XVII HISPANO-PORTUGUESE INTERNATIONAL SYMPOSIUM ON WATER RELATIONS

PAMPLONA

FEBRUARY 25-27th, 2026

XVII Spanish-Portuguese Symposium on Plant Water Relations

Water relations in plants to address current challenges for plants

Pamplona, February 25-27th, 2026

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Symposium Program

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Program– XVII Hispano-Portuguese International Symposium on Water Relations

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Wednesday, 25th February

- **08:15 – 09:30** Registration and documentation handout.
- **09:30 – 10:00 Opening session. Luis J. Fernández** (Director UNED Tudela), **Iker Aranjuelo** (CSIC-IdAB) **& Ismael Aranda** (INIA-CSIC)
- **10:00 – 11:00 Inaugural lecture. Ian Dodd** (University of Lancaster, UK): "*Root-to-shoot signalling: Rest in Peace*"
- **11:00 – 12:00** Coffee break
- **12:00 – 13:30 Session 1**: **Physiological and molecular mechanisms of water relations**. Moderators: Drs. Ricardo Aroca (Zaidín-CSIC) and Celia Rodríguez Domínguez (IRNAS-CSIC). Alternate: Rosana López (ISIFOR).

    - **Jeroni Galmés** (Universitat de les Illes Balears, España): "*Water as an evolutionary driver of photosynthesis: co-adaptation of internal CO_2 diffusion and Rubisco function during terrestrialization and aquatic recolonization of plants*"
    - **Antonio Díaz-Espejo** (IRNAS-CSIC, España): "The strange case of the wrong stomata and the caterpillars"
    - **Paulo Eduardo Ribeiro Marchiori** (Universidade Federal de Lavras, Brasil): "*Crosstalk between nitrogen remobilisation and carbon metabolism in sugarcane facing drought stress and rehydration*"
    - **José Javier Peguero-Pina** (CITA-Aragón, España): "*Age-dependent changes in cell-level anatomy and leaf hydraulic conductance triggers the decline in photosynthetic capacity in older leaves of the evergreen Mediterranean oak Quercus ilex subsp. rotundifolia*"
    - **María Varona** (Universidad Pública de Navarra, España): "*Efecto de poliaminas y oximas en la respuesta a estrés hídrico*"
    - **Nerea Urcola** (CITA-Aragón, España): *Gas exchange and Minimum Conductance Reveal Leaf Growth Plasticity Under Contrasting Vapor Pressure Deficit in Quercus ilex.*

- **13:30 – 15:30** Lunch

- **15:30 – 16:30 Session 2**: **Water relations in natural systems: from grasslands to forests**. Moderators: Luna Morcillo (CEAM-Alicante) and Teresa Gimeno (CREAF).

    - **Juan Pedro Ferrio** (EEAD-CSIC, España): "*Differential response of stem CO_2 efflux to soil and stem temperature in two Mediterranean oaks with contrasting water use strategy*"
    - **M. Rocío Martín-Peláez** (Universidad de Sevilla, España): "*Integrating drought history into plant water relations: eco-physiological acclimation in a xerohalophyte*"

- **Rosana López** (INIA-CSIC, España): "*Can mother tree age modulate the functional strategy in response to severe water stress in seedlings of Pinus uncinata?*"
- **Vera Prazeres** (Universidade de Lisboa, Portugal): "*Intraspecific variability in Quercus suber L. resistance to embolism in relation to turgor loss in leaves*"

- **16:30 – 17:30 Poster session**
- **17:30 – 18:00** Coffee break
- **18:00 – 19:00 Session 3: Water relations in agriculturally relevant species.** Moderators: Drs. Josefina Bota (UiB) and Paula Paredes (Instituto Superior de Agronomia- Lisbon). Alternate: Angie L. Gámez (IdAB-CSIC).

