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Plastic covering and double pruning mitigate climate challenges in cv. Sauvignon blanc vineyards

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ABSTRACT

Advances in viticultural management techniques have become increasingly important for mitigating the effects of challenging climatic conditions. The double-pruning technique is already widely used in Brazilian vineyards, enabling a shift of the harvest season from summer to winter. This shift allows key phenological stages, such as flowering, ripening, and harvest, to occur under milder temperatures and reduced precipitation. In contrast, the use of plastic coverings allows growers to maintain the conventional grapevine cycle while providing a physical barrier that protects the plants. Although both techniques are known and applied in some regions of the country, little information is available on their combined effects, particularly regarding plant physiology. Considering this gap, the present study evaluated the combined effects of double pruning and plastic coverings on the internal and external morphology and physiology of cv. Sauvignon blanc grapevines. The plastic covering prevented the direct incidence of rainwater on leaves and reduced the wash-off of applied fungicides. This led to lower disease incidence and fewer pesticide applications. Additionally, plastic covering reduced the photosynthetic photon flux density (PPFD) to levels close to the light saturation point of the grapevine, while also altering the light spectrum by attenuating UV-B radiation (280–315 nm). Together, these factors enhanced key physiological processes, including photosynthesis, transpiration, stomatal conductance, and chlorophyll accumulation. Leaf area is high in covered plants, and anatomical analyses revealed plastic responses in uncovered vines to excess light and UV-B exposure, such as thicker epidermal and palisade tissues and the deposition of epicuticular wax around stomata. However, these structural adjustments did not result in greater CO_2 assimilation. Overall, this study demonstrates that the combination of simple management practices, as plastic covering, can improve and facilitate grapevine cultivation under challenging weather conditions, while also providing valuable insights into the physiological responses of the plant.

KEYWORDS: *Vitis vinifera*, physiology, anatomy, vineyard management

INTRODUCTION

Grapevine *(Vitis vinifera)* is one of the most economically valuable and resilient crops in the world. This species originates from temperate climate regions and has a dormancy period of three–five months, typically followed by one pruning and one harvest per year (Xu *et al.*, 2011). However, with advances in technology and new research approaches, it is now possible to cultivate this species in different regions (Limier *et al.*, 2018). Brazil, a tropical country, has become one of the main grape producers in South America, showing significant growth in recent decades, mainly due to the adoption of grafted seedlings, advancements in management techniques, and the development of new cultivars (Favero *et al.*, 2011).

The cultivation of grapevines has become increasingly feasible in tropical regions, but some limiting factors remain. High temperatures and excessive precipitation, for example, can negatively affect important stages of the grapevine cycle, such as flowering and berry ripening, by accelerating these processes and even reducing their duration (Poni *et al.*, 2020; Rafique *et al.*, 2024). As a consequence, negative impacts can be observed in wine characteristics, such as increased alcohol content (de Orduña, 2010) or, conversely, lower sugar accumulation and, consequently, lower alcohol content. The latter occurs because the grape cuticle is more resistant to water entry than to water loss, resulting in dilution of berry contents (Becker & Knoche, 2011). In addition, rainfall directly on the leaves, combined with high temperatures, increases the incidence of diseases such as downy mildew *(Plasmopara viticola)*, which can significantly reduce productivity (Salazar-Parra *et al.*, 2010).

The challenging weather conditions for grapevine cultivation are not restricted to tropical regions, as climate change is causing similar or even more severe effects in many parts of the world (Rafique *et al.*, 2024). Grapevine productivity and fruit quality are directly affected by climatic fluctuations during their growth and development, especially in critical phases such as berry ripening (Nistor *et al.*, 2018; Shah *et al.*, 2021). Due to rising temperatures over time, famous vineyards in the northern hemisphere are considering relocating their cultivation areas to higher altitudes in search of milder climates (Kurtural & Gambetta, 2021). In this context, viticultural practices can be useful to mitigate these challenges, either by preventing problems or by facilitating production through the acclimatisation of plants to such conditions. These practices could be valuable not only in tropical regions but also in many areas worldwide that are affected by climate change.

Two important management techniques are known to improve grapevine cultivation under challenging environmental conditions: double pruning and plastic covering. Double pruning is a technique developed to modify the grapevine cycle, shifting the harvest from summer to winter and resulting in improved quality indices, such as soluble solids, pH, and titratable acidity, in areas where conventional cycle production is often hindered (Regina *et al.*, 2011). This is achieved by performing two pruning within the grapevine cycle: the conventional one after winter and a second during summer (Amorim *et al.*, 2005), when harvest would normally occur. Plastic covering, on the other hand, allows the maintenance of the grapevine cycle by improving microclimatic conditions, preventing the direct incidence of rainfall on the leaves, and reducing the consequent removal of applied fungicides (Holcman *et al.*, 2018).

The benefits of these two management techniques are already known and applied in many regions of Brazil. However, the effects of double pruning under protected cultivation have not been thoroughly investigated, particularly regarding morphological, physiological, and anatomical aspects of grapevine development. This gap in knowledge is highly relevant, since wine quality ultimately depends on the proper establishment of the plant. Therefore, this study aimed to evaluate the addition of plastic covering in a double-pruning vineyard on grapevine physiology, anatomy, and morphology in a tropical region, seeking an effective strategy to optimise production under challenging weather conditions.

MATERIALS AND METHODS

1. Experimental design

The experiment was conducted in the experimental vineyard of the University of São Paulo, Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ), Department of Plant Production, in Piracicaba, SP, Brazil (22° 42' 25" S 47° 37' 41" W; 546 m altitude). The regional climate is classified as Cwa, characterised by a humid subtropical climate with hot, wet summers and dry, mild winters (Peel *et al.*, 2007). The two-year-old vines cv. Sauvignon blanc *(Vitis vinifera)* grafted onto Paulsen 1103 rootstock (*Vitis berlandieri* × *Vitis rupestris*) were planted 1.0 meters between plants and 2.5 meters between rows, in rows of 25 meters. The vines were conducted on a vertical shoot positioning (VSP) system. In parts of the rows, the vines were planted on VSP with a transparent plastic film installed over the plants, and their maintenance consisted of replacement in each cycle. This plastic covering was made of polypropylene film, with a thickness of 200 µm and a solar radiation transmittance rate of 70–80 %.

The experiment was organised into five blocks, with each block containing five vines, resulting in a total of 25 vines per treatment (with and without plastic covering). All the plants were cultivated under a double-pruning management system, totalling four growing cycles (winter 2022, summer 2023, winter 2023, summer 2024). Each cycle was labelled according to the season in which the harvest occurred. As this technique involves two harvests by altering the grapevine cycle, and since many producers in different regions of the country adopt it to harvest twice, this study evaluated plant growth and development across all cycles. All phenological stages of the grapevine were monitored throughout the winter and summer cycles.

2. Environmental monitoring in the vineyard

For temperature measurements, a thermohygrometer was installed both under the plastic covering and outside. Light intensity was monitored using a luxmeter (AK305) in both conditions. Light quality was assessed with a spectrometer (USB2000+RAD/Ocean Optics; wavelength range: 200–850 nm; spectral resolution: 2.0 nm) to verify the spectral distribution of light under both conditions. General meteorological data, such as air temperature and precipitation, were obtained from the meteorological station of ESALQ.

3. Biometric traits and phenological stages

The growth of vines under covered and uncovered conditions was recorded through weekly measurements of 10 vines (two of each block) until the shoots reached the last wire of the vertical trellis system or until growth stabilised. The leaf area (cm^2) of individual leaves from each condition, in each cycle, was measured using ImageJ® software. The leaves were collected from the same bud position after growth had stabilised. The phenological stages were determined according to the BBCH scale, and they were monitored and photographed throughout all cycles (Lorenz *et al.*, 1995).

4. Physiological traits

The physiological parameters of the vines were analysed through leaf gas exchange measurements conducted on sun-exposed leaves from the main shoot of 10 randomly selected vines (two of each block). An infrared gas analyzer (IRGA, LI-6400XT/Li-Cor) was used to measure the photosynthetic rate (A), stomatal conductance (Gs), and leaf transpiration (E). The light saturation point in both covered and uncovered vines was determined by performing a light response curve. The chosen light intensities were 0, 50, 100, 200, 300, 500, 800, 1,000, 1,200, 1,600, 1,800, and 2,000 μmol m^{-2} s^{-1}, with measurements starting from the highest intensity. Regarding pigment content, the chlorophyll *a* and *b* indices were determined at the ripening stage in all cycles using a ClorofiLOG (CFL1030). For this analysis, 15 leaves at the same height on the shoot were selected.

5. Anatomical analyses

Leaves from the middle region of the main shoot of five vines from each condition (covered and uncovered) were randomly selected for anatomical analyses. For this purpose, samples from the fourth quadrant of each leaf were cut and fixed in formaldehyde–glutaraldehyde solution (2.5 % glutaraldehyde and 2.5 % formaldehyde in 0.05 M sodium cacodylate buffer with 0.001 M $CaCl_2$). After dehydration in a graded ethanol series (30 %, 50 %, 70 %, and 100 %, for 15 minutes each), the samples were sectioned into 5 μm slices using a manual rotary microtome (RM2235-Leica) (Marques & Soares, 2022). All images were obtained using a microscope equipped with a camera (ZEISS Axioskop). For scanning electron microscopy (SEM), 1 cm^2 leaf samples were dehydrated in a graded acetone series (30 %, 50 %, 70 %, and 100 %). Analyses were performed with a scanning electron microscope (JSM-IT300LV-JEOL), allowing for detailed imaging of the leaf epidermis. To quantify stomatal density more efficiently, the leaf impression method was employed by applying nail polish to the abaxial surface of the leaves.

