



## **Unveiling stomata 24/7: can we use carbonyl sulfide (COS) and oxygen isotopes ( $\delta^{18}\text{O}$ ) to constrain estimates of nocturnal transpiration across different evolutionary plant forms?**

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Numerous studies have reported a continued flux of water through plants at night, suggesting that stomata are not fully closed. Growing evidence indicates that this nocturnal flux of transpiration might constitute an important fraction of total ecosystem water use in certain environments. However, because evaporative demand is usually low at night, nocturnal transpiration fluxes are generally an order of magnitude lower than rates measured during the day and perilously close to the measurement error of traditional gas-exchange porometers. Thus estimating rates of stomatal conductance in the dark ( $g_{\text{night}}$ ) precisely poses a significant methodological challenge. As a result, we lack accurate field estimates of  $g_{\text{night}}$  and how it responds to different atmospheric drivers, indicating the need for a different measurement approach. In this presentation we propose a novel method to obtain detectable and robust estimates of  $g_{\text{night}}$ . We will demonstrate using mechanistic theory how independent tracers including the oxygen isotope composition of  $\text{CO}_2$  ( $\delta^{18}\text{O}$ ) and carbonyl sulfide (COS) can be combined to obtain robust estimates of  $g_{\text{night}}$ . This is because COS and  $\text{CO}^{18}\text{O}$  exchange within leaves are controlled by the light insensitive enzyme carbonic anhydrase. Thus, if plant stomata are open in the dark we will continue to observe COS and  $\text{CO}^{18}\text{O}$  exchange. Using our theoretical model we will demonstrate that the exchange of these tracers can now be measured using advances in laser spectrometry techniques at a precision high enough to determine robust estimates of  $g_{\text{night}}$ . We will also present our novel experimental approach designed to measure simultaneously the exchange of  $\text{CO}^{18}\text{O}$  and COS alongside the conventional technique that relies on measuring the total water flux from leaves in the dark. Using our theoretical approach we will additionally explore the feasibility of our proposed experimental design to detect variations in  $g_{\text{night}}$  during drought stress and across a variety of plant types that have evolved diverse strategies to control water loss from leaf tissues.