

Dying Light: Detecting Tree Mortality Risk with Chlorophyll Fluorescence

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Abstract: Increased greenhouse emissions have altered Earth's climate, resulting in rapid climate change which has brought warm droughts—especially in semi-arid regions—leading to forest die-off and dieback. Physiological stresses associated with climate change pose a great risk to tree survival, as future climate changes are likely to increase the intensity of drought. Some of these effects can be observed in species such as piñon pine (*Pinus edulis*), which typically have a high drought tolerance but have succumbed to widespread drought-induced mortality in recent decades, likely due to hydraulic failure. Currently, remotely sensing hydraulic failure is not possible. However, our results suggest chlorophyll fluorescence in dark-adapted samples is an indicator of water stress and the variation of chlorophyll fluorescence within a tree increases as drought stress prolongs. With continued observation, we believe chlorophyll fluorescence will show to be a useful proxy for hydraulic failure to aid mortality predictions as well as predict how future climate changes will impact terrestrial vegetation and climate feedback loops.

Keywords: *Pinus edulis*, Chlorophyll Fluorescence, Tree Mortality, Drought

Introduction

Trees are necessary for the regulation of biological processes, such as Earth's carbon cycle (Macalady and Bugmann 2014). Researchers have observed tree mortality events—large-scale die-off of trees—on every forested biome of Earth in recent decades (Adams et al. 2017). Increasing tree mortality poses a threat to biodiversity (McDowell et al. 2008) and carbon regulation in the atmosphere (Anderegg et al. 2013). Increased greenhouse emissions have altered the earth's climate (Allen et al. 2010), and rapid climate change has brought warm droughts—especially in semi-arid regions (Clifford et al. 2013). Physiological stresses associated with climate change pose a great risk to tree survival (Allen et al. 2010), as future climate changes are likely to increase the intensity of droughts (Duan et al. 2015). Current prediction models are limited by gaps in the knowledge of a tree's physiological response to drought and the impact climate change has on tree-mortality and other vegetation models (Macalady and Bugmann 2014).

Trees have varied protective physiological mechanisms in place to respond to water stress. However, the initiation of many of these responses does not occur until the tree meets a certain level of stress, often quantified in water potential (ψ) (Ryan 2011). Since plant species adapt to their environment and the climate in that environment through evolution (Kremer et al. 2012), response to drought is relative to each plant species and to the severity of the drought (Ryan 2011). Piñon pines (*Pinus edulis*) occur in semi-arid regions of the southwestern United States and are typically considered resistant to drought, but have recently experienced wide-spread die-off. (Clifford et al. 2013, McDowell et al. 2008). Critical analysis of a tree's physiological mechanisms is necessary to understand the causes of tree mortality after exposure to lethal levels of water stress.

Researchers in previous studies have proposed two physiological mechanisms as causal factors of drought-induced tree mortality: carbon starvation and hydraulic failure (McDowell et al. 2008). Due to their immobility, trees are unable to avoid various stressors, such as drought stress, and instead tolerate stress by

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allocating their nutrients to increase survival, which may result in depletion of carbon stores (e.g., carbon starvation) (Hartmann et al. 2018). However, recent global synthesis of tree mortality data has only found universal support for hydraulic failure in explaining tree death (Adams et al. 2017). Our research focused specifically on xylem hydraulic failure and its impact on tree mortality. Despite its importance, the definition of hydraulic failure remains contentious among researchers. Urli et al. (2013) define hydraulic failure as occurring when the disruption of water transport in embolized vessels is greater than water transport in working vessels due to drought-induced xylem tensions. Other researchers refer to hydraulic failure as the point at which complete embolization of the xylem occurs and no water is present in the xylem (McDowell et al. 2008). In both of these definitions, if the water potential surpasses a species-specific threshold, the water column ruptures and results in xylem embolism (Zweifel and Zeugin 2008).