  - **Francisco Javier Cano** (INIA-CSIC, España): "*From Leaf to Canopy: Optimizing iWUE and resource acquisition in C_4 crops under climate change*"
  - **MªCarmen Ruiz Sánchez** (CEBAS-CSIC, España): "*Rootstock influences persimmon water status indicators: ground-based and remote sensing approaches*"
  - **María Tasa** (IVIA-Generalitat Valenciana, España): "*Effects of high boron in irrigation water on plant water relations and gas exchange in Citrus macrophylla*"
  - **Nazareth Torres** (Universidad Pública de Navarra, España): "*An assessment of the influence of graft vascular alignment on grapevine physiological performance and growths*"

Thursday, 26th February

- **09:00 – 10:00 Guest lecture: Cristina Máguas** (University of Lisbon, Portugal): *Understanding plant responses to water availability across ecosystems - why do we require multiple approaches and collaborative efforts?*
- **10:00 – 11:30 Session 4: New methodologies and technologies for monitoring plant water relations**. Moderators: Drs. José Javier Pigero (Aula Dei) and Raquel Lobo do Vale (Universidad de Ébora). Alternate: María Ancín (IdAB-CSIC).

  - **Lola Fariñas** (Universidad de Deusto, España): "Non-contact ultrasonic sensing of leaf water relations in field-grown avocado"
  - **Irene Galindo Lao** (Universidad de Sevilla, España): "*Continuous stem microtensiometry assessment for irrigation decision-support in mature orange orchards under regulated deficit irrigation*"
  - **Angie L. Gámez** (CSIC, España): "In situ estimation of δ^{13}C, δ^{15}N in alfalfa using canopy reflectance under contrasting water regimes"
  - **Virginia Hernández-Santana** (IRNAS-CSIC, España): "*Towards a mechanistic calibration of ¨optical dendrometers" for the construction of pressure volume curves and the prediction of leaf water potential*"
  - **Andreu Mairata** (Universitat de les Illes Balears, España): "Modelling of dynamics of stomatal conductance and hydraulics traits derived from optical dendrometry in woody crops"
  - **Beng P. Umali** (ICT International, Australia): "*In-situ Water Potential Measurement using Stem Psychrometer*"

- **11:30 – 12:15** Coffee break

- **12:15 – 13:30 Session 5: Integrated water management in agricultural and forest systems.** Moderator: Iker Aranjuelo (IdAB-CSIC) and Ismael Aranda (INIA-CSIC).

  - **Yolanda Gogorcena** (EEAD-CSIC, España): "*Climate Change Effects on Prunus spp.: Physiological-Metabolic Crosstalk with Signaling Molecules*"
  - **Alícia Pou** (ICVV-CSIC, España): "*Heat Waves Collapse Rootstock-Mediated Drought Tolerance in Grapevine*"
  - **Jaime Sebastián-Azcona** (IRNAS-CSIC, España): "*Tree ring evidence of interactive effects of climate variability and groundwater depth on Pinus pinea growth in Doñana National Park*"
  - **Olfa Zarrouk** (Instituto Politécnico de Santarém, Portugal): "*Drought-induced transgenerational memory primes grapevine progenies for enhanced tolerance to water stress and recovery*"

- **13:30 – 15:00** Lunch
- **15:00 – 16:00 Poster session**
- **16:00 – 16:30** Coffee break
- **16:30 – 17:15 Awards and recognition for a long research career** on plant water relations: Robert Save, Manuela Chaves and Hipólito Medrano.
- **17:15– 18:15 Official reception and presentation** of the **VI National Research Award of the Best PhD on Water Relations** and the **Best Project for a Young Researcher on Water Relations**.
- **18:15– 18:45** Meeting of the Water Relations Group
- **20:00 – 20:45** Guided tour of the historic center of Pamplona
- **21:00 – 23:00** Social dinner at Restaurante La Capilla**.**

Friday, 27th February

- **09:00 – 10:00 Lecture by José M. Torres-Ruíz (IRNAS-CSIC, Spain):** *How plant water traits help us to elucidate when, why, and which trees succumb to drought*
- **10:00 – 11:00 Session 6: Water relations in the context of global change**. Moderators: Clara Pinto (INIAV) and Francisco J. Cano (ISIFOR).

