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## Research Article

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The evolution of life history strategies in plants and its relationship to the evolution of protective cellular mechanisms against reactive oxygen species

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#### Author Contributions

Abraam Zakhary conducted the phylogenetic analysis and part of the germination rate experiment. Aashika Nagarajan conducted the electrolyte leakage experiment. Charlotte Ngo conducted the chlorophyll degradation experiment. Marwa Saidajan conducted part of the germination rate experiment. Supreet Babbar conducted part of the germination rate experiment. Jason C. L. Brown conceived the objectives and design of the study and wrote the manuscript. All authors contributed to editing the manuscript.

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## Abstract

*Purpose.* The Oxidative Stress Theory of Aging (OSTA) states that accumulation of oxidative damage is a major contributor to aging; however, until now, no studies have examined whether perennial plants exhibit cellular mechanisms to better protect themselves against oxidative damage than annual plants, nor how these mechanisms may have evolved.

*Methods.* We undertook three approaches to evaluate the capacity for annual and perennial plants to resist oxidative damage. The first approach involved using an electrolyte leakage assay to assess the rate of cellular damage in leaves exposed to exogenous  $H_2O_2$ . The second approach involved determining the concentration of exogenous  $H_2O_2$  required to maximize germination rates, which provides insight about the antioxidant levels in seeds. The third approach involved assessing the susceptibility of chlorophyll a and chlorophyll b to exogenous  $H_2O_2$  and determining chlorophyll a/b ratios. We also conducted an ancestral state reconstruction of life history strategies in order to interpret our results in an evolutionary context.

*Results.* Leaves from deciduous and evergreen perennials showed a lower rate of cellular damage than leaves from annuals when exposed to exogenous  $H_2O_2$ . Seeds from deciduous perennials—but not biennials or evergreen perennials—required a higher  $H_2O_2$  concentration to maximize germination rate compared to seeds from annuals, suggesting that seeds from deciduous perennials have higher antioxidant levels. Although chlorophyll b was found to be more susceptible to damage from exogenous  $H_2O_2$ , chlorophyll a/b ratios did not differ among life history strategies. Ancestral state reconstruction revealed that the ancestral plant was most likely an evergreen perennial.

*Conclusion.* Our results showcase that resistance to oxidative stress is necessary for perennial plants to survive over multiple years. The mechanisms responsible for the increased tolerance of perennial species to oxidative stress has not been fully elucidated by this study, but it does not involve changes to chlorophyll a/b ratios, as such changes could disrupt photosynthesis. The developmental onset of these protective mechanisms was delayed in evergreen perennials compared to deciduous perennials, perhaps because the ancestral evergreens were primarily focused on rapid colonization of the terrestrial environment, which requires faster germination rates induced by higher  $H_2O_2$  levels.

Keywords: longevity, germination, electrolyte leakage, reactive oxygen species, life history, maximum lifespan

## Declarations

### Funding

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### Conflicts of Interest/Competing Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

### Availability of Data and Material

Not applicable.

### Code Availability

The R coding used to construct the phylogenetic tree and perform the ancestral state reconstruction has been provided in an appendix to this paper.

### Ethics Approval

Not applicable.

### Consent to Participate

Not applicable.

### Consent for Publication

Not applicable.

## Introduction

Charlesworth (2001) noted that “senescence of multicellular plants and animals is an almost universal phenomenon; it needs to be explained both in terms of cellular and physiological mechanisms, and of evolutionary forces.” While there has been an abundance of research on the ecological forces driving the evolution of annual, biennial, deciduous perennial, and evergreen perennial life histories in plants (e.g., Klinkhamer et al. 1997), and even some recent work on genetic factors regulating those developmental traits that differ among life histories in plants, such as flowering (Friedman 2020), there has been a dearth of research examining the underlying cellular and physiological mechanisms that permit these changes in life history strategies to occur. The present study—and our research program, in general—seeks to rectify this disparity.

According to the Oxidative Stress Theory of Aging (OSTA), the maximum lifespan of an organism is determined by the rate at which it accumulates oxidative damage to its biological molecules (Golden et al. 2002). Such damage is caused by interactions between reactive oxygen species (ROS; such as superoxide, singlet oxygen,  $\text{H}_2\text{O}_2$ , and hydroxyl radicals) and lipids, proteins, and nucleic acids (Bandyopadhyay et al. 1999). The mitochondrial electron transport system (ETS) is a major source of ROS in all aerobic organisms, but significant amounts of ROS are also produced by peroxisomes, endoplasmic reticulum, and cellular oxidases (Snezhkina et al. 2019). In plants, the photosynthetic ETS in chloroplasts is also a major source of ROS, with smaller amounts of ROS arising from the apoplast, plasma membrane, and cell walls (Das and Roychoudhury 2014).

Based on OSTA, there are four principal ways by which organisms could increase their maximum lifespan: i) reduce the rate at which they produce ROS, esp. from mitochondria and chloroplasts; ii) increase their activity and/or amount of antioxidants, which are compounds or enzymes capable of eliminating ROS after their formation; iii) construct their tissues from biological molecules that are more resistant to oxidative damage; and/or iv) repair their biological molecules after they suffer oxidative damage. While a few researchers have proclaimed the OSTA “dead” (e.g., Doonan et al. 2008; Pérez et al. 2009), we believe that such declarations are premature since they have been entirely based on just one facet of the OSTA: antioxidants. The OSTA can only be rejected when, for a given species, its lifespan cannot be explained by any facets of the OSTA; however, such a comprehensive investigation has never, to our knowledge, been undertaken. Indeed, there are several studies which provide evidence for many of the predictions of the OSTA in animals. For example, Robert et al. (2007) showed that liver mitochondria isolated from long-lived colubrid snakes (e.g., common king snake) produced  $\text{H}_2\text{O}_2$  at a slower rate than those isolated from short-lived confamilials, and Herrero and Barja (1998) showed that rates of  $\text{H}_2\text{O}_2$  production by heart mitochondria isolated from

parakeets and canaries, which have maximum lifespans of 21 and 24 years, respectively, were significantly lower than that from similar-sized mice, which have a maximum lifespan of 3.5 years. Moreover, methionine residues in proteins are known to be particularly susceptible to oxidative damage (Stadtman et al. 2003), and Pamplona et al. (2005) showed that proteins from parakeets and canaries had lower methionine content than those from mice. Similarly, it is well established that polyunsaturated fatty acids (PUFA) are more susceptible to oxidative attack than either monounsaturated or saturated fatty acids, and there is evidence in mammals that long-lived species frequently have lower levels of PUFA in their cell membranes than short-lived species (Pamplona et al. 1999). Furthermore, MacRae et al. (2015) showed that humans and naked mole rats, with maximum lifespans of 120 and 30 years, respectively, exhibited higher expression of DNA repair genes than mice, and Salway et al. (2011) showed a significant correlation between expression of heat shock proteins (HSPs) and maximum lifespan among 13 mammalian and avian species, reflecting that HSPs have been shown to refold denatured proteins resulting from oxidative damage (Perišić et al. 2007).

By contrast, to our knowledge, studies related to OSTA in plants are far less common and have been largely restricted to intraspecific comparisons. For example, Munné-Bosch and Alegre (2002) showed that 7-year-old individuals of *Cistus clusii* had higher malondialdehyde levels (a marker of lipid oxidation) in their leaves and chloroplasts, and lower chloroplast antioxidant levels, than 1- and 3-year old conspecifics. Similarly, Kurepa et al. (1998) showed that late-flowering *Arabidopsis* mutants, which had an extended lifespan because their vegetative phase was protracted, were more tolerant of paraquat, which stimulates superoxide production in organisms. In contrast to these studies, our laboratory has begun to explore whether interspecific differences in lifespan among plant species can be explained by OSTA. Most commonly, plants are classified as being annuals (lifespan < 1 year) or perennials (lifespan > 1 year). Annuals complete their entire life cycle—including vegetative growth and reproduction—within a single year, propagating among seasons via seed production only (Bazzaz and Morse 1991). Among perennials, there is considerable variation in lifespan, both within and among species. Within species, while a perennial plant may live for thousands of years, its leaves, flowers, and fruits may only live for a few months (Robbins 1957). Among species, biennials live for two years, usually accumulating resources via vegetative growth in their first season and reproducing in their second season (Hart 1977). Deciduous perennials have regions that persist for several years (e.g., roots) but have leaves that only persist for one year, falling each autumn in a synchronous fashion as low temperatures and short days preclude net photosynthetic gain of carbon (Estiarte and Peñuelas 2015). Evergreen perennials have leaves that persist for several years, being shed at a constant, low rate throughout the year so that the plant always

possesses some leaves (Williams-Linera 1997), though they may or may not be able to conduct photosynthesis in winter (Taneda and Tateno 2005). These differences in life history make studying the interspecific differences in plant aging more complex than similar studies in animals, which could explain the paucity of interspecific studies in plant aging to date.

One study, conducted by Brown et al. (2012), found that catalase, an antioxidant enzyme, was significantly higher in both the cotyledons and roots of perennial flax species compared to annual congeners. We are unaware of any other studies comparing aspects of ROS metabolism and/or oxidative damage between annual and perennial species; therefore, it remains uncertain whether the differences observed between annual and perennial flax in this previous study are broadly applicable within plants. On this basis, for the present study, we decided to investigate the effects of exogenous  $H_2O_2$  in a wide array of plant species, covering annuals, biennials, deciduous perennials, and evergreen perennials. Moreover, we decided to utilize three independent approaches in order to ensure that any observations made were robust, and we also decided to undertake an ancestral state reconstruction of the species used in our study so that we could gain insight into how ROS handling by plants may have evolved over time. Based on OSTA, we predicted that perennials would have a greater resistance to exogenous  $H_2O_2$  than annuals. Moreover, given that evergreen leaves have a minimum lifespan that is 3- to 4-fold higher than that of annual and deciduous perennial leaves (Tessier 2008), we also predicted that leaves from evergreen perennials would have greater resistance to exogenous  $H_2O_2$  than leaves from deciduous perennials.

