore, the soil rest
518 permanently protected, the water infiltration increases, and the root system develops
519 freely close to the soil surface. In addition, the mulch reduces soil temperature and
520 water losses by evaporation in summer (Van Huyssteen et al. 1984; Possingham 1992;
521 Rodrigues et al. 2011).

522 The mycorrhizal associations have been studied due to their advantages in arid
523 environments. These associations vary widely in structure and functions, but arbus-
524 cular mycorrhizas (AM) are by far the most prevalent of all mycorrhizal categories. It is
525 estimated that 80 % of all terrestrial plants form this type of association, including
526 many agriculturally and horticulturally important crop species (Peterson 2004;
527 Mohammadi Goltapeh et al. 2008). Inoculate soils with appropriate AM fungi may

528 directly enhance root water uptake, contributing to preserve physiological activity in
529 plants under severe drought conditions. Mycorrhizal fungi improved WUE in species
530 such as safflower and wheat (Bryla and Duniway 1997) and watermelon (Kaya et al.
531 2003). Among the mechanisms involved, many are related to the physiology of the host
532 plant, but some are related to the extraradical mycelia (Liu et al. 2007).

533 **6.5.2 At Plant Level**

534 At plant level, assuming that the plant breeding programs already took into
535 account the greater intrinsic water use capacity, there are numerous cultural
536 practices that can be implemented from the beginning of crop cycle to adult stage,
537 to improve this attribute. Normally, these cultural strategies have in common the
538 achievement of high environmental resources efficiency (light, temperature, CO₂,
539 water and nutrients), with significant benefit to crop water economy. First, the
540 management of the crop training system has a fundamental role to reach this goal
541 (Smart 1974). This subject includes a set of cultural techniques selected by the
542 farmers to train and guide the structure of the plant to its best performance. Some
543 of these cultural practices have permanent effects for the crop life, such as
544 the plantation density, the row orientation, and the plant shape, while others are
545 susceptible to annual changes, e.g., all operations performed on the size of the
546 branches and total leaf area during dormancy and the growing season (Carbonneau
547 et al. 1981; Huglin and Schneider 1998). The row orientation has an important
548 influence on WUE_C, both because of the light and thermal canopy microclimate
549 and the thermal soil microclimate effects. Specifically for vineyards in dry and hot
550 regions, some authors (Carbonneau et al. 1981; Intrieri et al. 1998) have proposed
551 an N-S orientation rather than an E-W one, to improve light interception early in
552 the morning (east side) and late in the afternoon (west side). This leads to higher
553 photosynthetic rates, mostly in the morning, when the environmental conditions
554 are relatively more favorable. On the other hand, at midday, the total leaf surface
555 exposed to light is reduced (just the top receives sunlight), which may be helpful in
556 reducing transpiration and preventing the leaf water potential dropping below the
557 threshold values for runaway embolism (Moutinho-Pereira et al. 2001).

558 As referred, the plant density has a crucial role for soil moisture exploitation.
559 Usually, for rainfed fruit tree orchards and vineyards grown in the driest areas, the
560 plant density must be lowered for each plant to have a greater rootzone and thus
561 increasing the available water for each plant. However, it may increase the water
562 loss by ET because the total leaf area and the soil area exposed to direct solar
563 radiation are higher (Yuste 2007).

564 Pruning and removal of shaded leaves influence plant vigor, affecting the ratio
565 between the total leaf area and sun exposed leaf area (Baeza et al. 2005). When
566 this ratio increases, WUE decreases. In very hot and dry regions, given the weak
567 vigor that plants usually present, the maintenance of the leaf surface in good health
568 guarantees greater photosynthetic productivity and allows lower water loss

569 because the integrity of protective tissues, the epidermis and cuticle, is better
 570 safeguarded.