6. Chemical control

Throughout all experimental cycles, disease and pest control were necessary, particularly due to challenging weather conditions that favoured their occurrence. Downy mildew *(Plasmopara viticola)* was managed through the application of fungicides, including Amistar© (azoxystrobin, 240 g/ha), Ranman© (cyazofamid, 300 mL/ha), and Revus© (mandipropamid, 500 mL/ha).

7. Statistical analyses

For comparison between the covered and uncovered treatments across all experimental cycles, a two-sample *t*-test was employed. Statistical analyses were performed using Minitab software (version 19.1), ensuring robust and accurate computations. Graphs were generated in Python (version 3.11.5) using visualisation libraries such as Matplotlib and Seaborn.

RESULTS AND DISCUSSION

1. Environmental conditions

The maximum and minimum air temperatures were generally similar in both experimental years, showing the expected seasonal pattern of increasing temperatures in summer and decreasing temperatures in winter (Figure 1). However, only three days exceeded 35 °C in 2022, whereas in 2023, there were 32 days with such extreme temperatures, likely related to several heat waves during the second semester, coinciding with the fourth cycle. During the experimental years of 2022 and 2023, the highest precipitation levels occurred in the summer, primarily in January, coinciding with the harvest period of the conventional cycle. In contrast, the lowest precipitation levels were recorded in July of both years, corresponding to the winter cycle harvest. Summer rainfall accounted for approximately 65 % of the total precipitation in 2022 and around 48 % in 2023, coinciding with critical stages of the summer cycle, such as fruit ripening and harvest (Figure 1).

Excessive rainfall can negatively affect production due to direct water contact with leaves, as the combination of high moisture and elevated temperatures creates ideal conditions for pathogen development, increasing disease incidence. For example, *Plasmopara viticola*, the causal agent of downy mildew, completes its life cycle in 5–18 days and can significantly reduce yield (Koledenkova *et al.*, 2022). Consequently, management strategies that mitigate these conditions, such as the use of plastic coverings, have become increasingly necessary.

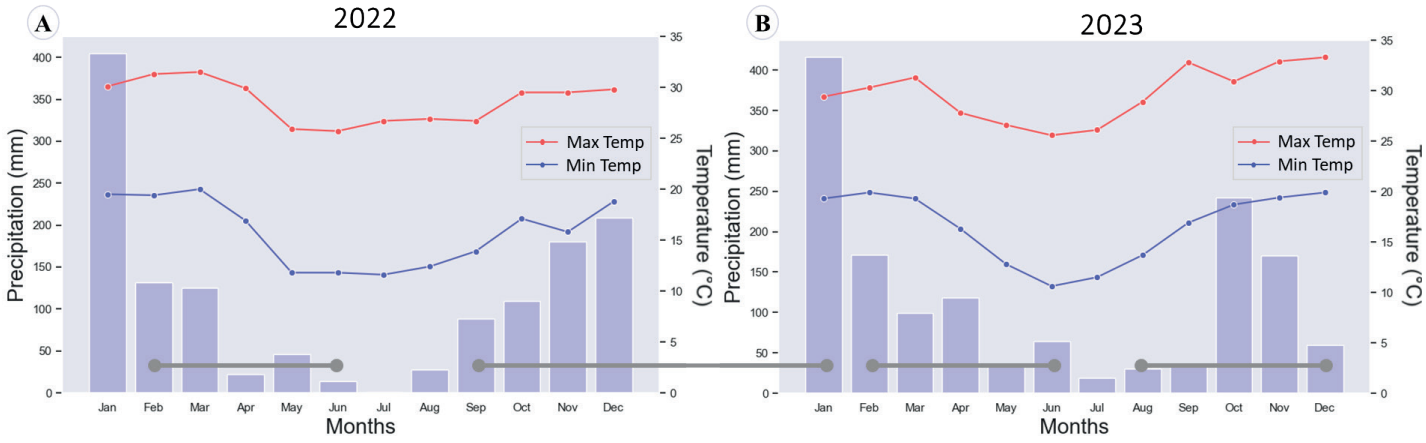


FIGURE 1. Variation of precipitation and temperature over the two years in the vineyard with the cv. Sauvignon blanc. A: 2022, B: 2023. The grey lines at the base of the graph represent the production cycles. From left to right, the first line represents cycle 1 (winter 2022), the second line represents cycle 2 (summer 2023), the third line represents cycle 3 (winter 2023), and the fourth line represents cycle 4 (summer 2024). The circle on the left end of each line indicates pruning, while the circle on the right end indicates harvesting.

The maximum photosynthetic photon flux density (PPFD) recorded outside the plastic covering was 1,820 µmol m^{-2} s^{-1} at 1 p.m., while under the plastic covering, the maximum was 1,362 µmol m^{-2} s^{-1} at the same time, indicating that the plastic reduced PPFD by approximately 30 %. Since the light saturation range for grapevines is 800–1,200 µmol m^{-2} s^{-1} (Carvalho *et al.*, 2016), plants outside the plastic were exposed to excessive light levels (Figure 2). Plants can mitigate light stress by activating photoprotective mechanisms in response to reactive oxygen species (ROS) generated in the photosynthetic apparatus (Mullineaux *et al.*, 2006). When these mechanisms are insufficient, photoinhibition can occur, reducing photosynthetic efficiency (Rizhsky *et al.*, 2002). Consequently, plastic coverings have increasingly been used in different regions of tropical areas to reduce excessive sunlight. For example, studies in different Brazilian states have reported PPFD reductions of up to 45 % in Rio de Janeiro (de Almeida *et al.*, 2020), 15 % in Minas Gerais (de Souza *et al.*, 2015), and 34 % in Rio Grande do Sul (Comiran *et al.*, 2012). This reduction can enhance vine growth and development by lowering the risk of photoinhibition.

The temperature dynamics at the experimental site were also notable. During the warmest hours of the day (9 a.m.–3 p.m.), air temperatures outside the plastic covering were higher than those inside, while at the beginning and end of the day, temperatures under the plastic were approximately 3 °C higher than outside. Under peak conditions, the plastic covering could reduce temperature by up to 3 °C (Figure 2). The literature reports variable effects of plastic coverings on vineyard microclimates. De Almeida *et al.* (2020), for instance, observed a ~7 % increase in maximum air temperature in covered vineyards, while Pedro Júnior *et al.* (2011) reported no differences compared to uncovered systems. Marigliano *et al.* (2022) observed that during a heatwave, temperatures in the berry region of uncovered vines reached 58 °C, while plastic covering mitigated this extreme heat. These discrepancies likely result from differences in plastic type, transmission rate, and thickness. Nonetheless, it is clear that plastic coverings alter vineyard microclimates.

Regarding light quality, differences were observed only in the UV-B range (280–315 nm), while the visible spectrum necessary for photosynthesis remained unaffected (Figure 3). Similarly, Chavarria *et al.* (2009) reported a 50 % reduction in UV radiation under plastic coverings. Higher UV-B exposure in uncovered conditions can negatively affect plants by reducing photosynthetic rate, stomatal conductance, and photosystem II efficiency, among other processes (Martínez-Lüscher *et al.*, 2013). Therefore, strategies that mitigate UV-B radiation are useful not only in tropical regions but also in areas increasingly affected by climate change.

2. Biometric traits

Interestingly, at the beginning of the cycle, vines in both conditions exhibited very similar growth rates, and in some cycles, no significant differences were observed (Figure 4). However, as the vines developed, differences in growth rate became increasingly apparent. Across all experimental cycles, the highest growth rates were consistently recorded in vines grown under plastic covering, with the greatest difference observed in the final cycle (summer 2024), where covered plants in the last week of analysis were 117.3 % taller than uncovered plants (Figure 4).

