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Canopy Position Influences Cultivar Differences in Leaf Gas Exchange in Apple Trees

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Abstract

The canopy light environment creates contrasting microclimatic conditions that influence leaf physiological acclimation within fruit tree canopies. However, whether cultivar differences in leaf gas exchange are expressed similarly across canopy positions remains unclear. This study evaluated the influence of canopy position on leaf gas exchange in two commercial apple (*Malus x domestica* Borkh.) cultivars, 'Golden' and 'Braeburn', under well-irrigated conditions during two growing seasons. Stomatal conductance (g_s) and transpiration (E) were monitored on exterior (Ex) and interior (In) leaves of the canopy, while photosynthetic light-response curves characterized their physiological acclimation. The two seasons differed markedly in atmospheric evaporative demand, while soil water availability remained non-limiting. Exterior leaves showed significantly higher maximum photosynthetic rates, light saturation points, g_s, and E than interior leaves in both cultivars. Golden exhibited a substantially higher P_{max} in exterior leaves (22.89 ± 1.79) than in interior leaves (10.56 ± 1.56), whereas P_{max} remained relatively similar between exterior (13.88 ± 1.63) and interior leaves (12.70 ± 0.33) of Braeburn. Braeburn also exhibited higher g_s and E than Golden in interior leaves, particularly in 2022. Seasonal differences in cultivar responses were associated with contrasting atmospheric evaporative demand rather than soil water availability. Overall, the results of this study indicate that cultivar physiological performance cannot be fully characterized without considering canopy position and environmental context.

Keywords: canopy microclimate; *Malus x domestica* Borkh.; photosynthesis; stomatal conductance; vapor pressure deficit

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1. Introduction

Climate change is altering the environmental conditions experienced by fruit trees through increasing temperatures, changes in air humidity, and shifts in radiation regimes, all of which modify the microclimate within orchard canopies [1,2]. At the same time, modern orchard systems are increasingly based on high planting densities, narrow planar hedgerows, and optimized training systems that further modify light distribution and air movement within the canopy [3–5]. Consequently, leaves developing at different canopy

positions experience contrasting irradiance, temperature, and atmospheric evaporative demand, resulting in distinct physiological environments. Exterior leaves in a canopy generally operate under higher irradiance and vapor pressure deficit (VPD), whereas interior leaves experience lower evaporative demand and reduced transpiration [6–9]. These differences lead to structural and functional acclimation of leaves within the canopy and ultimately influence carbon assimilation and tree functioning.

Leaf gas exchange is regulated by the interaction between environmental conditions and genotype. Stomatal conductance and transpiration respond rapidly to changes in atmospheric demand, while photosynthetic capacity reflects longer-term acclimation to the prevailing light environment. Apple cultivars differ in vigor, canopy architecture, and stomatal behavior, which may modify their physiological responses to changing environmental conditions [10–13]. Increasing atmospheric dryness associated with climate change is expected to further influence these responses, as stomatal regulation represents the primary mechanism controlling the balance between carbon gain and water loss [14,15]. Although stomatal conductance generally declines with increasing VPD, the magnitude of this response varies among species and cultivars, and the underlying mechanisms remain poorly understood [16,17].

Within apple canopies, light availability is a major determinant of leaf physiology and fruit development. Leaves located on the outer part of the canopy (exterior leaves) and leaves located within the inner canopy (interior leaves) differ in their photosynthetic capacity, transpiration, and stomatal conductance because they develop under contrasting irradiance regimes [8,9,18]. Canopy architecture also influences the distribution of light and humidity within the tree, generating heterogeneous physiological environments that vary among cultivars [19]. While numerous studies have examined the effects of canopy structure on light interception, fruit quality, and orchard productivity [20–22], considerably less attention has been given to whether cultivar-specific differences in leaf gas exchange are expressed similarly in exterior- and interior-located leaves under contrasting seasonal conditions. Understanding these within-canopy physiological responses is important both for interpreting leaf gas-exchange measurements and for improving our understanding of cultivar adaptation to increasingly variable climatic conditions.

The objective of this study was to determine whether canopy position influences cultivar-specific differences in leaf gas exchange in two commercial apple cultivars, 'Golden' and 'Braeburn'. Measurements were conducted during two growing seasons to assess the consistency of these responses under different atmospheric conditions. Light-response curves were used to characterize the physiological acclimation of leaves to contrasting irradiance environments associated with the leaves' positions in the canopy (i.e., exterior leaves versus interior leaves). We hypothesized that cultivar differences in leaf gas exchange would depend on canopy position, reflecting physiological acclimation to the contrasting microenvironments experienced by exterior and interior leaves.