Our first approach involved assessing the capacity of exogenous  $H_2O_2$  to cause cellular damage to leaf cells. For this approach, we utilized a protocol that measures the rate of electrolyte leakage in leaf samples subjected to abiotic stressors, which has been used in several previous studies to assess, for example, freezing tolerance (Nunes and Smith 2003) and disease resistance (Scheffer and Livingston 1980). This assay is based on the principle that abiotic stressors cause cellular damage primarily via disruption of cell membrane integrity, which causes the release intracellular contents, including ions, into the external medium; therefore, by measuring the rate at which the conductivity of this external medium increases, we can infer the rate at which cellular damage occurs in leaf cells in response to exogenous  $H_2O_2$ .

Our second approach took advantage of the known effects of exogenous  $H_2O_2$  on germination rate. Exogenous  $H_2O_2$  has been found to accelerate germination rate in many species (Barba-Epsín and Hernández 2012; Riffle and Springfield 1968), likely via oxidation of germination inhibitors (Ogawa and Iwabuchi 2001). Various antioxidants are expressed in germinating seeds, and their activities are known to increase shortly following imbibition

(Cakmak et al. 1993). These antioxidants would eliminate some proportion of exogenous  $H_2O_2$ , thereby preventing its stimulatory effects on germination rate. On this basis, we sought to determine the concentration of exogenous  $H_2O_2$  that would maximally stimulate germination rate as a proxy for seed(ling) antioxidant activity.

Our third approach was inspired by animal studies showing that biomolecules can differ in terms of their susceptibility to oxidative damage, allowing animals to alter their maximal lifespan via changes in body composition and/or diet. While PUFA composition of cellular membranes (Hulbert et al. 2014) and/or methionine composition of cellular proteins (Sun et al. 2009) are the canonical markers for biomolecular susceptibility to oxidative damage, we decided to focus on chlorophyll composition in the present study. While it is known that semi-synthetic versions of chlorophyll have antioxidant properties (Hsu et al. 2005), suggesting that chlorophyll can interact with ROS, it is largely unknown whether chlorophyll a and chlorophyll b differ in their susceptibility to oxidative damage and, therefore, whether alterations of chlorophyll a/b ratio can serve as a mechanism to promote longevity in perennial plants.

While it has been acknowledged that ROS likely play a significant role in shaping the evolution of life history strategies in animals (Dowling and Simmons 2009), there appears to be little understanding of how the physiological mechanisms related to ROS production, consumption, resistance, and repair may have evolved in plants, and whether they could similarly constrain the evolution of life history in this group. For this reason, we sought to evaluate the results of our study from an evolutionary perspective. Most studies examining the evolution of life history strategies in plants have focused on lower taxonomic levels, especially orders (e.g., Saxifragales; Soltis et al. 2013), tribes (Castillejinae; Tank and Olmstead 2008), and genera (e.g., *Medicago*; Bena et al. 1998; *Nemesia*; Datson et al. 2008), and have generally demonstrated that the perennial state is ancestral in plants. No studies, to our knowledge, have sought to determine whether evergreen or deciduous perennialism is ancestral in plants, at least at a broad taxonomic scale. Indeed, Kenrick and Crane (1997) discussed the early evolution of land plants but did not at all consider life history strategies. For these reasons, we conducted our study using a wide variety of plant species from numerous different families, covering ferns, gymnosperms, and angiosperms. Additionally, we built a phylogenetic tree using the species examined in this study to envision their evolutionary relationships and completed an ancestral state reconstruction to determine how life history strategies may have evolved in land plants at a broad level.



## Material and Methods

To investigate rates of cellular damage in leaves caused by exogenous  $\text{H}_2\text{O}_2$  exposure, we used an electrolyte leakage assay (Hatsugai and Katagiri 2018). Leaves were harvested in early autumn from plants growing naturally on the University of Toronto Scarborough campus grounds as well as public and private (with permission) lands in the adjacent neighbourhoods. Only green leaves were harvested, which reduced the likelihood that senescent leaves were included in our study since it has been shown that loss of greenness is one of the earliest markers of leaf senescence (Bertold et al. 2019). When not already known, plants were identified using publicly-available databases (e.g., [ontariotrees.com](http://ontariotrees.com)). For plants known to exhibit different life history strategies in different regions, we classified them according to the life history strategy that they most commonly exhibit when grown in southern Ontario. The species used for this experiment are listed in Table 1. Once harvested, leaves were subjected to the electrolyte leakage assay as soon as possible. Multiple species, with different life history strategies, were processed in parallel to minimize variation in the results due to disparity in the concentration of the prepared  $\text{H}_2\text{O}_2$  solutions, which were made fresh just prior to each assay being conducted. For each species, two  $1\text{ cm}^2$  samples of leaf tissue were obtained. For species with large leaves, scissors were used to excise the sample from the leaf, and for species with small leaves, multiple leaves were used together. Surface area is a major factor determining the rate of electrolyte leakage from leaves (Whitlow et al. 1991), which is why we used a standardized leaf surface area for all our species. One leaf sample was placed into 7 mL of distilled water (i.e., control medium), and the other leaf sample was placed in 7 mL of 30 mM  $\text{H}_2\text{O}_2$  (i.e., experimental medium). We used this concentration of  $\text{H}_2\text{O}_2$  because previous studies in our laboratory have shown that it reduces vegetative growth without preventing flowering (Dave and Brown, unpublished observations), suggesting that it causes mild oxidative stress in plants. The conductivity of the media was immediately measured using a conductivity meter (Extech EC400), and then every 10 minutes thereafter for 60 minutes. Both leaf samples were kept at room temperature. The rate of cellular damage (estimated from the rate of change in the conductivity of the medium, in  $\mu\text{S min}^{-1}$ ) for both control and experimental leaves was calculated from these measurements and corrected for changes in the conductivity of the external medium without leaves, which were measured simultaneously. The rate of cellular damage due solely to  $\text{H}_2\text{O}_2$  was determined as the difference between the experimental and control rates of electrolyte leakage, with the control rate of electrolyte leakage accounting for any differences in electrolyte permeability and/or harvest-induced cellular damage that might occur among different species.

To investigate the effects of exogenous  $\text{H}_2\text{O}_2$  on germination rate, we purchased seeds from various commercial suppliers. The species used for this experiment are listed in Table 2. Seeds were germinated in a

temperature- and humidity-controlled greenhouse in plastic Petri dishes lined with filter paper and filled with tap water or  $\text{H}_2\text{O}_2$  (10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, or 80 mM; diluted in tap water). Petri dishes were refilled as necessary to prevent seeds from dehydrating. Petri dishes were covered with an opaque material so that germination occurred in the dark. Seeds were examined for evidence of germination at approximately 12-hour intervals. Germination was scored when the radicle had emerged from seed and was visible, as radicle emergence has been shown to be a repeatable and reproduceable test of seed vigour (Matthews et al. 2011). Germination was scored until no further germination was observed for at least two days. A magnifying glass was used where necessary to examine germination in smaller seeds. In order to determine the  $\text{H}_2\text{O}_2$  concentration that brought about the fastest time to 50 % germination in each species (i.e., hereafter called ‘optimal  $[\text{H}_2\text{O}_2]$ ’), we fit the data for time since sowing and number of germinated seeds to a four-parameter logistic curve and determined the inflection point, which was interpreted as the time required for 50 % of the seeds to germinate. The curve fitting was accomplished using AAT Bioquest’s Four Parameter Logistic Curve Calculator, which is available at: <https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator>.

To investigate the susceptibility of chlorophyll a and chlorophyll b to exogenous  $\text{H}_2\text{O}_2$ , as well as chlorophyll a/b ratios, leaf samples were harvested in early autumn from plants growing naturally on the University of Toronto Scarborough campus grounds, as well as public and private (with permission) lands in the adjacent neighbourhoods and nearby commercial buildings. As for the electrolyte leakage assay above, only green leaves were harvested. When not already known, plants were identified using publicly-available databases (e.g., [ontariotrees.com](http://ontariotrees.com)). For plants known to exhibit different life history strategies in different regions, we classified them according to the life history strategies that they most commonly exhibit when grown in southern Ontario (or the particular region from which they were harvested). The species used for this experiment are listed in Table 3. The leaf samples were ground in 5mL of 100% with acetone using a mortar and pestle. Subsequently, the ground leaf samples were filtered (using coffee filters inserted into funnels) in order to remove any insoluble debris (e.g., unbroken leaves). The resulting chlorophyll extract was partitioned into two aliquots. To one aliquot, 1mL of tap water was added (as a control); to the other aliquot, 1mL of 30mM  $\text{H}_2\text{O}_2$  was added. Both aliquots were mixed and, immediately, the absorbance of each aliquot was measured at 662nm and 644nm using a visible spectrophotometer (Vinmax LDC, Model 721), which allowed for the calculation of both chlorophyll a and b concentrations using previously-published equations (Holm, 1954). The aliquots were then placed in the dark, and their absorbance at these same two wavelengths was remeasured again after 30 and 60 minutes,

which allowed us to calculate the rate of degradation of both chlorophyll a and chlorophyll b. Chlorophyll a/b ratios were calculated from time 0 measurements made from the aliquots to which water had been added.

