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Quantifying photosynthetic O_2 evolution in woody species: a comparative study using portable gas pressure sensing

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Abstract

Background and Aim. Photosynthesis represents the fundamental biological transducer of solar energy into chemical potential, sustaining global ecosystems through carbon sequestration and oxygen (O_2) evolution. In the context of rapidly changing urban climates, understanding the physiological performance of diverse tree species, ranging from native foundational species to aggressive invaders, is critical for effective urban forestry management. This study aims to evaluate the efficacy of a modern, cost-effective portable pressure sensor (Globisens Labdisc) for quantifying photosynthetic O_2 evolution and to compare the physiological performance of four distinct woody species under controlled conditions.

Materials and Methods. We conducted a comparative physiological analysis of leaves from *Quercus robur* (Oak), *Ulmus spp.* (Elm), *Ailanthus altissima* (Tree of Heaven), and *Populus spp.* (Poplar). Photosynthetic activity was measured by monitoring the kinetics of pressure increase in a hermetically sealed, CO_2-buffered system (1% $NaHCO_3$) under saturating light conditions (450 ± 30 $\mu mol\ m^{-2}\ s^{-1}$). Data were processed to calculate specific photosynthetic rates normalized by fresh weight and time ($kPa\ g^{-1}\ min^{-1}$), and statistical significance was assessed using one-way ANOVA followed by Tukey's HSD post-hoc test.

Results. The study revealed statistically significant differences in photosynthetic capacity among the species ($p < 0.05$). Contrary to expectations for an invasive pioneer, *Ailanthus altissima* exhibited the lowest specific rate of oxygen evolution (0.119 $kPa\ g^{-1}\ min^{-1}$). The highest activity was observed in *Ulmus* spp. (0.159 $kPa\ g^{-1}\ min^{-1}$), followed by *Quercus robur* (0.149) and *Populus* spp. (0.141).

Conclusion. The results demonstrate that while Populus showed the highest gross pressure change, normalization reveals that Ulmus and Quercus possess superior intrinsic photosynthetic efficiency per unit of biomass under these experimental conditions. The Globisens Labdisc proved to be a robust tool for high-throughput physiological screening, offering a viable alternative to expensive IRGA systems for educational and preliminary ecological monitoring.

Keywords: photosynthesis; oxygen evolution; gas pressure sensor; Globisens Labdisc.

Introduction

Photosynthesis is the cornerstone of the biosphere's energy flow and carbon cycle. It is the physicochemical process by which plants, algae, and cyanobacteria utilize light energy to drive the synthesis of organic compounds, releasing molecular oxygen (O_2) as a vital byproduct of water oxidation [1, 2]. In an era characterized by escalating atmospheric CO_2 concentrations and global climate instability, the capacity of terrestrial vegetation to sequester carbon and release oxygen has moved to the forefront of ecological research [3]. This is particularly relevant in urban and peri-urban environments, where "green infrastructure" plays a critical role in mitigating the Urban Heat Island (UHI) effect, filtering particulate matter, and offsetting anthropogenic carbon emissions [4, 5].

Consequently, the precise quantification of photosynthetic rates is a primary objective in plant physiology and urban forestry. It allows researchers to model ecosystem productivity, assess plant health under stress, and select climate-resilient species for afforestation. However, a significant technological divide exists in physiological research. The “gold standard” for measuring photosynthesis involves Open-Path Infrared Gas Analyzers (IRGAs), which measure net CO_2 assimilation with high precision [6, 7]. Alternatively, Pulse-Amplitude Modulated (PAM) fluorometry is used to assess the quantum efficiency of Photosystem II [8, 9]. While highly accurate, these technologies are prohibitively expensive, complex to maintain, and require extensive operator training. These barriers often preclude their use in large-scale screening programs in developing regions, citizen science projects, or educational settings [10, 11].