  - **María José Gómez-Bellot** (CEBAS-CSIC, España): "*Effects of irrigation with reclaimed water and brine on water relations, photosynthetic efficiency, nutrient uptake and ornamental indicators of two salinity-resistant plants*"
  - **Iván Jáuregui** (Universidad Pública de Navarra, España): "*Permanent crop cover as a strategy for drought-resistant viticulture: insights on how rhizosphere metagenomics influences leaf-level -omics for an enhanced overall plant response*"
  - **María Muñoz Santesteban** (IRNAS-CSIC, España): "*Saving water without sacrificing yield: a stomatal conductance-based irrigation strategy for Olive trees*"
  - **Clara Maria de Assunção Pinto** (Instituto Nacional de Investigação Agrária e Veterinária, I.P, Portugal): ¨*Competition for water drives hydraulic adjustment in Mediterranean cork oak–stone pine woodlands*¨.
  - **Eduardo Vargas-Fernández** (CEBAS-CSIC, España): "*Irrigation efficiency and prevention of deep percolation in grapefruit through soil sensor monitoring and agronomic evaluation in the Mar Menor watershed basin*"

- **11:00 – 12:00 Poster session**
- **12:00 – 12:30** Coffee break
- **13:00 –** Farewell

Session 1: Physiological and molecular mechanisms of water relations.

Physiologia Plantarum

Water as an evolutionary driver of photosynthesis: co-adaptation of internal CO_2 diffusion and Rubisco function during terrestrialization and aquatic recolonization of plants

Jeroni Galmés[1], Gabriel Silva Pires[1], Marcel Font-Carrascosa[1], Pere Aguiló-Nicolau[1], José Antonio Jurado-Rivera[2], Joan Pons[3], Mateu Fullana-Pericàs[4], Jaume Flexas[1], Concepción Íñiguez[1], Miquel Ribas-Carbó[1], Sebastià Capó-Bauçà, Hipólito Medrano[1]

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Introduction and objectives

Water has been a fundamental driver of plant evolution, not only by constraining growth and survival, but also by shaping the mechanisms through which plants acquire carbon. The evolution of photosynthesis, therefore, cannot be fully understood without considering water as a selective force acting on leaf structure, gas diffusion pathways, and enzymatic function. Changes in water availability and in the physical properties of the surrounding medium have repeatedly imposed limitations on CO_2 diffusion, forcing photosynthetic organisms to adjust both anatomical traits and biochemical processes to sustain carbon gain.

Two major evolutionary transitions exemplify the intimate link between water relations and photosynthesis. The first is the colonization of land by photosynthetic organisms, which exposed early embryophytes to intermittent water availability, desiccation risk, and a gaseous environment where CO_2 diffusion was faster but more variable than in water. The second is the recolonization of aquatic environments by terrestrial angiosperms, particularly marine angiosperms, which re-entered a medium where water is abundant but CO_2 diffusion is severely constrained. These transitions provide a unique evolutionary framework to examine how photosynthesis responds to contrasting hydric and gas diffusive environments.

In this context, two components emerge as central determinants of photosynthetic performance. The first is the internal diffusion of CO_2 within leaves, commonly described by mesophyll conductance (g_m), which links leaf anatomy, water-related structural traits, and CO_2 availability at the sites of carboxylation. The second is Rubisco, the enzyme responsible for CO_2 fixation, whose catalytic properties determine the efficiency and cost of carbon assimilation under given internal CO_2 and O_2 concentrations. Both g_m and Rubisco function are known to vary widely across photosynthetic lineages, yet their coordinated evolution across major hydric transitions remains insufficiently explored.

