Gas exchange and hydraulics during drought in crops: who drives whom?

Jaume Flexas*, Marc Carriquí1, and Miquel Nadal1

1 Research Group in Plant Biology under Mediterranean Conditions, Universitat de les Illes Balears-Instituto de Agroecología y Economía del Agua (INAGEA), Palma 07122, Illes Balears, Spain
* Correspondence: jaume.flexas@uib.es

The correlation between stomatal, mesophyll and leaf hydraulic conductance (Kleaf), and the timing of each during regulation under drought, are not fully understood. Studies which make precise, parallel measurement of these variables during progressive imposition of drought are needed. Wang et al. (2018) provide novel insights, showing that, in rice, a decline of Kleaf is the earliest response to decreasing water availability, and they propose that it triggers the later decline of stomatal and mesophyll conductance. Comparison with results from other species intensifies the debate about the relationships between these variables, as well as between photosynthesis (i.e. productivity) and hydraulic failure (death).

Drought stress is one of the largest threats to crop productivity and survival worldwide (Boyer, 1982; Ciais et al., 2005), hence the importance of unveiling the relationships between the different physiological mechanisms and traits that confer resistance in plants (McDowell et al., 2013). Water stress causes the decrease in leaf water potential (Ψleaf), which in turn causes the activation of turgor-related signals (Rodriguez-Dominguez et al., 2016) and/or hormonal signals. Abscisic acid (ABA) is considered the main plant hormone involved in the water stress response, although there is still debate as to whether the fraction of the total hormone pool involved in signalling is synthesized mostly in the roots (Dodd, 2005) or in the same leaf (McAdam et al., 2016). These hydraulic and non-hydraulic factors regulate stomatal but apparently also mesophyll conductances to control both transpiration (i.e. reduce hydraulic tension in the atmosphere–plant–soil continuum) and CO2 supply for the optimization of gas exchange (Nadal and Flexas, 2018). These signals are coupled with the supply capacity of the hydraulic system, otherwise extreme water loss and/or hydraulic failure could lead to complete desiccation of the plant (Sperry, 2004; Hochberg et al., 2017). However, this general scheme of drought response may vary between species depending on the degree of iso-or anisosohdry (Martinez-Vilalta and García-Forner, 2017). Signals induced by Ψleaf also regulate leaf hydraulic conductance (Kleaf) (Couplé-Ledru et al., 2017), in tight coordination with gas exchange (Brodríbb et al., 2014; Gleason et al., 2017). Decreases of Kleaf are generally associated with hydraulic failures, such as embolism, but also with other forms of regulation (Hochberg et al., 2017). However, the relative importance and mechanisms of regulation of its components – the conductance within the xylem (Kx) and the outside-xylem conductance (Ko) – during drought remain unresolved (Trifiló et al., 2016). If the drought worsens, the physiological effects on the leaves are incrementally increased, which may lead to the death of the leaf (e.g. full hydraulic failure, or 100% embolism; Martin-StPaul et al., 2017), and the whole plant may depend on the existence of safety margins among plant organs (Liu et al., 2015; Skelton et al., 2017; Rodriguez-Dominguez et al., 2018). Although the main processes that occur during drought are clear, knowledge of the general timescale of response and the importance of each parameter is limited because most studies do not monitor the same variables simultaneously, and few consider so many parameters during a prolonged drought as do Wang et al. (2018). So what do we really know about these inter-relationships and why is the work by Wang et al. important?

Variability in the physiological responses of crops to drought stress

There are very few interspecific studies on limitations to photosynthesis under drought, thus precluding broad generalizations. For instance, although a pattern has been suggested in which diffusion conductances limit photosynthesis under mild and moderate stress, while biochemical limitations appear only at the later stages (reviewed in Nadal and Flexas, 2018), some studies have found differences among species, especially regarding the relative importance of stomatal and mesophyll limitations (Galmés et al., 2007; Flexas et al., 2009; Galle et al., 2011) but also concerning the early appearance of biochemical limitations (Ennahli and Earl, 2005). Similarly, while it seems that a general coordination among both conductances occurs during drought, recent studies suggest that the nature of the relationship may be species-specific. In this sense, Flexas et al. (2013a) showed that the relationship between g and g in varies across crops under well-watered and water-stressed conditions: although most of them show a tight coordination between these two conductances, some (e.g. poplar) did not show such relationship.
Box 1. Limitations to net assimilation in relation to the vulnerability of its constraints ($g_s$, $g_m$, biochemistry and $K_{leaf}$) in different crops