2. Materials and Methods

The study was conducted during the 2022 and 2023 growing seasons in an experimental apple orchard (*Malus x domestica* Borkh.) established in 2008 at the Agroscope Research Centre in Conthey, Switzerland (46°13′ N, 7°18′ E; 504 m a.s.l.). Two commercial apple cultivars, 'Golden' and 'Braeburn', grafted onto 'M9' rootstock, were used. Trees were trained as a Vertical Axis (slender spindle-type) canopy system, producing a narrow, vertically oriented canopy with a spacing of 4.0 × 1.3 m. The orchard was managed according to standard commercial practices, including manual fruit thinning to achieve optimal crop load, mineral fertilization, pruning, pest, and disease control. Pruning consisted exclusively of long renewal pruning during the winter, with no summer pruning applied during the

experimental period. Irrigation was supplied using overhead sprinklers with a flow rate of 4.5 mm.h^{-1} to avoid severe soil water deficits and to maintain comparable tree water status throughout the experiment.

The experiment was conducted in an established orchard in which Golden and Braeburn trees were planted in separate rows. Within each cultivar row, four trees were randomly selected from the available trees and used as experimental units. The selected trees were distributed spatially along the row rather than being restricted to a single consecutive section. For each selected tree, one exterior and one interior leaf were selected at each measurement date, allowing the two canopy positions to be compared within the same tree. The individual tree was considered the biological experimental unit for statistical analyses.

Environmental conditions

Environmental conditions, including rainfall, air temperature (T_{air}), relative humidity (RH), and global solar radiation, were continuously recorded using a weather station (GRWS100 Research Weather Station, Campbell Scientific Inc., Logan, UT, USA) located adjacent to the experimental orchard (www.agrometeo.ch (accessed 01 November 2023)). Air vapor pressure deficit (VPD_{air}) was calculated from air temperature and relative humidity according to the standard equation of Allen et al. [23]:

$$VPDair = es(T)\ (1 - (100/RH)) \quad (1)$$

where the following applies:

es(T) is the saturation vapor pressure (kPa) at air temperature and RH is the relative humidity (%).

The two growing seasons differed markedly in rainfall and atmospheric conditions and therefore provided contrasting environmental contexts for evaluating cultivar responses under different canopy light environments.

Plant water status

Plant water status was monitored throughout the experiment to verify that trees remained well irrigated and did not experience severe water deficits during the measurement period. Stem water potential (Ψ_x) was measured weekly around midday (11:00–12:00 h) on four fully expanded mature leaves collected from the middle portion of the canopy. Leaves were enclosed in aluminum-coated plastic bags for at least 2 h to allow equilibration with the stem water potential before being excised and measured using a pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA) according to Scholander et al. [24].

Soil water availability was monitored weekly from late June to late August using WATERMARK 200SS sensors (Irrometer Co., Riverside, CA, USA) installed at depths of 30, 50, and 80 cm in each experimental block. These measurements were used to monitor soil water availability throughout the experiment.

Stomatal conductance and transpiration

Leaf stomatal conductance (g_s) and transpiration (E) were measured periodically during the growing seasons between 18 July (DOY 199) and 25 August (DOY 237) in 2022 and between 29 June (DOY 180) and 23 August (DOY 235) in 2023. Measurements were performed between 10:30 h and 12:00 h on clear days on healthy, fully expanded leaves located in two contrasting canopy positions. Exterior leaves were selected from the outer canopy and were exposed to direct sunlight, whereas interior leaves were selected from the inner canopy and experienced lower irradiance due to canopy shading. Two exterior and two interior leaves were selected from each tree at each measurement date, so that both canopy positions were represented within the same experimental trees. The two leaves measured within each canopy position were averaged for each tree, resulting in one mean value

per tree and canopy position. Thus, the tree was considered the biological experimental unit, with four biological replicates per cultivar and canopy position (n = 4 trees).

Measurements were performed using a portable porometer (LI-600, LI-COR Biosciences, Lincoln, NE, USA).

Vegetative parameters and yield

The leaf area was calculated based on the allometric relationships of leaf area and branch diameter and the measured branches per tree (number and diameter) obtained in previous experiments from the same varieties in similar commercial orchards [25,26].

Trunk diameter was measured using a caliper at a height of 30 cm at the beginning of each growing season and used to calculate trunk cross-sectional area (TCSA). At harvest, which took place on 4 September for Golden and 30 September for Braeburn in both seasons, the four experimental trees of each cultivar were harvested individually, and total yield was recorded as kg. tree^{-1}. Tree productivity was calculated as the ratio of total yield to TCSA (kg. cm^{-2}).

Photosynthetic light-response curves

Photosynthetic light-response curves were obtained for each selected tree at both canopy positions. One leaf from the exterior and one leaf from the interior canopy were measured per tree, resulting in one light-response curve for each tree × canopy-position combination. Because each curve required approximately 40 min to complete, measurements were distributed across several measurement days, with two to three curves performed per day. All measurements were conducted during the morning to minimize variation in environmental conditions. The parameters derived from the fitted curves were therefore obtained at the tree level, with four biological replicates per cultivar and canopy position.

