Spontaneous generation of exogenous hydrogen peroxide by plants

Saman Samadi
University of Victoria

Shabnam Sharifyazd
University of Victoria

Ludwig Cabling
University of Victoria

Isaac Dekker
University of Victoria

Barbara Hawkins
University of Victoria

Kristian Dubrawski (kdubrawski@uvic.ca)
University of Victoria

Article

Keywords:

Posted Date: March 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2635805/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: There is NO Competing Interest.
Spontaneous generation of exogenous hydrogen peroxide by plants
Saman Samadi¹, Shabnam Sharifyazd¹, Ludwig Paul B. Cabling¹, Isaac Dekker², Barbara J. Hawkins³, Kristian L. Dubrawski¹,²*¹Department of Civil Engineering, University of Victoria, Victoria, BC V8W 2Y2, Canada.
²Department of Geography, University of Victoria, Victoria, BC V8W 2Y2, Canada.
³Department of Biology, University of Victoria, Victoria, BC V8W 2Y2, Canada.
*Corresponding author. Email: kdubrawski@uvic.ca

Plants play important roles in maintaining air quality and biogeochemical cycles, although many mechanisms remain poorly understood. Recently, spontaneous production of hydrogen peroxide (H₂O₂) has been reported from the condensation of water vapor as microdroplets. Here, we report detection of H₂O₂ in proximity to plants undergoing photosynthesis in a closed environment, confirmed using commercial peroxide test strips and spectrophotometric titration. Our results have potential major implications for the role of plant-mediated atmospheric cleansing, climate change, and urban and indoor air quality.
Plants play a key role in air quality and biogeochemical cycles \(^1,^2\), although any role of exogenous hydrogen peroxide (H\(_2\)O\(_2\)) produced by plants has yet to be considered. H\(_2\)O\(_2\) contributes to atmospheric cleansing as a reservoir for hydroxyl radicals \(^3\), and as an oxidant of organic and biological contaminants. Endogenous H\(_2\)O\(_2\) production by plants has been well documented for signalling in cellular function, but exogenous H\(_2\)O\(_2\) production by plants has not yet been demonstrated. Recently, it has been shown that H\(_2\)O\(_2\) is spontaneously produced from water microdroplets (1-20 μm) without the addition of catalysts or additives, although the mechanistic explanation remains to be explained fully \(^4,^5,^6\). In vegetated ecosystems, the release of water as a byproduct of photosynthesis typically requires a vapour pressure deficit (VPD), where transpired water vapour typically mixes in the atmospheric column, contributing to local and global water cycles and climate dynamics. Whether plant transpiration and the recently discovered microdroplet condensation mechanism can contribute to local or atmospheric H\(_2\)O\(_2\) concentrations has yet to be explored. In this study, we report the first findings of H\(_2\)O\(_2\) production via transpired water vapour, and show that, surprisingly, all transpiring plants are likely contributing to local, and thus global, atmospheric H\(_2\)O\(_2\) concentrations.

Implications of H\(_2\)O\(_2\) generated from plants include local effects, such as impacting indoor air quality, and global effects, such as potential for climate change mitigation. For indoor air quality, H\(_2\)O\(_2\) is a strong oxidizing agent that denatures cellular components \(^7\), suggesting that indoor plants could contribute to deactivation of pathogenic bacteria and viruses (such as airborne COVID-19 in homes and workplaces). In global biogeochemical cycles, H\(_2\)O\(_2\) produced in vegetated areas may directly (via photochemical reactions), or indirectly (as a reservoir for HO\(_2\) radicals) contribute to the production of hydroxyl radicals (-OH\(^\bullet\)): the central oxidant of the lower atmosphere which controls the persistence of methane, carbon monoxide, nitrous oxide, and some ozone-depleting gases \(^8,^9\). While we demonstrate the first evidence for plant mediated exogenous H\(_2\)O\(_2\) production, the implications and applications of these findings require further study.