Vines in uncovered conditions were exposed to challenging environmental factors, including excessive light, high midday temperatures, and direct rainfall. Extreme light and temperature can negatively affect plant physiology, causing disorders such as sunburn, degradation of photosynthetic pigments, increased polyphenol accumulation, and loss of aroma compounds (Gambetta *et al.*, 2021). Additionally,

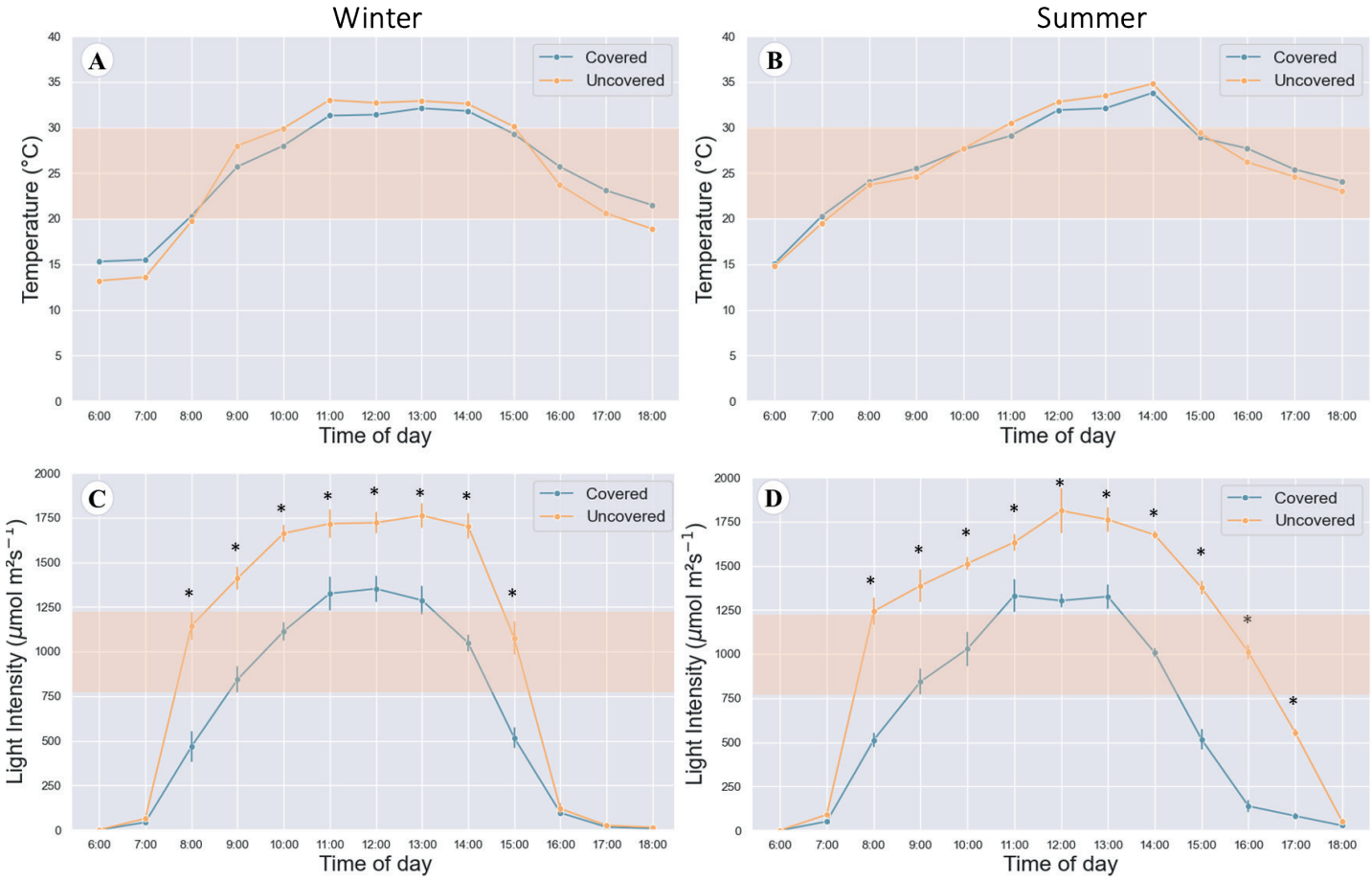


FIGURE 2. Variation in temperature and light intensity throughout the day in the experimental field. Environmental variables were monitored throughout the day under plastic (covered) and outdoors (uncovered) during the winter cycle (A and C) and the summer cycle (B and D). The red band indicates the light intensity range at which grapevine leaves generally reach light saturation and the temperature range considered ideal for the growth and development of grapevines. Asterisks indicate significant differences according to the *t*-test ($p < 0.05$). Error bars represent the standard deviation.

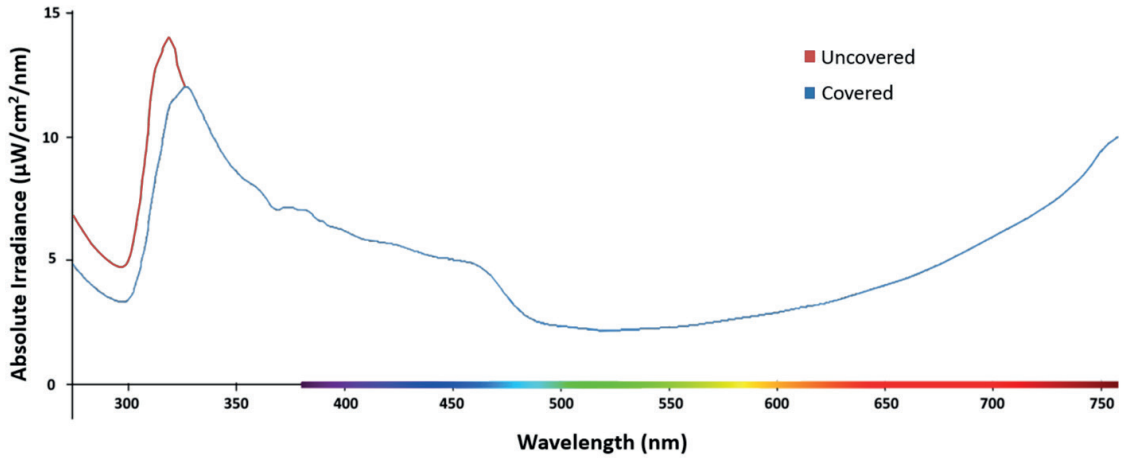


FIGURE 3. The light wavelengths under plastic (covered) and outdoor conditions (uncovered).

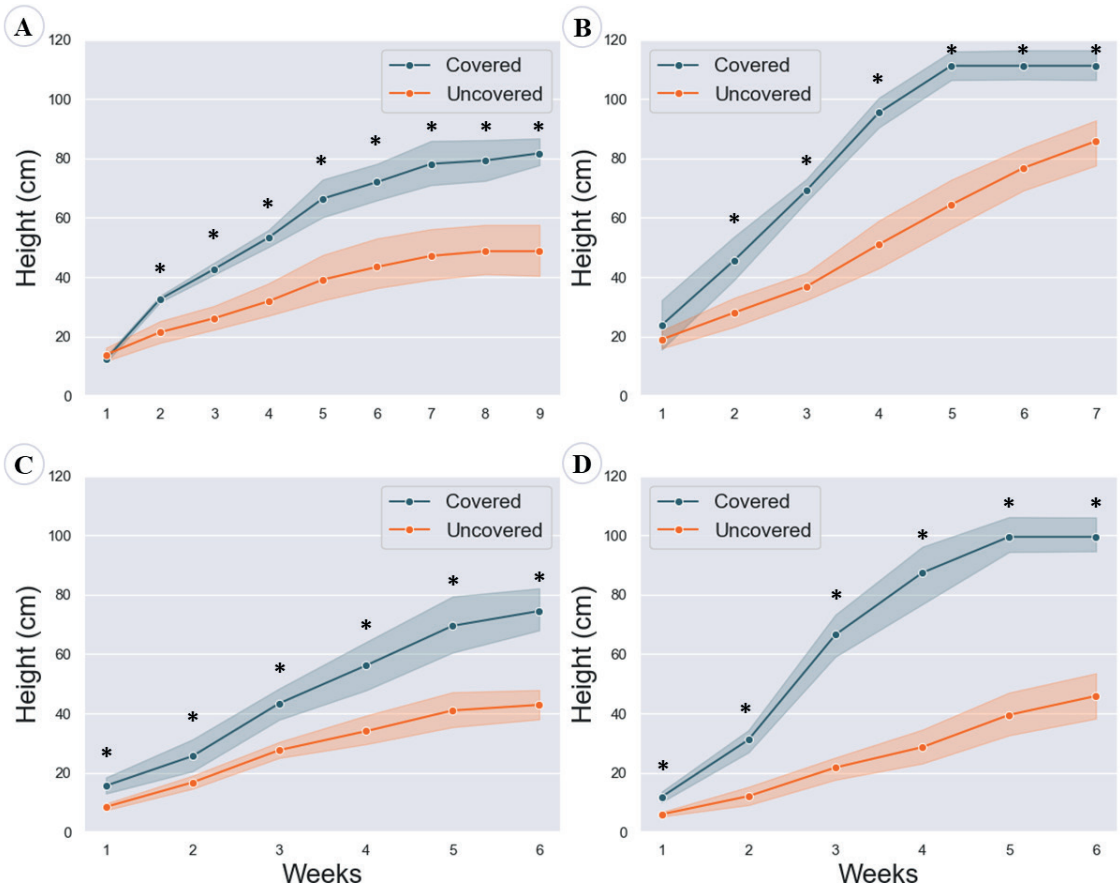


FIGURE 4. Growth of cv. Sauvignon blanc, over time cultivated under plastic (covered) and outside (uncovered) in different cycles. A: Winter (2022), B: Summer (2023), C: Winter (2023), D: Summer (2024). Asterisks indicate significant differences according to the *t*-test ($p < 0.05$). Error bands represent the standard deviation.

