Title: Physiological responses of *Arundo donax* ecotypes to drought: a common garden study

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ABSTRACT

Genetic analyses have suggested that the clonal reproduction of *Arundo donax* has resulted in low genetic diversity. However, an earlier common garden phenotyping experiment identified specimens of *A. donax* with contrasting biomass yields (ecotypes 6 and 20). We
utilised the same well-established stands to investigate the photosynthetic and stress physiology of the *A. donax* ecotypes under irrigated and drought conditions. Ecotype 6 produced the largest yields in both treatments. The *A. donax* ecotypes exhibited identical high leaf-level rates of photosynthesis (*P*<sub>N</sub>) and stomatal conductance (*G*<sub>s</sub>) in the well-watered treatment. Soil drying induced reductions in *P*<sub>N</sub> and *G*<sub>s</sub>, decreased use of light energy for photochemistry, impaired function of photosystem II and increased heat dissipation similarly in the two ecotypes. Levels of biologically active free-abscisic acid (ABA) and fixed glycosylated-ABA increased earlier in response to the onset of water deficit in ecotype 6; however, as drought progressed, the ecotypes showed similar increases in both forms of ABA. This may suggest that because of the low genetic variability in *A. donax* the genes responding to drought might have been activated similarly in the two ecotypes, resulting in identical physiological responses to water deficit. Despite the lack of physiological ecotypic differences that could be associated with yield, *A. donax* retained a high degree of *P*<sub>N</sub> and biomass gain under water deficit stress conditions. This may enable utilisation of *A. donax* as a fast growing biomass crop in rain-fed marginal lands in hot drought prone climates.

Key-words: biomass; bioenergy; water deficit; abscisic acid (ABA); isoprene; photosynthesis; stomatal conductance.

**INTRODUCTION**

The development of giant reed (*Arundo donax*) as a perennial biomass crop in drought-prone marginal lands requires physiological analysis of its responses to water deficit and identification of those traits that confer resistance to drought. The rapid growth of *A. donax* is sustained by high leaf-level rates of photosynthesis (*P*<sub>N</sub>) and accompanied by significant
transpirative water-loss due to concurrent stomatal opening (Rossa et al., 1998, Haworth et al., 2016). However, the clonal reproduction of *A. donax* has resulted in a low genetic diversity (Ahmad et al., 2008, Mariani et al., 2010, Saltonstall et al., 2010, Pilu et al., 2014) that may account for observations of similarities in productivity and drought tolerance in plants collected from different regions (Cosentino et al., 2006, Sánchez et al., 2015). Low genetic variability may also constrain the development of more productive and/or drought tolerant *A. donax* varieties. Indeed, the genetic background of *A. donax* is much less explored than other biomass crops (eg. Chen et al., 1997, Souch & Stephens, 1998, Zhang et al., 2004). For example, it is currently unclear whether sufficient genetic variation occurs in *A. donax* to warrant the use of genotype or ecotype as a descriptive term (eg. Khudamrongsawat et al., 2004, Ahmad et al., 2008). Here, we will use the term ecotype in respect of the ecological differences in habitat and any associated genetic variation represented by the *A. donax* accessions. Analysis of the photosynthetic and stress physiology of *A. donax* ecotypes under field conditions is crucial to the characterisation of the species and understanding its potential as a biomass crop.

Soil drying induces an increase in the flow of abscisic acid (ABA) from the roots to the leaves (Davies & Zhang, 1991), and increased conversion of biologically inactive ‘fixed’ glucose-conjugated ABA (GE-ABA) in the vacuole to biologically active free ABA within the cytosol of the leaves (Dietz et al., 2000, Seiler et al., 2011). This increased foliar concentration of [ABA] causes stomatal closure (Zhang & Davies, 1990) reducing transpirative water-loss, but also leading to a reduction in the uptake of CO$_2$ for photosynthesis (Pinheiro & Chaves, 2011, Lauteri et al., 2014). *Arundo donax* grown in 5 L pots exhibited declines in $P_N$ from ~20-30 μmol m$^{-2}$ s$^{-1}$ to ~1-2 μmol m$^{-2}$ s$^{-1}$ that corresponded to reductions in $G_s$ of 0.35-0.45 mol m$^{-2}$ s$^{-1}$ to ~0.01 mol m$^{-2}$ s$^{-1}$ after constant
water deficit was maintained for 60 days (Sánchez et al., 2015). Arundo donax ecotypes from an arid and warm-humid environment grown in 50 L pots in a common garden experiment exhibited identical declines in $P_N$ and $G_s$ after water was withheld over a three-week period. Despite the contrasting nature of their habitats no difference was observed in leaf gas-exchange, but a difference in xylem traits was recorded, suggesting that despite the low genetic diversity of A. donax, some ecotypic differences in response to water deficit do occur (Haworth et al., 2016). These studies indicate that A. donax possesses highly effective physiological stomatal control to prevent desiccation (Haworth et al., 2015, Haworth et al., 2016). However, at present there is little information regarding the possible role of ABA in the drought response of A. donax.

As $P_N$ decreases under drought, the available photosynthetic sinks for intercepted light energy decline. Consequently, energy dissipation as heat via non-photochemical quenching (NPQ) increases (Demmig-Adams & Adams, 2000). Pot grown A. donax exhibited concomitant reductions in foliar relative water content (RWC) and the performance of photosystem II (Nackley et al., 2014). Extended drought may degrade the protective systems leading to damage to the photosynthetic apparatus (Pinheiro & Chaves, 2011). During drought, A. donax undergoes morphological changes such as leaf-roll and loss to reduce transpiration by decreasing the area of exposed leaf surface (Cosentino et al., 2014). If the severity of drought increases, A. donax loses the above ground parts of the plant and sequesters carbohydrates and nutrients in the rhizome below-ground until growth conditions improve (Mann et al., 2013).