Here, we used several plant species in a closed container (Fig. 1) and examined the impacts of light intensity, humidity, distance from the leaf surface, and species on the resultant concentration of produced H\(_2\)O\(_2\) using commercial peroxide test strips and potassium titanium oxalate (PTO, K\(_2\)TiO(C\(_2\)O\(_4\))\(_2\)-H\(_2\)O) titration (See Methods and Materials in Supplementary).
Fig. 1. | Experimental set-up scheme. A) Schematic of experimental set-up for detecting plant-mediated H$_2$O$_2$. Commercial strips were located at 0, 10, and 40 cm from leaf surface. Condensate was collected for spectrophotometric titration. B) Photograph of peroxide detection strip after 2 hr, strip at 10 cm from leaf surface.

We observed H$_2$O$_2$ produced from transpiring *Saintpaulia ionantha* at concentrations of up to 5 ppm (~150 μM) after 2 hours of transpiration (Fig. 2A). As no peroxide was observed in controls with no plant present, nor with a plant present without a light source (Fig. 2B), we believe the observed H$_2$O$_2$ is generated via transpired water vapour that condenses in proximity to the leaf surfaces or on nearby surfaces.
Water vapour is a byproduct of photosynthesis in plants; gas exchange via plant stomata takes in CO₂ and releases H₂O. At some distance from the leaf, depending on temperature, relative humidity (RH), and presence of seed nuclei, this water vapour will condense. Lee et al. (2019) found that if the diameter of H₂O microdroplets is less than approximately 20 μm, high local electric field effects will cause autoionization of H₂O, releasing a solvated electron and forming OH⁻ radicals, two of which recombine to form H₂O₂. Other researchers propose a role of ultrasonic humidification in the formation of H₂O₂. Regardless of mechanism, previous reports of H₂O₂ concentration and persistence are variable. Previous research has detected H₂O₂ concentrations up to 30 μM (~1 part per million) by H₂O nebulization. Conversely, Lee et al. (2020) generated H₂O microdroplets by substrate cooling, reporting H₂O₂ concentrations up to 4 ppm, although with persistence of only 5 minutes. In our work, we report H₂O₂ concentrations produced via plant transpiration of up to 6 ppm, with persistence of several hours.

In our work, RH played a role in H₂O₂ concentration, possibly via controlling transpiration rate. Starting RH was ~37%, increasing to ~90% RH after 2 hours, and remaining at approximately that value for the duration of the experiments (Fig. S4). As bulk RH rose, the vapour pressure deficit between bulk air and the boundary layer next to the stomata surface decreased, with a corresponding decrease in transpiration as per the Penman-Monteith equation. We observed a sharp decrease in H₂O₂ concentration after 2 hrs, consistent with the increase and plateau of RH at 2 hr; strongly indicating that the observed H₂O₂ is a result of transpiration. When the RH increase was controlled by removing plant leaves, no H₂O₂ was observed (Fig. 2B).

We observed a sharp decrease in H₂O₂ concentration after 2 hr (Fig 2A), suggesting that the generated H₂O₂ is reduced back to H₂O via breaking of the weak peroxide bond, or via photochemical reactions. Previous findings have shown a similar decrease in H₂O₂ concentration after microdroplet condensation on Peltier-cooled substrates, with a persistence of < 5 min. Alternatively, decreasing H₂O₂ could be a result of redox reactions with plant mediated VOCs (not...
measured). Uncontrolled constituents in our experiment may also explain why H$_2$O$_2$ was persistent after several hours, far longer than the persistence observed in other experiments.

When comparing light intensity (Fig 3A), we observe an intermediate intensity (385 µmol m$^{-2}$ s$^{-2}$) resulted in higher measured H$_2$O$_2$ concentration (2.2 ± 0.2 ppm) than either low light intensity (230 µmol m$^{-2}$ s$^{-2}$, 1.6 ± 0.0 ppm), or high light intensity (600 µmol m$^{-2}$ s$^{-2}$, 0.8 ± 0.0 ppm). *Saintpaulia ionantha* may light saturate at an intermediate intensity, or the lower H$_2$O$_2$ concentration observed at a higher light intensity is a result of faster RH saturation, leading to a more rapid drop in H$_2$O$_2$ concentrations as described above.

