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Plants play important roles in maintaining air quality and biogeochemical cycles, although many mechanisms remain poorly understood. Recently, spontaneous production of hydrogen peroxide (H_2O_2) has been reported from the condensation of water vapor as microdroplets. Here, we report detection of H_2O_2 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_2O_2) produced by plants has yet to be considered. H_2O_2 contributes to atmospheric cleansing as a reservoir for hydroxyl radicals [3], and as an oxidant of organic and biological contaminants. Endogenous H_2O_2 production by plants has been well documented for signalling in cellular function, but exogenous H_2O_2 production by plants has not yet been demonstrated. Recently, it has been shown that H_2O_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_2O_2 concentrations has yet to be explored. In this study, we report the first findings of H_2O_2 production via transpired water vapour, and show that, surprisingly, all transpiring plants are likely contributing to local, and thus global, atmospheric H_2O_2 concentrations.

Implications of H_2O_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_2O_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_2O_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*): 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_2O_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_2O_2 using commercial peroxide test strips and potassium titanium oxalate (PTO, $K_2TiO(C_2O_4)_2 \cdot H_2O$) titration (See Methods and Materials in Supplementary).
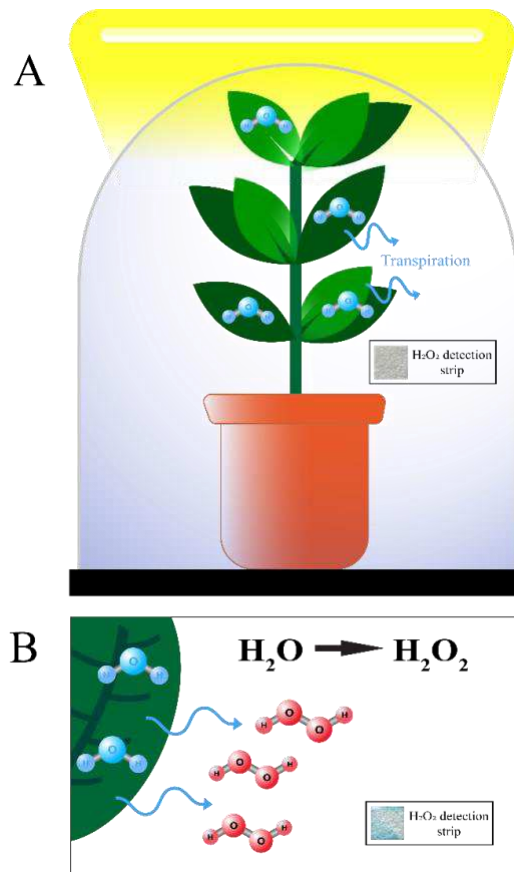
Fig. 1. | **Experimental set-up scheme.** A) Schematic of experimental set-up for detecting plant-mediated H_2O_2. Commercial strips were located at 0, 10, and 40 cm from leaf surface. Condensate was collected for spectrophotometric titration. B) Photograph of peroxide detection strip after 2 hr, strip at 10 cm from leaf surface.