Light-response curves were determined on fully expanded, healthy leaves between 09:00 h and 12:00 h (solar time) using a portable infrared gas analyzer (LI-6800, LI-COR Biosciences, Lincoln, NE, USA) equipped with a 3 × 3 cm leaf chamber. Leaves were exposed to progressively decreasing levels of photosynthetic photon flux density (PPFD), allowing 2–3 min for stabilization before each measurement. Chamber conditions were maintained at an ambient CO_2 concentration of 400 µmol mol^{-1}, a flow rate of 500 µmol s^{-1}, and a leaf temperature between 27 and 30 °C.

Statistical analysis

Statistical analyses were performed using the individual tree as the experimental unit. For gas-exchange measurements and photosynthetic light-response parameters, the two leaves measured within each canopy position were averaged for each tree, resulting in one value per tree and canopy position. Data were analyzed using a linear mixed-effects model with cultivar, canopy position (exterior vs. interior), and their interaction as fixed effects, and tree as a random effect to account for the repeated measurements of canopy positions within the same tree. Model assumptions were assessed by examination of residuals for normality and homogeneity of variance. When significant effects were detected, means were compared using appropriate pairwise comparisons with adjustment for multiple testing. Statistical significance was accepted at $p < 0.05$.

For plant-level variables, including stem water potential, total leaf area, trunk cross-sectional area (TCSA), yield, and productivity, the individual tree was considered the experimental unit. Because these measurements represented whole-tree characteristics and were not assigned to specific canopy positions, only cultivar was included as a fixed factor in the analysis. The two cultivars were compared separately for each growing season using one-way analysis of variance (ANOVA), with cultivar as the explanatory factor. Results

were expressed as means ± standard error (SE), and statistical significance was assessed at $p < 0.05$.

Light-response curves were fitted separately using the non-rectangular hyperbola model of Marshall and Biscoe [27], from which the maximum net photosynthetic rate ($P_n$sat), apparent quantum yield (α), curve convexity (θ), dark respiration (R_d), light saturation point (I_s), and light compensation point (I_c) were estimated following Zufferey et al. [28] and Pasian and Lieth [29]. The tree was considered the biological experimental unit; therefore, the four trees within each cultivar × canopy-position combination constituted the four biological replicates used for statistical analysis. For each light-response parameter, a linear mixed-effects model was used to test the effects of cultivar, canopy position, and their interaction, with tree included as the experimental unit to account for the paired measurements of exterior and interior leaves within the same tree. When significant effects were detected, pairwise comparisons among cultivar × canopy-position combinations were performed using Holm-adjusted comparisons at $p < 0.05$.

All statistical analyses were performed using R 4.4.2. (R Core Team, Vienna, Austria), and complementary analyses were conducted using InfoStat version 2017 (Universidad Nacional de Córdoba, Argentina).

3. Results

3.1. Seasonal Environmental Conditions

The two growing seasons provided contrasting environmental conditions during the measurement period (Figure 1). Cumulative precipitation from mid-July to the end of August was 86.6 mm in 2022 and 273.4 mm in 2023 (Figure 1A,B). Mean and maximum air temperatures followed similar seasonal patterns in both years, with maximum temperatures ranging between 25 and 35 °C and mean temperatures generally remaining below 25 °C (Figure 1C,D).

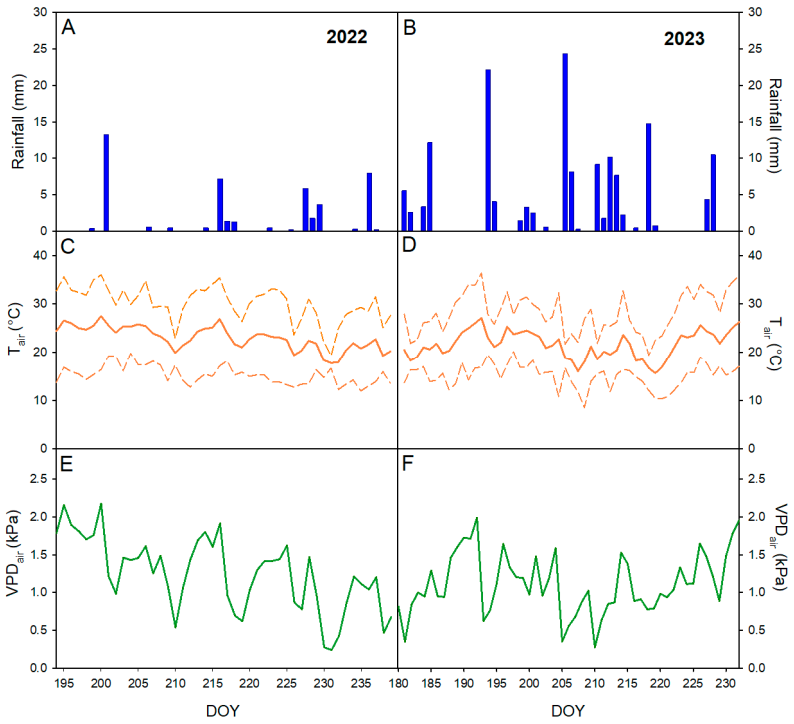


Figure 1. Cumulated rainfall (**A**,**B**), air mean (solid line), minimum and maximum temperatures (dashed lines; (**C**,**D**)), and air vapor pressure deficit (VPD_{air}; (**E**,**F**)) during the months the experiments were conducted in 2022 (DOY 194 to 239) and 2023 (DOY 180 to 233).