![Fig. 3](image.png)

**Fig. 3 | The effect of light intensity and distance on H$_2$O$_2$ concentration.** (A) Relationship between light intensity and generation of H$_2$O$_2$ by *Saintpaulia ionantha* after 8 h (n = 2). (B) Effects of distance from leaves of *Saintpaulia ionantha* on hydrogen peroxide condensation after 8h. Strips were positioned: (i) attached to the leaves, (ii) 10 cm from the leaves, and (iii) 40 cm from the leaves. (iv) shows the control with no leaf.

Plants endogenously produce intracellular H$_2$O$_2$ for signalling and function and with metabolic stress. While we cannot rule out leakage of endogenous H$_2$O$_2$, leaf tissue concentrations are on the order of 15-25 µM, we report concentrations up to an order of magnitude higher. Leakage and accumulation of endogenous H$_2$O$_2$ to this extent is unlikely. Photochemical mechanisms are also known in H$_2$O$_2$ production, e.g., hydroperoxyl radical or alkene / ozone photochemistry. However, we observe a distance-dependent concentration of H$_2$O$_2$ (discussed below) that would be inconsistent with photochemical production, provided that the equilibrium concentration of VOCs is homogenous in our closed system.

We report consistent H$_2$O$_2$ production with different plants of the same species (Fig. S2) and plants of different species (Fig. S3), although different plants and different species produced different quantities of H$_2$O$_2$. While H$_2$O$_2$ production was consistent, variation among plants and species was likely due to differences in photosynthesis and transpiration rates from differing leaf surface area, stomata size and morphology, leading to different H$_2$O$_2$ formation and persistence kinetics as previously described. Spatial heterogeneity in H$_2$O$_2$ formation was examined by placing peroxide test strips at various distances from the leaf surface (Fig. 3B), showing significantly higher H$_2$O$_2$ concentrations close to the leaf surface (0-10 cm) than further away (40 cm). Water vapour
transpired from leaf surfaces will condense closer to the leaf surface due to the higher RH gradient, assuming the leaf continues to transpire water at relatively consistent rates, and turbulent air mixing is negligible in a closed system. As the water vapour begins to condense and coalesce into microdroplets, H$_2$O$_2$ concentrations will depend on the size of the resultant microdroplet, with little H$_2$O$_2$ formation at sizes >10 um. In our experiment, at distances far from leaf surfaces (40 cm), spatial dilution of water vapour in air likely explained our lower observed H$_2$O$_2$ concentrations. Alternatively, H$_2$O$_2$ may have already been formed and reduced back to H$_2$O at greater distances from the leaf.

The higher H$_2$O$_2$ concentrations in proximity to leaf surfaces have important implications in both air quality and biogeochemical cycles. For air quality, exogenous H$_2$O$_2$ production by plants may have implications in indoor air quality (e.g., hospitals); high-density regions (such as megacities) and rural regions impacted by forest fires. Our work further implicates plants as a viable nature-based solution for air quality improvement and to defend against pathogenic outbreaks of infectious diseases. The findings of this study may also complement current research on plant-sourced biogenic volatile organic compounds, alkaloids, and polyphenols which have been known. In biogeochemical cycles, H$_2$O$_2$ generation by plants may impact boundary layer atmospheric H$_2$O$_2$ concentrations. The established major mechanism for the formation of hydrogen peroxide in the troposphere is the bimolecular self-reaction of the hydroperoxyl radical (HO$_2$) via photochemical chain reactions in the troposphere. However, tropospheric H$_2$O$_2$ concentrations have been found to decrease with increasing latitude, increase with increasing vegetation density (i.e., inside a forest vs. the forest perimeter) and vary by time of day and seasonality. These imply greater H$_2$O$_2$ concentrations near vegetation with high net primary productivity (NPP), suggesting, at least in theory, a potential role of exogenous H$_2$O$_2$ production in atmospheric H$_2$O$_2$ concentrations at the landscape scale. However, the relative magnitude of our results on landscape and global processes remains to be explored. Further studies to elucidate the contribution of exogenous H$_2$O$_2$ production by plants require considering the photochemical dynamics, including the role of seasonal and diurnal variation on the formation of hydroxyl radicals, the reaction of alkenes and ozone (O$_3$) in the presence of water vapour, and the self-reaction of the hydroperoxyl radical. If the contribution of exogenous H$_2$O$_2$ production by plants is found to be significant at a global scale, transpiration may be an overlooked negative feedback process affecting methane concentration in the atmosphere, and could potentially impact climate change models and forecasts.