Statistical analysis of the data was conducted using the Univariate General Linear Model procedure of SPSS (version 23) with lifespan as the fixed factor and electrolyte leakage rate, optimal [H<sub>2</sub>O<sub>2</sub>], chlorophyll degradation rate, or chlorophyll a/b ratio as the dependent variable, as appropriate. The least significant difference post-hoc test was used to determine specific differences among annuals, biennials, deciduous perennials, and evergreen perennials, where appropriate. For chlorophyll degradation rates, a covariate (chlorophyll concentration at time = 0) was included in the analysis since chlorophyll degradation rates were likely to be dependent on the amount of chlorophyll present initially. In addition, for chlorophyll degradation rates, we used the t-test function in Google Sheets to determine the P-value for comparisons between water- and H<sub>2</sub>O<sub>2</sub>-induced chlorophyll degradation rates as well as degradation rates between chlorophyll a and b. For all tests, differences were considered significant if  $P < 0.05$ .

The construction of a phylogenetic tree and its utilization for ancestral state reconstruction of life history strategy was accomplished using R (R Core Team 2017) and its ‘brranching’ package, which permits the creation of supertrees using Phylomatic (Webb and Donoghue 2004). A list of the 84 species used in this study, as well as their life history strategies, were input and read by the packages installed. Using the Zanne et. al (2014) phylogeny database as a reference, a circular phylogenetic tree was constructed with each species assigned to its corresponding life history. After ensuring the tree was rooted by having a common ancestor, we used a continuous-time Markov chain model to perform ancestral state reconstruction (Pagel et al. 2004). Scaled likelihoods were measured at each root of the tree. For a visual representation of the tree after the analysis of the ancestral state was determined, each root included the likelihood pie graphs of each life history. The margin of error for these likelihood values is 0.05%. The R code used for this entire procedure is provided in an Appendix for reference.

## Results

The rate of electrolyte leakage, which is a common marker of cellular damage, of leaves exposed to distilled water was above zero in all plant species used in this study, but did not differ significantly among life history strategy, being the same in annuals, deciduous perennials, and evergreen perennials ( $P = 0.892$ ; Fig. 1a). By contrast, the rate of electrolyte leakage of leaves due solely to exogenous  $H_2O_2$  exposure, which was the difference in electrolyte leakage rate between  $H_2O_2$ - and distilled water-exposed leaves, was 7.5- and 13-fold higher in annuals compared to deciduous perennials ( $P = 0.043$ ) and evergreen perennials ( $P = 0.035$ ), respectively (Fig. 1b).

To determine the  $H_2O_2$  concentration that brought about the fastest time to 50 % germination (i.e., optimal  $[H_2O_2]$ ), for each species, we measured the number of germinated seeds at roughly 12-hour intervals until either all seeds had germinated or no further germination had occurred for several days. Subsequently, we fit these data using a 4-parameter logistic curve, using the inflection point as the time to 50 % germination. Representative data sets for one annual, one biennial, one deciduous perennial, and one evergreen perennial are shown in Fig. 2. Time to 50% germination for seeds sown in water did not differ significantly among life history strategies ( $P = 0.13$ ; Fig. 3a), though it tended to be fastest in annuals. As expected, exogenous  $H_2O_2$  reduced the time to 50 % germination in all species (except *Dianthus barbatus*). The overall effect of lifespan on optimal  $[H_2O_2]$  was significant ( $P = 0.03$ ). Post-hoc analysis revealed that the optimal  $[H_2O_2]$  was 2-fold higher for deciduous perennials than annuals ( $P = 0.010$ ) and biennials ( $P = 0.017$ ) but was not higher than evergreen perennials ( $P = 0.122$ ; Fig. 3b). Optimal  $[H_2O_2]$  did not differ among annuals, biennials, and evergreen perennials ( $P > 0.05$  for all comparisons).

Both chlorophyll a and chlorophyll b showed a significantly greater rate of degradation when exposed to  $H_2O_2$  compared to water ( $P = 0.017$  and  $P = 0.005$ , respectively; data not shown), confirming that  $H_2O_2$  reacts with and alters the chemical structure of chlorophyll. We calculated the  $H_2O_2$ -specific rate of chlorophyll degradation as the difference between these two chlorophyll degradation rates (i.e., water- and  $H_2O_2$ -exposed). The  $H_2O_2$ -specific degradation rate of chlorophyll b was found to be nearly 2-fold higher than that of chlorophyll a (compare Figs. 4a, 4b;  $P = 0.007$ ). No significant difference among life history strategy was observed for chlorophyll a/b ratio ( $P = 0.612$ ; Fig. 4c).

For all three of our experiments combined, we analyzed a total of 84 different species (including variants and subspecies). A phylogenetic tree was constructed to illustrate the evolutionary relationships among these species (Fig. 5). Only 78 species are shown on this tree because i) two species (*Abies balsamea* and *Allium porrum*) were not found in the Zanne et al. (2014) database used to construct this tree, and ii) the four variants of *Brassica oleracea* and two

variants of *Beta vulgaris* were not identified separately in the database. An ancestral state reconstruction for these species revealed that the perennial evergreen condition is most likely ancestral (39.4% likelihood vs. 24.2% for deciduous perennial, 18.8% for annual, and 17.6% for biennial). From this evergreen perennial ancestor, there evolved a fern lineage, an evergreen perennial lineage, and a deciduous perennial lineage. The evergreen perennial lineage gave rise to various evergreen perennial species but also *Ginkgo biloba*, which is a deciduous perennial species. No annuals or biennial species have descended from this evergreen lineage. The deciduous perennial lineage has given rise to species with all four life history strategies, with annual and biennial species having evolved only relatively recently. Notably, within the lineage, we only observed two instances where a suspected annual ancestor gave rise to a perennial descendant (*Iberis sempervirens* and *Mentha spicata*).

## Discussion

Our ancestral state reconstruction of the 84 species used in this study yielded two major findings. First, the evergreen perennial condition is the most likely ancestral life history strategy in plants. From this evergreen ancestor, there arose a fern lineage; an evergreen plant lineage, which may have given rise to the gymnosperms; and a deciduous plant lineage, which may have given rise to the flowering plants (Taylor and Hickey 1992). Second, at multiple, independent times, annuals and biennials evolved within this deciduous perennial lineage, suggesting a transition to shorter lifespans in plants over time. Several factors are known to favour the annual strategy in plants, including short growing season length, low habitat reliability, and low storage efficiency (Iwasa and Cohen 1989); however, it is not clear how these factors have changed over time on Earth since plants first arose. Notwithstanding, we will discuss our findings on the effects of exogenous  $H_2O_2$  on plants in the context of this phylogenetic information as such an approach will permit us to understand how the physiological mechanisms associated with ROS tolerance in plants may have evolved. What is particularly striking about our ancestral state reconstruction is how infrequently perennial species re-evolved from annual ancestors: in our analysis, it happened only twice (with *Iberis sempervirens*, an evergreen perennial, and *Mentha spicata*, a deciduous perennial, both having evolved independently from an annual ancestor). While evidence for annual-to-perennial transitions is not absent (Tank and Olmstead 2008), it does seem to be relatively rare. We ponder whether the physiological changes that occur within plants during the perennial-to-annual transition are difficult to reverse, and we address this possibility as well.

The rate of cellular damage of excised leaves due solely to exogenous  $H_2O_2$  exposure was significantly lower in both deciduous and evergreen perennials compared to annuals. While we had expected the leaves of evergreen perennials to be more resistant to exogenous  $H_2O_2$  exposure than those of annuals, since the leaves of evergreen perennials can live as long as 40 years (Aerts 1995), we had not expected the leaves of deciduous perennials to be equally resistant to  $H_2O_2$  exposure since the leaves of deciduous perennials persist for less than one year. It seems, therefore, that the resistance of leaves to exogenous  $H_2O_2$  depends on the lifespan of the entire plant, not the lifespan of the leaves themselves. One possible explanation for this observation is that ROS produced within one plant tissue—whether directly or via free radical chain reactions, which can produce secondary ROS that have been shown to be particularly important in aging (Montgomery et al. 2011)—can freely diffuse to other plant tissues, causing oxidative damage at locations distant to the site of ROS production; therefore, even though the leaves of deciduous perennials do not, themselves, survive longer than one year, they may have mechanisms to eliminate any ROS produced within them in order to prevent ROS from diffusing to the perennating tissues, such as buds, roots, and tubers. Indeed, it has

been shown that  $\text{H}_2\text{O}_2$ , whose chemical structure is similar to that of water, uses certain aquaporins (called ‘peroxiporins’) to cross cell membranes (Henzler and Steudle 2000), though it is unclear how far leaf-derived ROS could travel within a plant. We have routinely grown flax in diluted  $\text{H}_2\text{O}_2$  solutions in our greenhouse and have observed that it negatively impacts not only root growth but also shoot growth, suggesting that  $\text{H}_2\text{O}_2$  can travel from the roots to the apical meristem (personal observations).

