

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

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The correlation between stomatal, mesophyll and leaf hydraulic conductance (K_{leaf}), 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 K_{leaf} 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 (Ψ_{leaf}), which in turn causes the activation of turgor-related signals (Rodríguez-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 plants depending on the degree of iso- or anisohydry (Martínez-Vilalta and García-Forner, 2017). Signals induced by Ψ_{leaf} also regulate leaf hydraulic conductance (K_{leaf}) (Coupel-Ledru *et al.*, 2017), in tight coordination with gas exchange (Brodrigg *et al.*, 2014; Gleason *et al.*, 2017). Decreases of K_{leaf} are generally associated

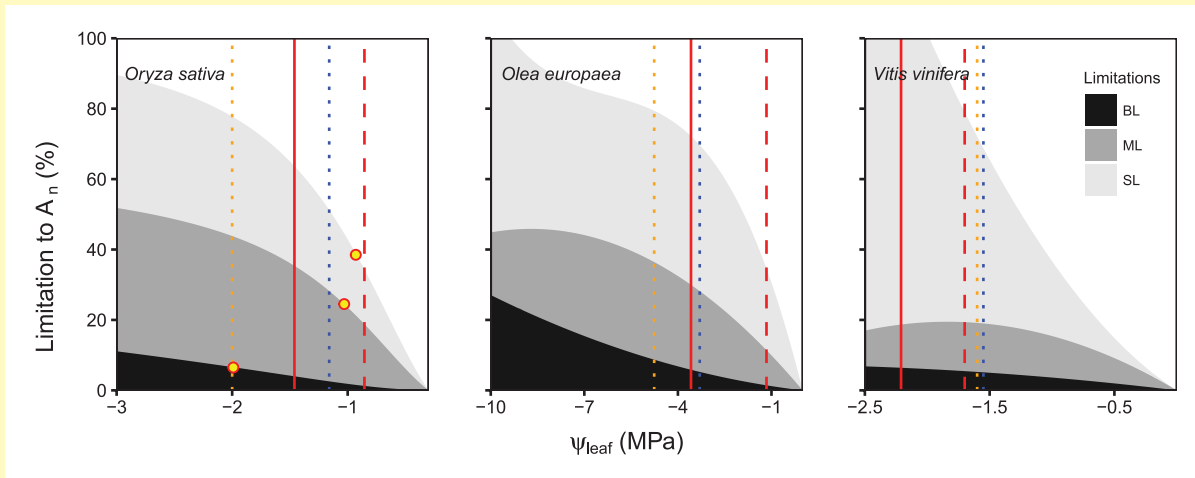
with hydraulic failures, such as embolism, but also with other forms of regulation (Hochberg *et al.*, 2017). However, the relative importance and mechanisms of regulation of its components – the conductance within the xylem (K_x) and the outside-xylem conductance (K_{ox}) – during drought remain unresolved (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; Rodríguez-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_s and g_m 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.

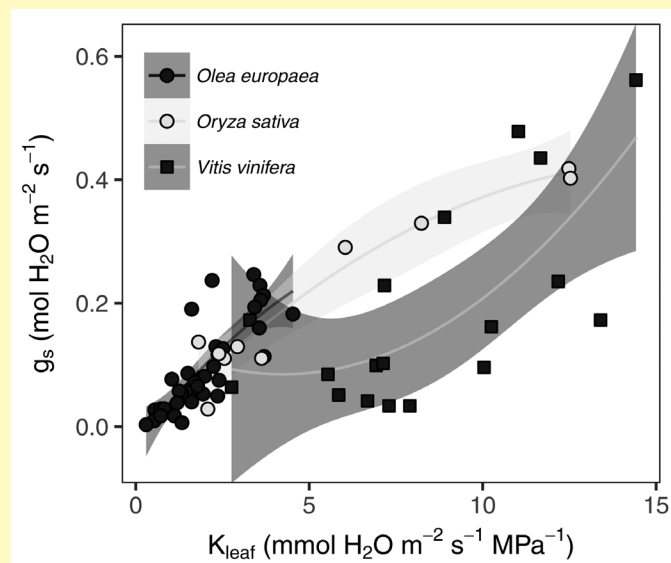
Box 1. Limitations to net assimilation in relation to the vulnerability of its constraints (g_s , g_m , biochemistry and K_{leaf}) in different crops

Response of limitations to photosynthesis – stomatal (SL), mesophyll conductance (ML) and biochemical (BL) limitations – to decreasing leaf water potentials (Ψ_{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 $K_x P_{50}$ in *O. sativa* (value from Stiller et al., 2003) or the Ψ_{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*).