Materials and Methods

Our initial aim was to determine whether plants produce exogenous H$_2$O$_2$ via transpiration. To investigate this, we placed a plant, initially Saintpaulia ionantha, in a closed container (Fig. 1A). The soil surface of the pot was covered with foil and the plant was placed inside a water and airtight polyethylene chamber. In experiments where peroxide test strips were used, strips were affixed to either the plant leaf surface, or to investigate spatial heterogeneity, strips were positioned at certain distances (0, 10, 40 cm) from the plant leaf surface. The chamber was then exposed to light at different intensities (300-600 µmol m$^{-2}$ s$^{-2}$) by a commercial LED plant growth bulb (SFR6 LED TUBE- 24W). The light intensity was measured by an LCA-4 (ADC Ltd., Hoddesdon, Herts, UK) - a portable leaf chamber gas analyser. Relative humidity and temperature inside the chamber were monitored (Omega OM-62). The plant was left in the lit
chamber for 1.5-8 hours, after which the chamber was opened, and the strip or condensate was collected for H₂O₂ analysis. Controls included: a chamber with no plant present; a chamber with a plant present but shielded from light; a chamber with a plant and light present, but with the leaves of the plant removed. All experiments were performed in duplicate.

H₂O₂ was quantified in two ways. Firstly, condensate was collected in the ranges of 100 to 300 µL on the chamber surface for analysis by chemical titration⁴. A 0.1 M potassium titanium oxalate (PTO, K₂TiO(C₂O₄)₂·H₂O; ≥ 99.0%; Sigma-Aldrich) solution was prepared before each batch of experiments. To develop a calibration curve, 300 µl of a hydrogen peroxide standard solution (H₂O₂, 30%; Fisher Scientific Co) with concentration between 0 to 20 ppm was added to 300 µl of PTO solution. Absorbance (400 nm) was measured by a SpectraMax M5 spectrophotometer (Molecular Devices). For experiments, 300 µl of collected condensate was added to 300 µl PTO solution and [H₂O₂] was determined by the calibration curve. The average time from collection and measurement was approximately two minutes. In experiments where insufficient condensate was generated, or where we preferred not to open the chamber, peroxide test strips were used (0.5-25 ppm H₂O₂, Quantofix; Macherey-Nagel).
References

Acknowledgments: We are grateful for Rebecca Hof at University of Victoria CAMTEC Biocore for experimental assistance. We acknowledge Richard Zare (Stanford University) and Jae Kyoo Lee (Seoul National University) for sharing their experimental methods.

Funding: This work was supported by the Canada Research Chairs program for KLD.

Author contributions:

Conceptualization: SSA, SSH, KLD
Methodology: SSA, SSH
Experimentation: SSA, SSH
Data analysis: SSA, SSH, LPBC, ID
Visualization: SFB, MJM, JLS, EH
Funding acquisition: KLD
Project administration: KLD
Supervision: KLD
Writing – original draft: SSA, SSH, KLD, LPBC, ID
Writing – review & editing: SSA, SSH, KLD, LPBC, ID, BH

Competing interests: The authors declare no competing interests.

Data and materials availability: All data are available in the main text or the supplementary materials, raw analytical data is available by request.

Supplementary Materials

Figs. S1 to S4
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PlantH2O2NatureCommunicationsSupplementaryMaterials.pdf