571 In arid regions, irrigation is undoubtedly a cultural practice with enormous
 572 benefits for the plant growth and development (Feres and Evans 2006). However,
 573 under limited water resources, the technical, economic, and cultural irrigation
 574 managements require careful decisions. The efficient management of limited water
 575 resources requires irrigation scheduling techniques based on deficit supplies with
 576 respect to crop water needs (Tognetti et al. 2005). Deficit irrigation, defined as the
 577 application of water below full crop water requirements, is an important tool to
 578 achieve the goal of reducing irrigation water use (Feres and Soriano 2007).
 579 Techniques such as RDI, where a water deficit is applied in a closely controlled way
 580 in low water stress sensitivity periods (Chalmers et al. 1981; Kriedemann and
 581 Goodwin 2003), and Partial Root-zone Drying (PRD), in which half of the root-zone
 582 is irrigated alternatively in scheduled irrigation events (Dry and Loveys 1998), are
 583 being increasingly valued because they allow to reconcile the advantages of a
 584 moderate water stress for a more efficient water use and an adequate development of
 585 the plants (Santos et al. 2007). In Australia, PRD has resulted in water savings of up
 586 to 50 % compared with the conventional irrigation of grapevines. An additional
 587 response of crops to PRD appears to be a shift of root growth to deeper soil layers,
 588 whereas under standard drip irrigation roots are often concentrated in the surface
 589 soil, which makes crops less drought resistant (Keller 2005).

590 Without disregarding the importance that irrigation can have for improving
 591 crop performance, it is crucial to develop mitigation alternatives, not only in
 592 economic terms but also in terms of environmental sustainability. Among these
 593 mitigation alternatives, there has been a major effort by the scientific community
 594 to study the effect of inorganic substances on the improvement of light micro-
 595 climate and water relations of leaves. Reflective antitranspirants (e.g., kaolin,
 596 Bordeaux mixture), have interesting properties because they can lower leaf
 597 temperature by increasing reflection of infrared radiation (Moutinho-Pereira et al.
 598 2001) (Fig. 6.1). Lowered leaf temperature reduces the vapor pressure gradient
 599 between the leaf and the bulk air. Since this is the driving force behind trans-
 600 spiration, the plant water consumption is reduced (Glenn and Puterka 2005).
 601 Jifon and Syvertsen (2003) found that midday WUE of grapefruit kaolin-sprayed
 602 leaves was 25 % higher than that of control leaves, indicating improved WUE
 603 under high light intensity. However, the results of kaolin application require
 604 further demonstration since they have often been contradictory, ranging from
 605 increased to decreased yields and photosynthetic rates.

606 6.6 Water Use Strategies of Native and Invasive Plants

607 In xeric environments, WUE is likely to be a target of natural selection (Heschel
 608 et al. 2002). As previously discussed in this chapter, plants under natural condi-
 609 tions need to greatly improve their WUE to increase their chances of survival in