The emission of isoprenoid biogenic volatile organic compounds reduces the deleterious impact of heat and oxidative stress (Sharkey et al., 2008). Not all plants emit isoprenoids, but
emitters possess greater tolerance of oxidative stress associated with excess excitation energy (Centritto et al., 2014) and especially characterises fast-growing plants (Loreto & Fineschi, 2015). Analysis of $^{13}$C labelling, in leaves fumigated with $^{13}$C, indicates that isoprene is derived from recently synthesised photosynthate in well-watered plants (Brilli et al., 2007). Isoprene emission may also be linked to the production of foliar ABA via the methyl-erythritol phosphate pathway (Barta & Loreto, 2006). The rate of emission of isoprene is positively related to the level of $P_N$ (Loreto & Sharkey, 1990), and this relationship is observed not only in A. donax (Hewitt et al., 1990) but across the Arundineae tribe of the Poaceae (Ahrar et al., 2015). Arundo donax occupies habitats that receive high levels of photosynthetically active radiation and also exhibits high rates of $P_N$ (Rossa et al., 1998). However, rates of isoprene emission in pot-grown A. donax in a glasshouse (Ahrar et al., 2015) were not significantly greater than other fast growing species such as Populus nigra with similar rates of $P_N$ (Centritto et al., 2011). The role of isoprene emission in the water deficit response of A. donax and any protective function is currently unclear.

Since April 1997, the University of Catania has hosted a collection of 39 ecotypes of A. donax collected from contrasting habitats in Southern Italy. These A. donax plants have grown in a common garden phenotyping experiment for 18 years in a semi-arid Mediterranean climate. The results from the first two-years of growth indicated that ecotype 20 was one of the two most productive A. donax specimens, while ecotype 6 was amongst the least productive (Cosentino et al., 2006). Thus, we investigated the gas-exchange responses and yield of ecotypes 6 and 20 under irrigated and water deficit conditions. The identification of crop varieties with high leaf-level rates of $P_N$ is important to the development of more productive cultivars (Hubbart et al., 2007, Lauteri et al., 2014). We hypothesised that the more productive ecotype 20 might exhibit greater leaf-level rates of $P_N$.
and stomatal conductance ($G_s$), while the less productive ecotype 6 might be less susceptible to water stress, possibly due to lower $G_s$ and growth rate. The aim of this study was to: i) assess physiological and morphological response to drought in ecotypes 6 and 20; ii) quantify isoprene emission rates and changes in amounts of foliar free-ABA and GE-ABA during water deficit, and iii) identify any physiological traits in ecotypes 6 and 20 that may be related to biomass production under well-watered and water deficit conditions.

MATERIALS AND METHODS

Plant material and growth conditions

In 1997, rhizomes of 39 ecotypes of *A. donax* were collected from a range of sites (lakes, rivers and uncultivated areas) in Sicily and Calabria, Southern Italy. These rhizomes were planted in a common garden experiment at the experimental field of the Faculty of Agriculture, University of Catania (37° 25′N 15′03′E). The rhizomes were planted directly into the soil at a depth of 30 cm in 2.5 × 3.0 m plots at a density of 2.67 plants / m$^2$ with plants placed at a distance of 0.75 m between rows and 0.5 m in each row. In the first year the plots were fertilised with 100 kg ha$^{-1}$ of phosphate, and 80 of kg ha$^{-1}$ nitrogen were supplied during the first and second years. Supplementary irrigation was also provided during the first two years to enable the rhizomes to more fully establish. Irrigation and fertilization were not applied for the next fifteen years. Biomass production and morphology of the *A. donax* specimens was assessed from 1997 to 1998. Full details of the site, soil and experimental set-up are given in Cosentino *et al.* (2006).

In the first two years of the study, ecotype 20 produced the greatest biomass yield, while ecotype 6 was amongst the least productive. The two ecotypes were collected from contrasting environments: ecotype 20 from a coastal habitat and ecotype 6 from the slopes of
Mount Etna (Fig 1). In the summer of 2014 all plots received supplementary irrigation equivalent to 100% of potential evapotranspiration during July to August. Evapotranspiration (ETc) was calculated each day as:

$$ETc = E_o \times K_p \times K_c$$

Where $E_o$ is the evaporation of water from a class-A pan (mm); $K_p$ is the pan coefficient, and; $K_c$, the crop growth stage (between 0.7 and 1.1). Daily rain-fall was subtracted from the daily calculation of water to be supplied as irrigation (Allen et al., 1998, Cosentino et al., 2015). On average 4.494 mm ha$^{-1}$ of water was supplied each day. On the 20$^{th}$ of September (day 20 in Figure 2) irrigation was ceased in one of the two plots for each ecotype and allowed to continue in the remainder. Measurements were conducted prior to the cessation of irrigation (time 0) and over the next 40 days as the soil dried (time 1 and 2). Soil samples were collected from three depths (0 to 30 cm; 30 to 60 cm, and; 60 to 90 cm) at the mid-point of each time period, the soil water content determined gravimetrically by drying in a ventilated oven at 105˚C. The available soil water content for plant growth was then determined following Klute (1986) and Killi et al. (2014)(Fig 2).

**Leaf gas-exchange and chlorophyll-fluorescence**

Measurements of $P_N$, $G_s$, the internal sub-stomatal concentration of $[CO_2]$ ($C_i$) and electron transport rate (ETR) were performed on the mid-section of the second fully expanded leaf from the flag leaf using a LiCor Li6400XT fitted with a 2 cm$^2$ leaf cuvette (Li-Cor, Inc., Nebraska, USA). Four replicate plants from the centre of each plot were analysed for each ecotype and treatment. Cuvette settings of 400 ppm [CO$_2$], 2000 μmol m$^{-2}$ s$^{-1}$ of photosynthetically active radiation (10% blue and 90% red light) and a leaf temperature of 30˚C were used. To reduce diffusive leaks through the chamber gasket, a supplementary gasket was added and the IRGA exhaust air was fed into the interspace between the chamber

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and the supplementary gaskets (Rodeghiero et al., 2007). Gas-exchange measurements were performed between 10.00 and 12.00 each day when the plants exhibited the highest levels of $P_N$ and $G_s$. The maximum ($F_v/F_m$) and the actual quantum yield of photosystem II ($\Phi_{PSII: \Delta F/F_m}$), and the dissipation of light energy as non-photochemical quenching (NPQ) were recorded using a Hansatech FMS-2 (saturating pulse of 10,000 $\mu$mol m$^{-2}$ s$^{-2}$) and dark adaptation clips (Hansatech, King’s Lynn, UK) after 30 minutes of dark adaptation and exposure to actinic light of 2000 $\mu$mol m$^{-2}$ s$^{-2}$ for a minimum of 10 minutes after the first saturating pulse (Genty et al., 1989, Maxwell & Johnson, 2000).