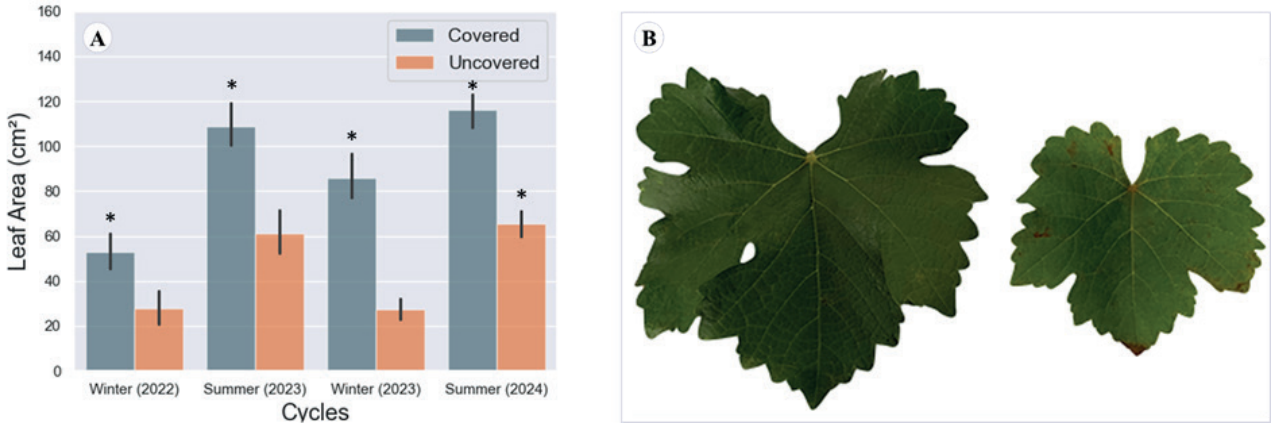


FIGURE 5. Comparison of leaf area and visual differences of cv. Sauvignon blanc cultivated under plastic (covered) and outdoors (uncovered) across different cycles. A: Graph showing the leaf area across different growth cycles; B: Photograph comparing a leaf from the covered treatment (left) and a leaf from the uncovered treatment (right). Asterisks indicate significant differences according to the *t*-test ($p < 0.05$). Error bars represent the standard deviation.

high temperatures combined with direct rainfall increase the incidence of diseases, such as downy mildew (González-Rodríguez *et al.*, 2011). Under such stress, plants often reduce or suspend growth through a finely tuned "growth-defence trade-off" (Figueroa-Macías *et al.*, 2021), which likely explains the reduced growth rate observed in uncovered vines. Several studies support these observations. Comiran *et al.* (2012) reported faster growth in covered vines, associated with higher thermal accumulation, whereas higher disease incidence was observed in uncovered vines. Similarly, de Souza *et al.* (2015) found that covered vines grew approximately 6 cm per day, compared to less than 4 cm per day in uncovered vines. These findings are consistent with the present study, highlighting that microclimate modification via plastic covering can improve growth conditions and promote vine development.

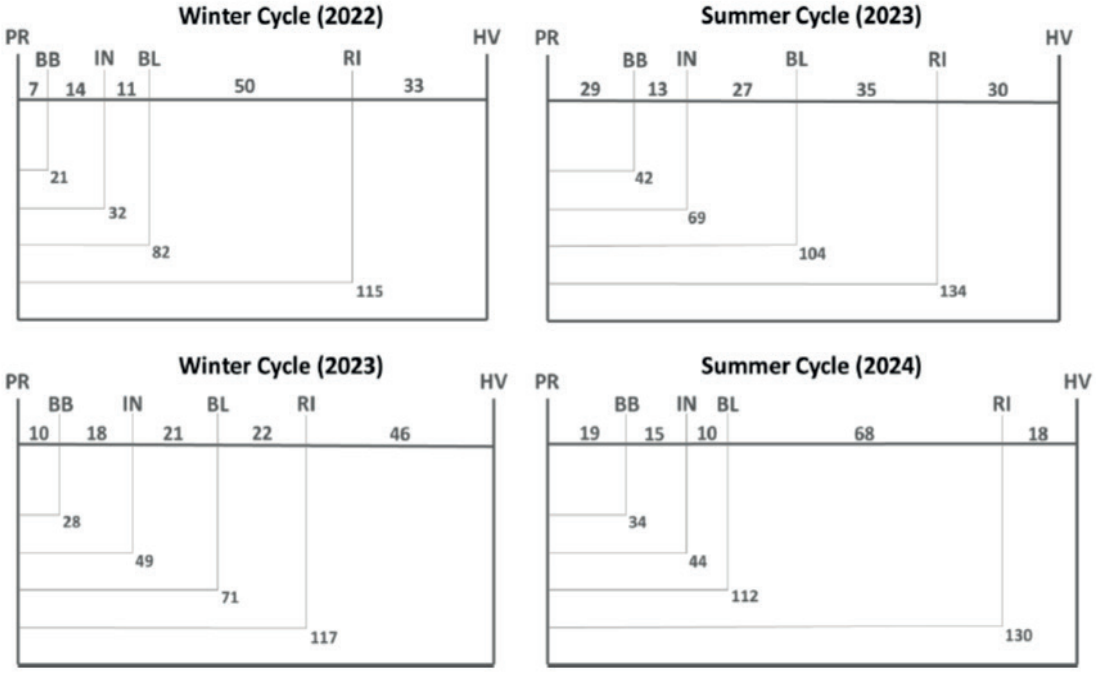


FIGURE 6. The phenological stages of the covered grapevines were influenced by the seasons of each cycle, while the uncovered grapevines failed to produce fruit. Duration in days of the main phenological stages of cv. Sauvignon blanc, according to the BBCH scale, is produced in different production cycles. PR: Pruning, BB: 09—bud break, IN: 57—inflorescence appearance, BL: 65—full bloom, RI: 81—beginning of ripening, HV: 89—harvest.

Vines grown under plastic covering consistently produced larger, darker green leaves compared to uncovered vines across all experimental cycles. The final cycle (summer) recorded the maximum leaf area, with covered vines reaching approximately 115 cm^2, whereas uncovered vines reached about 65 cm^2 (Figure 5). Similarly, a study conducted in southeastern Brazil reported larger leaf area in covered vines, which was associated with higher carbon assimilation and increased yield (de Souza *et al.*, 2015). Another study in the same region also observed greater carbon assimilation in covered vines, attributed to their larger leaf area (de Almeida *et al.*, 2020).

The winter cycles had similar overall durations: the first cycle occurred in 2022, lasting 115 days, and the second occurred in 2023, lasting 117 days. The summer cycles had a longer duration, with the 2023 cycle lasting 134 days and the 2024 cycle lasting 130 days (Figure 6). However, fruit ripening was faster during the summer cycles, taking 18–30 days, compared to 33–46 days in the winter cycles. Sauvignon blanc is a cultivar characterised by late bud break, and its growth cycle can vary between 115 and 170 days in Brazil (Borghezan *et al.*, 2011; Radünz *et al.*, 2015; Leão *et al.*, 2024). This period can vary depending on the specific climate and growing conditions of the vineyard. A longer time to bud break was observed in the summer cycles (19–29 DAP; Days After Pruning), compared to the winter cycle (7–10 DAP) (Figure 6). Since winter cycle pruning is done during the summer, when temperatures are higher, and pruning is performed on a higher bud (5th–6th), these factors likely accelerated the beginning of bud break.

The phenological stages of grapevines vary depending on the cultivar, management techniques, and prevailing weather conditions (Rafique *et al.*, 2024). The maximum temperatures influence significantly important stages, as budbreak and bloom, while average temperatures are more important for later season events, as fruit ripening and harvest (Alikadic *et al.*, 2019). In fact, this could be observed in this study, where budbreak occurred faster in winter cycles, with higher maximum temperatures (~32 °C) during this stage, if compared with summer cycles, where the pruning was done during maximum temperatures lower (~26 °C). Consequently, this early budbreak probably influenced the inflorescences to appear earlier. On the other hand, the fruit ripening was faster during summer cycles, where the average temperature during this stage was constantly higher.

One of the few experiments studying the cultivation of cv. Sauvignon blanc and other cultivars under double-pruning management showed that harvest during the conventional grapevine cycle (summer cycle) had to be advanced due to the incidence of diseases in the bunches (da Mota *et al.*, 2011). The authors noted that the higher incidence of diseases was attributed to the elevated precipitation commonly experienced in the summer. In fact, higher precipitation was observed during the harvest of the summer cycles (Figure 2), but the use of plastic covering prevented the onset of bunch diseases. As a result, ripening was influenced solely by temperature. This suggests that plastic covering can mitigate the negative impacts of excessive rainfall on grape quality.

