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Commentary

What stops stomata reopening after a drought?

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This scientific commentary refers to 'Ethylene constrains stomatal reopening in Fraxinus chinensis post moderate drought' by Bi et al. (doi: 10.1093/treephys/tpac144).

Drought is an existential threat to terrestrial plant life (Brodribb et al. 2020). The severing of the liquid continuum between the soil and the evaporating surfaces of the leaf by xylem embolism at low water potentials is often lethal (Urli et al. 2013). Droughts can be long, severe and ecologically destabilizing, as is becoming increasingly common with changing climates (Choat et al. 2018), but by far the most common form of drought is the intermittent, non-lethal episode of water deficit that can occur more than once in a growing season. Stomatal closure is one of the first physiological responses to any drought, and is very effective at arresting water potential decline (Cochard et al. 2021). For many species, drought-induced stomatal closure is a frequent occurrence over the life of an individual; in some long-lived trees, the tree-ring record suggests a pattern of repeated stomatal closure following drought, and delayed growth recovery for many years (Anderegg et al. 2015). Given the repeated occurrence of droughts, and the potential lethality of each drought, a question that has long entertained plant biologists is: does a drought induce water-conservative stomata function (Darwin 1898)? It could be hypothesized that a conservative stomatal recovery from drought (stomata not reopening to maximum apertures as fast as water potentials recover) might be advantageous if there are frequent drought events in quick succession, or the break in water limitation is temporary, by limiting the waste of a soon-to-be scarce nutrient. A similar behavior is seen developmentally in response to drought, with leaves initiated under water deficit having fewer stomata (Yoo et al. 2010). In this issue, Bi et al. (2022) explores the physiological mechanism restricting gas exchange recovery following drought in *Fraxinus chinensis*. Bi et al. (2022) report that on rewatering from a drought severe enough to close stomata, the complete recovery of leaf gas exchange took >7 days, yet water potentials recovered within 12 h of rewatering. Bi et al. (2022) explored two hypotheses that could explain this delayed stomatal recovery following drought, given that reduced xylem hydraulic conductivity (Brodribb and Cochard 2009) was ruled out because water potentials during drought did not drop to a level that would induce embolism. The first theory tested was that the levels of the phytohormone abscisic acid (ABA), which is critical for closing stomata during drought in seed plants (Mittelheuser and Van Steveninck 1969, Raschke 1975), remained high following recovery from drought and gradually declined. Bi et al. (2022) found that this was not the case and that like leaf water potential, which rapidly recovered, ABA levels declined rapidly on rewatering. This left a final hypothesis that another metabolic signal was keeping stomata closed on recovery from drought. Bi et al. (2022) found that in *F. chinensis* this was the gaseous, fruit-ripening hormone ethylene. Bi et al. (2022) provide a compelling series of experiments, including gas exchange in plants treated with ethylene antagonists, to measuring ethylene emission rates, to demonstrate that ethylene keeps the stomata of *F. chinensis* closed on recovery from drought. Bi et al. (2022) conclude that ethylene acts as a conservative water-use signal for stomata following an intermittent and non-lethal drought (Figure 1).

One of the most impactful conclusions of the recent work by Bi et al. (2022) is that this study resolves a perplexing