Isoprene emission

The emission of isoprene was measured in the field from the same leaves of A. donax used for gas-exchange analysis, but a LiCor Li6400 fitted with a 6 cm$^2$ cuvette and LED light unit was used. Four replicate plants from the centre of each plot were analysed for each ecotype and treatment. Cuvette settings of 400 ppm [CO$_2$], 2000 $\mu$mol m$^{-2}$ s$^{-1}$ of photosynthetically active radiation (10% blue and 90% red light) and a leaf temperature of 30°C were used. The match tube on the LiCor cuvette was detached, and air from the leaf passed through a biphasic adsorbent trap containing 30 mg of Tenax and 20 mg of Carboxen (GERSTEL GmbH & Co.KG, Germany). A pump (Elite 5, A.P. Buck, Orlando, Florida, USA) was used to pass 2 L of air through each trap at a rate of 200 ml min$^{-1}$. Measurements of the concentration of isoprene in the ambient air (blanks) were performed using an empty leaf cuvette before and after each measurement. The traps were then stored at 4°C prior to analysis in the laboratory. The volatiles collected in the traps were analysed using a gas chromatographer – mass spectrometer (GC-MS) with an Agilent HP-INNOWAX (30 m x 0.32 mm x 0.15 $\mu$m) GC column. A 5977A mass selective detector with electron ionization operating at 70eV was used for analysis. Isoprene was identified by matching the spectrum
peak with a library spectral database (NIST 11.L) and through comparison of the retention time and mass spectrum with an isoprene analytical standard (Sigma Aldrich, St. Louis, Missouri, USA) injected into the GC-MS at different concentrations. The data was analysed using Agilent MassHunter Workstation software (Agilent 7890A, Agilent Technologies, Santa Clara, California, USA).

Abscisic acid analysis
At the end of each measurement period, leaf samples were collected from four replicates for each treatment and ecotype using the second and third fully expanded leaves from the flag leaf of stems from the rhizomes adjacent to those used for gas-exchange and isoprene analyses. Leaf samples for time 0 were collected on the 20th September, time 1 on the 9th October and time 2 on the 31st October between 10:00 and 12:00 hours. The leaves were frozen in liquid nitrogen and then stored at -80˚C. To determine concentrations of free-ABA and GE-ABA, 300-350 mg of leaf tissue was added to 40 ng of d6-ABA and 40 ng of d5-ABA-GE (ABA and derivatives provided by Prof. Zaharia, National Research Council of Canada) and ground in liquid nitrogen using a pestle and mortar. Once ground, 3 mL of CH₃OH:H₂O (50:50; pH 2.5 with HCOOH) was added to the leaf homogenate and left at 2°C for 30 minutes. The extract was then purified using Sep-Pak C18 cartridges (Waters, Massachusetts, USA), and the eluate dried and rinsed with 500 µL CH₃OH:H₂O (50:50) pH 2.5 (modified protocol of Xiong et al., 2014). To quantify ABA and ABA-metabolites, 3 µL of the eluate was then injected into a LC-ESI-MS/MS, using UPLC (Nexera UPLC Shimadzu Corporation) coupled with an MS/MS detector (TQ 8030) equipped with an ESI source (all from Shimadzu Corporation, Kyoto, Japan). Analyses were performed in the negative ion mode. The compounds were separated in a Poroshell C₁₈ column (3.0 x 100 mm, 2.7 µm i.d., Agilent, USA) using a binary solvent system consisting of water with 0.1 % of HCOOH (solvent A) and acetonitrile/methanol (1/1) (added with 0.1 % of HCOOH, solvent B). The
solvent gradient was programmed to change linearly from 95% A to 100% B during a 30-min run at a flow-rate of 0.3 mL min\(^{-1}\). The GC operated at a He flow rate of 1 ml min\(^{-1}\). The GC was programmed with an initial oven temperature of 40°C (5 min hold), which was then increased 5°C min\(^{-1}\) up to 250°C (4 min hold). Quantification was conducted in the multiple reaction mode (MRM) using a calibration curve constructed from analysis of standards following the protocol of López-Carbonell et al. (2009).

**Foliar water content and biomass harvest**

The relative water content (RWC) of leaves from the A. donax ecotypes was measured for time 0 on the 20\(^{th}\) September, time 1 on the 9\(^{th}\) October and time 2 on the 31\(^{st}\) October. The second fully expanded leaf from the flag leaf was removed from four plants for each ecotype and treatment (plants adjacent to those used for gas-exchange were sampled) and the RWC determined using the approach of Diaz-Pérez et al. (1995). The biomass and morphological parameters of the A. donax ecotypes were measured in February 2015 – the period of the year when the moisture content of the stems is lowest and drying of the plants would require the least energy, a critical consideration in the commercial exploitation of A. donax (Cosentino et al., 2006). A border of 50 cm was removed from each plot, to eliminate border effects influencing the determination of yield. Three central sub-plots of 1 m\(^2\) were then sampled for each ecotype and treatment. Stem density was determined by counting the total number of stems in each sub-plot. Three stems in each sub-plot were randomly selected and stem height and basal stem diameter were measured. Dry mass yield was calculated by collecting sub-samples of the stems and leaves from sub-plots of each ecotype and treatment and then oven drying them at 105°C until their weight remained constant. The susceptibility to drought of each ecotype was calculated using the susceptibility index (S) of Fischer and Maurer (1978):
\[ S = \frac{1 - \left( \frac{Y}{Y_0} \right)}{1 - \left( \frac{X}{X_0} \right)} \]

Where: \( Y \) = yield under stress; \( Y_0 \) = yield without stress, and; \( X \) and \( X_0 \) signify the yield of all ecotypes under stress and non-stress conditions, in this case values of 13.2 and 17.9 t ha\(^{-1}\) were utilised for \( A. \) donax under irrigated and drought conditions, respectively, as reported by Cosentino \textit{et al.} (2014) in a study performed at the same site over three growing seasons.