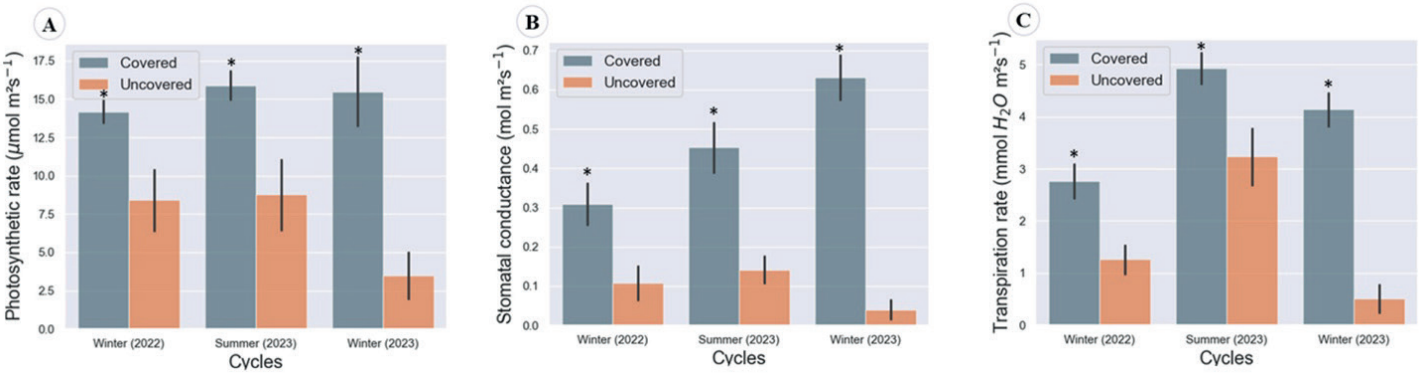


FIGURE 7. Physiological traits of 'Sauvignon blanc' cultivated under plastic and outside in different cycles. * Significant differences according to the *t*-test ($p < 0.05$). Error bars represent the standard deviation.

3. Physiological traits

Covered and uncovered vines exhibited distinct physiological traits throughout the experimental cycle. Across all cycles, covered vines showed higher photosynthetic rate (A), stomatal conductance (Gs), and transpiration rate (E) compared with uncovered vines (Figure 7). This indicates that covered vines were more actively photosynthesising and transpiring. All these processes are strongly influenced by light, of which 80–90 % of the transmitted spectrum is required to sustain photosynthesis, flower differentiation, and fruit ripening (Novello & de Palma, 2008). These findings suggest that the microclimatic alterations caused by plastic covering did not impair key physiological processes such as photosynthesis.

Several studies have also investigated whether plastic covering in vineyards impacts plant physiology. For example, an experiment conducted in Minas Gerais state with cv. Syrah demonstrated that covered vines did not show differences in A, Gs, and E compared with uncovered vines (de Souza *et al.*, 2015). Similarly, another study in Brazilian vineyards found no effect of plastic covering on these parameters (de Almeida *et al.*, 2020). In California, USA, an experiment with cv. Cabernet-Sauvignon under different types of plastic coverings also reported no significant effects on photosynthetic performance (Marigliano *et al.*, 2022). On the other hand, a study conducted in southern Brazil with cv. Cabernet-Sauvignon observed a higher maximum photosynthetic rate in covered vines (16.7 μmol m^{-2} s^{-1}) compared to uncovered vines (14.2 μmol m^{-2} s^{-1}), along with increased stomatal conductance (Mota *et al.*, 2009). The authors associated this improvement with a 44 % reduction in UV radiation under the covering, consistent with the findings of the present study.

Interestingly, the light response curves developed under both conditions showed that covered and uncovered vines reached similar light saturation levels, at 1,200 μmol m^{-2} s^{-1} (Figure 8). However, differences emerged at this saturation point, where covered vines reached a higher photosynthetic rate (17.5 μmol m^{-2} s^{-1}) compared with uncovered vines (13.5 μmol m^{-2} s^{-1}) (Figure 8). The lower photosynthetic rate observed in uncovered vines is likely associated with unfavourable conditions such as excess light, direct rainfall, and increased disease incidence. Furthermore, uncovered vines were more exposed to UV-B radiation, which is known to negatively affect photosynthesis and stomatal conductance (Martínez-Lüscher *et al.*, 2013).

Covered vines also exhibited significantly higher chlorophyll (chl) a and b indices throughout all experimental cycles compared with uncovered vines (Figure 9). This likely explains the darker green leaf colouration observed under plastic covering. Similar patterns were reported in southern Brazil, where cv. Moscato Giallo grown under plastic covering showed increased chl a content (Chavarria *et al.*, 2009). A comparable trend was observed with cv. Syrah in southeastern Brazil, where covered vines exhibited higher leaf chl content (de Souza *et al.*, 2015). The authors attributed this effect to reduced light transmission through the covering material. Interestingly, a study conducted in Egypt also reported higher chlorophyll content in covered vines (Doaa, 2018).

These changes in pigment indices under plastic covering were expected. Chlorophyll plays a fundamental role in photosynthesis by absorbing the light energy required for the process (Mao *et al.*, 2007). However, under unfavourable conditions such as excessive sunlight, damage to the chlorophyll reaction centres can occur due to excess energy (Dai *et al.*, 2009). In prolonged exposure, plants may not be able to efficiently dissipate this energy. Thus, the significant differences between covered and uncovered conditions in the present study are consistent with this mechanism, supporting the positive relationship observed between leaf chl indices, leaf area, and higher photosynthetic rates in covered vines (Chavarria *et al.*, 2012).

4. Anatomical analyses

Anatomical analyses revealed that the palisade parenchyma, spongy parenchyma, and adaxial epidermis were more developed in covered leaves during most experimental cycles compared with uncovered leaves (Table 1). Raphides, needle-shaped crystals, were frequently observed in uncovered vines (Figure 10). These anatomical differences are likely influenced by light intensity.

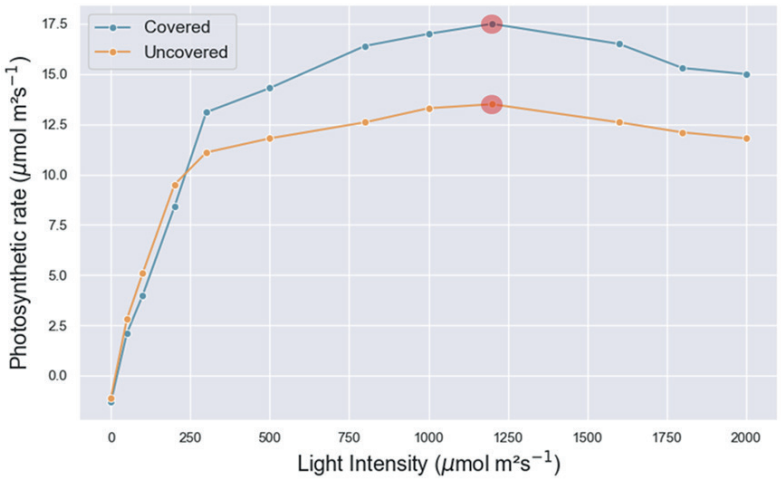


FIGURE 8. Light response curves from IRGA 409 of cv. Sauvignon blanc grapevines grown under plastic (covered) and outdoors (uncovered). The red circles indicate light saturation.

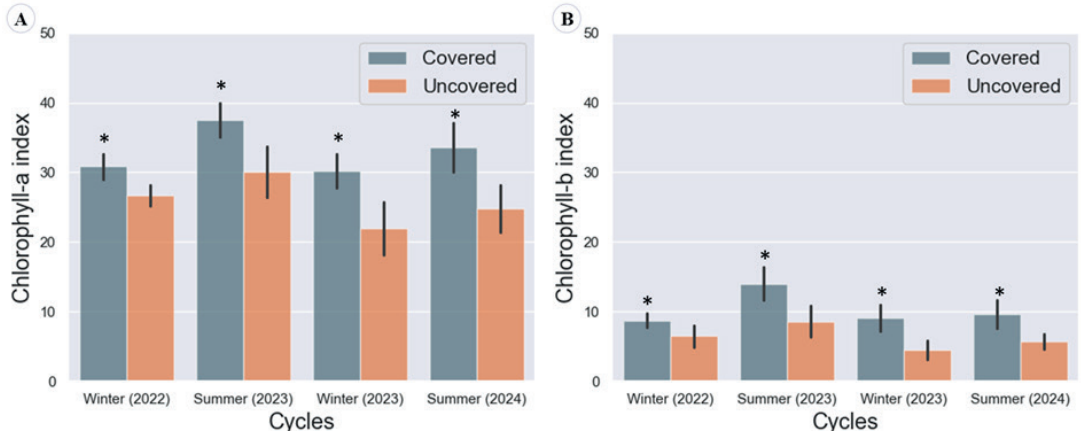


FIGURE 9. Chlorophyll index of leaves under the cover and outside the cover. Chlorophyll a (A) and b (B) indices of cv. Sauvignon blanc cultivated under plastic (covered) and outdoors (uncovered) in different cycles. Asterisks indicate significant differences according to the *t*-test ($p < 0.05$). Error bars represent the standard deviation.

TABLE 1. Leaf anatomical characteristics of cv. Sauvignon blanc cultivated under plastic (covered) and outdoors (uncovered), in a double-pruning system across four different cycles.