We observed H_2O_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_2O_2 is generated via transpired water vapour that condenses in proximity to the leaf surfaces or on nearby surfaces.
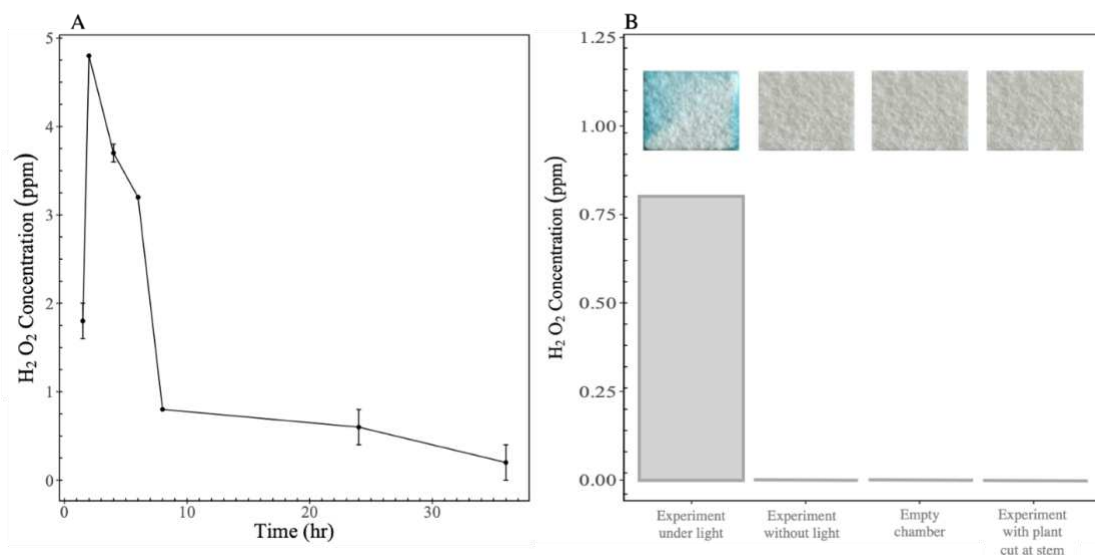
Fig. 2 | The effect of time and different conditions on hydrogen peroxide concentration. (**A**) Concentration of hydrogen peroxide produced by *Saintpaulia ionantha* under the light at 600 µmol m^{-2} s^{-2}. (n=2). (**B**) Concentration of H_2O_2 produced at different condition (T1: exposed to light at 600 µmol m^{-2} s^{-2}, T2: no light (dark), T3: empty chamber - control, T4: experiment with plant cut at stem).

Water vapour is a byproduct of photosynthesis in plants; gas exchange via plant stomata takes in CO_2 and releases H_2O. 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 H_2O microdroplets is less than approximately 20 µm, high local electric field effects will cause autoionization of H_2O, releasing a solvated electron and forming OH^- radicals, two of which recombine to form H_2O_2. Other researchers propose a role of ultrasonic humidification in the formation of H_2O_2 [5]. Regardless of mechanism, previous reports of H_2O_2 concentration and persistence are variable. Previous research has detected H_2O_2 concentrations up to 30 µM (~1 part per million) by H_2O nebulization [4]. Conversely, Lee et al. (2020) generated H_2O microdroplets by substrate cooling, reporting H_2O_2 concentrations up to 4 ppm, although with persistence of only 5 minutes. In our work, we report H_2O_2 concentrations produced via plant transpiration of up to 6 ppm, with persistence of several hours.

In our work, RH played a role in H_2O_2 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 [10]. We observed a sharp decrease in H_2O_2 concentration after 2 hrs, consistent with the increase and plateau of RH at 2 hr; strongly indicating that the observed H_2O_2 is a result of transpiration. When the RH increase was controlled by removing plant leaves, no H_2O_2 was observed (Fig. 2B).

We observed a sharp decrease in H_2O_2 concentration after 2 hr (Fig 2A), suggesting that the generated H_2O_2 is reduced back to H_2O via breaking of the weak peroxide bond, or via photochemical reactions [8]. Previous findings have shown a similar decrease in H_2O_2 concentration after microdroplet condensation on Peltier-cooled substrates, with a persistence of < 5 min [6]. Alternatively, decreasing H_2O_2 could be a result of redox reactions with plant mediated VOCs (not

measured). Uncontrolled constituents in our experiment may also explain why H_2O_2 was persistent after several hours, far longer than the persistence observed in other experiments [6].