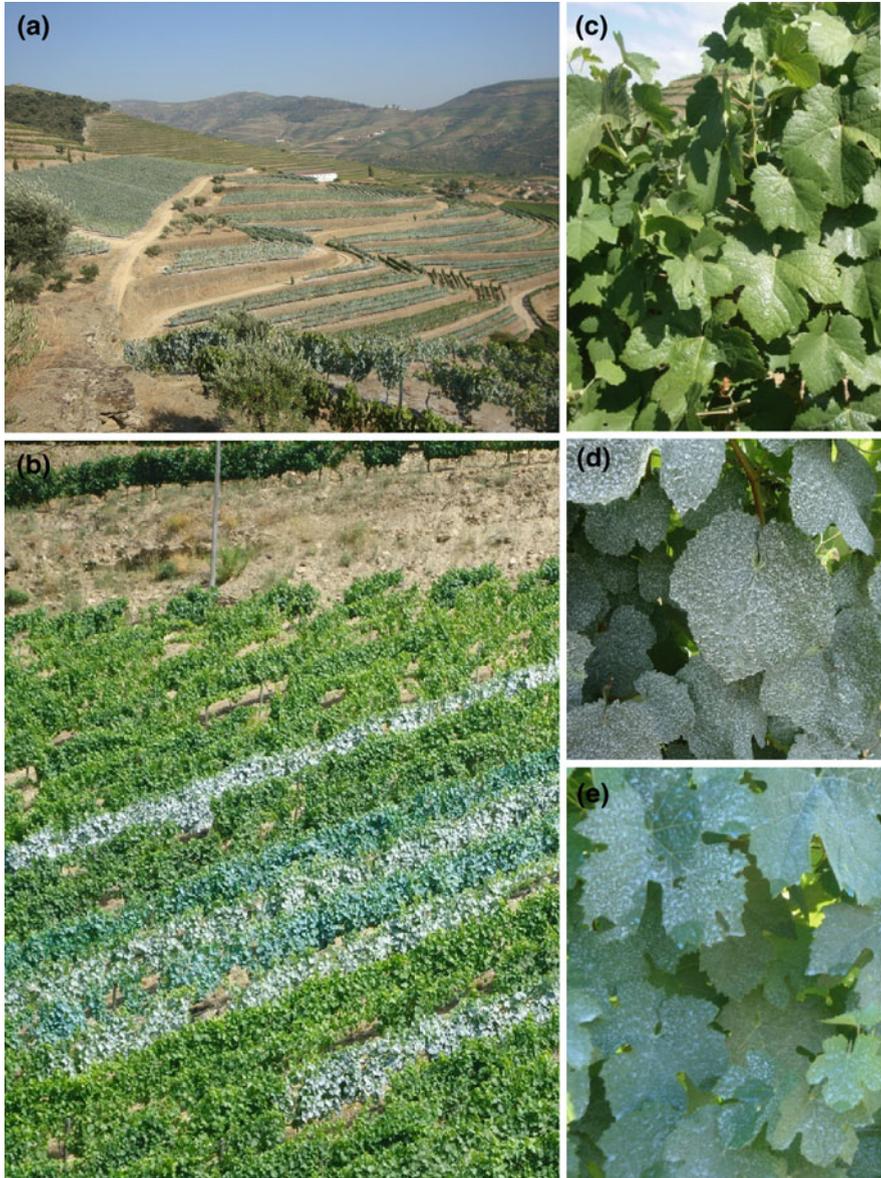


Fig. 1 Kaolin and Bordeaux mixture application in grapevine: **a** and **b** field trials in the Douro Demarcated Region; treatments: **c** control; **d** kaolin and **e** Bordeaux mixture

610 water -scarce environments. Due to climate change, biological invasions may
611 become a serious problem under natural conditions. Competitive strategies are
612 important for the successful establishment and persistence of invasive plants (Vilá
613 and Weiner 2004). Tolerance strategies may also help in the successful

614 establishment or dominance of invasive species (Allred et al. 2010). An important
615 trait is the ability of seedlings to develop root systems quickly, before the dry
616 summer begins (Roché et al. 1994). Greater competitive ability could include
617 greater use of limiting resources, such as water, thereby inhibiting establishment,
618 survival, and reproduction of native species (Blicker et al. 2003). According to
619 McDowell (2002) invasive species increase the efficiency of resource capture by
620 maintaining a high rate of photosynthesis per unit water loss.

621 To identify the factors that make some plant species troublesome invaders,
622 many studies have compared native and invasive plant performance (Daehler
623 2003). Hill et al. (2006) found that invasive species had lower diurnal variation in
624 leaf water potential than native ones despite the high transpiration. This capacity
625 may be due to the ability of water uptake through deeper roots (Hill et al. 2006).