Season 2022 was characterized by higher atmospheric evaporative demand than 2023, resulting in generally higher air vapor pressure deficit (VPD_{air}) values at the beginning of the measurement period, which gradually decreased toward the end of August (Figure 1E). In contrast, VPD_{air} remained lower during most of the 2023 season, with only brief increases observed in mid-July and near the end of the measurement period (Figure 1F). These climatic differences provided contrasting environmental conditions under which cultivar responses were evaluated.

3.2. Plant Water Status

Soil moisture and stem water potential (Ψ_x) were monitored throughout the experiment to verify that irrigation prevented soil water limitation (Figures 2 and S1). Soil water potential remained above −30 kPa during both growing seasons (Figure S1), indicating that soil moisture was maintained within the target range despite the contrasting rainfall conditions.

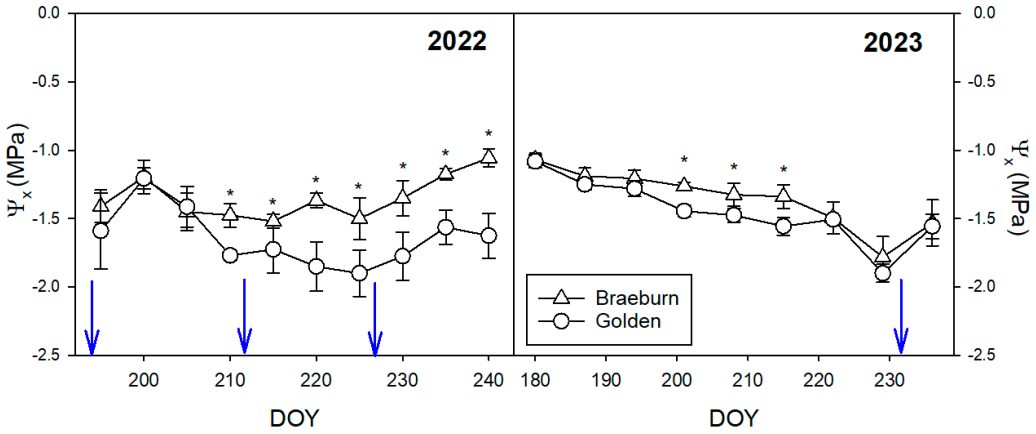


Figure 2. Stem water potential (Ψ_x; MPa) in well-watered apple trees, 'Golden' and 'Braeburn', during the 2022 and 2023 growing seasons. Each point represents the mean ± SE of four trees. Asterisks indicate statistically significant differences between treatments at $p \leq 0.05$. Blue arrows indicate the days when irrigation was applied.

Seasonal variation in Ψ_x reflected differences in atmospheric conditions between years (Figure 2). In 2022, Golden exhibited lower midday Ψ_x values than Braeburn, whereas no significant differences between cultivars were observed in 2023. Although midday Ψ_x occasionally approached −2.0 MPa during periods of high evaporative demand, these measurements occurred under well-irrigated conditions and therefore reflected the effect of atmospheric demand rather than soil water deficit.

3.3. Canopy Development

Golden exhibited a significantly greater total leaf area than Braeburn in both seasons (Figure 3), indicating greater vegetative development under the experimental conditions.

Differences in tree size and productivity were further assessed using trunk cross-sectional area (TCSA), yield (kg. $tree^{-1}$), and productivity calculated as the yield per TCSA (kg. cm^{-2}; Table S1).

Golden trees had significantly greater TCSA than Braeburn in both 2022 ($p = 0.046$) and 2023 ($p = 0.033$). Yield did not differ significantly between cultivars in either year, although Golden showed numerically higher yields in both years. Yield/TCSA was also not significantly different between cultivars in either year (Table S1).