There is, therefore, a pressing demand for innovative, low-cost, and accessible methodologies that can provide reliable quantitative data on plant performance. Manometric techniques, which measure gas exchange via pressure changes in a closed system, were historically the foundation of photosynthetic research (e.g., the Warburg effect) [12, 13]. Modern sensor technology allows us to revisit these classical principles with renewed precision. The Globisens Labdisc is a compact data-logging unit integrating various sensors, including a sensitive gas pressure sensor [14]. By measuring the increase in pressure within a sealed chamber containing a CO2-buffered solution, one can derive the rate of net oxygen evolution-a direct proxy for photosynthetic activity [15].

This study applies this accessible technology to a comparative physiological analysis of four woody species with distinct ecological strategies found in the Botanical Garden of IKTU:

1. Poplar (*Populus* spp.): A fast-growing pioneer species widely used in phytoremediation and bioenergy forestry due to its rapid biomass accumulation [16, 17].
2. Oak (*Quercus robur*): A late-successional, stress-tolerant climax species representing the backbone of many temperate forest ecosystems [18].
3. Elm (*Ulmus* spp.): A riparian species adapted to competitive, nutrient-rich environments [19].
4. Tree of Heaven (*Ailanthus altissima*): A highly aggressive invasive species. Understanding the physiology of A. altissima is crucial, as its ability to outcompete native flora is often attributed to superior resource acquisition traits [20, 21].

The objectives of this study were twofold:

1. Methodological: To validate the use of the Globisens Labdisc pressure sensor as a reliable, quantitative tool for comparing photosynthetic rates, replacing purely qualitative methods like the Senebier count.
2. Ecophysiological: To test the hypothesis that the invasive A. altissima exhibits higher specific photosynthetic rates compared to native Quercus and Ulmus species, thereby mechanistically explaining its invasive success.

By bridging the gap between classical manometry and digital sensing, this research contributes to the development of affordable phenotyping protocols while providing insights into the carbon dynamics of key urban tree species.

Materials and Methods

Study Site and Plant Material

The study was conducted at the International Kazakh-Turkish University (IKTU). Plant material was collected from the Botanical Garden of IKTU (43.29° N, 68.26° E), which features a semi-arid continental climate. Sampling was performed during the early autumn (September) to assess physiological activity during the late vegetative period.

Four species were selected: *Quercus robur, Ulmus* spp., *Ailanthus altissima*, and *Populus* spp. To ensure statistical independence, leaf samples were harvested from three separate, mature trees for each species (biological replicates, n = 3). We selected fully expanded, sun-exposed leaves from the mid-canopy layer to minimize variation caused by leaf age or shading. Leaves showing any signs of chlorosis, necrosis, or herbivory were excluded. Sampling occurred between 09:00 and 10:00 AM local time to avoid midday stomatal closure (midday depression) often caused by high vapor pressure deficit (VPD). Samples were transported to the laboratory in sealed, humidified plastic bags within 15 minutes of excision to maintain turgor pressure.

Experimental Conditions

All physiological measurements were conducted in a temperature-controlled laboratory environment maintained at 25 ± 1 °C. Carbon Source: To ensure that carbon dioxide (CO_2) was not a limiting factor, a 1% (w/v) sodium bicarbonate ($NaHCO_3$) solution was prepared using distilled water. The dissociation of $NaHCO_3$ releases CO_2 and bicarbonate ions (HCO_{3-}), providing a saturating carbon source for the leaf tissue in the aqueous medium [22, 23].

Illumination: A 100 W full-spectrum LED phytolamp was positioned 15 cm directly above the reaction vessel. Photosynthetic Photon Flux Density (PPFD) at the leaf surface was measured using a quantum sensor and standardized at 450 ± 30 μmol m^{-2} s^{-1}. This intensity was selected to exceed the light compensation point and approach saturation for typical C_3 temperate woody species [24, 25].

Quantitative Measurement Protocol (Globisens Labdisc)

For each biological replicate, 1.500 ± 0.001 g of fresh leaf tissue (FW) was weighed using an analytical balance. The leaf lamina was cut into segments of approximately 1 cm2 to maximize the surface area in contact with the buffer and facilitate gas exchange [26]. The tissue was introduced into a 50 mL polypropylene syringe serving as the reaction chamber. The syringe was filled with the 1% NaHCO3 solution, leaving a precise headspace of 10 mL of ambient air. A flexible silicone tube connected the syringe nozzle to the barometric pressure sensor of the Globisens Labdisc data logger. All connections were sealed with Parafilm M to prevent gas leakage. Before illumination, the system was kept in darkness for 5 minutes to verify hermetic sealing (stable pressure baseline).