The objectives of this work are therefore:

1. To assess how internal CO_2 diffusion capacity and Rubisco properties have co-evolved during the terrestrialization of photosynthesis, spanning the major phylogenetic groups of land plants.
2. To disentangle the relative contributions of quantitative changes (Rubisco abundance) versus qualitative changes (Rubisco catalytic traits) to the evolutionary increase in photosynthetic capacity under terrestrial conditions.
3. To evaluate whether these terrestrial adaptations were reversed or reconfigured during the recolonization of aquatic environments by angiosperms.
4. To frame these evolutionary trajectories within a plant water-relations perspective, highlighting how water availability and diffusion constraints act as indirect but powerful drivers of photosynthetic evolution.

Terrestrialization of photosynthesis: water constraints, internal CO_2 diffusion, and Rubisco investment

The colonization of land by embryophytes during the Paleozoic represented a profound shift in the physical and physiological environment of photosynthesis. On land, CO_2 diffusion from the atmosphere is rapid, but its delivery to chloroplasts depends on a complex internal pathway shaped by leaf anatomy, hydration status, and tissue organization. At the same time, water availability became spatially and temporally heterogeneous, imposing selective pressure on leaf structure and hydraulic function.

Comparative analyses across bryophytes, lycophytes, ferns, and angiosperms reveal a progressive increase in mesophyll conductance to CO_2 (g_m) during land plant evolution. This increase is tightly associated with anatomical changes such as thinner mesophyll cell walls and a greater chloroplast surface area exposed to intercellular airspaces. These traits not only facilitate CO_2 diffusion but also reflect structural adjustments constrained by water transport, mechanical support, and cellular hydration.

Parallel to the increase in g_m, terrestrial plants exhibit a marked evolutionary increase in Rubisco concentration per unit leaf mass or area. Phylogenetic analyses indicate that Rubisco abundance, nitrogen investment in Rubisco, and total soluble protein content are conserved traits within lineages, suggesting that the evolution of higher photosynthetic capacity relied primarily on quantitative increases in carboxylation potential. Importantly, Rubisco concentration correlates strongly with g_m and with anatomical traits governing diffusion, indicating a coordinated optimization between substrate supply and enzymatic demand.

Although Rubisco catalytic parameters also show evolutionary trends—such as higher specificity for CO_2 and increased affinity for substrates in diffusion-limited systems—their contribution to increased photosynthetic capacity is secondary compared with Rubisco abundance. These results support the view that terrestrialization favored anatomical and diffusive innovations that allowed greater Rubisco investment to be effectively exploited, rather than a radical redesign of Rubisco catalysis itself.

Recolonization of aquatic environments: diffusion limitation, CCMs, and Rubisco reconfiguration

The return of angiosperms to aquatic environments, particularly marine systems, imposed a radically different set of constraints. While water availability is no longer limiting, CO_2 diffusion in water is orders of magnitude slower than in air, and inorganic carbon is predominantly present as bicarbonate. Marine angiosperms therefore face extreme limitations on CO_2 acquisition, despite inhabiting water-rich environments.

Anatomically, these species display traits that sharply contrast with terrestrial angiosperms: absence of stomata, lack of internal airspaces connected to the atmosphere, extremely thick cell walls, and reduced chloroplast numbers positioned near the leaf surface. These features result in extraordinarily low mesophyll conductance to CO_2. However, in aquatic angiosperms, low g_m serves a different function than in terrestrial plants: it limits CO_2 leakage from tissues, thereby enhancing the efficiency of carbon-concentrating mechanisms (CCMs).

Our analyses reveal that marine angiosperms possess highly effective CCMs, as demonstrated by large discrepancies between *in vitro* Rubisco K_c values and *in vivo* apparent K_m for CO_2. Concomitant with the evolution of CCMs, Rubisco in these species exhibits lower catalytic efficiency and reduced affinity for CO_2 and O_2. Modeling of Rubisco performance under elevated internal O_2 concentrations—likely resulting from restricted O_2 diffusion—shows that these catalytic shifts reduce the inhibitory effects of high O_2 on photosynthesis, illustrating a clear case of adaptive enzyme evolution driven by internal gas composition.