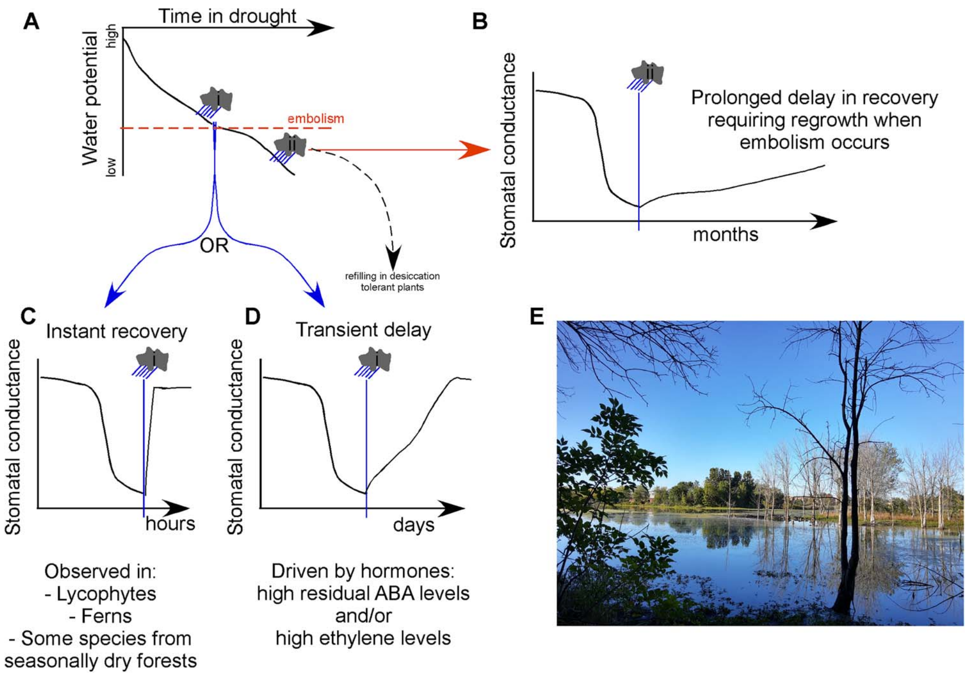

Figure 1. There is a wide diversity of stomatal responses to recovery from drought. (A) As leaf water potential declines during drought, different responses of stomata occur if rainfall events happen before incipient embolism in the xylem (dashed horizontal red line) (rain cloud i), or after embolism has formed in the xylem (rain cloud ii). (B) When relief from a drought occurs after embolism has formed in the xylem then gas exchange recovery can take many months in species that must construct new xylem and organs to regain pre-drought gas exchange capacity (Brodribb and Cochard 2009, Anderegg et al. 2015). In desiccation-tolerant species in which xylem refilling can freely occur, gas exchange recovery often experiences a transient delay due to the recovery of hydraulic conductivity (Holmlund et al. 2019). When relief from a drought occurs prior to the onset of embolism then gas exchange recovery can follow one of two paths: either, (C) recovery is rapid, occurring over the span of hours as water potentials recover, this response is commonly observed in lycophytes and ferns which have stomata that are passively responsive to changes in leaf water status, and not regulated by endogenous hormone levels (McAdam and Brodribb 2012); but has also been observed in some seasonally dry forest communities (Skelton et al. 2017). Alternatively, (D) stomata may take many days to reopen to maximum apertures after recovery from a mild drought, and this suppression of stomatal reopening has traditionally been attributed to the hormone ABA (Davies et al. 1981), but Bi et al. (2022) show that in *Fraxinus chinensis*, suppression of stomatal reopening may be due to high levels of ethylene. (E) There is an urgent need to characterize this physiological diversity across land plants before extinction from human impact leads to the permanent loss of these adaptations. The work by Bi et al. (2022) is timely because in many areas of the Northern Hemisphere, species of *Fraxinus* are threatened with extinction from human impact (Musolin et al. 2017), like this now standing dead population of *Fraxinus pennsylvanica* extirpated from Celery Bog Nature Area, West Lafayette, IN, USA by the Emerald Ash Borer. With a continued loss of species from human impact, it is unknown how many novel discoveries about stomatal function we will never have the chance to make.