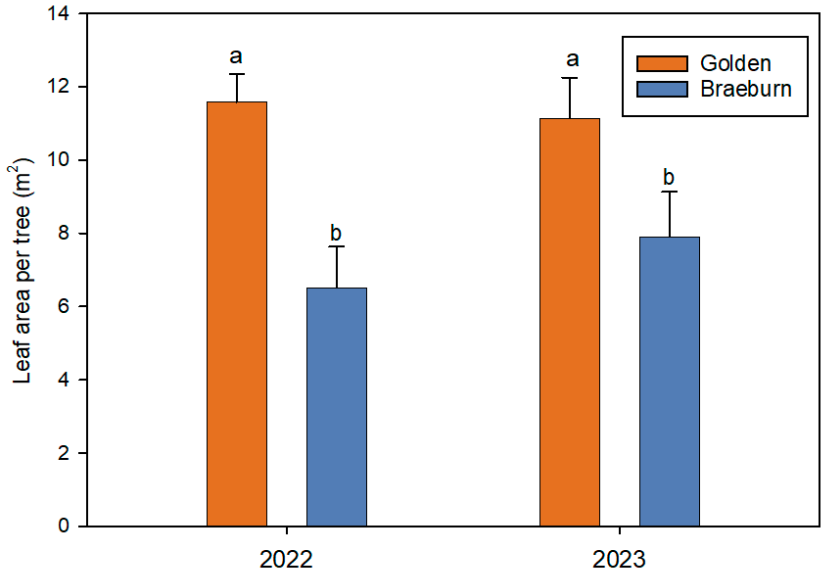


Figure 3. Total leaf area per tree (m^2) in 'Golden' and 'Braeburn' apple varieties measured using allometric relationships during the 2022 and 2023 seasons. Different letters indicate statistically significant differences between treatments at $p \leq 0.05$.

3.4. Photosynthetic Acclimation to Canopy Light Environment

Photosynthetic light-response curves showed distinct responses between exterior and interior leaves in both cultivars (Figure 4; Table 1). Exterior leaves generally reached higher photosynthetic rates at high irradiance and exhibited higher light saturation indices (I_s) than interior leaves, consistent with greater photosynthetic capacity under high irradiance. The fitted light-response parameters confirmed a significant effect of canopy position on I_s ($p = 0.026$), whereas the cultivar × canopy-position interaction was not significant ($p = 0.108$).

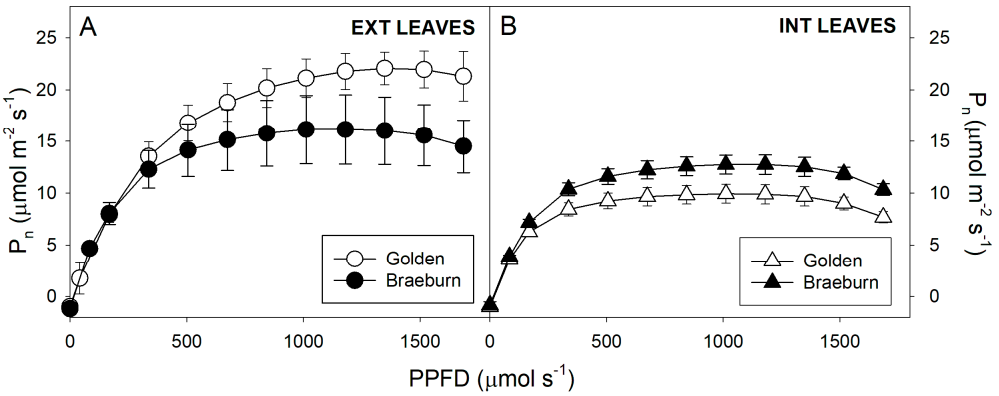


Figure 4. Photosynthetic light-response curves in exterior (**A**) and interior leaves (**B**) of 'Golden' (empty symbols) and 'Braeburn' (full symbols) apple trees during the 2022 growing season. Each point represents the mean ± SE of four trees. For each tree and canopy position, parameter estimates obtained from multiple light-response curves measured on different dates were first averaged to obtain a single tree-level value.

The response of photosynthetic capacity to canopy position differed between cultivars for maximum net photosynthetic rate (P_{max}). A significant cultivar × canopy-position interaction was detected for P_{max} ($p = 0.007$). Golden exhibited a substantially higher P_{max} in exterior leaves (22.89 ± 1.79) than in interior leaves (10.56 ± 1.56), whereas P_{max} remained relatively similar between exterior (13.88 ± 1.63) and interior leaves (12.70 ± 0.33) of Braeburn.

Other light-response parameters showed cultivar effects but no significant cultivar × canopy-position interactions. Dark respiration (R_d) was significantly higher in Braeburn than Golden (p = 0.029), whereas neither canopy position nor the interaction between cultivar and canopy position was significant. Similarly, the light compensation index (I_c) was significantly higher in Braeburn than Golden (p = 0.038), with no significant effects of canopy position or the cultivar × canopy-position interaction. No significant effects of cultivar, canopy position, or their interaction were detected for apparent quantum yield (Φ) or photochemical efficiency (α).

Table 1. Parameters derived from photosynthetic light-response curves measured on exterior and interior leaves of 'Golden' and 'Braeburn' apple trees: α [μmol (CO_2) μmol photon^{-1}], photochemical efficiency; P_{max} [μmol (CO_2) m^{-2} s^{-1}], maximum net photosynthetic rate; Φ, apparent quantum yield; R_d [μmol (CO_2) m^{-2} s^{-1}], dark respiration rate; I_s [μmol (photon) m^{-2} s^{-1}], light saturation index; I_c [μmol (photon) m^{-2} s^{-1}].