Response of limitations to photosynthesis – stomatal (SL), mesophyll conductance (ML) and biochemical (BL) limitations – to decreasing leaf water potentials ($\Psi_{leaf}$) in *Oryza sativa* (Wang et al., 2018), *Olea europaea* (data combined from Perez-Martin et al., 2009, and Varone et al., 2012) and *Vitis vinifera* (from El Aou-ouad et al., 2016). $K_{leaf}$ $P_{50}$ and $P_{80}$ are represented by red dashed and solid lines (data from Wang et al., 2018, for rice, and data combined from Torres-Ruiz et al., 2015, and Hernandez-Santana et al., 2016, for *O. europaea*, and from Martorell et al., 2015, for *V. vinifera*). Yellow points in *O. sativa* represent the $P_{50}$ of $g_s$, $g_m$ and electron transport rate (ETR) (each of them situated over the upper line of its limitation – SL, ML or BL, respectively – data from Wang et al., 2018). The blue dotted line represents the turgor loss point (data from Wang et al., 2018, for rice, and value from Hernandez-Santana et al., 2016, for *O. europaea* and from Martorell et al., 2015, for *V. vinifera*). The orange dotted line accounts for either $K_{leaf}$ $P_{50}$ in *O. sativa* (value from Stiller et al., 2003) or the $\Psi_{leaf}$ in which approximately 50% embolism occurs in the leaf midrib (based on optical measurements; data from Rodriguez-Dominguez et al., 2018, for *O. europaea* and from Hochberg et al., 2017 for *V. vinifera*).

![Graph showing the relationships between stomatal and hydraulic conductance in different crops](image)

Box 2. Interrelationships between stomatal and hydraulic conductance in different crops

The graph shows the relationships between stomatal ($g_s$) and leaf hydraulic ($K_{leaf}$) conductances and the magnitudes of each for the same crop species considered in Box 1: *Oryza sativa* (mean data from Wang et al., 2018), *Olea europaea* (data combined from Fernandes-Silva et al., 2016; Hernandez-Santana et al., 2016) and *Vitis vinifera* (data combined from Pou et al., 2012, 2013; El Aou-ouad et al., 2017). Lines represent quadratic polynomial fittings for each species and shaded areas are their 95% confidence intervals.

![Graph showing the interrelationships between stomatal and hydraulic conductance](image)
In two rice cultivars, Wang et al. show that there is strong coordination between $K_{\text{leaf}}$, $g_s$ and $g_{sm}$ during their decrease under drought. Indeed, a similar sequence of events can also be observed for olive when combining data from several studies (Box 1), although olive seems to operate along a wider range of $\Psi_{\text{leaf}}$. On the other hand, this early decline in all three conductances is not observed in grapevine, where the decline of $K_{\text{leaf}}$ ($P_{50}$) occurs at the latest stages of water stress, after a previous progressive and strong decrease in photosynthesis, mainly due to limitation by stomatal conductance. The three examples displayed in Box 1 suggest different possibilities regarding limitations to photosynthesis and coordination of conductances across species.