After exposure to drought, tree responses to rewatering vary with the severity of the water stress. In mild to severe levels of water stress, photosynthesis typically resumes pre-drought levels following relief of drought (Xu et al. 2010); trees beyond a point of no return will fail to recover and die. While previous studies have identified water potentials that result in tree mortality (Gleason et al. 2012, Barigah *et al.* 2013, Adams et al. 2017), recent observations have quantified lethal levels of hydraulic failure for the first time (Hammond et al., in press). Despite these advances, hydraulic failure presently cannot be detected via remote sensing and is time-consuming to measure on individual trees, creating the need for reliable proxies of hydraulic failure.

In the present study, we used chlorophyll fluorescence to measure photosynthetic efficiency, a metric shown to correlate with xylem conductivity and hydraulic failure (Maxwell and Johnson 2000). Chlorophyll fluorescence (F_v/F_m)—with F_v being the difference between the maximum and minimum fluorescence and F_m denoting the maximum

fluorescence—is a measurement of light re-emitted from Photosystem II (PSII) within a plant's thylakoid membrane (Murchie and Lawson 2013). Considering plants require water—along with light energy and carbon dioxide—to perform photosynthesis (Pineiro and Chaves 2011), measuring F_v/F_m provides a useful proxy for the amount of water stress a plant experiences. We hypothesized that photosynthetic capacity would be predictive of which trees survive or die after a period of drought. In addition, we predicted as the trees became exposed to increasing levels of water stress and water potential decreased, the photosynthetic capacity would decrease as well.

Methods

For this project, we used mature piñon pine trees—estimated to be 20 to 25 years old—transplanted from a forest in southwest Colorado. We then potted the trees in their native sandy loam soil, using 38 L easy-fill planter bags in a greenhouse at Oklahoma State University. We logged environmental conditions (temperature, relative humidity, and incoming solar radiation) using a weather station (Onset Computer Corporation, Bourne, Massachusetts, USA). We placed white flagging tape on 5 sunlit branches on the South-facing side of each tree—including only the two most recent needle cohorts—and measured the ratio of chlorophyll fluorescence of these branches throughout the experiment to account for natural variation within tree canopies. To determine a lethal level of hydraulic failure, we used Program R to randomly assign mature Piñon pine trees to 6 different rewatering groups with varying water-stress levels. The control group ($n=4$) was not exposed to any water stress and received water weekly. We exposed another group of trees ($n=4$) to extreme water stress and only rewatered these trees after determining they were completely desiccated (no water left in the xylem). In the other 4 groups ($n=5$ and $n=10$ per group), we exposed each group to varying levels of drought stress—ranging from little to severe stress. We designed the treatment groups to show a range of water stress at the time of rewatering, rather

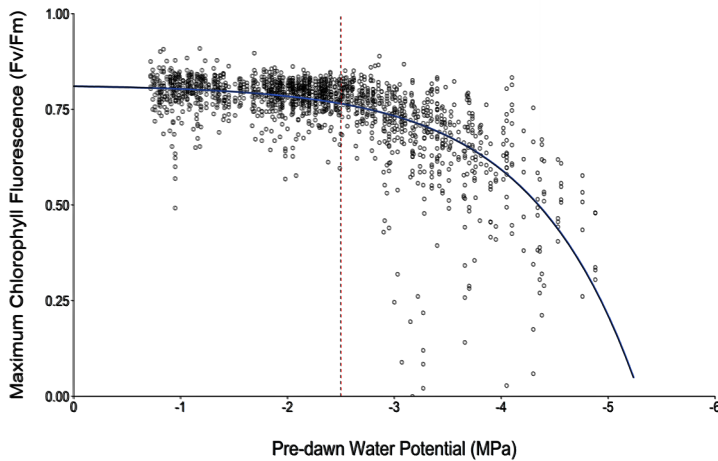


Figure 1: We compiled all of the data points from throughout the experiment and graphed the ratios of chlorophyll fluorescence (F_v/F_m) as a response to the tree's respective water potential (MPa) measurement. We fit the data with an exponential equation (blue line) to indicate the relationship between water potential and F_v/F_m . A dashed line at -2.5 MPa was added to represent the pre-dawn water potential at which drought stress induced stomatal closure.