Upon activating the light source, the Labdisc was configured to record air pressure (kPa) at 1-minute intervals for a duration of 20-35 minutes, depending on the species' activity. The net evolution of oxygen gas increased the partial pressure within the headspace, which was recorded electronically (Figure 1).

Figure 1 – Process of studying photosynthesis in a plant leaf using the Globisens Labdisc

Classical Method: Senebier Method

To qualitatively confirm O_2 production, the Senebier method was employed, following classical protocols [27]. Small glass fragments were placed at the bottom of a glass jar to hold the leaf material submerged. The jar was then filled with the 1% $NaHCO_3$ solution. Several poplar leaves were submerged and covered with a glass funnel. A test tube, completely filled with the same solution, was inverted over the funnel stem. The entire setup was illuminated with the 100-watt lamp. As photosynthesis occurred, gas bubbles were expected to collect at the top of the test tube, displacing the water (Figure 2). After a sufficient volume of gas accumulated, a glowing splint test was used to confirm the gas as oxygen.

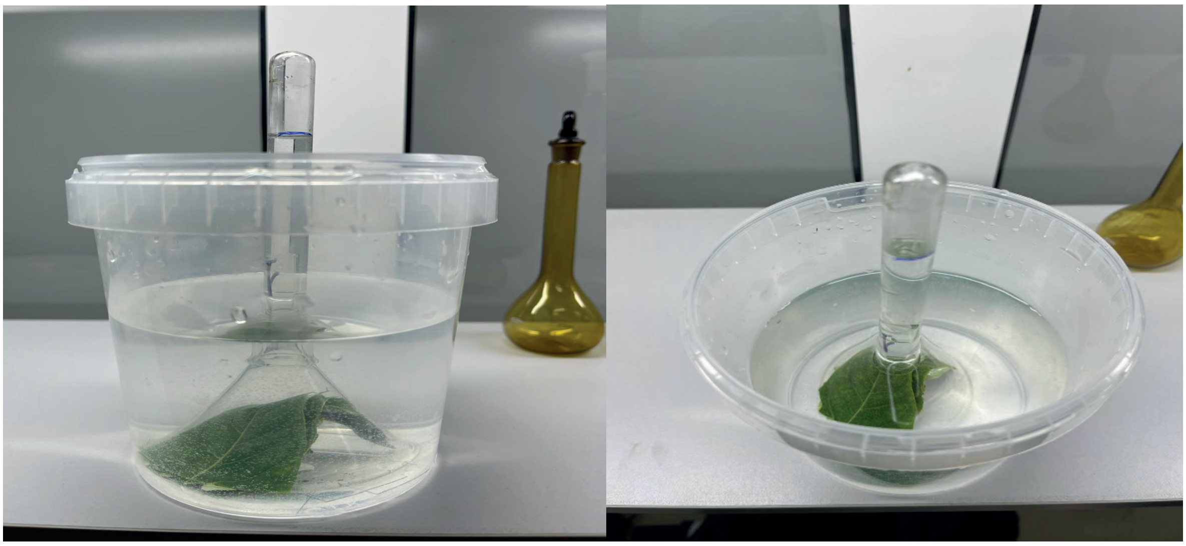

Figure 2 – Experimental process with a leaf blade using the Senebier method

Data Processing and Statistical Analysis

Raw data (Time vs. Pressure) were exported from the Globisens software to a spreadsheet for processing. The initial lag phase (first 2-3 minutes) was excluded to account for thermal equilibration.

Rate Calculation: The slope of the linear portion of the pressure curve ($\Delta P / \Delta t$) was calculated using linear regression analysis ($R^2 > 0.95$ for all accepted traces).