Electrolyte leakage from plant cells in response to abiotic stress (e.g., exogenous  $\text{H}_2\text{O}_2$ ) is thought to reflect the loss of cell membrane integrity, which is a common definition of cell death. Therefore, it is likely that exogenous  $\text{H}_2\text{O}_2$  accelerated the rate of electrolyte leakage from the leaves in our study because it induced cell death. Although  $\text{H}_2\text{O}_2$  is known to induce apoptosis in plants (Gadjev et al. 2008), at the high concentrations used in this experiment, it is more likely that  $\text{H}_2\text{O}_2$  caused cellular death via necrosis (Saito et al. 2006). Brunk et al. (1995) have shown that, when cells are exposed to high levels of  $\text{H}_2\text{O}_2$ , damage to lysosomal membranes occurs, causing the leakage of destructive enzymes (possibly including phospholipases) that degrade the cell within the 30 minutes, which is shorter than the length of time to which our leaves were exposed to exogenous  $\text{H}_2\text{O}_2$ . Given the short duration that our leaves were exposed to exogenous  $\text{H}_2\text{O}_2$ , we believe that there are two possible reasons why deciduous and evergreen perennial leaves experienced less  $\text{H}_2\text{O}_2$ -induced cellular damage than annuals. First, deciduous and evergreen perennial leaves may have higher antioxidant activities than annual leaves, permitting them to better detoxify any exogenous  $\text{H}_2\text{O}_2$ . Consistent with this hypothesis, Brown et al. (2012) showed that perennial flax (which are deciduous) had higher catalase activity than annual flax. Second, the cell membranes of deciduous and evergreen perennial leaves may have a lower proportion of polyunsaturated fatty acids (PUFA) than those of annual leaves. It is well established that PUFA are more susceptible to oxidative attack via ROS, and there is evidence in animals that long-lived animals frequently have lower levels of PUFA than short-lived animals (Pamplona et al. 1999). We suspect that the former possibility is more likely than the latter because, given that evergreen leaves must be able to endure low temperatures during winter, we would expect that their cell membranes would be enriched with PUFA, as these fatty acids help maintain membrane fluidity during cold exposure (Samala et al. 1998).

Bulk membranes are thought to be relatively impermeable to  $\text{H}_2\text{O}_2$  (Bienert et al. 2006), so it seems likely that exogenous  $\text{H}_2\text{O}_2$  enters leaves through either stomata or aquaporin channels expressed in the leaf epidermal cells (Bienert and Chaumont 2014; Hachez et al. 2008), in the same manner as water. On this basis, it is possible that the lower rate of  $\text{H}_2\text{O}_2$ -induced cellular damage in deciduous and evergreen perennials observed in this study resulted from their lower  $\text{H}_2\text{O}_2$  permeability compared to annual leaves; however, we believe that this possibility is unlikely.

With regards to stomatal density, Wang et al. (2015) showed that stomatal density was nearly 2-fold higher in broadleaf deciduous leaves than evergreen conifers, and Liu et al. (2019) showed that stomatal density was lowest in  $C_3$  annuals, with  $C_4$  annuals as well as perennials being higher but not different from each other. With regards to aquaporin expression, Brodribb et al. (2004) showed that the hydraulic conductance of angiosperms (both annuals and perennials) was 3- to 4-fold higher than that of conifers, likely reflecting a significant difference in leaf aquaporin expression. Consequently, it is clear that the patterns of ROS-induced cellular damage observed in this study do not match previously-established patterns in either stomatal density and/or aquaporin expression, leading us to conclude that differential leaf  $H_2O_2$  permeability cannot likely explain our results.

For all species examined, there was significant electrolyte leakage from leaves soaked in distilled water (i.e., in the absence of  $H_2O_2$ ). This electrolyte leakage from control leaves is not likely due to leaves beginning autumnal senescence prior to the time of harvesting because i) only green leaves were used, as discussed in the Methods and Materials, and ii) electrolyte leakage was observed in annuals and deciduous perennials to same extent as in evergreen leaves, even though the latter do not exhibit autumnal senescence (Chapin and Kedrowski 1983). Some previous studies have shown that necrosis in leaf samples does occur even in the absence of perturbing factors (Maxwell et al. 1997), so it is possible that the electrolyte leakage observed for leaves in distilled water in our study reflects the onset of cellular damage following leaf excision. Bar-Dror et al. (2011) have shown that, following leaf abscission, there are changes in nuclear morphology and nuclear fragmentation—hallmarks of apoptosis—that occur in the leaf cells distal to the abscission zone. Separation of a leaf from its mother plant, whether naturally-occurring or forceful, may induce cellular damage, leading to electrolyte leakage. Regardless of the underlying mechanism by which electrolyte leakage occurred in leaves exposed to distilled water in this study, there were no differences in the rate of electrolyte leakage among annuals, deciduous perennials, and evergreen perennials. This reinforces that the differences in rates of cellular damage that we observed in response to  $H_2O_2$  reflected a lesser capacity for annuals to mitigate the effects of exogenous ROS exposure compared to perennials, rather than simply inherent differences in the invocation of cell death responses.

Given that the evergreen perennial strategy is likely ancestral in plants, our observation that evergreen perennial leaves are better able to resist damage in the face of exogenous  $H_2O_2$  suggests that cellular mechanisms to protect leaf cells from ROS damage have likely existed since plants first evolved. This finding is consistent with work by Inupakutika et al. (2016) who showed that genes encoding ROS scavenging mechanisms originated as early as 4.1 billion years ago, prior to the first great oxygenation event, suggesting that localized oxygen originating from



photosynthesis is likely sufficient to allow for ROS production, necessitating the endogenous synthesis of antioxidant enzymes. Deciduous perennial leaves would likely have retained this inherited trait, despite their transition to an annual leaf-dropping character, in order to limit their production of ROS and its possible transfer to perennating tissues, as described above. By contrast, annuals may have abandoned this trait due to its cost, allowing them to direct more energy towards reproduction. Indeed, Vaanholt et al. (2008) showed that the daily energy expenditure of mice was positively correlated with antioxidant levels in the liver, suggesting that antioxidant synthesis (and likely other ROS protective measures) may entail a substantial energetic burden for organisms.

Compared to annuals, we observed that deciduous perennials—but not evergreen perennials or biennials—required a higher concentration of exogenous  $\text{H}_2\text{O}_2$  in order to bring about maximal germination rate, which is consistent with the notion that the seeds of these species have an increased capacity to eliminate ROS, most likely via elevated antioxidant levels, as predicted by OSTA. It is important to note that the seeds used for this experiment were germinated within a few months of being purchased, so their antioxidant levels should have been maximal. This is a relevant consideration because it is known that antioxidant levels in seeds decrease with storage time (Pukacka 1991).

The fact that evergreen perennials did not also require higher  $\text{H}_2\text{O}_2$  concentrations to maximize their germination rate is a curious observation given their comparable longevity to deciduous perennials, but we believe that our ancestral state reconstruction may offer some insight here. While high seed antioxidant levels may better protect developing plants against oxidative damage, they may also retard germination rate, as  $\text{H}_2\text{O}_2$  is thought to facilitate several processes necessary for germination. Given that evergreen perennials were likely the first plants to evolve, they would have felt pressure to rapidly colonize the terrestrial environment in order to establish themselves. Consistent with this notion, Wainwright and Cleland (2013) found that invasions by exotic plant species were facilitated by their faster germination rates compared to native species. On this basis, evergreen perennials may only begin to express antioxidants following germination, as their tissues begin to develop and mature, in order to facilitate rapid germination and colonization of their landscape. When deciduous perennials subsequently evolved, they would have found themselves in direct competition with evergreen perennials, which occurs even in modern habitats (Álvarez-Yépiz et al. 2017). Deciduous perennials may have advanced the timing of their antioxidant expression so that their seeds were better endowed with protection against ROS. While this would have slowed down their germination rate, it would also have significantly increased their seed viability during storage (and soil bank dormancy) compared to evergreen perennials (Panetta 2008), which, in turn, would have increased their ability to survive periods of climatic variability (Morris et al. 2008).

Biennials were found to be like annuals, from the perspective of exogenous  $\text{H}_2\text{O}_2$  concentrations required to maximize germination rate. Although biennials have a longer lifespan than annuals (2 years vs. 1 year), biennials have a much shorter lifespan than deciduous and evergreen perennials, the average lifespan of which is 35 years (Ehrlén and Lehtilä 2002); therefore, from the perspective of OSTA, it is not surprising that biennials would be more similar to annuals than perennials. Moreover, our ancestral state reconstruction revealed that, similar to the annual condition, the biennial condition evolved only recently in the history of plants, further suggesting that biennials are more annual-like than perennial-like.

We did not observe a significant difference in germination time among annuals, biennials, deciduous perennials, and evergreen perennials in the present study, although germination time tended to be lower in annuals, consistent with previous work (Shipley and Parent 1991). Even if we presume that annuals generally do have faster germination rates than other plant species, it remains unlikely that this observation would account for the results of our study for two reasons. First, the optimal  $[\text{H}_2\text{O}_2]$  for annuals was approximately 25mM in the present study, which is substantially higher than the endogenous  $[\text{H}_2\text{O}_2]$  reported previously for dry and germinating annual seeds (2-3 $\mu\text{M}$  in *Pisum sativum*; Barba-Espín et al. 2011). Given the large concentration of exogenous  $\text{H}_2\text{O}_2$  required to maximize germination rate, it is unlikely that endogenous variation in  $\text{H}_2\text{O}_2$  levels among seeds from different species would have any significant effect on germination rate and/or optimal  $[\text{H}_2\text{O}_2]$ . Second, evergreen perennials, despite tending to have longer germination times than annuals, had the same optimal  $[\text{H}_2\text{O}_2]$  as annuals, again suggesting that differences in endogenous  $\text{H}_2\text{O}_2$  levels (or germination rate) did not determine the amount of exogenous  $\text{H}_2\text{O}_2$  required to maximize germination rate. The most likely explanation for the differences observed in terms of optimal  $[\text{H}_2\text{O}_2]$  in the present study is that seeds from deciduous perennials have a greater  $\text{H}_2\text{O}_2$  degradation capacity than seeds from other plants, which helps them to keep ROS production under control during early development, thereby permitting increased longevity as predicted by the OSTA.