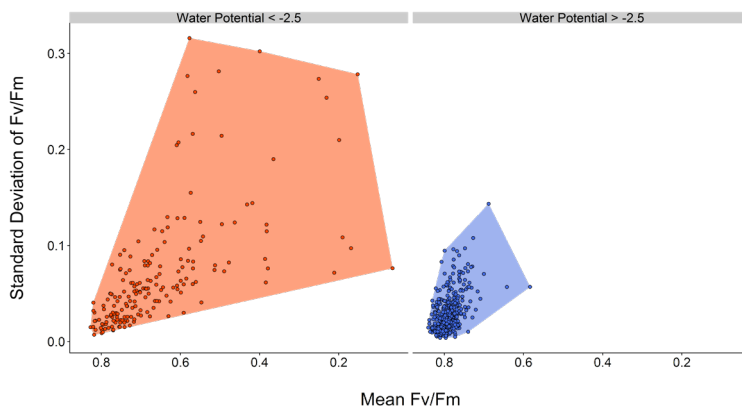


Figure 2: Variation of chlorophyll fluorescence within a tree after undergoing drought stress. Panel A demonstrates the variation of F_v/F_m within a tree in the trees that are undergoing moderate to severe drought-induced water stress. Panel B shows the variation of F_v/F_m within trees that are undergoing mild to no drought-induced water stress.

than definite quantifications, as the time spent dry would differ for each individual tree. We rewatered the 4 treatment groups to field capacity once they reached their treatment's target water potential. We measured

chlorophyll fluorescence and the water potential of each tree once a week during the drought and recovery phases of the experiment using an OS 30P+ handheld chlorophyll fluorometer (Opti-Sciences Inc., Hudson, New Hampshire, USA). Both measurement types were taken before dawn, when photosynthetic capacity is at its maximum, to analyze the speed of recovery in each treatment during the relaxed physiological state of *P. edulis*. We directly measured the percent loss of hydraulic conductivity (PLC) at the rewatering point, which is a measure of hydraulic failure (Hietz *et al.* 2008, Klein *et al.* 2016). By analyzing the rate of recovery, we attempted to quantify what level of drought stress is lethal to *P. edulis* (no recovery), as well as the carbon-cycle cost of lost photosynthetic capacity at varying levels of drought stress.

Progress to Date:

In our experiment, we have so far measured the ratio of chlorophyll fluorescence (F_v/F_m) of 5 branches per tree as well as the water potential (-MPa) of each tree and analyzed the relationship between the water potential and chlorophyll fluorescence ratio using Program R. Currently, we only have data from 10 weeks of observations, and the drought is ongoing—we expect trees to reach lethal levels of stress in the coming months. With this data, we fit an exponential model to show the relationship between water potential and chlorophyll fluorescence measurements (Figure 1). We also modeled the variation of chlorophyll fluorescence within a tree using standard deviation calculations between the 5 measurements we took for each tree as water stress prolonged (Figure 2).

Discussion:

Our observations indicate chlorophyll fluorescence can be used to predict drought-induced water stress in *P. edulis*. Our results also indicated that variation in chlorophyll fluorescence increases within a tree the longer drought stress prolongs. With further

observations, we predict F_v/F_m will decrease exponentially toward zero as drought continues and will be a useful proxy of hydraulic failure, and therefore could be useful for determining if a tree will survive after drought stress is relieved. Continued research may allow us to quantify a lethal limit of drought stress as well as predict which trees will undergo the phenomenon of dieback and then recover rather than face mortality. Limitations of this project include the factor that we conducted this experiment in a greenhouse rather than in a natural forest. Since trees could die from factors other than drought in nature (e.g., insect attack, pathogenic fungi), this project may not fully explain the actual extent of observed tree-mortality from water stress. Additionally, in this experiment, we excluded the tree's opportunity to get water from other sources.

While our study was focused on the photosynthetic capacity of dark-adapted samples, solar-induced fluorescence (SIF) could be used to predict chlorophyll fluorescence remotely. Future satellite observations of SIF could provide means of linking our research to measurements at the ecosystem scale. Continued research on drought-induced tree mortality will improve our ability to more accurately predict when and where trees will die, furthering our understanding of climate change impacts on forests as well as feedbacks between terrestrial vegetation and climate.