Leaf anatomical characteristics	Winter (2022)				Summer (2023)			
	Covered		Uncovered		Covered		Uncovered	
Mesophyll (μm)	102.83 (± 9)	a	109.35 (± 7.54)	a	107.11 (± 6.22)	b	131.14 (± 7.03)	a
Adaxial epidermis (μm)	131.51 (± 0.85)	b	15.64 (± 1.56)	a	13.67 (± 1.83)	b	15.90 (± 1.45)	a
Palisade parenchyma (μm)	45.15 (± 6.44)	b	55.93 (± 4.97)	a	44.16 (± 2.64)	b	58.62 (± 5.78)	a
Spongy parenchyma (μm)	60.22 (± 6.25)	a	54.15 (± 7.25)	a	62.04 (± 6.83)	b	70.61 (± 4.53)	a
Stomatal density (stomata/mm²)	113.73 (± 9.21)	b	174.8 (± 11.4)	a	147.40 (± 4.20)	b	168.70 (± 8.27)	a
	Winter (2023)				**Summer (2024)**			
	Covered		**Uncovered**		**Covered**		**Uncovered**	
Mesophyll (μm)	98.81 (± 2.78)	b	104.57 (± 4.55)	a	105.60 (± 13.5)	b	122.68 (± 4.04)	a
Adaxial epidermis (μm)	10.36 (± 0.81)	b	13.76 (± 0.63)	a	10.56 (± 1.36)	b	14.74 (± 1.33)	a
Palisade parenchyma (μm)	40.94 (± 2.46)	b	48.03 (± 0.61)	a	47.26 (± 3.39)	b	53.69 (± 3.11)	a
Spongy parenchyma (μm)	50.17 (± 2.52)	b	55.50 (± 4.38)	a	57.5 (± 11.7)	b	69.97 (± 4.54)	a
Stomatal density (stomata/mm²)	128.67 (± 4.55)	b	152.50 (± 10.9)	a	122.2 (± 13.2)	a	128.3 (± 11.5)	a
Cuticle (μm)	–		–		1.24 (± 0.11)	a	1.18 (± 0.17)	b

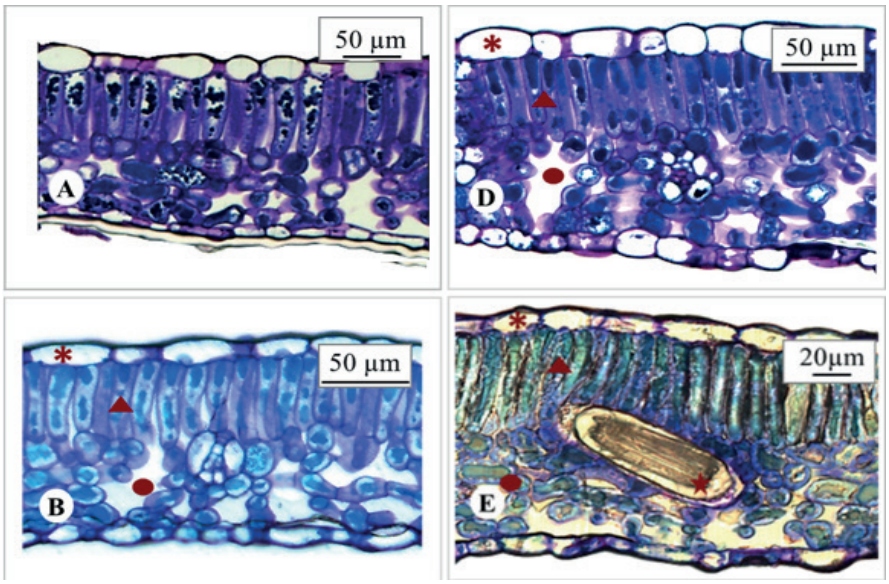


FIGURE 10. Images of leaf anatomy of covered and uncovered leaves. Light microscopy of cv. Sauvignon blanc leaves cultivated under plastic (D, E and F – covered) and outdoors (A, B and C – uncovered). Arrows point to the cuticle. *—epidermis adaxial; ▲—palisade parenchyma; ●—spongy parenchyma; ★—raphides.

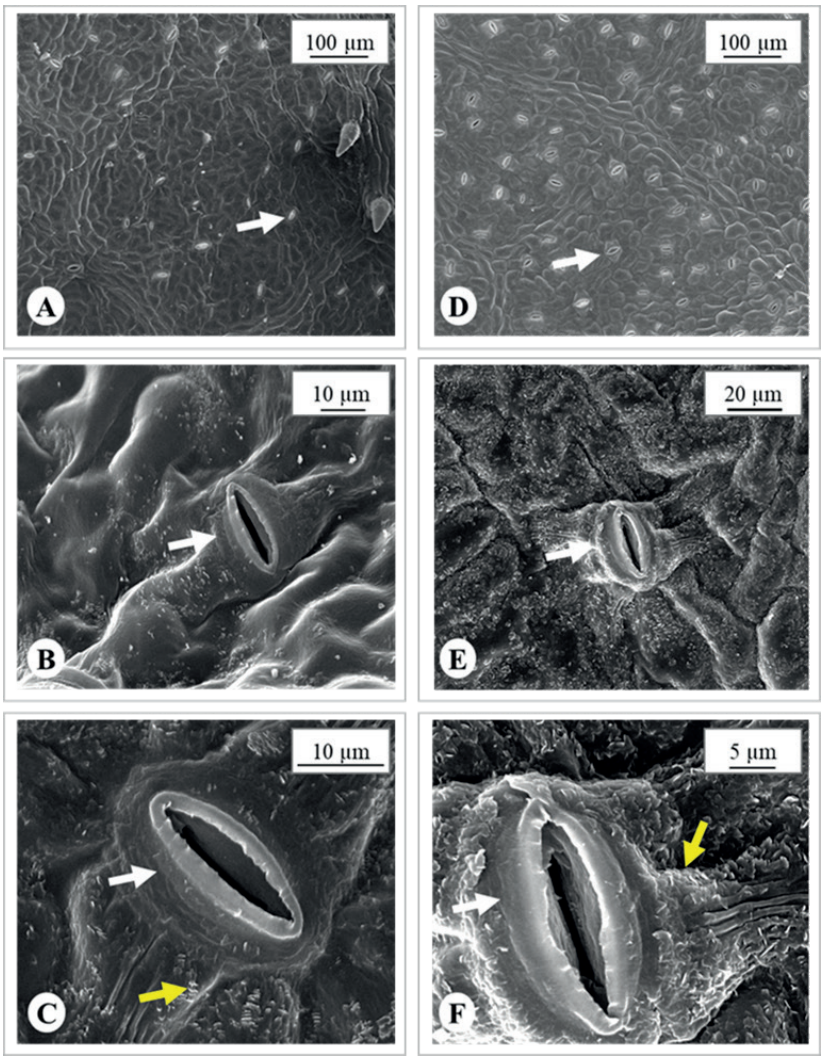


FIGURE 11. Epidermis of covered and uncovered leaves. Scanning electron microscopy of the abaxial epidermis of cv. Sauvignon blanc leaves grown covered (A–C) and conventionally (uncovered) (D–F). White arrows indicate stomata. Yellow arrows point to the epicuticular wax.

Interestingly, the leaf anatomy of covered vines resembled the typical characteristics of shade leaves, such as thinner leaf blades with reduced palisade tissue and higher pigment accumulation (Zhang *et al.*, 2022). Thinner leaves are known to enhance light interception compared to thicker ones (Terashima *et al.*, 2011). This pattern was consistent with the findings of this study, where covered leaves were larger, had smaller palisade parenchyma, and exhibited higher chlorophyll indices. In contrast, uncovered leaves likely adjusted their structure in response to excessive light and lack of protection, thereby minimising damage to the photosynthetic apparatus. Under such conditions, leaves typically develop a thicker palisade parenchyma, sometimes with additional cell layers or elongated cells (Poorter *et al.*, 2019), which aligns with the observations recorded in this study (Figure 10).

Means followed by the same letter in the row, in each cycle, indicate no significant difference among them according to the *t*-test ($p < 0.05$).

Stomatal density (SD), defined as the number of stomata per square millimetre of leaf surface, varied significantly across all cycles except for cycle 4, with uncovered vines consistently showing higher SD. Scanning electron microscopy analysis of the abaxial epidermis further revealed that uncovered leaves had a greater deposition of epicuticular wax on the ordinary epidermal cells and guard-cells in the form of platelets (Figure 11).

The micromorphology of the grapevine leaf cuticles of the covered and uncovered plants shows a plasticity pattern of the epicuticular wax production. The epicuticular wax plays a key role in reducing UV damage, non-stomatal dehydration, the effect of strong sunlight, and preventing fungal invasion (Guo *et al.*, 2011; Marques *et al.*, 2016; Guan *et al.*, 2024). The effect of light on epicuticular wax micromorphology was investigated in *Hosta* spp. genotypes, indicating that higher light intensities modify wax crystal organisation and are associated with changes in cuticular structure (Guan *et al.*, 2024). Similar results were observed herein, showing that direct light exposition alters cuticle micro morphology and its protection may prevent epicuticular wax changes. SD is strongly influenced by environmental factors affecting both developing and mature leaves. For example, when mature leaves are exposed to shade or elevated CO_2 levels, newly emerging leaves typically develop a reduced SD (Engineer *et al.,* 2016; Lake *et al.,* 2001).