When comparing light intensity (Fig 3A), we observe an intermediate intensity (385 µmol m^{-2} s^{-2}) resulted in higher measured H_2O_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 [11], or the lower H_2O_2 concentration observed at a higher light intensity is a result of faster RH saturation, leading to a more rapid drop in H_2O_2 concentrations as described above.
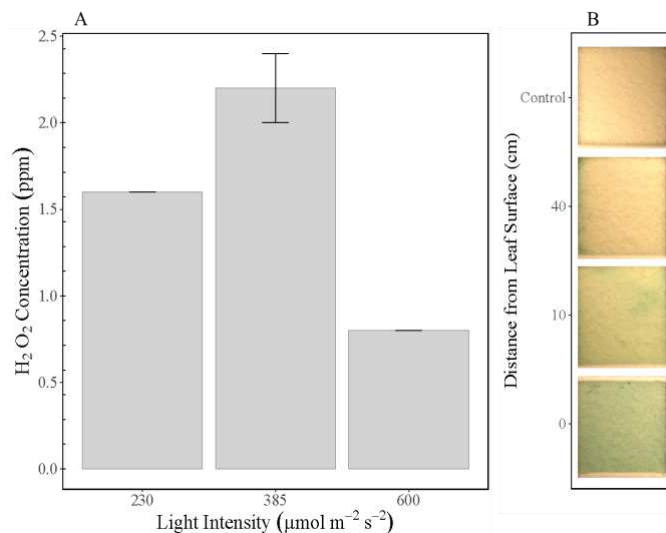

Fig. 3 | The effect of light intensity and distance on H_2O_2 concentration. (**A**) Relationship between light intensity and generation of H_2O_2 by *Saintpaulia ionantha* after 8 h (n = 2). (**B**) Effects of distance from leaves of *Saintpaulia ionantha* on hydrogen peroxide condensation after 8h. 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_2O_2 for signalling and function and with metabolic stress. While we cannot rule out leakage of endogenous H_2O_2, leaf tissue concentrations are on the order of 15-25 µM [12], we report concentrations up to an order of magnitude higher. Leakage and accumulation of endogenous H_2O_2 to this extent is unlikely. Photochemical mechanisms are also known in H_2O_2 production, e.g., hydroperoxyl radical or alkene / ozone photochemistry [13]. However, we observe a distance-dependent concentration of H_2O_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_2O_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_2O_2. While H_2O_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 [14], leading to different H_2O_2 formation and persistence kinetics as previously described. Spatial heterogeneity in H_2O_2 formation was examined by placing peroxide test strips at various distances from the leaf surface (Fig. 3B), showing significantly higher H_2O_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_2O_2 concentrations will depend on the size of the resultant microdroplet, with little H_2O_2 formation at sizes >10 um [4]. In our experiment, at distances far from leaf surfaces (40 cm), spatial dilution of water vapour in air likely explained our lower observed H_2O_2 concentrations. Alternatively, H_2O_2 may have already been formed and reduced back to H_2O at greater distances from the leaf.

The higher H_2O_2 concentrations in proximity to leaf surfaces have important implications in both air quality and biogeochemical cycles. For air quality, exogenous H_2O_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 [15-17]. In biogeochemical cycles, H_2O_2 generation by plants may impact boundary layer atmospheric H_2O_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 [18]. However, tropospheric H_2O_2 concentrations have been found to decrease with increasing latitude [19], increase with increasing vegetation density (i.e., inside a forest vs. the forest perimeter) [20] and vary by time of day and seasonality [8]. These imply greater H_2O_2 concentrations near vegetation with high net primary productivity (NPP), suggesting, at least in theory, a potential role of exogenous H_2O_2 production in atmospheric H_2O_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_2O_2 production by plants require considering the photochemical dynamics, including the role of seasonal and diurnal variation on the formation of hydroxyl radicals [21], the reaction of alkenes and ozone (O_3) in the presence of water vapour [13], and the self-reaction of the hydroperoxyl radical [18]. If the contribution of exogenous H_2O_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_2O_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 (5FR6 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 H_2O_2 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.

H_2O_2 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 titration [4]. A 0.1 M potassium titanium oxalate (PTO, $K_2TiO(C_2O_4)_2 \cdot H_2O$; ≥ 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 (H_2O_2, 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 $[H_2O_2]$ 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 H_2O_2, Quantofix; Macherey-Nagel).

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Author contributions:

Conceptualization: SSA, SSH, KLD

Methodology: SSA, SSH

Experimentation: SSA, SSH

Data analysis: SSA, SSH, LPBC, ID

Visualization: SFB, MJM, JLS, EH

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Writing – original draft: SSA, SSH, KLD, LPBC, ID

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Supplementary Materials

Figs. S1 to S4

Supplementary Files

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