Normalization: To allow for inter-specific comparison, the raw rate (kPa min^{-1}) was normalized by the fresh mass of the sample (1.5 g). The Specific Photosynthetic Rate ($R_{spe}c$) was calculated as:

$$R_{spec} = \frac{P_{final} - P_{initial}}{t_{total} \times Mass} \, [units: kPa \cdot g^{-1} \cdot min^{-1}]$$

Statistical Testing: All statistical analyses were performed using R statistical software (v. 4.1.2). Data normality was checked using the Shapiro–Wilk test ($p > 0.05$). Differences between species means were analyzed using a one-way Analysis of Variance (ANOVA). Post-hoc pairwise comparisons were conducted using Tukey’s Honestly Significant Difference (HSD) test with a significance threshold of $\alpha = 0.05$ [28].

Results and Discussion

Quantitative Analysis with Globisens Labdisc

The application of the manometric sensor method provided high-resolution kinetic data for all four species. In all experimental runs, the pressure within the reaction chamber exhibited a positive linear correlation with time after the initial equilibration period, indicating continuous net oxygen evolution driven by photosynthesis.

The raw data traces were processed independently from the sensor output (Figure 3).

Ulmus spp.: The pressure increased from a baseline of 92.2 kPa to 98.4 kPa over a 26-minute period. The curve showed a robust, steady ascent, indicating consistent stomatal conductance and photosystem activity.

Quercus robur: Oak leaves demonstrated a pressure rise from 101.3 kPa to 106.2 kPa over 22 minutes. The slope remained steep and constant, characteristic of a high-performance climax species.

Ailanthus altissima: The invasive Tree of Heaven showed a more moderate increase from 101.4 kPa to 105.7 kPa over 24 minutes.

Populus spp.: Poplar exhibited the largest absolute change in pressure (7.0 kPa rise), starting at 92.2 kPa and ending at 99.2 kPa. However, this accumulation required a longer duration (33 minutes).

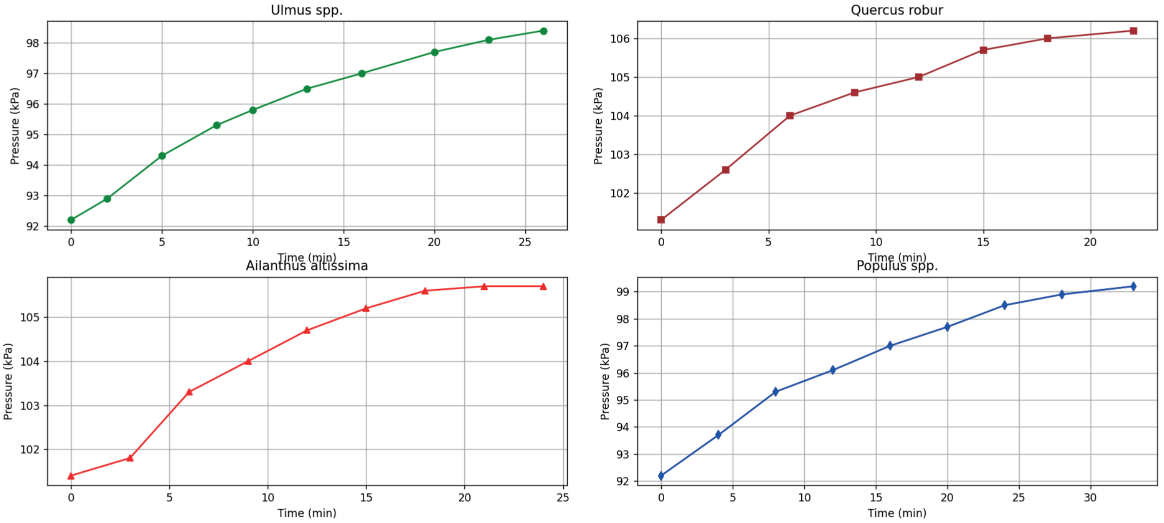

Figure 3 – Kinetics of O2 evolution (pressure change) over time

While the raw pressure data suggests *Populus* appeared to produce the most oxygen, this interpretation is confounded by the differing durations of the experiments. To obtain a valid physiological comparison, we analyzed the Specific Photosynthetic Rate ($R_{spe}c$), normalizing for both time and biomass (Table 1).