Neither chlorophyll a nor chlorophyll b, following its extraction from leaves, showed any difference in susceptibility to exogenous  $\text{H}_2\text{O}_2$  exposure among species with different life history strategies. Given that the structure of chlorophyll a and chlorophyll b is the same in all the species investigated in this study, this finding is unsurprising. Our study did reveal, however, that isolated chlorophyll b was approximately 2-fold more susceptible to exogenous  $\text{H}_2\text{O}_2$  exposure than isolated chlorophyll a, suggesting that the formyl group may be more susceptible to ROS attack than the methyl group, as this is the only structural difference between these two pigments. To our knowledge, little work has been done to assess the ROS susceptibility of the formyl group, though it has been shown that tocopherols

with a higher number of methyl groups serve as better antioxidants, being more capable of interacting with ROS (Sharma et al. 2012), so we suspect that the formyl group must be even more susceptible to ROS attack. Given that chlorophyll b is more susceptible to degradation in the face of exogenous H<sub>2</sub>O<sub>2</sub> than chlorophyll a, it might be expected that perennial plants would minimize the amount of chlorophyll b within their photosystems, resulting in a higher chlorophyll a/b ratio; however, this was not observed in the present study. Reger and Krauss (1970) used illumination intensity to modify chlorophyll a/b ratios in *Chlorella vannielli* and found that higher chlorophyll a/b ratios caused a significant decline in photophosphorylation capacity, requiring supplementation via oxidative phosphorylation to maintain ATP balance. On this basis, perennials may be constrained with regard to their ability to modulate chlorophyll a/b ratios to prevent oxidative stress. Consistent with this notion, Smeets et al. (2005) found that chlorophyll a/b ratio was not altered following Cd exposure in beans, a condition which is known to cause oxidative stress.

Chlorophyll is bound to various light-harvesting proteins *in vivo* (Büchel 2015), so it is possible that these proteins serve to shield chlorophyll from ROS exposure. It is even possible that the amino acid sequence of these proteins may differ between annuals and perennials in a way that maximizes the ability of proteins from the latter to react with, and thereby neutralize, ROS that would otherwise inflict damage upon chlorophyll. Moreover, there is evidence that certain plant secondary metabolites can function as antioxidants to mitigate the effects of ROS (Franklin et al. 2009), so their presence within chloroplasts could also serve to protect chlorophyll from ROS exposure. Furthermore, Nisbett et al. (submitted) have shown that perennials have a greater capacity to synthesize and repair chlorophyll following exposure to oxidative stress, so it is also possible that perennials rely upon their repair capabilities to replenish their chlorophyll stores in the face of any oxidative damage.

Our ancestral state reconstruction revealed that the evolution of perennial species from annual ancestors is quite rare, having occurred only twice in the evolutionary history of the 84 species that we examined in this study. While recent climatic shifts to conditions, such as aridity and drought, that favour the annual condition have been proposed to explain this observation (Evans et al. 2007), it prompted us to consider whether there may be physiological constraints that prevent a reversion to perennialism from annualism. Most of our results have suggested that the longevity of perennial plants may result from their higher levels of antioxidants. Hasanuzzaman et al. (2014) reported that in rice, which is normally grown as an annual, upregulation of the antioxidant defense system in the face of oxidative stress is limited by the lack of biosynthetic capacity of regulatory molecules, such as proline and glycine betaine, and further demonstrated that exogenous supplementation of these compounds could significantly boost antioxidant expression. It is possible that annual plants have reduced their proline and/or glycine betaine biosynthetic

capacity in order to redirect resources to reproduction. Perhaps, then, transition from the annual to perennial condition is constrained by lack of environmental availability of such molecules or their precursors. Indeed, Hayat et al. (2012) found that both proline and antioxidant levels could be boosted in tomatoes, an annual species, via exogenous application of sodium nitroprusside, a nitrogen donor, consistent with the notion that, in annuals, their ROS resistance may be intimately tied to environmental nutrient supply.

While our study is the first, to our knowledge, to provide evidence that perennial plant species have physiological mechanisms to minimize the accumulation of oxidative damage, consistent with the OSTA, we do recognize that our study has three significant limitations. First, many aging studies conducted in animals treat maximum lifespan as a continuous variable and look for correlations between maximum lifespan and potential aging-related variables. This is possible because there are databases from which the maximum lifespan of most animal species can be easily extracted (e.g., AnAge: <https://genomics.senescence.info/species/>). To our knowledge, no similar database is available for plants, which requires studies conducted in plants to treat lifespan as a discrete variable (annual, biennial, deciduous perennial, evergreen perennial), as we did. One of the drawbacks to this approach is that some plants straddle the line between different categories, growing as annuals in some regions but as perennials in other regions. Indeed, nearly half of the plants that we classified as annual plants in our study are known to exhibit perennialism under some growing conditions, though not in the region where our study was conducted, which is why we classified them as annuals. Franco and Silvertown (1996) have shown that continuous variables, such as adult mortality rate, can account for the different life histories found in plants, and future aging studies in plants should examine whether there are relationships between these variables and resistance to exogenous  $H_2O_2$  exposure. The construction of databases for such information would be a helpful first stage in this process. Second, we only made a single measurement of  $H_2O_2$  resistance for each plant species in our experiments. One of us (JCLB) has experience with conducting aging studies in animals (Brown et al. 2009), and the most common critique of such studies is their lack of phylogenetic coverage; therefore, we sought to maximize our phylogenetic coverage in the present study, and this would not have been feasible if we had made multiple measurements for each plant species. Certainly, we recognize that neglecting intraspecific variation—while common practice in comparative studies—is problematic (Garamszegi and Møller 2010), but we feel that it was justified because our stated objective for this study was to extend our observations from flax to the broadest possible range of species. Third, the plant material used in this study was obtained from various sources, i.e., plants growing naturally or artificially in the local area and/or seeds available for purchase from commercial suppliers. We utilized this approach because it would be impossible for us to grow

plants from the 84 different species investigated in this study under the same conditions in such a manner where not only leaves but also seeds could be harvested and analyzed. We recognize that this approach precludes our ability to account for certain factors on our measurements; for example, electrolyte leakage is known to increase with age for potato tubers (De Weerd et al. 1995), so knowing the age of the leaves used in our electrolyte leakage experiment could have allowed us to control for its effects on our results. Nevertheless, we submit that our inability to control for these factors should have increased the variability in our dataset, thereby making it more difficult for us to obtain statistically significant results (Biau et al. 2008); therefore, the fact that we observed significant differences among life history strategies for both cellular damage rate and optimal  $\text{H}_2\text{O}_2$  for germination rate underscores the robustness of these findings.

Overall, our results demonstrate support for OSTA in plants as we have shown that both deciduous and evergreen perennials exhibit greater resistance to the deleterious effects of exogenous  $\text{H}_2\text{O}_2$  compared to annuals during at least some point in their life. Furthermore, our ancestral state reconstruction has shown that the evergreen perennial state is ancestral in plants, suggesting that mechanisms to resist ROS-induced biomolecular damage are ancient. Future studies in our laboratory will focus on investigating the underlying mechanisms responsible for this increased ROS tolerance. In particular, we plan to measure cellular antioxidant levels in annuals, deciduous perennials, and evergreen perennials, especially since our data have suggested that antioxidant levels may be higher in these latter two groups.

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Table 1. List of species used for the electrolyte leakage experiment.

Scientific Name	Common Name
Annuals (16 species)	
<i>Abelmoschus esculentus</i>	Okra
<i>Alliaria petiolata</i>	Garlic Mustard
<i>Ambrosia artemisiifolia</i>	Ragweed
<i>Brassica nigra</i>	Black Mustard
<i>Brassica oleracea</i> var. <i>sabellica</i>	Green Kale
<i>Capsicum annuum</i>	Green Bell Pepper
<i>Capsicum annuum</i>	Green Chili Pepper
<i>Erigeron canadensis</i>	Canadian Horseweed
<i>Helianthus annuus</i>	Sunflower
<i>Ipomoea purpurea</i>	Morning Glory
<i>Ocimum basilicum</i>	Basil
<i>Pelargonium</i> sp.	Geranium
<i>Salvia officinalis</i>	Sage
<i>Solanum lycopersicum</i>	Tomato
<i>Solanum melongena</i>	Eggplant
<i>Vigna radiata</i>	Mung Bean
Deciduous Perennials (11 species)	
<i>Acer saccharum</i>	Sugar Maple
<i>Aegopodium podagraria</i>	Bishop's Goutweed
<i>Cornus florida</i>	Flowering Dogwood
<i>Dianthus caryophyllus</i>	Carnation
<i>Melissa officinalis</i>	Lemon Balm
<i>Mentha piperita</i>	Peppermint
<i>Mentha spicata</i>	Spearmint

<i>Rosa sp.</i>	Rose
<i>Syringa vulgaris</i>	Common Lilac
<i>Taraxacum officinale</i>	Common Dandelion
<i>Toxicodendron diversilobum</i>	Poison Oak

Evergreen Perennials (12 species)

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<i>Abies balsamea</i>	Dwarf Balsam Fir
<i>Abies concolor</i>	White Fir
<i>Hibiscus rosa-sinensis</i>	Rose Mallow Hibiscus
<i>Juniperus horizontalis</i>	Creeping Juniper
<i>Picea glauca</i>	White Spruce
<i>Picea pungens</i>	Blue Spruce
<i>Pinus halepensis</i>	Aleppo Pine
<i>Pinus strobus</i>	White Pine
<i>Podocarpus macrophyllus</i>	Buddhist Pine
<i>Taxus baccata</i>	English Yew
<i>Taxus brevifolia</i>	Pacific Yew
<i>Thuja occidentalis</i>	Eastern White Cedar

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Table 2. List of species used for the germination rate experiment.