626 Increased WUE may also be an important contribution to the success of
627 invasive species in areas where water is limiting (Hill et al. 2006). The efficiency
628 of water use can be caused by variations in the environment or leaf functional traits
629 (Seibt et al. 2008). Invasive *Rubus* plants had higher instantaneous WUE than
630 native ones, probably due to their thicker leaves (McDowell 2002). However, Hill
631 et al. (2006) found that WUE was higher in invasive species than in natives only
632 under the most unusually dry conditions. Leaf carbon isotope composition allowed
633 McAlpine et al. (2008) to conclude that the higher WUE of invasive plants/species
634 resulted from increased carbon gain and not from strategies that limit water loss.
635 Blicker et al. (2003) found that mature invasive plants may prolong their growing
636 season by increasing WUE during mid- and late summer, when neighboring semi-
637 arid plants are quiescent. On the other hand, Funk and Vitousek (2007) and
638 Cavaleri and Sack (2010) reported similar values of WUE among invasive and
639 native species.

640 Some studies support the hypothesis that high photosynthetic rate is an
641 important strategy of invasive ability (Kloeppel and Abrams 1995; Baruch and
642 Goldstein 1999; McDowell 2002; Hill et al. 2006; Jiang et al. 2009; Allred et al.
643 2010). Invasive species can maintain higher photosynthetic rates by modifying leaf
644 anatomy and structure. Specific leaf area (SLA) represents light-capturing area
645 deployed per dry mass allocated (Hamilton et al. 2005). A high SLA is a trait often
646 associated with invasiveness (Baruch and Goldstein 1999; Lake and Leishman
647 2004; Hamilton et al. 2005; Burns and Winn 2006; Grotkopp and Rajmánek 2007;
648 Feng et al. 2008; Allred et al. 2010). A high SLA reflects the occurrence of thinner
649 leaves, which are less costly to produce and to maintain (Lee and Graham 1986)
650 and, due to the associated rapid leaf production, allows an opportunistic capture of
651 solar energy to invasive plants (Grotkopp and Rajmánek 2007). Thus, species with
652 a high SLA have a shorter investment return rate and greater potential for fast
653 growth (Lake and Leishman 2004). However, some authors defend that a high
654 SLA favors invasive species only under some conditions, such as when in new
655 environments with non-limiting resources (Burns 2006; Burns and Winn 2006;
656 Leishman et al. 2007).

657 In general, invasive species had greater WUE and adaptations at root level that
658 allow a more efficient water uptake. The most relevant attributes that favor the

659 invaders are the greater photosynthetic rates associated to a high SLA. However,
660 these attributes may vary according to the species and the environment in which
661 they are established. This subject is still controversial, and the problem of what
662 makes a species invasive is far from solved.

663 6.7 Conclusions

664 The available freshwater is limited in many parts of the planet due to multiple
665 factors, such as climate change, population increase, and rapid economic devel-
666 opment. The greatest potential savings can be made in agriculture. In fact, small
667 improvements in WUE by human manipulation such as breeding and agricultural
668 management techniques can have a significant effect on water availability. How-
669 ever, the concept of WUE is of limited application when discussing the fitness of
670 plants for water-limited environments (Jones 2004). Intrinsic WUE_{leaf} presents
671 substantial intra- and interspecific variation, and depends on environmental con-
672 ditions. Moreover, improving WUE_{leaf} may not necessarily result in improved
673 WUE_{WP} , WUE_C and WUE_Y . For natural ecosystems, improvements in WUE are
674 only valuable if there is little competition. In fact is not useful for a species to have
675 a conservative water use, with a high WUE, if the competitors will use all the
676 water available first.

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Change to lower case	Encircle matter to be changed	≡
Change italic to upright type	(As above)	⊕
Change bold to non-bold type	(As above)	⊖
Insert 'superior' character	/ through character or ∧ where required	Y or Y under character e.g. Y or Y
Insert 'inferior' character	(As above)	∧ over character e.g. ∧
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	Y or Y and/or Y or Y
Insert double quotation marks	(As above)	Y or Y and/or Y or Y
Insert hyphen	(As above)	⊥
Start new paragraph	┌	┌
No new paragraph	┐	┐
Transpose	┌┐	┌┐
Close up	linking ○ characters	○
Insert or substitute space between characters or words	/ through character or ∧ where required	Y
Reduce space between characters or words		↑