Despite these findings, the role of SD in photosynthesis and plant growth remains controversial. Some studies have shown that reduced SD can promote greater plant height and biomass under constant light and favourable water and temperature conditions, as stomatal development requires considerable energy (Wang *et al.*, 2016). Conversely, other studies have reported that lower SD is associated with reduced photosynthetic rate and stomatal conductance (Lawson & Blatt, 2014). In this study, the higher SD observed in uncovered vines was not sufficient to enhance the photosynthetic rate, most likely due to the unfavourable conditions these plants experienced, such as elevated temperatures, excess light, and increased disease incidence.

5. Chemical control

The plastic covering prevented the direct incidence of rainwater on the leaves of covered vines, thereby reducing the wash-off of applied chemicals such as fungicides and insecticides. As a result, the frequency of pesticide application differed between treatments. Across all cycles, only two sprayings were required in the covered system, whereas in the uncovered system, the number varied between 7 and 0, depending on the cycle (Table 2). Consequently, protected cultivation reduced both the number of chemical applications and the incidence of diseases. Interestingly, caterpillar attacks were observed only in uncovered rows. These findings reinforce that management strategies that modify environmental conditions represent an effective approach to disease control by creating environments less favourable to pathogens.

Other studies have also reported differences in chemical control strategies in vineyards due to plastic covering. For instance, Chavarria *et al.* (2007) recorded 17 pesticide applications in uncovered vineyards compared to only 2 in covered vineyards. Similarly, Genta *et al.* (2010) reported a 75 % reduction in pesticide spraying under protected cultivation. Taken together, these results indicate that the use of plastic covering is an effective strategy for disease management, mitigating the challenges posed by unfavourable weather conditions.

TABLE 2. Number of sprayings during the cycle in cv. Sauvignon blanc vines conducted under covered and uncovered conditions.

Cycles	Number of sprayings Covered	Number of sprayings Uncovered
Winter (2022)	2	7
Summer (2023)	2	10
Winter (2023)	2	7
Summer (2024)	2	9

CONCLUSION

The use of plastic covering created a favourable microclimate that positively influenced vine growth and development, while also reducing disease incidence. In the studied region of Brazil, the double-pruning management technique alone was insufficient, as it likely required multiple fungicide applications to maintain vine health. Plastic covering reduced PPFD levels to values sufficient for vines to reach light saturation, while simultaneously decreasing UV-B radiation. Together, these effects contributed to improved physiological processes, such as photosynthesis.

REFERENCES

Alikadic, A., Pertot, I., Eccel, E., Dolci, C., Zarbo, C., Caffarra, A., de Filippi, R., & Furlanello, C. (2019). The impact of climate change on grapevine phenology and the influence of altitude: A regional study. *Agricultural and Forest Meteorology*, *271*, 73–82. https://doi.org/10.1016/j.agrformet.2019.02.030

Amorim, D. A. D. E., Favero, A. N. A. C., & Regina, M. D. E. A. (2005). Produção extemporânea da videira, cultivar Syrah, nas condições do sul de Minas Gerais. *Revista Brasileira de Fruticultura*, *27*(2), 327–331. https://doi.org/10.1590/S0100-29452005000200036

Becker, T., & Knoche, M. (2011). Water movement through the surfaces of the grape berry and its stem. *American Journal of Enology and Viticulture*, *62*(3), 340–350. https://doi.org/10.5344/ajev.2011.10056

Borghezan, M., Gavioli, O., Pit, F. A., & da Silva, A. L. (2011). Comportamento vegetativo e produtivo da videira e composição da uva em São Joaquim, Santa Catarina. *Pesquisa Agropecuária Brasileira*, *46*(4), 398–405. https://doi.org/10.1590/S0100-204X2011000400009

Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Chaves, M. M., & Amâncio, S. (2016). Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. *Plant Biology*, *18*(S1), 101–111. https://doi.org/10.1111/plb.12410

Chavarria, G., Cardoso, L. S., Bergamaschi, H., dos Santos, H. P., Mandelli, F., & Marodin, G. A. B. (2009). Microclima de vinhedos sob cultivo protegido. *Ciência Rural*, *39*(7), 2029–2034. https://doi.org/10.1590/S0103-84782009005000147

Chavarria, G., dos Santos, H. P., Castro, L. A. S., Marodin, G. A. B., & Bergamaschi, H. (2012). Anatomy, chlorophyll content and photosynthetic potential in grapevine leaves under plastic cover. *Revista Brasileira de Fruticultura*, *34*(3), 661–668. https://doi.org/10.1590/S0100-29452012000300003

Chavarria, G., dos Santos, H. P., Sônego, O. R., Marodin, G. A. B., Bergamaschi, H., & Cardoso, L. S. (2007). Incidência de doenças e necessidade de controle em cultivo protegido de videira. *Revista Brasileira de Fruticultura*, *29*(3), 477–482. https://doi.org/10.1590/S0100-29452007000300014

Comiran, F., Bergamaschi, H., Heckler, B. M. M., Pessoa, H., dos Santos, D., Alba, D., & Saretta, E. (2012). Microclima e produção de videiras 'Niágara Rosada' em cultivo orgânico sob cobertura plástica. *Revista Brasileira de Fruticultura*, *34*(1), 120–126. https://doi.org/10.1590/S0100-29452012000100021

Dai, Y., Shen, Z., Liu, Y., Wang, L., Hannaway, D., & Lu, H. (2009). Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environmental and Experimental Botany*, *65*(2–3), 177–182. https://doi.org/10.1016/j.envexpbot.2008.12.008

da Mota, R. V., Silva, C. P. C., Favero, A. C., Purgatto, E., Shiga, T. M., & Regina, M. de A. (2011). Composição físico-química de uvas para vinho fino em ciclos de verão e inverno. *Revista Brasileira de Fruticultura*, *32*(4), 1127–1137. https://doi.org/10.1590/S0100-29452011005000001

de Almeida, L. V. B., Rodrigues, W. P., da Silva, J. R., de Assis Figueiredo, F. A. M. M., da Silva de Deus, B. C., Ruas, K. F., Ferraz, T. M., de Oliveira Martins, A., Griffin, K. L., Bressan-Smith, R., & Campostrini, E. (2020). Transparent polyethylene covering film in tropical grapevines does not alter photosynthesis, plant growth, fruit quality or yield. *Theoretical and Experimental Plant Physiology*, *32*(3), 255–270. https://doi.org/10.1007/s40626-020-00177-8

de Orduña, R. M. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*, *43*, 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

de Souza, C. R., da Mota, R. V., Dias, F. A. N., de Melo, E. T., Pimentel, R. M. de A., de Souza, L. C., & Regina, M. de A. (2015). Physiological and agronomical responses of Syrah grapevine under protected cultivation. *Bragantia*, *74*(3), 270–278. https://doi.org/10.1590/1678-4499.0047

Doaa, M. H. (2018). Effect of plastic covering and rootstocks on yield, physical and chemical characteristics of Superior grapevines (*Vitis vinifera* L.). *Journal of Plant Production*, *9*(10), 813–818. https://doi.org/10.21608/jpp.2018.36444

Engineer, C. B., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordström, M., Azoulay-Shemer, T., Rappel, W. J., Iba, K., & Schroeder, J. I. (2016). CO_2 sensing and CO_2 regulation of stomatal conductance: Advances and open questions. *Trends in Plant Science*, *21*(1), 16–30. https://doi.org/10.1016/j.tplants.2015.08.014

Favero, A. C., Amorim, D. A., Mota, R. V., Soares, A. M., Souza, C. R., & Regina, M. (2011). Double-pruning of Syrah grapevines: A management strategy to harvest wine grapes during the winter in the Brazilian Southeast. *Vitis - Journal of Grapevine Research*, *50*(4), 151–158.

Figueroa-Macías, J. P., García, Y. C., Núñez, M., Díaz, K., Olea, A. F., & Espinoza, L. (2021). Plant growth-defense trade-offs: Molecular processes leading to physiological changes. *International Journal of Molecular Sciences*, *22*(2), 693. https://doi.org/10.3390/ijms22020693

Gambetta, J. M., Romat, V., Schmidtke, L. M., & Holzapfel, B. P. (2021). Secondary metabolites coordinately protect grapes from excessive light and sunburn damage during development. *Biomolecules*, *12*(1), 42. https://doi.org/10.3390/biom12010042

Genta, W., Tessmann, D. J., Roberto, S. R., Vida, J. B., Colombo, L. A., Scapin, C. R., Ricce, W. da S., & Clovis, L. R. (2010). Manejo de míldio no cultivo protegido de videira de mesa 'BRS Clara'. *Pesquisa Agropecuária Brasileira, 45*(12), 1388–1395. https://doi.org/10.1590/S0100-204X2010001200008

González-Rodríguez, R. M., Cancho-Grande, B., & Simal-Gándara, J. (2011). Decay of fungicide residues during vinification of white grapes harvested after the application of some new active substances against downy mildew. *Food Chemistry*, *125*(2), 549–560. https://doi.org/10.1016/j.foodchem.2010.09.047

Guan, P., Chen, S., Sun, J., Zhao, S., Fan, R., Xu, Y., & Qu, B. (2024). Effects of different shade treatments on the epidermal wax deposition of *Hosta* genotypes with different glaucousness of leaf surface. *Horticulturae*, *10*(9), 981. https://doi.org/10.3390/horticulturae10090981