Parameter	Golden Ext	Golden Int	Braeburn Ext	Braeburn Int	Cultivar	Canopy Position	Cultivar × Canopy Position
α	0.059 ± 0.011	0.054 ± 0.004	0.047 ± 0.001	0.054 ± 0.004	0.299	0.906	0.396
P_{max}	22.89 ± 1.79 ^{a}	10.56 ± 1.56 ^{b}	13.88 ± 1.63 ^{b}	12.70 ± 0.33 ^{b}	0.062	0.029	0.007
Φ	0.809 ± 0.065	0.892 ± 0.039	0.916 ± 0.007	0.862 ± 0.027	0.259	0.781	0.203
R_d	0.304 ± 0.094 ^{a}	0.634 ± 0.132 ^{ab}	0.894 ± 0.176 ^{b}	0.769 ± 0.151 ^{ab}	0.029	0.559	0.191
I_s	877.1 ± 169.1 ^{a}	412.1 ± 23.5 ^{b}	631.7 ± 47.5 ^{ab}	506.7 ± 29.1 ^{ab}	0.433	0.026	0.108
I_c	5.87 ± 1.99 ^{a}	12.24 ± 2.77 ^{ab}	19.61 ± 4.26 ^{b}	14.50 ± 2.77 ^{ab}	0.038	0.866	0.113

Values are means ± SE (n = 4 trees). Different superscript letters indicate significant differences among cultivar × canopy-position combinations based on Holm-adjusted pairwise comparisons ($p < 0.05$). α, photochemical efficiency; P_{max}, maximum net photosynthetic rate; Φ, apparent quantum yield; R_d, dark respiration rate; I_s, light saturation index; I_c, light compensation index.

Overall, the light-response curves and derived parameters indicate that canopy position was associated with differences in photosynthetic capacity, particularly in Golden, which showed a pronounced reduction in P_{max} from exterior to interior leaves. In contrast, Braeburn showed more similar P_{max} between canopy positions. Thus, the results support a canopy-position-dependent cultivar response in maximum photosynthetic capacity, while differences in the other light-response parameters were primarily associated with cultivar or canopy position independently.

3.5. Seasonal Stomatal Conductance and Transpiration

Throughout the study, exterior leaves exhibited significantly higher stomatal conductance (g_s) and transpiration (E) than interior leaves in both cultivars (Figure 5). These differences were consistent across the two growing seasons, reflecting the contrasting physiological behavior of leaves developed under different canopy light environments.

Seasonal fluctuations in g_s and E followed the prevailing environmental conditions, with generally higher values recorded during the 2023 growing season than in 2022. However, the relative differences between exterior- and interior-developed leaves remained consistent across seasons.

Differences between cultivars were mainly observed in interior leaves. In 2022, Braeburn exhibited significantly higher g_s and E than Golden in the inner canopy, whereas no significant cultivar differences were detected in exterior leaves (Figure 5A,C). These differences were less pronounced during the 2023 growing season (Figure 5B,D).

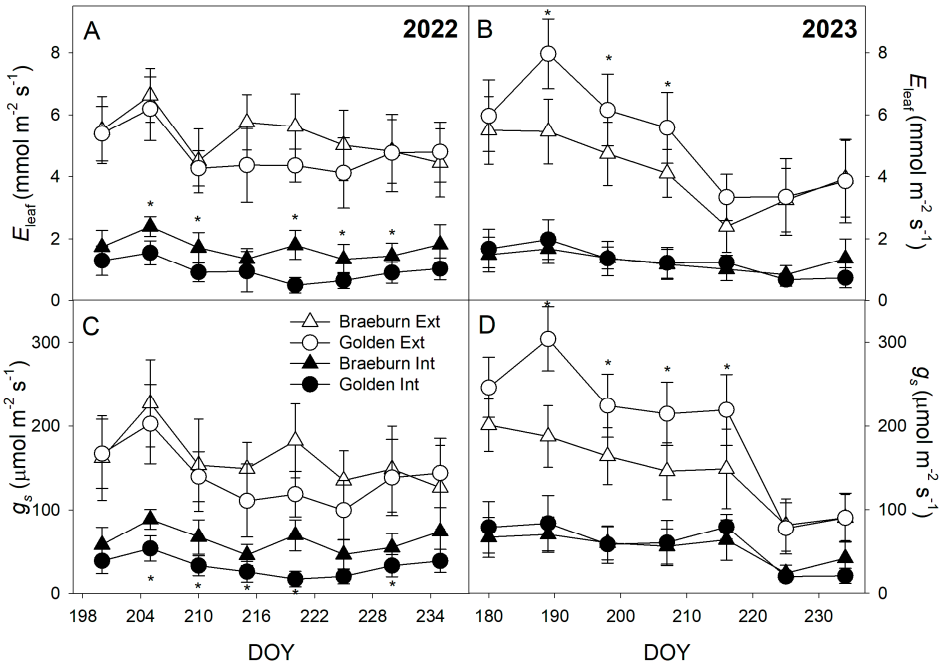


Figure 5. Seasonal dynamics of (**A**,**B**) leaf transpiration (E) and (**C**,**D**) stomatal conductance (g_s) measured in exterior (empty symbols) and interior leaves (full symbols) of 'Golden' (circles) and 'Braeburn' (triangles) apple varieties and two growing seasons, 2022 and 2023. Each point is the mean of 4 trees ± SE. Asterisks indicate statistically significant differences between treatments at $p \leq 0.05$.