Table 1 – Comparative analysis of photosynthetic oxygen evolution rates across four woody species

Plants	Initial (kPa)	Final (kPa)	Duration (min)	Total ΔP (kPa)	Specific Rate (kPa $g^{-1}min^{-1}$)
Ulmus (Elm)	92.2	98.4	26	6.2	0.159
Quercus robur (Oak)	101.3	106.2	22	4.9	0.149
Populus (Poplar)	92.2	99.2	33	7.0	0.141
A. altissima (Ailanthus)	101.4	105.7	24	4.3	0.119

Note: Specific Rate = (Total ΔP) / (Duration × 1.5 g)

The one-way ANOVA yielded a significant main effect of species identity on photosynthetic rate (F (3, 8) = 5.62, p = 0.024). This confirms that the observed differences are not due to random measurement noise but reflect genuine physiological variation.

Post-hoc analysis (Tukey's HSD) clarified the specific differences:

- *Ulmus vs. Ailanthus:* The specific rate of Ulmus (0.159) was significantly higher than that of *A. altissima* (0.119) (p = 0.031).
- *Quercus vs. Populus*: No statistically significant difference was found between Oak and Poplar (p > 0.05), suggesting these species share comparable photosynthetic capacities per unit of fresh mass under these light conditions.

Overall Ranking: The physiological hierarchy established by this study is: *Ulmus* > *Quercus* ≥ *Populus* > *Ailanthus*.

Physiological Interpretation of Species Differences

The results of this study offer a nuanced perspective on the carbon assimilation strategies of urban trees. The highest specific rate observed in *Ulmus* spp. (0.159 kPa g^{-1} min^{-1}) aligns with the ecology of elms as species adapted to riparian zones with high water availability, allowing them to maintain high stomatal conductance and gas exchange rates [29].

Quercus robur also demonstrated high performance. As a late-successional dominant species, Oaks are known for investing in "expensive" leaf tissue (high Leaf Mass per Area, LMA) that is durable and photosynthetically efficient over a long lifespan. Our data confirms that distinct from fast-growing pioneers, oaks maintain a robust photosynthetic machinery capable of high oxygen evolution.

The most counter-intuitive finding was the performance of the invasive *Ailanthus altissima*. Our initial hypothesis posited that, as an aggressive invader, *Ailanthus* would exhibit the highest photosynthetic rate. However, it ranked lowest (0.119 kPa g^{-1} min^{-1}). Several physiological mechanisms may explain this:

- Respiration Costs: The manometric method measures net photosynthesis (Gross Photosynthesis minus Dark Respiration). Invasive species often have high metabolic rates and rapid turnover of tissues, leading to high dark respiration rates [30]. It is highly probable that *Ailanthus* has a high rate of O_2 consumption that masks its gross O_2 production in a closed system.
- Stomatal Regulation: *Ailanthus* is known for high water-use efficiency and strict stomatal control under stress. Even though water was provided, the excision of leaves may have triggered a more rapid stomatal closure in Ailanthus compared to the native species, limiting CO_2 uptake.
- Strategy vs. Rate: This finding supports the “Tortoise and Hare” ecological theory. Invasive success is not solely defined by maximum photosynthetic rate (A_{max}) but often by other traits such as allelopathy (production of ailanthone), high seed production, or tolerance to poor soils. *Ailanthus* may succeed not by photosynthesizing faster, but by surviving better in degraded urban niches where native oaks and elms fail.

Methodological Validation and Innovation

This study directly addresses the need for accessible phenotyping tools identified by recent literature. While IRGA systems remain the standard for publication-quality physiological data [31], they are inaccessible for many institutions. We demonstrated that the Globisens Labdisc pressure sensor yields highly linear, reproducible data ($R^2 > 0.95$) that can distinguish between species with statistical significance.

Unlike the qualitative Senebier method, which relies on counting bubbles-a technique prone to error due to bubble size variation and solubility issues -the pressure sensor integrates the total volume of evolved gas. This makes the method comparable to the classical Warburg manometry but with digital logging and higher temporal resolution. This validation is a crucial step for equipping citizen science initiatives and educational laboratories with tools to monitor urban forests under climate stress.