question that has plagued stomatal biologists for half a century, namely: does ethylene control stomata? Since the work of Pallaghy and Raschke (1972), there has been an ongoing debate about whether ethylene closes stomata. The stomata of some species are sensitive to ethylene, yet some are not (Pallas and Kays 1982, Madhavan et al. 1983). In the absence of ecophysiological studies like that of Bi et al. (2022), the answer to whether ethylene regulates stomata has been an unsatisfying, maybe. Bi et al. (2022) demonstrate that ethylene keeps stomata closed in *F. chinensis* after drought recovery, but in the context of the past 50 years of work on stomatal regulation by ethylene, it appears that this response is likely one of a wide, and still largely uncharacterized diversity of stomatal responses to drought and recovery that exist across land plants. Each of these very different responses are likely to be adaptively relevant and play an important role in determining the ecological strategies of particular species (McAdam and Brodribb 2015), and how they compete with each other through periods of drought (Haberstroh and Werner 2022). In a phylogenetic context, the drought-stressed leaves of lycophytes and ferns can be rehydrated and stomata instantaneously reopen as soon as leaf water potential recovers (McAdam and Brodribb 2012), because in species from these lineages we see no evidence of an endogenous metabolic regulator of stomatal closure during drought (Brodribb and McAdam 2011, Cardoso et al. 2019, Gong et al. 2021). Similarly, in conifers which have a peaking type ABA dynamic during drought, in which ABA levels decline once plants reach −4 MPa during a drought, rehydration of leaves following a long-term drought results in rapidly reopening stomata, indicating no role of a metabolic regulator closing stomata (Brodribb and McAdam 2013, Brodribb et al. 2014). In some forest systems, stomata have also been observed to reopen rapidly on recovery from drought, with delays in gas exchange recovery only observed once embolism-induced declines in hydraulic conductance occur (Skelton et al. 2017). In contrast to these reports in which no evidence of a metabolic inhibitor of stomatal reopening is present, there are studies which find a prolonged period of reduced rates of gas exchange following recovery from a mild drought even when water potentials recover to pre-stressed levels, indicative of a metabolic signal keeping stomata closed (Raschke 1975, Galmés et al. 2007). Thanks to the work of Bi et al. (2022), we now know that this could be due to high levels of ethylene in the leaves, and not just high levels of ABA as has long been posited (Davies et al. 1981). Things we do not yet know include which species use ABA and which use ethylene to keep stomata shut on drought recovery, and why some plants use ethylene and not ABA to keep stomata closed during drought, or why some species have evolved this conservative strategy in the first place when others have not. Recent work over the past decade has suggested that diversity in stomatal function is considerable across land plants (Kubásek et al. 2021, McAdam et al. 2021), has shaped the evolution of land plants and plays a major role in defining ecological strategies; it seems that ecological diversity may be the explanation for 50 years of contradictory conclusions about whether ethylene controls stomata. More ecophysiological studies like that of Bi et al. (2022) will go a long way toward characterizing this diversity.

For those interested in modeling plant gas exchange and upscaling stomatal responses spatio-temporally, diversity in stomatal responses across land plants adds a level of complexity to this already challenging task (Wolz et al. 2017). In terms of characterizing the diversity of stomatal sensitivity to ethylene across land plants, the solution would be expedited if we were to know the key molecular elements of guard cell response to ethylene (Desikan et al. 2006), which would allow us to map these across phylogenies as the database of sequenced genomes continues in its exponential growth phase (Kress et al. 2022). This is likely to prove very challenging given the lack of phylogenetic pattern so far noted in the presence or absence of stomatal sensitivity to ethylene (Pallaghy and Raschke 1972, Pallas and Kays 1982, Madhavan et al. 1983), and the work on the stomatal signaling pathway for ethylene which has so far only been conducted in *Arabidopsis* (Desikan et al. 2006).

It will not be lost on readers from Europe and North America that the study species chosen by Bi et al. (2022) was *F. chinensis*, a member of a widespread Holarctic genus that in recent times has been driven functionally extinct in the Midwest of North America with the arrival of the Emerald Ash Borer (Ward et al. 2021) and threatened with extinction in Europe due to Ash dieback caused by a fungus (Goberville et al. 2016). The impact of these two pests has been linked to climate change and human activity (Musolin et al. 2017). As a community of plant physiologists, we face some critical questions that need to be answered, such as how much physiological and functional diversity exists in nature? How much stomatal diversity exists in nature, and how much has already been lost due to human impact? What is the effect of a physiologically diverse forest community on the responses of forests to climate change (Haberstroh and Werner 2022)? With the threat of extinction of *Fraxinus*, the discovery of Bi et al. (2022) is timely. As climate change progresses, we stand to lose many potentially transformative scientific discoveries stemming from functional diversity, like that of the importance of ethylene as a drought memory signal for stomata.

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