4. Discussion

Canopy position determines leaf physiological acclimation

Both cultivars showed differences in leaf physiology between canopy positions. Exterior leaves exhibited higher maximum photosynthetic rates and light saturation indices (I_s) than interior leaves, consistent with the acclimation of leaves to contrasting irradiance environments [8,9,18]. Similar positional differences in photosynthetic rate, stomatal conductance, and transpiration have been reported in apple. For example, leaves in the upper or exterior canopy generally showed greater photosynthetic activity and gas exchange than leaves located inside the canopy [6,8,9,20]. In slender-spindle 'Hongro' apple trees, the photosynthetic rate was highest in the external upper canopy and was approximately 4.5-fold greater than in the internal lower canopy; these differences were associated with light penetration and leaf anatomical traits, including palisade-tissue thickness [20]. Similarly, Kviklys et al. [22] reported higher photosynthetic rate, stomatal conductance, and transpiration in more exposed canopy positions, together with differences in fruit size and quality. These results support the view that canopy position is an important determinant of leaf physiological function. Differences between exterior and interior leaves likely arise from the contrasting light environments experienced during leaf development, which can influence leaf structure, nitrogen allocation, and photosynthetic capacity [30,31]. However, canopy position also encompasses associated gradients in leaf temperature, humidity, VPD, and boundary-layer conductance. Therefore, the positional differences observed in the present study should not be attributed to irradiance alone, but rather to the combined environmental conditions associated with the exterior and interior canopy.

Cultivar differences in gas exchange depend on canopy position

Seasonal measurements of stomatal conductance and transpiration showed that cultivar differences were mainly expressed in interior leaves. Braeburn exhibited higher g_s and E than Golden in the interior canopy in 2022, whereas differences were not detected in

exterior leaves and were less pronounced in 2023. This indicates that cultivar effects on gas exchange depend on the environmental conditions experienced by individual leaves. The higher g_s and E of interior Braeburn leaves may reflect differences in stomatal regulation under lower irradiance. Such cultivar-specific stomatal responses are consistent with previous work showing that apple cultivars can differ in the sensitivity of stomatal conductance to environmental drivers, including VPD [11].

The light-response curves provided complementary evidence of cultivar-dependent differences in canopy-position responses. Golden showed a pronounced reduction in P_{max} from exterior to interior leaves, whereas Braeburn exhibited relatively similar P_{max} between canopy positions. This pattern may indicate differences in the functional acclimation of leaves to heterogeneous canopy environments [32]. Fallahi et al. [30] reported cultivar differences in the acclimation of apple leaves to contrasting light regimes, while Li and Lakso [31] demonstrated that exposure to light can influence the photosynthetic characteristics of apple spur leaves. More recent work has also shown that cultivar and tree architecture can jointly affect the distribution of leaf photosynthetic traits and their contribution to whole-canopy photosynthesis and water-use efficiency [33]. Nevertheless, the present study did not measure leaf nitrogen, anatomy, or biochemical limitations, and therefore cannot identify the mechanisms responsible for the contrasting P_{max} responses. The results should consequently be interpreted as cultivar-dependent responses to canopy position rather than as evidence of a specific light-acclimation mechanism.

Golden also exhibited greater total leaf area and TCSA than Braeburn, indicating greater vegetative development under the experimental conditions. However, gas exchange was measured at the individual-leaf level, whereas leaf area, TCSA, yield, and productivity were evaluated at the tree level. Thus, the observed differences in g_s, E, and P_{max} cannot be directly attributed to differences in total leaf area. Rather, the results indicate that differences in canopy development and leaf physiological function coexist between cultivars. Canopy architecture determines the distribution of leaves across contrasting microenvironments and may therefore influence whole-tree carbon assimilation and water use. The relative contribution of leaf distribution and leaf function to canopy photosynthesis has been demonstrated in comparisons among apple cultivars and tree architectures [33]. This interaction was not directly quantified in the present study because measurements were conducted at the leaf level.

Environmental conditions were associated with the expression of cultivar differences

Although both cultivars were maintained under well-irrigated conditions, the two growing seasons differed substantially in rainfall and atmospheric evaporative demand. Soil water potential generally remained above −30 kPa, indicating adequate bulk soil moisture during the measurement periods. However, midday stem water potential occasionally approached −2.0 MPa, showing that adequate soil moisture did not necessarily prevent reductions in tree water status under high evaporative demand. Therefore, the seasonal differences observed in leaf physiology should not be interpreted as responses to soil drought alone, but rather as differences occurring under contrasting atmospheric and environmental conditions. The contribution of atmospheric demand and possible hydraulic limitations cannot be separated with the present measurements.