\textit{Statistical analyses}

Statistical analyses were performed using SPSS 20 (IBM, Armonk, New York, USA). To test the effect of water deficit on \( A. \) donax ecotypes 6 and 20 we used one-way ANOVAs with an LSD \textit{post-hoc} test to assess differences in variance between samples associated with either ecotype or treatment effects.

\textbf{RESULTS}

\textit{Arundo donax} ecotypes 6 and 20 exhibited high levels of \( P_N \) (~33-38 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)) and \( G_s \) (0.548-0.770 mol m\(^{-2}\) s\(^{-1}\)) at time 0 prior to the cessation of irrigation in the drought treatment; no statistical difference in \( P_N \) or \( G_s \) values was observed between the two well-watered ecotypes. These rates of \( P_N \) and \( G_s \) were maintained under well-watered conditions, with the exception of the latter stages of time 2 where temperatures were lower (Fig 3). The soil water content available for plant growth in all layers declined by 55.3 to 75.0\% over the course of the study; the lowest reduction was in the upper 30 cm where rain-falls were more effective in restoring higher soil water availability (Fig 2). As soil drying progressed, the two ecotypes showed identical declines in both \( P_N \) and \( G_s \). Photosynthesis reduced by 66.7\% (Fig 3a) and \( G_s \) fell by 91.2\% by time 2 (Fig 3b). Stomatal conductance during the entire study was positively related to \( P_N \) (Fig 4). Stomatal closure resulted in reductions in \( C_i \) of water-
stressed leaves, indicating increasing stomatal limitation of photosynthesis (Fig 3c). As drought progressed and $P_N$ declined the amount of electrons not utilised in photochemistry of photosynthesis rose, as signified by an increase in the ratio of the ETR to $P_N$ (Fig 3d); however, ETR/PN values were comparatively low, possibly due to high. Soil drying did not affect the maximal yield of photosystem II as indicated by the $F_v/F_m$ ratio (Fig 5a). However, the quantum yield of photosystem II in the light ($\Phi_{PSII}$) declined, most significantly in ecotype 20 (Fig 5b), while NPQ increased in both ecotypes (Fig 5c).

Levels of ABA during drought were different in the two ecotypes (Fig 6). In the first stage of soil drying, concentrations of free and GE-ABA were higher in the leaves of ecotype 6. However, as drought stress progressed in time 2, the leaves of ecotype 20 showed greater concentrations of both forms of ABA that correlated with a decline in $G_s$ values but no alteration in $P_N$ (Fig 3). It is noteworthy, that levels of ABA in the irrigated plants also rose during time 2, possibly due to a reduction in mean daily temperature (Fig 2) (Lim et al., 2007). However, this higher concentration of ABA also corresponded with reduced $G_s$ in time 2 (Fig 3 and 6). Rates of isoprene emission were relatively variable, preventing observation of any significant differences between treatment and ecotype (Fig 7); although the levels of isoprene release found in this experiment were consistent with those observed in previous studies (Hewitt et al., 1990, Ahrar et al., 2015). However, during the initial period of soil drying in time 1, ecotype 6 showed a 34.6% increase in mean isoprene emission, while ecotype 20 reduced isoprene emission by 15.1%; while these findings are not statistically significant, they do indicate proportional and directional difference in the response of isoprene emission to drought between the two ecotypes. In time 2 isoprene emission was broadly similar across treatment and ecotype.
Foliar RWC of the two *A. donax* ecotypes also differed at the mid-point of the study. At time 1, no significant effect of drought was observed in ecotype 20 while ecotype 6 exhibited a significant 3.6% reduction in RWC (Fig 8). At time 2 both *A. donax* ecotypes showed statistically identical leaf RWC values of 87.1-89.9%, which were significantly lower than in well-watered plants. The ecotypes did display differences in biomass and morphology, as ecotype 6 performed better than ecotype 20 under both well-watered and drought conditions (Fig 9). In ecotype 6, stem height was respectively 17.7 and 33.9% greater in the irrigated and drought treatments than in ecotype 20 (Fig 9a). The density (Fig 9c) and diameter (Fig 9b) of stems were not affected by ecotype or treatment: the greater stem height in ecotype 6 (Fig 9a) was not associated with any difference in the number or thickness of stems. Dry mass yield was also greater in ecotype 6 than 20 under both irrigated and drought conditions (Fig 9d). Calculation of the susceptibility index \( S \) suggests that ecotype 6 \( S = 1.067 \) was more strongly affected by drought than ecotype 20 \( S = 0.853 \). This signifies a greater proportional decline in yield in ecotype 6. However, drought yield in ecotype 6 is greater than that found in irrigated ecotype 20 during the study period; suggesting that the faster growing ecotype 6, while proportionally more vulnerable to water deficit, was the more effective biomass crop during drought experienced in this study period.

**DISCUSSION**

The two *A. donax* ecotypes analysed in this study exhibited contrasting biomass responses, with ecotype 6 more productive in both the irrigated and drought treatments (Fig 9d). This result contrasts with the observations of Cosentino *et al.* (2006) in the first two-years of the common garden study involving the *A. donax* ecotypes, where ecotype 20 generated the highest yields. This result may be due to a number of factors such as growth conditions favouring ecotype 6 during this study (Fig 2), more favourable establishment of ecotype 20 in
the first few years of the common garden experiment (eg. Mann et al., 2013), possible
differences in the physiology of the two ecotypes (Figs 6 and 7) or the general lack of genetic
diversity present in A. donax (eg. Mariani et al., 2010, Pilu et al., 2014) associated with high
phenotypic plasticity (Ghalambor et al., 2007) resulting in a lack of consistency in year-on-
year biomass during phenotyping studies (Cosentino et al., 2006). Both ecotypes showed
identical rates of $P_N$ and $G_s$ on a leaf-area basis under well-watered and drought conditions
(Fig 3). In irrigated conditions, the high rate of carbon assimilation accounts for the high
biomass production observed in A. donax in comparison to other species (Angelini et al.,
2009, Mantineo et al., 2009, Smith et al., 2015). As already observed when addressing the
issue of dry mass yield, similar $P_N$ and $G_s$ of the two ecotypes may confirm low genetic
diversity in A. donax (eg. Ahmad et al., 2008, Mariani et al., 2010, Pilu et al., 2014). The
obtained yields, both in rainfed and irrigated condition, are in accordance to the asymptotic
non-linear relationship developed by Cosentino et al. (2014) to predict biomass yields of
giant reed as function of crop water use in semi-arid Mediterranean environment.