The species-dependent coordination between stomatal and $K_{\text{leaf}}$ responses to drought could indicate different strategies regarding water conservation and safety of transport (see Box 2). As shown by Wang et al., rice presents a tight coordination between $K_{\text{leaf}}$ and $g_s$; in fact, the decrease of $g_s$ is mainly attributed to $K_{\text{leaf}}$. This has also been shown in woody crops (Hernández-Santana et al., 2016; Rodriguez-Dominguez et al., 2016). On the other hand, no such coordination has been observed in soybean (Locke and Ort, 2014). On a broader phylogenetic scale, clearer differences emerge; for example, $g_s$ presents a higher sensitivity to $\Psi_{\text{leaf}}$ in ferns compared to coexisting angiosperms (Brodribb and Holbrook, 2004). In ferns, stomata closed before any significant drop in $K_{\text{leaf}}$ whereas in the angiosperms studied there was a tighter coordination between $g_s$ and $K_{\text{leaf}}$. This was also observed when studying the different responses of $g_s$ and $K_{\text{leaf}}$ to drought but to varying light intensity (Xiong et al., 2018). Indeed, the differences in $P_{50}$ for $g_s$ and $K_{\text{leaf}}$ may be more related to phylogeny than to ambient conditions as no common pattern in $P_{50}$ was observed in co-occurring tree species (Liu et al., 2015). In the case of the drought-induced $g_{sm}$–$K_{\text{leaf}}$ relationship, significant variability has been reported even at the clone level (Théroux-Rancourt et al., 2015). Some degree of plasticity in these relationships has also been seen in grapevines, where $K_{\text{leaf}}$ presented a decreasing $P_{50}$ as summer progressed (Martorell et al., 2015). Moreover, even the mechanistic basis for the decline in $K_{\text{leaf}}$ (i.e. the relative importance of $K_{xy}$ and $K_{ox}$) may be species-dependent (Trifiló et al., 2016). All these examples of interspecific variation hinder disentanglement of the factors limiting photosynthesis and transpiration under water stress.
Role of, and relationships among, water conductances during drought: universal or species-specific?

Many theories have considered the stomata as the safety valves preventing hydraulic dysfunction under mild to moderate water stress conditions (Hochberg et al., 2017 and references therein), considering leaf xylem hydraulic vulnerability as the main component of leaf hydraulic vulnerability. However, results from Wang et al. challenge these theories. The fact that the $K_{\text{leaf}}$ of $P_{\text{g}}$ was achieved before the $g_{s}$ and $g_{m}$ of $P_{\text{g,s}}$ suggests that, in rice, the stomata do not function as a safety valve and therefore either: (i) if $K_{\text{leaf}}=K_{\text{m}}$, leaf xylem cavitated before stomata closed; or (ii) if $K_{\text{leaf}}=K_{\text{v,x}}$, outside-xylem hydraulic vulnerability protected against xylem failure instead of stomata (see Box 3 for a depiction of these two possibilities). The first hypothesis is unlikely as the xylem vulnerability $P_{\text{g,s}}$ reported by Stiller et al. (2003) is about $-2.0$ MPa. On the other hand, although Wang et al. measured $K_{\text{leaf}}$ without distinguishing $K_{\text{v}}$ from $K_{\text{m}}$, the second hypothesis may be more likely: indeed, Trifilò et al. (2016) and Scoffoni et al. (2017) showed that outside-xylem hydraulic vulnerability explains 75 to 100% of $K_{\text{leaf}}$ decline before reaching the turgor loss point in most of the species studied. However, this hypothesis cannot be considered confirmed yet, at least for all vascular plants, as measurements performed using new techniques (such as the leaf optical properties) and more experiments are carried out monitoring the multiple interrelated variables that act during drought for multiple species, a very interesting debate where (at least) two major hypotheses will continue. The work by Wang et al. (2018) adds important new data and ideas to this debate.

Acknowledgements

MC is supported by a predoctoral fellowship FPI/1700/2014 from the Conselleria d’Educació, Cultura i Universitats (Govern de les Illes Balears) and European Social Fund, and MN is supported by a predoctoral fellowship BES-2015-072578 from the Ministerio de Economía y Competitividad (MINECO, Spain) co-financed by the ESF. Research of JF, MC and MN is supported by the project CTM2014-53902-C2-1-P from the Ministerio de Economía y Competitividad (MINECO, Spain) and the ERDF (FEDER).

Keywords: Drought stress, gas exchange, leaf hydraulics, mesophyll conductance, photosynthesis, stomatal conductance.

Journal of Experimental Botany, Vol. 69 No. 16 pp. 3791-3795, 2018 doi:10.1093/jxb/ery235

References

Boyer JS. 1982. Plant productivity and environment. Science 218, 443–448.

Brodribb TJ, Holbrook NM. 2004. Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms. New Phytologist 162, 663–670.

Brodribb TJ, McAdam SAM, Jordan GJ, Martins SCV. 2014. Conifer species adapt to low-rainfall climates by following one of two divergent pathways. Proceedings of the National Academy of Sciences, USA 111, 14489–14493.