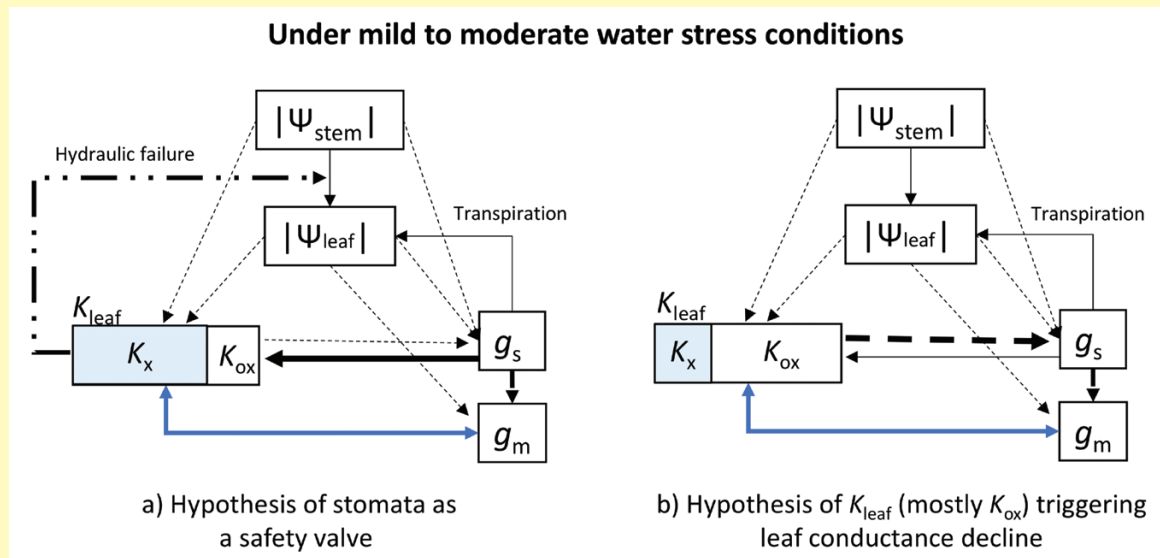
Box 2. Interrelationships between stomatal and hydraulic conductance in different crops

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.



Box 3. Variables and hypothetical relationships controlling physiological drought response in crops

Diagram showing the potential interrelations between water potential and leaf conductances under mild to moderate drought stress conditions. Solid lines indicate positive relationships between variables, whereas broken lines indicate negative relationships. The dotted broken line indicates the hydraulic disconnection between leaf and stem due to embolism. Left diagram (a) follows the hypothesis of the safety valve function of stomata to prevent hydraulic failure (K_{leaf} mostly constituted by leaf xylem conductance, K_x). In this scenario, stomatal and mesophyll conductance (g_s and g_m , respectively) are reduced to keep K_{leaf} within the safety margin to avoid hydraulic disconnection from the stem. Right diagram (b) reflects a hypothesis that can be derived from the suggestion by Wang *et al.* of outside-xylem conductance controlling K_{leaf} , which in turn triggers the decline of both g_s and g_m . In this case, cavitation would be of little magnitude because K_{leaf} would be governed mainly by K_{ox} . Notice the double-headed blue line linking g_m and K_{leaf} in both diagrams; this accounts for the coordinated nature of these two conductances (Flexas *et al.*, 2013b), which could emerge from a common structural basis (Xiong *et al.*, 2017), rather than by one being directly affected by the other.



In two rice cultivars, Wang *et al.* show that there is strong coordination between K_{leaf} , g_s and g_m 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 Ψ_{leaf} . On the other hand, this early decline in all three conductances is not observed in grapevine, where the decline of K_{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_{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_{leaf} and g_s ; in fact, the decrease of g_s is mainly attributed to K_{leaf} . This has also been shown in woody crops (Hernandez-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 Ψ_{leaf} in ferns compared to coexisting angiosperms (Brodribb and Holbrook, 2004). In ferns, stomata closed before any significant drop in K_{leaf} , whereas in the angiosperms studied there was a tighter coordination between g_s and K_{leaf} . This was also observed when studying the different responses of g_s and K_{leaf} not to drought but to varying light intensity (Xiong *et al.*, 2018). Indeed, the differences in P_{50} for g_s and K_{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_m - K_{leaf} relationship, significant variability has been reported even at the clone level (Th eroux-Rancourt *et al.*, 2015). Some degree of plasticity in these relationships has also been seen in grapevines, where K_{leaf} presented a decreasing P_{80} as summer progressed (Martorell *et al.*, 2015). Moreover, even the mechanistic basis for the decline in K_{leaf} (i.e. the relative importance of K_{ox} and K_x) may be species-dependent (Trifil o *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 $K_{\text{leaf}} P_{50}$ was achieved before the g_s and g_m P_{50} s suggests that, in rice, the stomata do not function as a safety valve and therefore either: (i) if $K_{\text{leaf}}=K_x$, leaf xylem cavitated before stomata closed; or (ii) if $K_{\text{leaf}}=K_{\text{ox}}$, 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_{50} reported by Stiller *et al.* (2003) is about -2.0 MPa. On the other hand, although Wang *et al.* measured K_{leaf} without distinguishing K_x from K_{ox} , the second hypothesis may be more likely: indeed, Trifiló *et al.* (2016) and Scoffoni *et al.* (2017) showed that outside-xylem hydraulic vulnerability explains 75 to 100% of K_{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 vulnerability; Brodribb *et al.*, 2016) that allow the simultaneous measurement of K_x and g_s (Hochberg *et al.*, 2017) provide new evidence supporting the hypothesis that K_{leaf} is mainly driven by K_x . Nonetheless, these two hypotheses are not necessarily irreconcilable; in fact, they may represent species- or even genotype-specific strategies for plants coping with water stress along the iso-anisohydric spectrum (Tombesi *et al.* 2014; Coupel-Ledru *et al.*, 2017).

In summary, until methodological limitations are improved, 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 are possible will continue. The work by Wang *et al.* (2018) adds important new data and ideas to this debate.

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