Guo, Y.-J., Ni, Y., Guo, Y.-J., Han, L., Tang, H., & Yu, Y.-X. (2011). Effect of soil water deficit and high temperature on leaf cuticular waxes and physiological indices in Alfalfa *(Medicago sativa)* leaf. *Acta Agronomica Sinica*, *37*(5), 911–917. https://doi.org/10.3724/SP.J.1006.2011.00911

Holcman, E., Sentelhas, P. C., Conceição, M. A. F., & Couto, H. T. Z. (2018). Vineyard microclimate and yield under different plastic covers. *International Journal of Biometeorology*, *62*(6), 925–937. https://doi.org/10.1007/s00484-017-1494-y

Koledenkova, K., Esmaeel, Q., Jacquard, C., Nowak, J., Clément, C., & Ait Barka, E. (2022). *Plasmopara viticola*, the causal agent of downy mildew of grapevine: From its taxonomy to disease management. *Frontiers in Microbiology*, *13*, 889472. https://doi.org/10.3389/fmicb.2022.889472

Kurtural, S. K., & Gambetta, G. A. (2021). Global warming and wine quality: Are we close to the tipping point? *OENO One*, *55*(3), 353–361. https://doi.org/10.20870/oeno-one.2021.55.3.4774

Lake, J. A., Quick, W. P., Beerling, D. J., & Woodward, F. I. (2001). Signals from mature to new leaves. *Nature*, *411*(6834), 154. https://doi.org/10.1038/35075660

Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology*, *164*(4), 1556–1570. https://doi.org/10.1104/pp.114.237107

Leão, P. C. de S., Telles Biasoto Marques, A., Moura da Silva, M., Badji, C. A., Barros, A. P. A., & Peroba de Oliveira Santos, J. (2024). *Circular técnica: Cultivares de videira para elaboração de vinhos finos no Agreste pernambucano*. Embrapa.

Limier, B., Ivorra, S., Bouby, L., Figueiral, I., Chabal, L., Cabanis, M., Ater, M., Lacombe, T., Ros, J., Brémond, L., & Terral, J. (2018). Documenting the history of the grapevine and viticulture: A quantitative eco-anatomical perspective applied to modern and archaeological charcoal. *Journal of Archaeological Science*, *100*, 45–61. https://doi.org/10.1016/j.jas.2018.10.001

Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., & Weber, E. (1995). Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*) – Codes and descriptions according to the extended BBCH scale. *Australian Journal of Grape and Wine Research*, *1*(2), 100–103. https://doi.org/10.1111/j.1755-0238.1995.tb00085.x

Mao, L. Z., Lu, H. F., Wang, Q., & Cai, M. M. (2007). Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*. *Photosynthetica*, *45*(4), 601–605. https://doi.org/10.1007/s11099-007-0103-4

Marigliano, L. E., Yu, R., Torres, N., Tanner, J. D., Battany, M., & Kurtural, S. K. (2022). Photoselective shade films mitigate heat wave damage by reducing anthocyanin and flavonol degradation in grapevine (*Vitis vinifera* L.) berries. *Frontiers in Agronomy*, *4*, 898870. https://doi.org/10.3389/fagro.2022.898870

Marques, J. P., Amorim, L., Spósito, M. B., & Appezzato-da-Glória, B. (2016). Ultrastructural changes in the epidermis of petals of the sweet orange infected by *Colletotrichum acutatum*. *Protoplasma*, *253*(5), 1233–1242. https://doi.org/10.1007/s00709-015-0877-3

Marques, J. P. R., & Soares, M. K. M. (2022). *Handbook of techniques in plant histopathology* (1st ed.). Springer Nature.

Martínez-Lüscher, J., Morales, F., Delrot, S., Sánchez-Díaz, M., Gomés, E., Aguirreolea, J., & Pascual, I. (2013). Short- and long-term physiological responses of grapevine leaves to UV-B radiation. *Plant Science*, *213*, 114–122. https://doi.org/10.1016/j.plantsci.2013.08.010

Mota, C. S., do Amarante, C. V. T., dos Santos, H. P., & Albuquerque, J. A. (2009). Disponibilidade hídrica, radiação solar e fotossíntese em videiras 'Cabernet Sauvignon' sob cultivo protegido. *Revista Brasileira de Fruticultura*, *31*(2), 432–439. https://doi.org/10.1590/S0100-29452009000200017

Mullineaux, P. M., Karpinski, S., & Baker, N. R. (2006). Spatial dependence for hydrogen peroxide-directed signaling in light-stressed plants. *Plant Physiology*, *141*(2), 346–350. https://doi.org/10.1104/pp.106.078162

Nistor, E., Dobrei, A., Dobrei, A., Camen, D., & Matti, G. B. (2018). Temperature and rainfall influence on shoot length in Pinot Noir, Merlot and Cabernet Sauvignon varieties. *Scientific Papers – Series B, Horticulture*, *LXII*, 267–274.

Novello, V., & de Palma, L. (2008). Growing grapes under cover. *Acta Horticulturae*, *785*, 353–362. https://doi.org/10.17660/ActaHortic.2008.785.44

Pedro Júnior, M. J., Hernandes, J. L., & Rolim, G. de S. (2011). Sistema de condução em Y com e sem cobertura plástica: microclima, produção, qualidade do cacho e ocorrência de doenças fúngicas na videira "Niagara Rosada". *Bragantia*, *70*(1), 228–233. https://doi.org/10.1590/S0006-87052011000100030

Peel, M. C., Finlayson, B. L., & McMahon, T. A. (2007). Updated world map of the Köppen–Geiger climate classification. *Hydrology and Earth System Sciences*, *11*(5), 1633–1644. https://doi.org/10.5194/hess-11-1633-2007

Poni, S., Gatti, M., Tombesi, S., Squeri, C., Sabbatini, P., Rodas, N. L., & Frioni, T. (2020). Double cropping in *Vitis vinifera* L. 'Pinot Noir': Myth or reality? *Agronomy*, *10*(6), 799. https://doi.org/10.3390/agronomy10060799

Poorter, H., Niinemets, Ü., Ntagkas, N., Siebenkäs, A., Mäenpää, M., Matsubara, S., & Pons, T. L. (2019). A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist*, *223*(3), 1073–1105. https://doi.org/10.1111/nph.15754

Radünz, A. L., Schöffel, E. R., Borges, C. T., Malgarim, M. B., & Pötter, G. H. (2015). Necessidades térmicas de videiras na região da Campanha do Rio Grande do Sul, Brasil. *Ciência Rural*, *45*(4), 626–632. https://doi.org/10.1590/0103-8478cr20140134

Rafique, R., Ahmad, T., Khan, M. A., Ahmed, M., & Hoogenboom, G. (2024). Developing a simple and efficient modeling solution for predicting key phenological stages of table grapes in a non-traditional viticulture zone in South Asia. *International Journal of Biometeorology*, *68*, 1587–1601. https://doi.org/10.1007/s00484-024-02686-6

Regina, M. A., Mota, R. V., Souza, C. R., & Favero, A. C. (2011). Viticulture for fine wines in Brazilian Southeast. *Acta Horticulturae*, *912*, 113–120. https://doi.org/10.17660/ActaHortic.2011.910.8

Rizhsky, L., Liang, H., & Mittler, R. (2002). The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiology*, *130*(3), 1143–1151. https://doi.org/10.1104/pp.006858

Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J. J., & Morales, F. (2010). Effects of climate change scenarios on Tempranillo grapevine (*Vitis vinifera* L.) ripening: Response to a combination of elevated CO_2 and temperature, and moderate drought. *Plant and Soil*, *337*, 179–191. https://doi.org/10.1007/s11104-010-0514-z

Shah, M. H., Rafique, R., Rafique, T., Naseer, M., Khalil, U., & Rafique, R. (2021). Effect of climate change on polyphenols accumulation in grapevine. In: *Phenolic compounds—chemistry, synthesis, diversity, non-conventional industrial, pharmaceutical and therapeutic applications*. IntechOpen. DOI: 10.5772/intechopen.99779

Terashima, I., Hanba, Y. T., Tholen, D., & Niinemets, Ü. (2011). Leaf functional anatomy in relation to photosynthesis. *Plant Physiology*, *155*(1), 108–116. https://doi.org/10.1104/pp.110.165472

Wang, C., Liu, S., Dong, Y., Zhao, Y., Geng, A., Xia, X., & Yin, W. (2016). *PdEPF1* regulates water-use efficiency and drought tolerance by modulating stomatal density in poplar. *Plant Biotechnology Journal*, *14*(3), 849–860. https://doi.org/10.1111/pbi.12434

Xu, C., Zhang, Y., Zhu, L., Huang, Y., & Lu, J. (2011). Influence of growing season on phenolic compounds and antioxidant properties of grape berries from vines grown in subtropical climate. *Journal of Agricultural Science*, *149*(6), 1078–1086. https://doi.org/10.1021/jf104157z

Zhang, Y., Chen, C., Jin, Z., Yang, Z., & Li, Y. (2022). Leaf anatomy, photosynthesis, and chloroplast ultrastructure of *Heptacodium miconioides* seedlings reveal adaptation to light environment. *Environmental and Experimental Botany*, *195*, 104780. https://doi.org/10.1016/j.envexpbot.2022.104780