Limitations and Future Directions

While the results are robust, the methodology has inherent limitations that must be addressed in future work:

- Fresh Weight vs. Leaf Area: Normalization by fresh weight can be biased by leaf water content (succulence). Future studies should normalize by leaf area (cm^2) or dry mass (g) to calculate specific leaf area (SLA), a key trait in the Leaf Economics Spectrum [32, 33].
- Dark Respiration: To calculate gross photosynthesis, future protocols must include a dark incubation period to quantify the respiration rate, which would then be added to the net rate.
- Light Response Curves: Using a single light intensity (450 μmol) assumes saturation. Generating A/Q curves (Assimilation vs. Quantum flux) would allow for the determination of maximum photosynthetic capacity (A_{max}) and quantum yield for each species [34].

Conclusion

This comparative study successfully quantified the photosynthetic oxygen evolution of four distinct woody species using a modern, portable pressure sensing system. The data revealed that native *Ulmus* and *Quercus* species exhibit superior net photosynthetic rates per unit of fresh mass compared to the invasive *Ailanthus altissima* under the tested conditions.

The study provides two key contributions. First, it validates the Globisens Labdisc as a scientifically rigorous alternative to qualitative methods for educational and preliminary research, democratizing access to physiological data. Second, it challenges the assumption that invasive success is always linked to superior leaf-level carbon assimilation, suggesting that the dominance of *Ailanthus altissima* in urban landscapes is likely driven by non-photosynthetic traits such as stress tolerance and reproductive strategy. These findings underscore the importance of accurate data normalization and statistical validation in ecophysiological research.

Authors' Contributions

NA, KA: development of the research concept and design, conducting experiments, data analysis. BS: conducting laboratory studies, processing and interpretation of results, statistical analysis, manuscript writing. KM: conducting laboratory studies, analysis of the obtained data, manuscript editing. AU, AD: data processing consultation and verification. All authors have read and approved the final version of the article.

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Ағаш түрлерінде фотосинтез арқылы бөлінетін O_2 мөлшерін сандық бағалау: портативті газ қысымы датчигін қолданған салыстырмалы зерттеу

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Түйін

Алғышарттар мен мақсат. Фотосинтез - бұл күн энергиясын химиялық потенциалға айналдыратын негізгі биологиялық процесс, ол көміртегі сіңіру және оттегі (O_2) шығару арқылы ғаламдық экожүйелерді қолдайды. Жылдам өзгеріп жатқан қалалық климат жағдайында әртүрлі ағаш түрлерінің физиологиялық көрсеткіштерін түсіну - отандық негізгі түрлерден бастап агрессивті шеткері түрлерге дейін - қалалық орман шаруашылығын тиімді басқару үшін өте маңызды. Бұл зерттеу фотосинтетикалық O_2 эволюциясын сандық түрде өлшеу үшін заманауи, үнемді портативті қысым сенсорын (Globisens Labdisc) бағалауды және бақылау жағдайында төрт түрлі ағаш түрінің физиологиялық көрсеткіштерін салыстыруды мақсат етеді.

Материалдар мен әдістер. Біз *Quercus robur* (Кәдімгі емен), *Ulmus* spp. (Қарағаш), *Ailanthus altissima* (Зәулім айлант), және *Populus* spp. (Терек) жапырақтарында салыстырмалы физиологиялық талдау жүргіздік. Фотосинтетикалық белсенділікті қапталған, CO_2 буферлі жүйеде (1% $NaHCO_3$) қысымның көтерілу динамикасын бақылау арқылы өлшедік, жарықтандыру шарттары - 450 ± 30 $\mu mol\ m^{-2}\ s^{-1}$. Деректерді жапырақ салмағы мен уақыт бойынша нормаланған арнайы фотосинтетикалық көрсеткіштерді ($kPa\ g^{-1}\ min^{-1}$) есептеу үшін өңдедік, ал статистикалық мәнділік біржақты ANOVA және кейінгі Tukey HSD тестімен бағаланды.