Brodribb TJ, Bienaimé D, Marmottant P. 2016. Revealing catastrophic failure of leaf networks under stress. Proceedings of the National Academy of Sciences, USA 113, 4965–4969.

Ciais P, Reichstein M, Viovy N, et al. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533.

Coupeil-Ledru A, Tyerman SD, Masclcf D, Lebon E, Christophe A, Edwards EJ, Simonneau T. 2017. Abscisic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only. Plant Physiology 175, 1121–1134.

Dodd IC. 2005. Root-to-shoot signalling: assessing the roles of ‘up’ in the up and down world of long-distance signalling in plants. Plant Soil 274, 251–270.

El Aou-Ouad H, Montero R, Medrano H, Bota J. 2016. Interactive effects of grapevine leafroll-associated virus 3 (GLRaV-3) and water stress on the physiology of Vitis vinifera L. cv. Malvasia de Banyalbufar and Giron-Ros. Journal of Plant Physiology 196-197, 106–115.

El Aou-Ouad H, Pou A, Tomás M, Montero R, Ribas-Carbo M, Medrano H, Bota J. 2017. Combined effect of virus infection and water stress on water flow and water economy in grapevines. Physiologia Plantarum 160, 171–184.

Ennahl S, Earl HJ. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. Crop Science 45, 2374–2382.

Fernandes-Silva AA, López-Bernal A, Ferreira TC, Villalobos FJ. 2016. Leaf water relations and gas exchange response to water deficit of olive (cv. Cobrançosa) in field grown conditions in Portugal. Plant Soil 402, 191–209.

Flexas J, Barón M, Bota J, et al. 2009. Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (V. berlandieriXV. rupestris). Journal of Experimental Botany 60, 2361–2377.

Flexas J, Niinemets U, Gallé A, et al. 2013a. Diffusional conductances to CO2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. Photosynthesis Research 117, 45–59.

Flexas J, Scoffoni C, Gago J, Sack L. 2013b. Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. Journal of Experimental Botany 64, 3965–3981.

Galle A, Florez-Sarasá I, Aououad HE, Flexas J. 2011. The Mediterranean evergreen Quercus ilex and the semi-deciduous Cistus albidus differ in their leaf gas exchange regulation and acclimation to repeated drought and re-wetting cycles. Journal of Experimental Botany 62, 5207–5216.

Galán M, Medrano H, Flexas J. 2007. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. New Phytologist 175, 81–93.

Gleason SM, Wiggans DR, Bliss CA, Comas LH, Cooper M, De Jonge KC, Young JS, Zhang H. 2017. Coordinated decline in photosynthesis and hydraulic conductance during drought stress in Zea mays. Flora 227, 1–9.

Hernandez-Santana V, Rodriguez-Dominguez CM, Fernández JE, Diaz-Espejo A. 2016. Role of leaf hydraulic conductance in the regulation of stomatal conductance in almond and olive in response to water stress. Tree Physiology 36, 725–735.

Hochberg U, Windt CW, Ponnamarenko A, Zhang YJ, Gersony J, Rockwell FE, Holbrook NM. 2017. Stomatal closure, basal leaf embolism, and shedding protect the hydraulic integrity of grape stems. Plant Physiology 174, 764–775.
Liu YY, Song J, Wang M, Li N, Niu CY, Hao GY. 2015. Coordination of xylem hydraulic and stomatal regulation in keeping the integrity of xylem water transport in shoots of two compound-leaved tree species. Tree Physiology 35, 1333–1342.

Locke AM, Ort DR. 2014. Leaf hydraulic conductance declines in coordination with photosynthesis, transpiration and leaf water status as soybean seedlings age regardless of soil moisture. Journal of Experimental Botany 65, 6617–6627.

Martin-StPaul N, Delzon S, Cochard H. 2017. Plant resistance to drought depends on timely stomatal closure. Ecology Letters 20, 1437–1447.

Martínez-Vilalta J, García-Forner N. 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant, Cell & Environment 40, 962–976.

Martorell S, Medrano H, Tomás M, Escalona JM, Flexas J, Díaz- Espejo A. 2015. Plasticity of vulnerability to leaf hydraulic dysfunction during acclimation to drought in grapevines: an osmotic-mediated process. Physiologia Plantarum 153, 381–391.