Scientific Name	Common Name
Annuals (11 species)	
<i>Beta vulgaris</i>	Beet
<i>Capsicum annuum</i>	Green Bell Pepper
<i>Celosia argentea</i>	Plumed Cockscomb
<i>Helianthus annuus</i>	Sunflower
<i>Impatiens balsamina</i>	Touch-Me-Not
<i>Lathyrus odoratus</i>	Sweet Pea
<i>Linum grandiflorum</i>	Scarlet Flax
<i>Linum usitatissimum</i>	Grain Flax
<i>Nigella damascena</i>	Love-in-a-Mist
<i>Papaver rhoeas</i>	Flanders Field Poppy
<i>Phaseolus vulgaris</i>	Pole Bean
Biennials (3 species)	
<i>Campanula medium</i>	Canterbury Bells
<i>Daucus carota</i>	Carrot
<i>Dianthus barbatus</i>	Chabauds Giant Carnation
Deciduous Perennials (9 species)	
<i>Asclepias tuberosa</i>	Butterfly Weed
<i>Catananche caerulea</i>	Cupid's Dart
<i>Dianthus caryophyllus</i>	Carnation
<i>Gypsophila paniculata</i>	Baby's Breath
<i>Lavandula officinalis</i>	Lavender
<i>Linum compactum</i>	Golden Flax
<i>Linum perenne</i>	Blue Flax

*Malva sylvestris*

Common Mallow

*Nepeta mussinii*

Catnip

## Evergreen Perennials (5 species)

*Iberis sempervirens*

Evergreen Candytuft

*Picea glauca*

White Spruce

*Picea pungens*

Blue Spruce

*Pinus strobus*

White Pine

*Pseudotsuga menziesii*

Douglas Fir



Table 3. List of species used for the chlorophyll degradation experiment.

Scientific Name	Common Name
Annuals (12 species)	
<i>Brassica oleracea</i> var. <i>palmifolia</i>	Black Kale
<i>Brassica oleracea</i> var. <i>sabellica</i>	Green Kale
<i>Beta vulgaris</i>	Beet
<i>Beta vulgaris</i> subsp. <i>vulgaris</i>	Swiss Chard
<i>Capsicum annuum</i>	Cayenne Pepper
<i>Capsicum annuum</i>	Gong Bao Pepper
<i>Dahlia pinnata</i>	Dahlia
<i>Glebionis coronaria</i>	Tong Ho
<i>Solanum lycopersicum</i>	Tomato
<i>Solanum melongena</i>	Eggplant
<i>Spinacia oleracea</i>	Spinach
<i>Zea mays</i>	Corn
Biennials (4 species)	
<i>Apium graveolens</i>	Celery
<i>Brassica oleracea</i> var. <i>italica</i>	Broccoli
<i>Brassica oleracea</i> var. <i>viridis</i>	Collard Green
<i>Daucus carota</i>	Carrot
Deciduous Perennials (10 species)	
<i>Acer saccharum</i>	Sugar Maple
<i>Allium fistulosum</i>	Green Onion
<i>Allium porrum</i>	Leek
<i>Asparagus officinalis</i>	Asparagus
<i>Euonymus fortunei</i>	Fortune's Spindle

<i>Ginkgo biloba</i>	Maidenhair Tree
<i>Hydrangea paniculate</i>	Panicle Hydrangea
<i>Rhus typhina</i>	Staghorn Sumac
<i>Salix integra</i>	Brocade Willow
<i>Symphyotrichum cordifolium</i>	Blue Wood-Aster

Evergreen Perennials (10 species)

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<i>Artemisia schmidtiana</i>	Silver Mound Artemisia
<i>Gerbera jamesonii</i>	Gerbera Daisy
<i>Hibiscus rosa-sinensis</i>	Rose Mallow and Orange Hibiscus
<i>Phegopteris hexagonoptera</i>	Broad Beech Fern
<i>Picea pungens</i>	Blue Spruce
<i>Pinus strobus</i>	White Pine
<i>Saintpaulia ionantha</i>	African Violet
<i>Taxus cuspidata</i>	Golden Japanese Yew
<i>Thuja occidentalis</i>	Eastern White Cedar

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## Figure Captions

**Fig. 1** Rate of electrolyte leakage from 1cm<sup>2</sup> leaf samples from annual, deciduous perennial, and evergreen perennial plants. Rate of electrolyte leakage, which was assessed by measuring changes in the conductivity of the external medium in which leaf samples were placed over a period of 60 minutes, measured every 10 minutes, was interpreted to reflect the rate of cellular damage in response to exogenous H<sub>2</sub>O<sub>2</sub>. a. Rate of electrolyte leakage from leaves exposed to distilled water. b. Rate of electrolyte leakage from leaves due solely to H<sub>2</sub>O<sub>2</sub> exposure (calculated as the difference between rate of electrolyte leakage from leaves exposed to H<sub>2</sub>O<sub>2</sub> and rate of electrolyte leakage from leaves exposed to distilled water). Values are mean  $\pm$  SEM. Means with different letters were significantly different ( $P < 0.05$ ). All values were corrected for the rate of change in conductivity of the external medium in the absence of any leaf samples, which was 0.025 $\mu$ S min<sup>-1</sup> for distilled water and 0.005 $\mu$ S min<sup>-1</sup> for H<sub>2</sub>O<sub>2</sub>. See Table 1 for list of species sampled for each life history strategy. Each species was sampled once

**Fig. 2** Cumulative germination of four representative species used in this experiment at their optimal [H<sub>2</sub>O<sub>2</sub>]. Optimal [H<sub>2</sub>O<sub>2</sub>] is the concentration of H<sub>2</sub>O<sub>2</sub> that caused the fastest time to 50% germination. Cumulative germination data were modeled using AAT Bioquest's Four Parameter Logistic Curve Calculator (<https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator>), with the inflection point (indicated by the dashed lines) being interpreted as the time to 50% germination. a. *Nigella damascena* (annual). b. *Duacus carota* (biennial). c. *Malva sylvestris* (deciduous perennial). d. *Picea glauca* (evergreen perennial). For each species, 10 seeds were sown, but not all seeds germinated

**Fig. 3** Effect of H<sub>2</sub>O<sub>2</sub> on the germination rate of seeds from annual, biennial, deciduous perennial, and evergreen perennial species. a. Time to 50% germination of seeds sown in water only. b. Concentration of exogenous H<sub>2</sub>O<sub>2</sub> that caused the fastest time to 50 % germination (called optimal [H<sub>2</sub>O<sub>2</sub>]). Germination rates excluded seeds for which no radicle emergence was ever observed. Values are mean  $\pm$  SEM. Means with different letters were significantly different ( $P < 0.05$ ). See Table 2 for list of species sampled for each life history. Each species was examined once at each of 8 different H<sub>2</sub>O<sub>2</sub> concentrations (10, 20, 30, 40, 50, 60, 70, 80 mM) and in water

**Fig. 4** Rate of chlorophyll degradation and chlorophyll a/b ratio of annuals, biennials, deciduous perennials, and evergreen perennials. Total chlorophyll was isolated from leaves using 100% acetone and subjected to 30mM exogenous  $H_2O_2$  (or water, as a control) to induce oxidative damage. Chlorophyll concentration was measured spectrophotometrically at 0, 30, and 60 minutes using absorbance measurements and previously published equations (Holm 1954). Chlorophyll degradation rate was calculated as the difference in the rate of change of chlorophyll concentration in 30mM  $H_2O_2$  samples and water samples over 60 minutes. All chlorophyll degradation rates were corrected for the initial chlorophyll concentration at time = 0 as higher initial chlorophyll concentrations led to higher rates of chlorophyll degradation rates. a. Degradation rate for chlorophyll a. b. Degradation rate for chlorophyll b. c. Chlorophyll a/b ratio for chlorophyll samples subjected to water only at time = 0. Values are mean  $\pm$  SEM. No significant differences among mean values were observed within any parameter, but the degradation rate for chlorophyll b was found to be significantly higher than that for chlorophyll a. See Table 3 for list of species sampled for each life history. Each species was sampled once

**Fig. 5** Ancestral state reconstruction of life history strategy for the 84 different species used in this study. Shown is maximum likelihood phylogeny inferred using seven gene sequences (Zanne et al. 2014). Ancestral state reconstruction of life history strategy was computed using a continuous-time Markov chain model. The pie chart at each node in the tree shows the scaled likelihood for the life history strategy of the ancestor, with a margin of error of 0.05%