Soil drying induced ~90% reductions in $G_s$, suggesting that the plants under drought
conditions experienced moderate to severe drought (Haworth et al., 2016). The internal sub-
stomatal concentration of [CO$_2$] of both A. donax ecotypes decreased under drought,
consistent with stomatal closure inducing increasing diffusive limitations to CO$_2$ entry in the
leaves, while suggesting no onset of biochemical limitations (known to invert the trend in $C_i$)
(Flexas et al., 2002). The reduction in $G_s$ induced by increased [ABA] may be indicative of a
biochemical protection to maintain foliar water content before soil water availability declines
to critical levels (Maseda & Fernandez, 2006). Water deficit did not affect $F_v/F_m$ values of
the ecotypes (Fig 5) despite the observed reductions in RWC and $G_s$, indicating stability of
photochemistry of photosynthesis. In fact, declines in the $F_v/F_m$ ratio of pot grown A. donax

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only occurred at RWCs below 60% (Nackley et al., 2014). Nonetheless, the quantum yield of photosystem II in the light and NPQ were more sensitive to reductions in water availability (eg. Souza et al., 2004). This would reflect a decrease in the proportion of energy utilised by photochemical reactions driving photosynthesis and photorespiration (Fig 3d) and increase in energy dissipated as heat (Fig 5c), whereas it does not represent permanent damage of the photosynthetic apparatus of water-stressed plants. As rates of $P_N$ are comparatively higher in A. donax than other species this may have reduced selective pressures related to carbon assimilation on a leaf-area basis between varieties with contrasting leaf-level rates of $P_N$ (Haworth et al., 2011, Galmes et al., 2014). A study of 87 A. donax specimens from Italy found no geographic genetic variability, but did observe variation and heritability in stem height and diameter (Pilu et al., 2014). This may indicate that while the photosynthetic physiology of A. donax shows little variability, ecotypes 6 and 20 may vary in terms of stem morphology (Fig 9a), if not photosynthetic physiology (Fig 3), thus accounting for the differences observed in yield (Fig 9d).

The rapid decline in $G_s$ of both A. donax ecotypes, with 70.9% of the reduction occurring five days after the cessation of irrigation (Fig 3b), suggests that A. donax does not progressively adjust its water use efficiency to the development of drought (eg. Mahmood et al., 2015) and instead employs a ‘use it or lose it’ water use strategy (Bacelar et al., 2012) dependent upon a high degree of stomatal functionality to prevent desiccation (Franks & Farquhar, 2007). This may reflect large changes of ABA, the hormone that is known to control stomatal closure in water stress conditions (Davies & Zhang, 1991). Indeed, stomatal closure occurred alongside increases in the concentration of free- and GE-ABA (Fig 6). Ecotype 6 exhibited more pronounced rises in free- and GE-ABA than ecotype 20 in the first stage of drought at time 1, indicating a more rapid root-to-shoot signal of soil drying (eg. Fujita et al., 2005), possibly
accounting for increased yield in the drought-stressed plants (eg. Chen et al., 1997). The rise in conjugated GE-ABA in ecotype 6 may also reflect greater drought tolerance through increased storage of the stress hormone ABA (Seiler et al., 2011). The concentrations of free- and GE-ABA in the drought-stressed plants were higher in both plants in time 2, suggesting a progression of drought (Liu et al., 2005), and/or a reduction in temperature towards the latter stages of the study (Fig 2) as the amount of ABA also increased in the well-watered plants (Lim et al., 2007). This rise in ABA in the plants grown under irrigated conditions in time 2 also corresponded to reduced $G_s$ (Fig 3). The higher amounts of free-ABA in ecotype 6 at time 1 may be related to lower foliar RWC developing more rapidly in this ecotype than in ecotype 20 (Fig 8). Increased leaf [ABA] during drought often occurs prior to any declines in RWC (Liu et al., 2005), but stomatal sensitivity to [ABA] is increased at low water potentials (Tardieu & Davies, 1992); possibly suggesting that the influence of free-ABA on stomatal closure in A. donax was more pronounced in time 2. The higher foliar concentrations of free- and GE-ABA in ecotype 6 at time 1 also coincided with a 34.6% increase in isoprene emission of drought-stressed plants, in respect to well-watered plants (Fig 7). In contrast, levels of ABA remained constant in ecotype 20, whereas isoprene emission declined, although non-significantly, in drought-stressed leaves at time 1 (Fig 7). This suggests that part of foliar ABA synthesized under early stress is generated by a labile pool of carbon that is also used to form isoprene within the methyl erythritol phosphate (MEP) pathway, confirming that isoprene may be used as a proxy of ABA formation under certain circumstances (Barta & Loreto, 2006). However, due to the variable nature of isoprene emissions (Sharkey & Loreto, 1993), further characterisation of isoprene emission in A. donax in response to stress under more highly controlled conditions would be required before any firm conclusion could be drawn.
The emission of isoprene plays a protective role in the reduction of oxidative and heat stress in many plants (Sharkey et al., 2008). As isoprene is derived from recently assimilated carbon, emissions generally do not increase during soil drying (Centritto et al., 2011, Brilli et al., 2013). However, in the A. donax ecotypes isoprene emission remained stable during the development of drought, indicating an increasing proportion of photosynthetic carbon feeding isoprene biosynthesis during the occurrence of stress. Only when water deficit stress is severe, as $P_N$ approaches zero, the emission of isoprene declines before increasing during re-watering (Brilli et al., 2007). In our case, increased emission in drought-stressed ecotype 6 at time 1 is not surprising as $P_N$ (17-21 μmol m$^{-2}$ s$^{-1}$) provided sufficient carbon for isoprene biosynthesis. However, as isoprene increases photoprotection of photosynthetic machinery under stress conditions (Velikova et al., 2011), we maintain that ecotype 6 might have developed a better strategy to protect photosynthesis from transient water stress.