McAdam SA, Sussmilch FC, Brodribb TJ. 2016. Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms. Plant, cell & environment 39, 435–450.

McDowell NG, Fisher RA, Xu C, et al. 2013. Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. New Phytologist 200, 304–321.

Nadal M, Flexas J. 2018. Mesophyll conductance to CO2 diffusion: effects of drought and opportunities for improvement. In: García-Tejero IF, Durán-Zuazo VH, eds. Water scarcity and sustainable agriculture in semiarid environment. London: Elsevier, 404–438.

Perez-Martín A, Flexas J, Ribas-Carbó M, Boto J, Tomás M, Infante JM, Díaz-Espejo A. 2009. Interactive effects of soil water deficit and air vapour pressure deficit on mesophyll conductance to CO2 in Vitis vinifera and Olea europaea. Journal of Experimental Botany 60, 2391–2405.

Pou A, Medrano H, Tomás M, Martorell S, Ribas-Carbó M, Flexas J. 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric. Plant Soil 359, 335–349.

Pou A, Medrano H, Flexas J, Tyerman SD. 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. Plant, Cell & Environment 36, 829–843.

Rodríguez-Dominguez CM, Buckley TN, Egea G, de Gires A, Hernandez-Santana V, Martorell S, Díaz-Espejo A. 2016. Most stomatal closure in woody species under moderate drought can be explained by stomatal responses to leaf turgor. Plant, Cell & Environment 39, 2014–2026.

Rodríguez-Dominguez CM, Carins Murphy MR, Lucani C, Brodribb TJ. 2018. Mapping xylem failure in disparate organs of whole plants reveals extreme resistance in olive roots. New Phytologist 218, 1025–1035.

Scoffoni C, Albuquerque C, Brodersen CR, Townes SV, John GP, Bartlett MK, Buckley TN, McElrone AJ, Sack L. 2017. Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. Plant Physiology 173, 1197–1210.

Skelton RP, Brodribb TJ, McAdam SAM, Mitchell PJ. 2017. Gas exchange recovery following natural drought is rapid unless limited by loss of leaf hydraulic conductance: evidence from an evergreen woodland. New Phytologist 215, 1399–1412.

Sperry JS. 2004. Coordinating stomatal and xylem functioning – an evolutionary perspective. New Phytologist 162, 568–570.

Stillier V, Lafitte HR, Sperry JS. 2003. Hydraulic properties of rice and the response of gas exchange to water stress. Plant Physiology 132, 1698–1706.

Théroux Rancourt G, Éthier G, Pepin S. 2015. Greater efficiency of water use in poplar clones having a delayed response of mesophyll conductance to drought. Tree Physiology 35, 172–184.

Tombesi S, Nardini A, Farinelli D, Palliotti A. 2014. Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of Vitis vinifera. Physiologia Plantarum 152, 453–464.

Torres-Ruiz JM, Díaz-Espejo A, Perez-Martin A, Hernandez- Santana V. 2015. Role of hydraulic and chemical signals in leaves, stems and roots in the stomatal behaviour of olive trees under water stress and recovery conditions. Tree Physiology 35, 415–424.

Trifiló P, Raimondo F, Savi T, Lo Gullo MA, Nardini A. 2016. The contribution of vascular and extra-vascular water pathways to drought-induced decline of leaf hydraulic conductance. Journal of Experimental Botany 67, 5029–5039.

Varone L, Ribas-carlo M, Cardona C, Gallé A, Medrano H, Gratani L, Flexas J. 2012. Stomatal and non-stomatal limitations to photosynthesis in seedlings and saplings of Mediterranean species pre-conditioned and aged in nurseries: Different response to water stress. Environmental and Experimental Botany 75, 235–247.

Wang X, Du T, Huang J, Peng S, Xiong D. 2018. Leaf hydraulic vulnerability triggers the decline in stomatal and mesophyll conductance during drought in rice (Oryza sativa). Journal of Experimental Botany 69, 4033–4045.

Xiong D, Flexas J, Yu T, Peng S, Huang J. 2017. Leaf anatomy mediates coordination of leaf hydraulic conductance and mesophyll conductance to CO2 in Oryza. New Phytologist 213, 572–583.

Xiong D, Douthe C, Flexas J. 2018. Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. Plant, Cell & Environment 41, 436–450.