## Appendix: R Code used for Phylogenetic Analysis and Ancestral State Reconstruction

These analyses were performed using the data analysis software R i386 4.0.2.

```
require(brranching)

require(ape)

require(phytools)

library("diversitree")

taxa <- read.csv('taxa_list_x.csv', header=T, sep=',', stringsAsFactors=F)

taxa_list <- c()

for (i in 1:nrow(taxa)) {
  family_name <- tolower(taxa$Family[i])
  split_binomial_name <- strsplit(taxa$Species[i], split=' ')
  genus_name <- tolower(split_binomial_name[[1]][1])
  species_name <- paste(split_binomial_name[[1]][1],
    split_binomial_name[[1]][2], sep='_')
  phylomatic_syntax <- paste(family_name, genus_name, species_name, sep='/')
  taxa_list <- c(taxa_list, phylomatic_syntax)
}

taxa_subset <- sample(taxa_list, size=84)

tree <- phylomatic(taxa=taxa_subset, storedtree='zanne2014')

taxa <- read.csv('taxa_list_order.csv', header=T, sep=',',
  stringsAsFactors=F)

x<-setNames(taxa[,3],taxa[,2])

cols<-setNames(palette()[1:length(unique(x))],sort(unique(x)))
tiplabels(pie=to.matrix(x,sort(unique(x))),piecol=cols,cex=0.3)
add.simmap.legend(colors=cols,prompt=FALSE,x=0.9*par()$usr[1],
  y=-max(nodeHeights(tree)),fsize=0.8)
```

```

is.rooted(tree)

tree<-multi2di(tree)
is.binary.tree(tree)

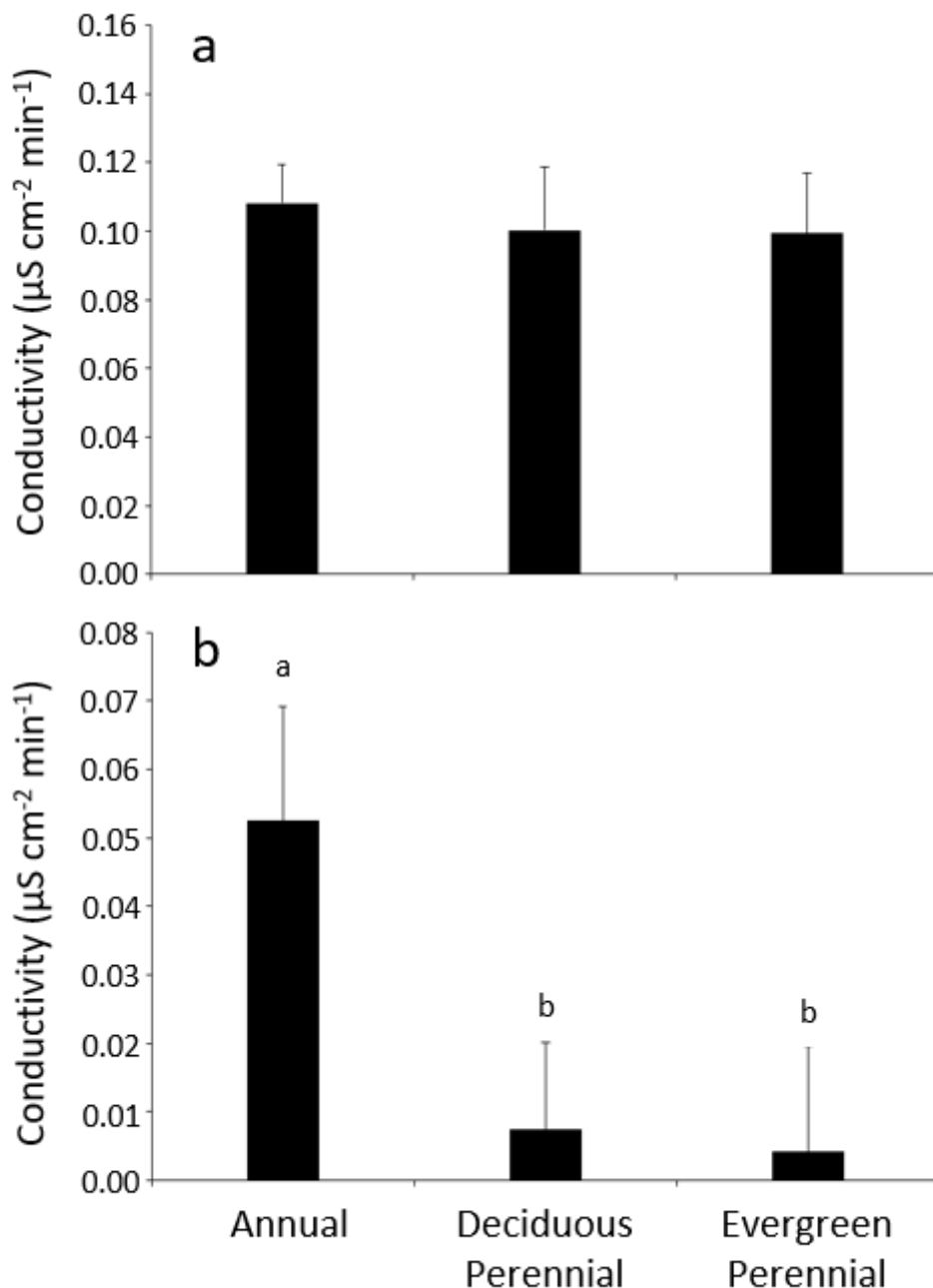
dst<-tree
dst$edge.length[dst$edge.length==0]<-max(nodeHeights(tree))*1e-6
fitER<-ace(x,dst,type="discrete",model="ER")

fitER

plotTree(tree,type="fan",fsize=0.8,ftype="i")
nodelabels(node=1:tree$Nnode+Ntip(tree),
  pie=fitER$lik.anc,piecol=cols,cex=0.5)
tiplabels(pie=to.matrix(x,sort(unique(x))),piecol=cols,cex=0.3)
add.simmap.legend(colors=cols,prompt=FALSE,x=0.9*par()$usr[1],
  y=-max(nodeHeights(tree)),fsize=0.8)

```

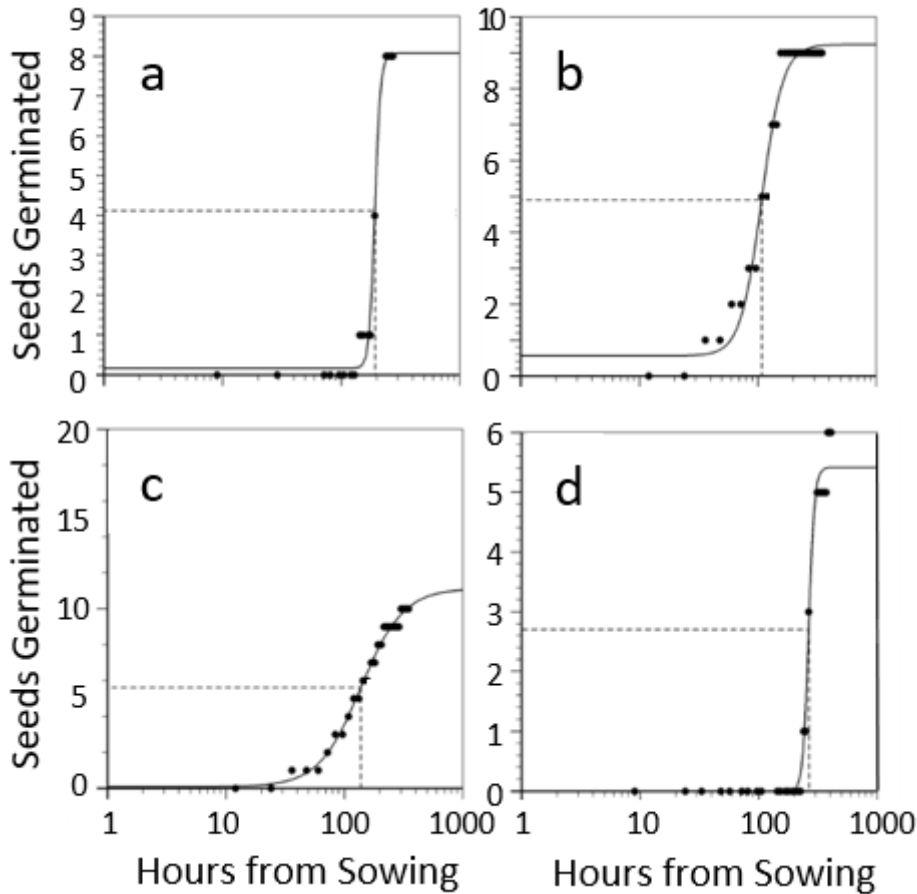
## Figures



**Figure 1**

Rate of electrolyte leakage from 1cm<sup>2</sup> leaf samples from annual, deciduous perennial, and evergreen perennial plants. Rate of electrolyte leakage, which was assessed by measuring changes in the conductivity of the external medium in which leaf samples were placed over a period of 60 minutes, measured every 10 minutes, was interpreted to reflect the rate of cellular damage in response to exogenous H<sub>2</sub>O<sub>2</sub>. a. Rate of electrolyte leakage from leaves exposed to distilled water. b. Rate of electrolyte leakage from leaves due solely to H<sub>2</sub>O<sub>2</sub> exposure (calculated as the difference between rate of