The two A. donax ecotypes analysed in this study exhibited largely identical photosynthetic and gas-exchange responses to growth under well-watered and drought conditions. Photosynthetic rates in drought-stressed A. donax remained comparatively high into time 2 (11-15 μmol m$^{-2}$ s$^{-1}$), possibly due to its extensive root system permitting extraction of water from deep within the soil profile (Mann et al., 2013). The protective dissipation of heat (Fig 5c) and emission of isoprene (Fig 7) permitted the retention of photosynthetic function (Fig 3a) and reductions in biomass yield of 30-35% (Fig 9) that are significantly lower than the 48.2% proportional loss experienced by 5 year-old Miscanthus x giganteus under similar conditions (Mantineo et al., 2009). In general, the yield of irrigated and drought-stressed A. donax was greater than that of other fast growing bioenergy crops such as Cynara cardunculus (Cosentino et al., 2007, Mantineo et al., 2009). This suggests that A. donax...
possesses the physiological adaptation to serve as an effective biomass crop in drought-prone arid areas with a hot Mediterranean climate.

CONCLUSIONS

Physiological analysis of the most (20) and least (6) productive A. donax ecotypes from the study of Cosentino et al. (2006) under rain-fed and irrigated conditions produced an unexpected result. Ecotype 6 generated higher biomass yields than ecotype 20 in both treatments during the study period. This faster growth in ecotype 6 was not associated with significant differences in leaf-area levels of $P_N$ or $G_s$, function of photosystem II or emission of isoprene. This lack of variation in photosynthetic physiological parameters may reflect low genetic diversity in A. donax. The contrast in biomass results from the earlier study may also reflect this lack of genetic variability, resulting in an absence of consistency in yield measurements when phenotyping A. donax ecotypes. Future studies should employ a wider geographical collection of specimens and present results over a greater time period to ensure that the most consistently productive ecotypes are identified. Despite the lack of a physiological ecotypic difference in this study, both ecotypes exhibited rapid rates of $P_N$ under well-watered conditions, and as soil dried the signalling of drought via ABA and protective mechanisms ensured that the rain-fed plants retained comparably high rates of $P_N$ and only experienced reductions in yield of 30-35%. The stress physiology of A. donax is adaptable to growth in arid hot Mediterranean climates, making it a viable crop species for biomass production in drought-prone marginal lands.

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LIST OF FIGURES

Figure 1: Map showing the original location where the Arundo donax clonal populations used in this study were collected. Ecotype 6 was collected from Piedimonte Etneo (37˚48N 15˚10E; 348 m a.s.l.), and ecotype 20 was collected from Capo D’Orlando (38˚08N 14˚43E; 8 m a.s.l.). Re-drawn from Cosentino et al. (2006).

Figure 2: Growth conditions during the study period: a) daily temperature at the site - the upper dashed line shows the maximum daily temperature, the solid line shows the mean daily temperature and the lower dashed line shows the minimum daily temperature; b) daily rainfall, and; c) gravimetric measurements of soil water content taken at the mid-point of each measurement period. The measurement period began on the 1st September (day 1), irrigation ceased on the 20th September (end of time 0) and analysis of the physiological response to soil drying of the Arundo donax ecotypes was performed during time 1 (24th September to 9th October) and time 2 (10th to 30th October).

Figure 3: Photosynthesis ($P_N$) (a), stomatal conductance ($G_s$) (b), internal sub-stomatal [CO$_2$] ($C_i$) (c) and the ratio of the electron transfer rate to photosynthesis (ETR/$P_N$) (d) of Arundo donax ecotypes 6 (square symbols) and 20 (triangle symbols) under irrigated (solid symbols,
solid line) and drought conditions (open symbols, dashed line). Error bars indicate one standard error either side of the mean.

Figure 4: The relationship between photosynthesis and stomatal conductance in irrigated (solid symbols) and drought (grey symbols at time 1; white symbols at time 2) *Arundo donax* ecotypes 6 and 20 grown in the field. In theory the relationship between $P_N$ and $G_s$ should originate at zero; however, drought stress under field conditions did not progress this far and we have decided to not extrapolate beyond the limits of our observable data-set and therefore a linear regression was applied: the solid black line indicates the linear regression and the grey lines either side of the linear regression indicate 95% confidence intervals of the regression line.

Figure 5: Chlorophyll fluorescence parameters at time 2 of *Arundo donax* ecotypes 6 (white) and 20 (grey) under drought (hatched) and irrigated (open) conditions: a) the efficiency of photosystem II ($F_v/F_m$); b) quantum yield of photosystem II ($\Phi_{PSII}: \Delta F/F'_m$), and c) dissipation of energy as non-photochemical quenching (NPQ). Error bars indicate one standard deviation either side of the mean. Letters indicate significant difference using a one-way ANOVA and LSD post-hoc test.

Figure 6: The amount of free- and GE-ABA in leaves of *Arundo donax* ecotypes 6 (white) and 20 (grey) per unit dry mass under drought (hatched) and irrigated (open) conditions. Error bars indicate one standard error either side of the mean. Letters indicate significant difference between free- and GE-ABA in all ecotypes / treatment using a one-way ANOVA and LSD post-hoc test.

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Figure 7: Rates of isoprene emission in field grown *Arundo donax* ecotypes 6 (white) and 20 (grey) under drought (hatched) and irrigated (open) conditions. Error bars indicate one standard error either side of the mean. Letters indicate significant difference using a one-way ANOVA and LSD *post-hoc* test.

Figure 8: Relative water content of leaves of *Arundo donax* ecotypes 6 (white) and 20 (grey) under drought (hatched) and irrigated (open) conditions. Error bars indicate one standard deviation either side of the mean. Letters indicate significant difference using a one-way ANOVA and LSD *post-hoc* test.

Figure 9: Morphological characteristics of *Arundo donax* ecotypes 6 (white) and 20 (grey) under drought (hatched) and irrigated (open) field conditions: a) stem height; b) stem diameter; c) the density of stems within the sub-plots, and; d) dry biomass yield. Error bars indicate one standard error either side of the mean. Letters indicate significant difference using a one-way ANOVA and LSD *post-hoc* test.

REFERENCES

Ahmad R, Liow P-S, Spencer DF, Jasieniuk M (2008) Molecular evidence for a single genetic clone of invasive *Arundo donax* in the United States. Aquatic Botany, 88, 113-120.