Differences were more pronounced during the season characterized by higher atmospheric demand, whereas they were generally less evident under lower VPD. This pattern is consistent with the important role of atmospheric dryness in regulating stomatal conductance and carbon assimilation, even when bulk soil moisture remains adequate [14,34]. Stomatal responses to VPD can differ among cultivars, and previous work in apple has demonstrated cultivar-specific thresholds and responses of stomatal conductance to increasing VPD [11]. The present results therefore indicate that the magnitude of cultivar

differences was associated with contrasting seasonal atmospheric conditions, although the specific contribution of VPD cannot be separated from the simultaneous effects of temperature, rainfall, tree developmental status, soil–plant hydraulic processes, and other year-specific factors.

This environmental dependence is particularly relevant when comparing physiological traits among cultivars. Evaluating cultivar responses across contrasting environmental conditions may provide a more robust characterization of their physiological behavior than measurements conducted under a single set of conditions. Future experiments combining measurement-specific VPD, soil and plant water status, and hydraulic traits would help disentangle the relative contributions of atmospheric demand and soil–plant hydraulic limitations to cultivar-specific physiological responses.

Canopy position, photosynthesis, and yield

Although the present experiment did not quantify yield separately for exterior and interior canopy positions, previous studies indicate that canopy light distribution can influence fruit development and yield components. In apple, fruit located in more exposed canopy positions often show greater fruit weight, soluble-solids concentration, and skin coloration than fruit developing in shaded interior positions [20,22,31]. Kviklys et al. [22] also reported differences in fruit yield between canopy positions, with lower yield in the inner canopy than in the top of the tree. These effects may result from differences in local carbon assimilation, assimilate availability, and reproductive development under contrasting light environments.

In the present study, yield was recorded for each tree and did not differ significantly between Golden and Braeburn in either season, despite the greater TCSA and total leaf area of Golden. The productivity (Yield/TCSA) also did not differ significantly between cultivars. This suggests that greater vegetative development in Golden did not translate into a statistically detectable increase in yield or productivity under the conditions of this experiment. Whole-tree yield integrates several processes, including flowering, fruit set, fruit thinning, fruit growth, carbon allocation, and canopy light interception, and cannot be inferred directly from leaf-level photosynthetic capacity [35]. Therefore, the higher P_{max} of Golden exterior leaves should not be interpreted as evidence of higher whole-tree yield. Future studies combining spatial measurements of leaf physiology, leaf area distribution, and fruit number and growth would be needed to determine how cultivar and canopy-position effects on leaf function translate into whole-tree productivity.

Implications for gas exchange measurements in apple canopies

An important practical implication of this study is that canopy position should be considered when comparing leaf gas exchange among apple cultivars. Measurements are often conducted on exterior, fully sun-exposed leaves because these provide relatively standardized conditions and facilitate comparisons among experiments. However, our results show that cultivar differences may vary with canopy position and growing conditions. For example, Braeburn exhibited higher g_s and E than Golden in interior leaves during 2022, whereas these differences were not detected in exterior leaves and were less pronounced in 2023. In addition, Golden exhibited a pronounced reduction in maximum photosynthetic rate from exterior to interior leaves, while Braeburn showed relatively similar values between canopy positions.

Canopy heterogeneity should therefore be considered in experimental design, as measurements from a single canopy position may capture only part of cultivar physiology. Including both exterior and interior leaves, together with canopy structure, can improve the interpretation and scaling of leaf-level responses to whole-canopy photosynthesis and water use [6,8,9,33].

5. Conclusions

This study demonstrates that canopy position influences the expression of cultivar-specific differences in apple leaf physiology. Golden exhibited greater photosynthetic capacity in exterior leaves, particularly under high irradiance, whereas Braeburn showed more similar photosynthetic capacity between exterior and interior leaves. The magnitude of cultivar differences in stomatal conductance and transpiration varied between the two contrasting growing seasons and coincided with differences in atmospheric evaporative demand. However, because VPD and other year-specific environmental factors varied simultaneously, the observed responses cannot be attributed to VPD alone. Moreover, although bulk soil moisture was generally adequate, this did not necessarily exclude transient hydraulic limitations under high evaporative demand.

Overall, the results indicate that cultivar physiological performance cannot be fully characterized without considering canopy position and environmental context. Accounting for both exterior and interior leaves may therefore improve the interpretation of cultivar differences in gas exchange and provide a more representative assessment of physiological responses under heterogeneous orchard conditions. Further studies incorporating measurement-specific atmospheric variables together with soil and plant water status and hydraulic measurements are needed to disentangle the mechanisms underlying these cultivar-specific responses.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae12101204/s1, Figure S1: Soil water potential (kPa) measured with a Watermark soil moisture sensor during season 2022 (A) and 2023 (B) for each variety. The sensors were installed at three soil depths: 30 cm, 50 cm, and 80 cm. Irrigation was applied to keep values above −30 kPa. Table S1: Trunk cross-sectional area (TCSA), yield, and productivity (yield/TCSA) of two apple cultivars, 'Golden' and 'Braeburn', in the 2022 and 2023 growing seasons.

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