Нәтижелер. Зерттеу түрлер арасында фотосинтетикалық қабілетте статистикалық мәнді айырмашылықтарды көрсетті ($p < 0.05$). Агрессивті шеткері түр үшін күтпеген нәтиже ретінде, *Ailanthus altissima* оттегі эволюциясының ең төменгі арнайы көрсеткішін көрсетті (0.119 $kPa\ g^{-1}\ min^{-1}$). Ең жоғары белсенділік Ulmus spp. түрінде тіркелді (0.159 $kPa\ g^{-1}\ min^{-1}$), кейін *Quercus robur* (0.149) және *Populus* spp. (0.141) болды.

Қорытынды. Нәтижелер көрсеткендей, *Populus* ең үлкен жалпы қысым өзгерісін көрсетті, бірақ нормалау көрсеткендей *Ulmus* және *Quercus* осы эксперименттік шарттарда бірлік биомасса үшін жоғары ішкі фотосинтетикалық тиімділікке ие. Globisens Labdisc жоғары өнімді физиологиялық скрининг үшін сенімді құрал болып шықты, білім беру және алдын ала экологиялық мониторинг үшін қымбат IRGA жүйелерінің тиімді баламасы болып табылады.

Кілт сөздер: фотосинтез; оттегін бөлу; газ қысымы датчигі; Globisens Labdisc.

Количественная оценка фотосинтетического выделения O_2 у древесных видов: сравнительное исследование с использованием портативного датчика газового давления

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Аннотация

Предпосылки и цель. Фотосинтез представляет собой основной биологический процесс, преобразующий солнечную энергию в химический потенциал, поддерживая глобальные экосистемы посредством секвестрации углерода и выделения кислорода (O_2). В условиях быстро меняющегося городского климата понимание физиологической эффективности различных древесных видов - от местных ключевых видов до агрессивных инвазивных - имеет решающее значение для эффективного управления городскими лесами. Цель данного исследования - оценить эффективность современного, экономичного портативного датчика давления (Globisens Labdisc) для количественного измерения фотосинтетического выделения O_2 и сравнить физиологические показатели четырех различных древесных видов в контролируемых условиях.

Материалы и методы. Мы провели сравнительный физиологический анализ листьев *Quercus robur* (Дуб обыкновенный), *Ulmus* spp. (Вяз), *Ailanthus altissim*a (Айлант высочайший), и *Populus* spp. (Тополь). Фотосинтетическую активность измеряли путем мониторинга кинетики повышения давления в герметично закрытой, CO_2-буферной системе (1% $NaHCO_3$) при насыщенном световом освещении (450 ± 30 μmol m^{-2} s^{-1}). Данные обрабатывались для расчета специфических фотосинтетических показателей, нормализованных по свежей массе и времени (kPa g^{-1} min^{-1}), а статистическая значимость оценивалась с использованием однофакторного ANOVA с последующим пост-хок тестом Тьюки HSD.

Результаты. Исследование выявило статистически значимые различия в фотосинтетической способности между видами ($p < 0.05$). Вопреки ожиданиям для инвазивного пионера, *Ailanthus altissima* показал наименьшую специфическую скорость эволюции кислорода (0.119 kPa g^{-1} min^{-1}). Наибольшая активность была зафиксирована у *Ulmus* spp. (0.159 kPa g^{-1} min^{-1}), за ней следовали *Quercus robur* (0.149) и *Populus* spp. (0.141).

Заключение. Результаты показывают, что, хотя *Populus* продемонстрировал наибольшее общее изменение давления, нормализация выявляет, что *Ulmus* и *Quercus* обладают более высокой внутренней фотосинтетической эффективностью на единицу биомассы в этих экспериментальных условиях. Globisens Labdisc оказался надежным инструментом для высокопроизводительного физиологического скрининга, предлагая жизнеспособную альтернативу дорогим системам IRGA для образовательного и предварительного экологического мониторинга.

Ключевые слова: фотосинтез; выделение кислорода; датчик газового давления; Globisens Labdisc.