Ahrar M, Doneva D, Koleva D, Romano A, Rodeghiero M, Tsonev T, Biasioli F, Stefanova M, Peeva V, Wohlfahrt G, Loreto F, Varotto C, Velikova V (2015) Isoprene emission in the monocot Arundineae tribe in relation to functional and structural organization of the photosynthetic apparatus. Environmental and Experimental Botany, 119, 87–95.

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. FAO, Rome, 300, D05109.

Angelini LG, Ceccarini L, Di Nassa NNO, Bonari E (2009) Comparison of *Arundo donax* L. and *Miscanthus x giganteus* in a long-term field experiment in Central Italy: Analysis
of productive characteristics and energy balance. Biomass and Bioenergy, 33, 635-643.

Bacelar EL, Moutinho-Pereira JM, Gonçalves BM, Brito CV, Gomes-Laranjo J, Ferreira HM, Correia CM (2012) Water use strategies of plants under drought conditions. In: Plant Responses to Drought Stress. pp 145-170. Springer.

Barta C, Loreto F (2006) The relationship between the methyl-erythritol phosphate pathway leading to emission of volatile isoprenoids and abscisic acid content in leaves. Plant Physiology, 141, 1676-1683.

Brilli F, Barta C, Fortunati A, Lerdau M, Loreto F, Centritto M (2007) Response of isoprene emission and carbon metabolism to drought in white poplar (Populus alba) saplings. New Phytologist, 175, 244-254.

Brilli F, Tsonev T, Mahmood T, Velikova V, Loreto F, Centritto M (2013) Ultradian variation of isoprene emission, photosynthesis, mesophyll conductance, and optimum temperature sensitivity for isoprene emission in water-stressed Eucalyptus citriodora saplings. Journal of Experimental Botany, 64, 519-528.

Centritto M, Brilli F, Fodale R, Loreto F (2011) Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (Populus nigra) saplings. Tree physiology, 31, 275-286.

Centritto M, Haworth M, Marino G, Pallozzi E, Tsonev T, Velikova V, Nogues I, Loreto F (2014) Isoprene emission aids recovery of photosynthetic performance in transgenic Nicotiana tabacum following high intensity acute UV-B exposure. Plant Science, 226, 82-91.

Chen S, Wang S, Altman A, Hütttermann A (1997) Genotypic variation in drought tolerance of poplar in relation to abscisic acid. Tree physiology, 17, 797-803.

Cosentino SL, Copani V, D’agosta GM, Sanzone E, Mantineo M (2006) First results on evaluation of Arundo donax L. clones collected in Southern Italy. Industrial Crops and Products, 23, 212-222.

Cosentino SL, Copani V, Testa G, Scordia D (2015) Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. a potential perennial grass for biomass production in marginal land in semi-arid Mediterranean environment. Industrial Crops and Products, 75, 93–102.

Cosentino SL, Patane C, Sanzone E, Copani V, Foti S (2007) Effects of soil water content and nitrogen supply on the productivity of Miscanthus giganteus Greef et Deu. in a Mediterranean environment. Industrial Crops and Products, 25, 75-88.

Cosentino SL, Scordia D, Sanzone E, Testa G, Copani V (2014) Response of giant reed (Arundo donax L.) to nitrogen fertilization and soil water availability in semi-arid Mediterranean environment. European Journal of Agronomy, 60, 22-32.

Davies WJ, Zhang JH (1991) Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Physiology and Plant Molecular Biology, 42, 55-76.

Demmig-Adams B, Adams WW (2000) Harvesting sunlight safely. Nature, 303, 371-374.

Diaz-Pérez JC, Shackel KA, Sutter EG (1995) Relative water content and water potential of tissue 1. Journal of Experimental Botany, 46, 111-118.

Dietz KJ, Sauter A, Wichert K, Messdaghi D, Hartung W (2000) Extracellular β-glucosidase activity in barley involved in the hydrolysis of ABA glucose conjugate in leaves. Journal of Experimental Botany, 51, 937-944.

Fischer R, Maurer R (1978) Drought resistance in spring wheat cultivars. I. Grain yield responses. Crop and Pasture Science, 29, 897-912.
Flexas J, Bota J, Escalona JM, Sampol B, Medrano H (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Functional Plant Biology, 29, 461-471.

Franks PJ, Farquhar GD (2007) The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiology, 143, 78-87.

Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. The Plant Cell, 17, 3470-3488.

Galmes J, Kapralov MV, Andralojc P, Conesa MÀ, Keys AJ, Parry MA, Flexas J (2014) Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. Plant, Cell and Environment, 37, 1989-2001.

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA) - General Subjects, 990, 87-92.

Ghalambor CK, Mckay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology, 21, 394-407.

Haworth M, Centritto M, Giovannelli A, Marino G, Proietti N, Capitani D, De Carlo A, Loreto F (2016) Xylem morphology determines the drought response of two Arundo donax ecotypes from contrasting habitats. GCB Bioenergy, In Press.

Haworth M, Elliott-Kingston C, Mcelwain JC (2011) Stomatal control as a driver of plant evolution. Journal of Experimental Botany, 62, 2419-2423.

Haworth M, Killi D, Materassi A, Raschi A (2015) Co-ordination of stomatal physiological behavior and morphology with carbon dioxide determines stomatal control. American Journal of Botany, 102, 677 – 688.

Hewitt CN, Monson RK, Fall R (1990) Isoprene emissions from the grass Arundo donax L. are not linked to photosynthesis. Plant Science, 66, 139-144.

Hubbart S, Peng S, Horton P, Chen Y, Murchie EH (2007) Trends in leaf photosynthesis in historical rice varieties developed in the Philippines since 1966. Journal of Experimental Botany, 58, 3429-3438.

Khudamrongwat J, Tayyar R, Holt JS (2004) Genetic diversity of giant reed (Arundo donax) in the Santa Ana River, California. Weed Science, 52, 395-405.

Killi D, Anlauf R, Kavdir Y, Haworth M (2014) Assessing the impact of agro-industrial olive wastes in soil water retention: Implications for remediation of degraded soils and water availability for plant growth. International Biodeterioration and Biodegradation, 94, 48-56.