Integrating terrestrialization and aquatic recolonization: a water-relations perspective

When considered together, terrestrialization and aquatic recolonization reveal a coherent evolutionary narrative in which water governs photosynthesis indirectly through its effects on diffusion pathways, tissue structure, and internal gas dynamics. During terrestrialization, increasing control over water loss and internal CO_2 diffusion enabled higher Rubisco investment and greater photosynthetic capacity. During aquatic recolonization, the physical properties of water imposed severe diffusion constraints that selected for CCMs, reduced internal conductance, and a reconfiguration of Rubisco catalytic traits.

These findings underscore the central role of the tandem mesophyll conductance – Rubisco as a nexus between water relations and carbon assimilation, and highlight the necessity of integrating anatomical, physiological, and biochemical perspectives to understand photosynthetic evolution.

Keywords: (about 6 words): Plant water relations, Mesophyll conductance, Rubisco evolution, CO_2 diffusion, Terrestrialization of photosynthesis, Aquatic angiosperms.

Funding: This work was financially supported by the Spanish Ministry of Sciences, Innovation and Universities, the Spanish State Research Agency and the European Regional Development Funds (project UNRAVENAR PID2023-148523NB-I00).

The strange case of the wrong stomata and the caterpillars

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Abstract:

Plants have evolved a wide array of mechanisms to interact with—and respond to—environmental change. Many of these mechanisms, associated with abiotic drivers such as radiation, temperature, vapor pressure deficit, and soil water deficit, have been studied in depth, providing a solid understanding of their functioning across organizational levels. Likewise, biotic factors—including pathogens, herbivores, plant–plant interactions, and plant–microbiome relationships—have been extensively characterized. Yet several processes central to integrated whole-plant coordination, and potentially to plant–plant communication, are receiving renewed attention. Among these, volatile compounds and, especially, electrical signals are increasingly recognized as rapid modulators of wound and herbivory responses, stress acclimation, systemic coordination, and hormonal regulation. Classical examples include rapid leaf movements in carnivorous plants and systemic defense activation during herbivory, while recent work points to complex gas-exchange responses to wounding in which electrical, hydraulic, and chemical cues coexist and propagate beyond the injured tissue.

In this talk, I will briefly discuss a stomatal behavior that has gone largely unnoticed by other researchers when interpreting their results. The stomatal response to wounding reveals subtle patterns that appear to be species-specific in certain plant families. Evolutionary pressures may have shaped these responses, and the resulting traits can now help us better understand fundamental physiological mechanisms related to plant water status and the regulation of leaf gas exchange.

Keywords: Signal transduction, hydraulic integration, stress physiology, phylogenetic constraints

Crosstalk between nitrogen remobilisation and carbon metabolism in sugarcane facing drought stress and rehydration

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Nitrogen (N) is a essential nutrient driving biomass production in C4 crops like sugarcane (*Saccharum* spp.), playing a critical role in the biosynthesis of chlorophyll and photosynthetic enzymes (Rubisco and PEPC). However, drought stress disrupts the synergy between Carbon (C) and N metabolism. Simplifing, water deficit restricts root N uptake due to reduced transpiration and impairs carbon fixation via stomatal closure. To survive these limitations, plants rely on the remobilization of internal N reserves from senescing tissues to sink organs. In sugarcane, the physiological response to drought is often non-uniform across the leaf blade due to the distinct developmental ages of tissue from the base (younger, expanding) to the tip (older, mature). While previous research has focused on drought onset, the dynamics of N redistribution during the rehydration phase remain less understood. Furthermore, the metabolic coupling between N remobilization and the enzymatic machinery—specifically Glutamine Synthetase (GS), Glutamate Synthase (GOGAT), and Nitrate/Nitrite Reductases (NR/NiR)—requires elucidation under fluctuating water regimes. This study investigates the crosstalk between remobilization and C metabolism in sugarcane. We tested the hypothesis that drought induces a "storage strategy," moving N to roots and sheath leaves via GS-mediated recycling, which is then remobilized to photosynthetic tissues upon rehydration to support the recovery of gas exchange at specific leaf segments (Base vs. Tip).Sugarcane plants (cv. CTC9001bt) were grown in a greenhouse. At 25 days after transplanting (DAT), the +1 leaf was pulse-labeled with ^{15}N-enriched ammonium sulfate (10% atoms excess). Plants were divided into two treatments: Well-Watered (WW), maintened at ~90% WHC, and Water-Deficit (WD), which was exposes to sequential water withholding during 11 days, reaching ~20% WHC. Stress was imposed from 27 DAT until Maximum Stress (MS) at 38 DAT, followed by rehydration (R) until 45 DAT. The gas