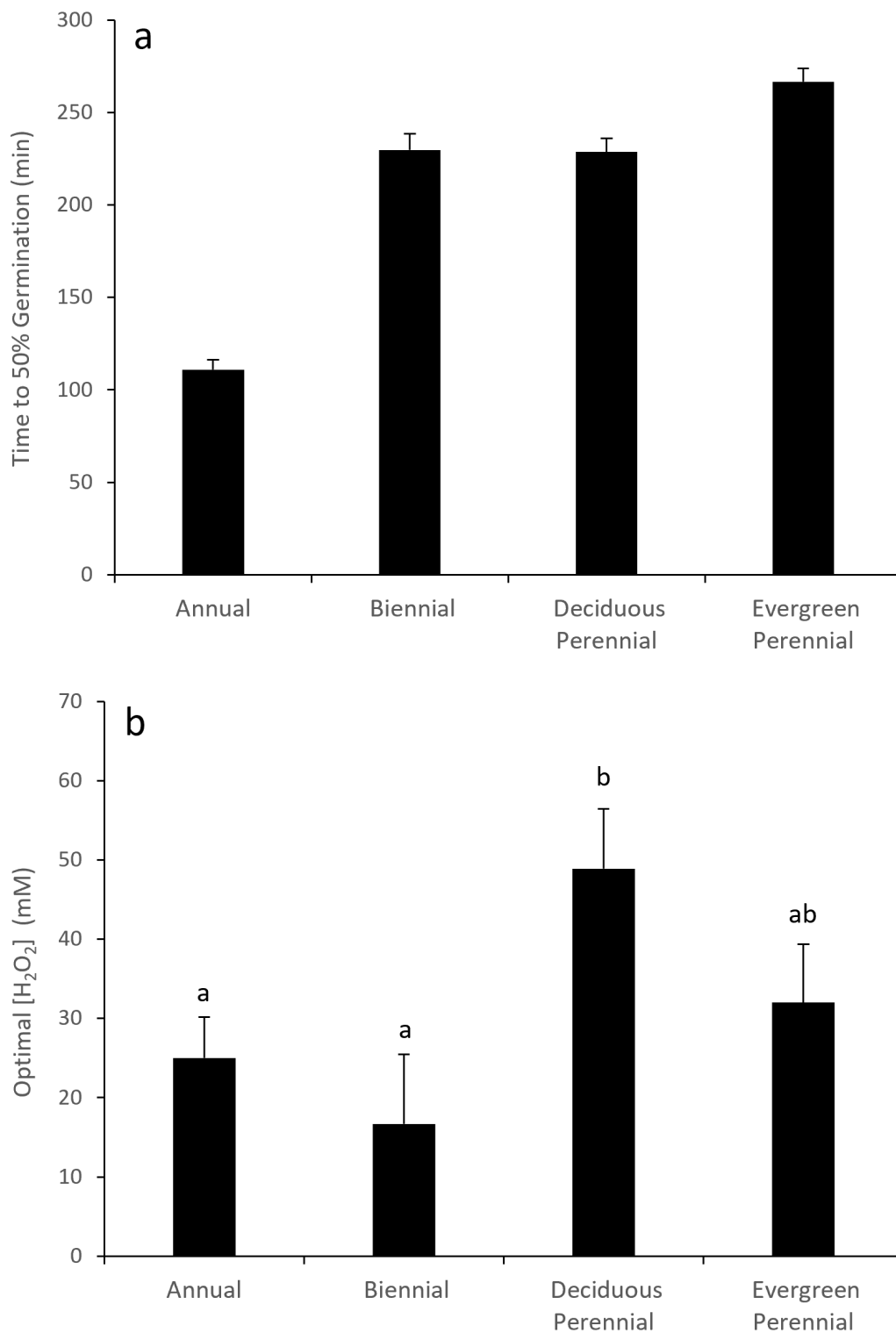
electrolyte leakage from leaves exposed to H<sub>2</sub>O<sub>2</sub> and rate of electrolyte leakage from leaves exposed to distilled water). Values are mean  $\pm$  SEM. Means with different letters were significantly different ( $P < 0.05$ ). All values were corrected for the rate of change in conductivity of the external medium in the absence of any leaf samples, which was 0.025  $\mu\text{S min}^{-1}$  for distilled water and 0.005  $\mu\text{S min}^{-1}$  for H<sub>2</sub>O<sub>2</sub>. See Table 1 for list of species sampled for each life history strategy. Each species was sampled once



**Figure 2**

Cumulative germination of four representative species used in this experiment at their optimal [H<sub>2</sub>O<sub>2</sub>]. Optimal [H<sub>2</sub>O<sub>2</sub>] is the concentration of H<sub>2</sub>O<sub>2</sub> that caused the fastest time to 50% germination. Cumulative germination data were modeled using AAT Bioquest's Four Parameter Logistic Curve Calculator (<https://www.aatbio.com/tools/fourparameter-logistic-4pl-curve-regression-online-calculator>), with the inflection point (indicated by the dashed lines) being interpreted as the time to 50% germination. a. *Nigella damascena* (annual). b. *Duacus carota* (biennial). c. *Malva sylvestris* (deciduous perennial). d. *Picea glauca* (evergreen perennial). For each species, 10 seeds were sown, but not all seeds germinated

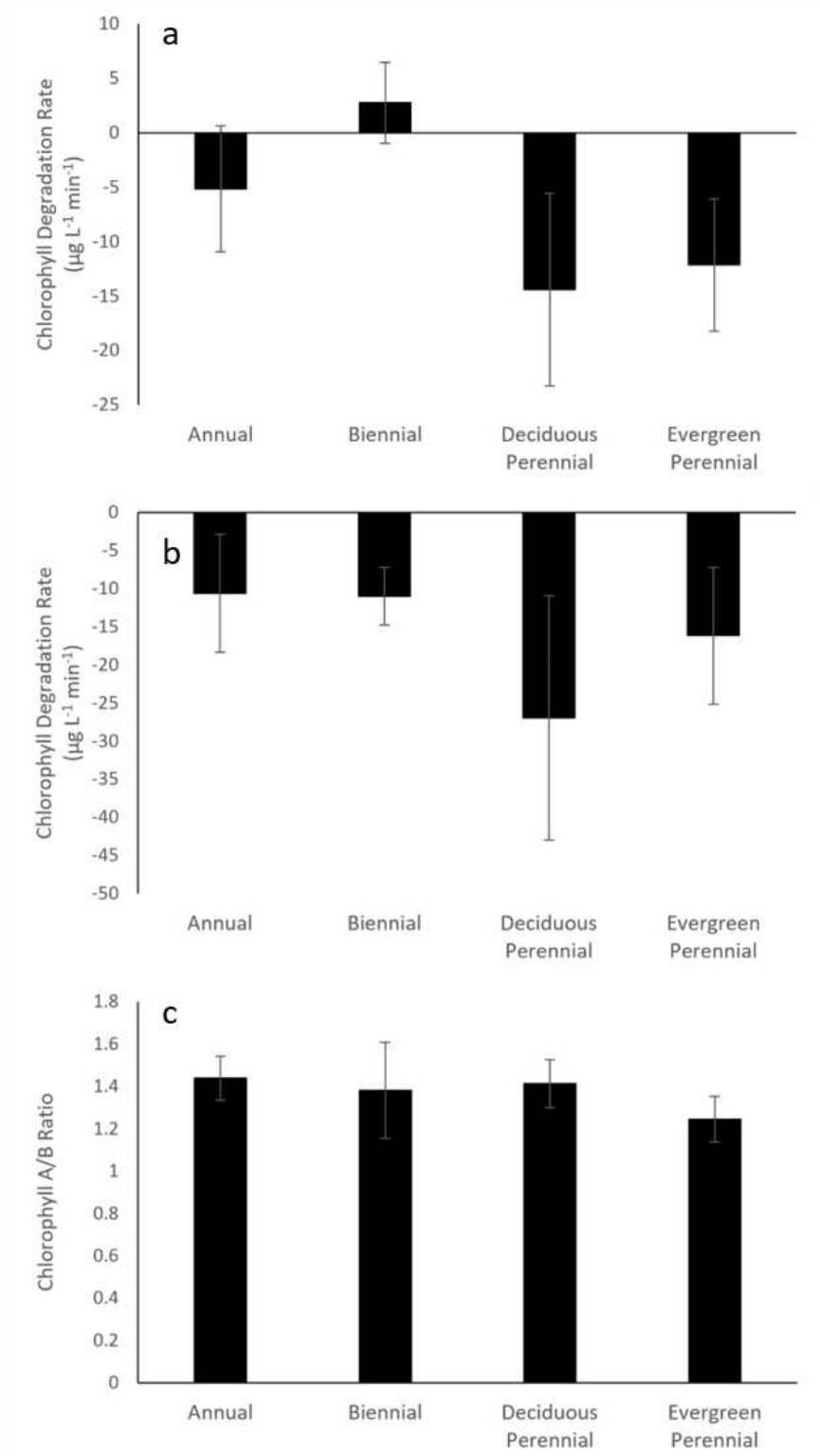




**Figure 3**

Effect of H<sub>2</sub>O<sub>2</sub> on the germination rate of seeds from annual, biennial, deciduous perennial, and evergreen perennial species. a. Time to 50% germination of seeds sown in water only. b. Concentration of exogenous H<sub>2</sub>O<sub>2</sub> that caused the fastest time to 50 % germination (called optimal [H<sub>2</sub>O<sub>2</sub>]). Germination rates excluded seeds for which no radicle emergence was ever observed. Values are mean  $\pm$  SEM. Means with different letters were significantly different ( $P < 0.05$ ). See Table 2 for list of species sampled for

each life history. Each species was examined once at each of 8 different H<sub>2</sub>O<sub>2</sub> concentrations (10, 20, 30, 40, 50, 60, 70, 80 mM) and in water



**Figure 4**

Rate of chlorophyll degradation and chlorophyll a/b ratio of annuals, biennials, deciduous perennials, and evergreen perennials. Total chlorophyll was isolated from leaves using 100% acetone and subjected to 30mM exogenous H<sub>2</sub>O<sub>2</sub> (or water, as a control) to induce oxidative damage. Chlorophyll concentration



Ancestral state reconstruction of life history strategy for the 84 different species used in this study. Shown is maximum likelihood phylogeny inferred using seven gene sequences (Zanne et al. 2014). Ancestral state reconstruction of life history strategy was computed using a continuous-time Markov chain model. The pie chart at each node in the tree shows the scaled likelihood for the life history strategy of the ancestor, with a margin of error of 0.05%