Klute A (1986) Methods of soil analysis. Part 1. Physical and mineralogical methods, American Society of Agronomy, Inc.

Lauteri M, Haworth M, Serraj R, Monteverdi MC, Centritto M (2014) Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. Plos One, 9, e109054.

Lim PO, Kim HJ, Gil Nam H (2007) Leaf senescence. Annual Review of Plant Biology, 58, 115-136.

Liu F, Jensen CR, Shahanzari A, Andersen MN, Jacobsen S-E (2005) ABA regulated stomatal control and photosynthetic water use efficiency of potato (Solanum tuberosum L.) during progressive soil drying. Plant Science, 168, 831-836.

López-Carbonell M, Gabasa M, Jáuregui O (2009) Enhanced determination of abscisic acid (ABA) and abscisic acid glucose ester (ABA-GE) in Cistus albidus plants by liquid
Chromatography–mass spectrometry in tandem mode. Plant Physiology and Biochemistry, 47, 256-261.

Loreto F, Fineschi S (2015) Reconciling functions and evolution of isoprene emission in higher plants. New Phytologist, 206, 578-582.

Loreto F, Sharkey TD (1990) A gas-exchange study of photosynthesis and isoprene emission in Quercus rubra L. Planta, 182, 523-531.

Mahmood T, Saeed A, Saleem AR, Rasool H, Haworth M, Centritto M (2015) Divergent gas-exchange, physiological, isotopic and compositional responses of two wood-crop species to water deficit: Ziziphus nummularia and Corymbia citriodora. International Journal of Agriculture and Biology, 17, 681-690.

Mann JJ, Barney JN, Kyser GB, Di Tomaso JM (2013) Miscanthus x giganteus and Arundo donax shoot and rhizome tolerance of extreme moisture stress. Global Change Biology Bioenergy, 5, 693-700.

Mantineo M, D’agosta GM, Copani V, Patanè C, Cosentino SL (2009) Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment. Field Crops Research, 114, 204-213.

Mariani C, Cabrini R, Danin A, Piffanelli P, Frigano A, Gomarasca S, Dicandilo M, Grassi F, Soave C (2010) Origin, diffusion and reproduction of the giant reed (Arundo donax L.): a promising weedy energy crop. Annals of Applied Biology, 157, 191-202.

Maseda PH, Fernandez RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. Journal of Experimental Botany, 57, 3963-3977.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany, 51, 659-668.

Nackley LL, Vogt KA, Kim S-H (2014) Arundo donax water use and photosynthetic responses to drought and elevated CO2. Agricultural Water Management, 136, 13-22.

Pilu R, Cassani E, Landoni M, Badone FC, Passera A, Cantaluppi E, Corno L, Adani F (2014) Genetic characterization of an Italian giant reed (Arundo donax L.) clones collection: exploiting clonal selection. Euphytica, 196, 169-181.

Pinheiro C, Chaves MM (2011) Photosynthesis and drought: can we make metabolic connections from available data? Journal of Experimental Botany, 62, 869-882.

Rodeghiero M, Niinemets Õ, Cescatti A (2007) Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimates of Farquhar et al. model parameters? Plant, Cell & Environment, 30, 1006-1022.

Rossa B, Tüffers A, Naidoo G, Willert D (1998) Arundo donax L.(Poaceae)—a C3 species with unusually high photosynthetic capacity. Botanica acta, 111, 216-221.

Saltonstall K, Lambert A, Meyerson LA (2010) Genetics and reproduction of common (Phragmites australis) and giant reed (Arundo donax). Invasive Plant Science and Management, 3, 495-505.

Sánchez E, Scordia D, Lino G, Arias C, Cosentino S, Nogués S (2015) Salinity and water stress effects on biomass production in different Arundo donax L. clones. Bioenergy Research, 8, 1461-1479.

Seiler C, Harshavardhan VT, Rajesh K, Reddy PS, Strickert M, Rolletschek H, Scholz U, Wobus U, Sreenivasulu N (2011) ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. Journal of Experimental Botany, 62, 2615-2632.

Sharkey TD, Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia, 95, 328-333.

Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: why and how. Annals of Botany, 101, 5-18.
Smith LL, Allen DJ, Barney JN (2015) Yield potential and stand establishment for 20 candidate bioenergy feedstocks. Biomass and Bioenergy, 73, 145-154.

Souch C, Stephens W (1998) Growth, productivity and water use in three hybrid poplar clones. Tree physiology, 18, 829-835.

Souza R, Machado E, Silva J, Lagôa A, Silveira J (2004) Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. Environmental and Experimental Botany, 51, 45-56.

Tardieu F, Davies WJ (1992) Stomatal response to abscisic acid is a function of current plant water status. Plant Physiology, 98, 540-545.

Velikova V, Varkonyi Z, Szabo M, Maslenkova L, Nogues I, Kovács L, Peeva V, Busheva M, Garab G, Sharkey TD, F. L (2011) Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. Plant Physiology, 157, 905-916.

Xiong D-M, Liu Z, Chen H, Xue J-T, Yang Y, Chen C, Ye L-M (2014) Profiling the dynamics of abscisic acid and ABA-glucose ester after using the glucosyltransferase UGT71C5 to mediate abscisic acid homeostasis in Arabidopsis thaliana by HPLC–ESI-MS/MS. Journal of Pharmaceutical Analysis, 4, 190-196.

Zhang J, Davies W (1990) Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. Plant, Cell and Environment, 13, 277-285.

Zhang X, Zang R, Li C (2004) Population differences in physiological and morphological adaptations of Populus davidiana seedlings in response to progressive drought stress. Plant Science, 166, 791-797.
The diagram illustrates the changes in several physiological parameters over time for ecotype 20 and ecotype 6 under irrigated and water deficit conditions. The parameters measured include:

- **(a)** Net photosynthetic rate ($P_N$) in μmol m$^{-2}$ s$^{-1}$
- **(b)** Stomatal conductance ($G_s$) in mol m$^{-2}$ s$^{-1}$
- **(c)** Interception (CI) in ppm
- **(d)** ETR/N ratio

The x-axis represents time in days, and the y-axis represents the values of the measured parameters. The data points are color-coded to distinguish between irrigated and water deficit conditions for each ecotype.
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