exchange was measured in three sections of the leaf +1, at the Base, Middle, and Tip of the leaf blade. We partitioned the biomass (roots, stems, sheaths, leaves) to determine the metabolic intermediates (ammonium, nitrate, proline, sugars), and the activity of key N-metabolism enzymes: Nitrate Reductase (NR), Glutamine Synthetase (GS), and GOGAT in these tissues. Drought imposed severe limitations on leaf physiology, but the impact was spatially heterogeneous. Until reach the maximum tress (MS) the stomatal conductance (gs) reduced significantly in WD plants, however in different rates among the parts of the leaf blade. The restriction was most severe at the leaf base and tip compared to the middle, which took 13 days to reach the minimum value, whereas the tip took only 7 days. As a consequence of the stomatal closure the photosynthesis and transpiration declined in a similar manner. The chlorophyll content reduced through the water deficit. In contrast to WW plants, which maintained relatively stable chlorophyll levels throughout the period, the chlorophyll degradation occurred during the water withholding. It is important to notice, the degradation was spatially heterogeneous, being significantly more pronounced in the leaf tip compared to the middle and base of the leaf blade. However, during rehydration, a recovery trajectory in pigment levels was observed in the WD plants. The leaf base demonstrated the highest recovery capacity, with chlorophyll *a* and *b* levels nearly returning to the levels observed in the control (WW) plants, whereas the leaf tip showed an incomplete recovery, suggesting that prolonged water stress caused more persistent damage or accelerated senescence in the older tissues of the leaf tip. This suggests that younger tissues at the base possess higher metabolic plasticity and repair capacity. The isotopic tracing revealed a strategic shift in N allocation driven by water status. Under WD conditions (MS), the plant prioritized N storage over vegetative growth. There was a marked remobilization of towards roots and sheath leaves, acting as temporary reservoirs. Concurrently, total N content decreased in leaf blades and increased in roots/sheaths. It seems they were acting as N storage organs, improving Nitrogen Use Efficiency (NUE) in them. Following rehydration, the dynamic reversed and total N were remobilized from the roots/sheaths back to the aerial photosynthetic tissues (marked and top leaves). This N return was crucial for synthesizing new chlorophyll and repairing the photosynthetic apparatus, correlating directly with the chlorophyll recovery of at the leaf base, which was faster than middle and tip of the blade. The movement of N was tightly coupled with the activity of N-assimilating enzymes, responding to the accumulation of ammonium and the reduction of nitrate. NR activity is highly energy-dependent, requiring reducing equivalents (NADH/NADPH) derived from respiration/photosynthesis. Consequently, NR activity declined sharply in WD roots and leaves during MS, following the photosynthetic limitation. This downregulation seems to prevents the accumulation of toxic ammonium when C-skeletons are scarce. Post-rehydration, NR activity recovered in roots, signaling a resumption of primary N uptake. In contrast the GS activity increased in WD plants, particularly in top leaves at MS and in roots after rehydration. Ammonium levels were significantly elevated under drought stress. The upregulation of GS suggests an active detoxification of the ammonium produced by protein degradation and/or photorespiration (which can occur in C4 sugarcane under stressful condition), recycling it into amino acids (glutamine) for transport to storage organs. GOGAT levels remained relatively stable, indicating that GS was the rate-limiting step for ammonium reassimilation under stress. The coordination between C and N is further evidenced by osmotic adjustment. WD plants accumulated significant amounts of proline (especially in roots/stems), soluble sugars, and amino acids during MS. This accumulation serves two functions: protecting cellular turgor and acting as C and N reserves. Upon rehydration, the concentration of these compatible solutes declined, suggesting their metabolic consumption to rebuilt photosynthetic machinery. The Carbon Isotope Discrimination ($D^{13}C$) decreased under stress, which is related to better Water Use Efficiency, but increased after rehydration, confirming the reopening of stomata. This study demonstrates that sugarcane drought tolerance relies on a tightly coordinated crosstalk between Nitrogen and Carbon metabolism.