Literature Cited

- Adams, H., M. Zeppel, W. Anderegg, H. Hartmann, S. Landhäusser, D. Tissue, T. Huxman, P. Hudson, T. Franz, and C. Allen. 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology and Evolution* 1:1285-1291.
- Allen, C., A. Macalady, H. Chenchounic, D. Bachelet, N. McDowell, M. Venetier, T. Kitzberger, A. Rigling, D. Breshears, E. Hodd, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J. Lim, G. Allard, S. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259:660-684.
- Anderegg, W., J. Kane, and L. Anderegg. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* 3:30-36.
- Barigah, T., O. Charrier, M. Douris, M. Bonhomme, S. Herbette, T. Améglio, R. Fichot, F. Brignolas, and H. Cochard. 2013. Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. *Annals of Botany* 112:1431-1437.
- Clifford, M., P. Royer, N. Cobb, D. Breshears, and P. Ford. 2013. Precipitation thresholds and drought-induced tree die-off: insights from patterns of *Pinus edulis* mortality along an environmental stress gradient. *New Phytologist* 200:413-421.
- Duan, H., A. O'Grady, R. Duursma, B. Choat, G. Huang, R. Smith, Y. Jiang, and D. Tissue. 2015. Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated $[CO_2]$ and temperature. *Tree Physiology* 35:756-770.
- Gleason, S., D. Butler, K. Ziemińska, P. Waryszak, and M. Westoby. 2012. Stem xylem conductivity is key to plant water balance across Australian angiosperm species. *Functional Ecology* 26:343-352.
- Hartmann, H., H. Adams, W. Hammond, G. Hoch, S. Landhäusser, E. Wiley, and S. Zährle. 2018. Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. *Environmental and Experimental Botany* 152:7-18.
- Hammond, W., K. Yu, L. Wilson, R. Will, W. Anderegg, and H. Adams. Dead or dying? Quantifying the point of no return from hydraulic failure in drought-induced tree mortality. *New Phytologist* In press. Accepted May, 2019.
- Hietz, P., S. Rosner, J. Sorz, and S. Mayr. 2008. Comparison of methods to quantify loss of hydraulic conductivity in Norway spruce. *Annals of Forest Science* 65:502.
- Klein, T., S. Cohen, I. Paudel, Y. Preisler, E. Rotenburg, and D. Yakir. 2016. Diurnal dynamics of water transport, storage and hydraulic conductivity in pine trees under seasonal drought. *iForest-Biogeosciences and Forestry* 9:e1-e10.
- Kremer, A., O. Ronce, J. Robledo-Arnuncio, F. Guillaume, G. Bohrer, R. Nathan, J. Bridle, R. Gomulkiewicz, E. Klein, K. Ritland, A. Kuparinen, S. Gerber, and S. Schueler. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters* 15:378-392.
- Macalady, A., and H. Bugmann. 2014. Growth-mortality relationships in piñon pine (*Pinus edulis*) during severe droughts of the past century: shifting processes in space and time. *PloS One* 9:e92770.
- Maxwell, K., and G. Johnson. 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* 51:659-668.
- McDowell, N., W. Pockman, C. Allen, D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D. Williams, and E. Yezpez. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178:719-739.
- Murchie, E., and T. Lawson. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany* 64:3983-3998.
- Pinheiro, C., and M. Chaves. 2011. Photosynthesis and drought: can we make metabolic connections from available data? *Journal of Experimental Botany* 62:869-882.
- Ryan, M. 2011. Tree responses to drought. *Tree Physiology* 31:237-239.
- Urli, M., A. Porté, H. Cochard, Y. Guengant, R. Burlett, and S. Delzon. 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiology* 33:1-12.
- Xu, Z., G. Zhou, and H. Shimizu. 2010. Plant responses to drought and rewatering. *Plant Signaling and Behavior* 5:649-654.
- Zweifel, R., and F. Zeugin. 2008. Ultrasonic acoustic emissions in drought-stressed trees – more than signals from cavitation? *New Phytologist* 179:1070-1079.