Keywords: Nitrogen remobilisation, ^{15}N isotopic labelling, drought stress, rehydration, nitrogen-use efficiency, sugarcane, *Saccharum* spp., carbon metabolism.
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Age-dependent changes in cell-level anatomy and leaf hydraulic conductance triggers the decline in photosynthetic capacity in older leaves of the evergreen Mediterranean oak *Quercus ilex* subsp. *rotundifolia*

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Abstract:

Leaves of Mediterranean evergreen tree species experience a reduction in the activity of the photosynthetic apparatus during aging and senescence associated with age-dependent changes in stomatal conductance (g_s), mesophyll conductance to CO_2 (g_m) and net CO_2 assimilation (A_N). This process would be influenced by changes in leaf anatomical traits at cell level and cytoplasmic nitrogen that finally led to the dismantling of the photosynthetic apparatus. However, this is a matter that is far from being elucidated in old non-senescent leaves of evergreen trees with long leaf lifespan. Moreover, it remains unclear the time course and the ultimate mechanistic factor that triggers this phenomenon. This study evaluated age-dependent changes in anatomical, biochemical, hydraulic and photosynthetic traits during three consecutive vegetative periods in current, 1-year and 2-year-old leaves of holm oak (*Quercus ilex* subsp. *rotundifolia* Lam.), an evergreen oak with high leaf longevity. We found that the maximum activity of the photosynthetic apparatus of current-year leaves occurred during early summer, together with the coordinated maturation of anatomical traits (i.e. mesophyll and chloroplast surface area exposed to intercellular air space, S_m/S and S_c/S) and the increased ability for CO_2 diffusion trough stomata (g_s) and mesophyll (g_m). Thereafter, we evidenced that all photosynthetic traits declined with increasing leaf age, mainly due to decreases S_c/S, which was a key anatomical trait explaining variations in

g_m and A_N among different age classes. The reduction of S_c/S were related to ultrastructural changes in chloroplasts associated to leaf aging, with a concomitant reduction in cytoplasmic nitrogen. Furthermore, 2-years old leaves experienced an abrupt decrease in leaf hydraulic conductance (K_{leaf}), which indicated a coordination between A_N and K_{leaf} that might be mediated by cell-level anatomical traits such as S_c/S. Our findings demonstrated for the first time that limitations of water transport through the leaves (i.e. K_{leaf}) acted as the triggering factor involved in the deterioration of the photosynthetic activity in older leaves of evergreen tree species with long leaf lifespan such as holm oak. However, contrary to expectations, abscisic acid (ABA) did not appear to play a main role as a signaling molecule in this process. This study contributes to a deeper understanding of the physiological and anatomical mechanisms underlying leaf development and aging in long-lived evergreen species, providing insights into the ecological strategies that govern canopy water consumption and CO_2 assimilation.

Keywords: abscisic acid, leaf aging, leaf anatomy, leaf hydraulic, mesophyll conductance, nitrogen, photosynthesis

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