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Physiological characteristics of expanding and expanded leaves of *Vitis vinifera* L. cv. Assyrtiko in climate change conditions

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Abstract: The impact of climatic change in viticulture is expected to be severe in the Mediterranean area in the future. The scope of this study is the evaluation of the leaf functional and optical properties of grapevine (*Vitis vinifera* L.) cultivar (cv.) Assyrtiko and its response to abiotic stress conditions (elevated temperature and water deficiency) caused by climatic change. Plants of grapevine cv. Assyrtiko were placed in a growth chamber in the Botany Department of the National and Kapodistrian University of Athens, Greece and four indoor environmental treatments were applied, concerning temperature (ambient versus ambient +2 °C) and water availability (well watered versus water stressed). The photosynthetic pigments (chlorophylls *a* and *b* and carotenoids) were determined as well as the leaf area, dry weight and specific leaf area in expanding and fully expanded leaves of the treated plants. Using a UV/VIS spectrophotometer (Perkin Elmer Lambda-950), equipped with an integrating sphere, the reflectance (R) and the transmittance (T), were measured *in situ*, between 250nm and 2500nm wavelength, in both adaxial and abaxial leaf surfaces of the grapevine cv. Assyrtiko and the absorbance (A) was calculated. It is likely that leaf chlorophyll content declined under drought and elevated temperature conditions.

Keywords: chlorophyll; leaf absorbance; specific leaf area; temperature; *Vitis vinifera* L. cv. Assyrtiko; water deficit.

1. Introduction

Global warming is defined in the report of IPCC (Intergovernmental Panel on Climate Change) as an average increase in combined surface air and sea surface temperatures over the globe and over a 30-year period. Various climate models have been released since 1992 from IPCC reporting the impact of emissions and air pollution on earth. The climatic models are constantly being updated, as different modeling groups around the world incorporate higher spatial resolution and new physical processes. According to the latest report of IPCC [1], the global temperature is likely to increase by 2 °C during the first half of 21st century due to climate change, and this increase is projected to be in the range between 2.6 °C and 4.8 °C for the second half of 21st century, according to the concentration-driven CMIP5 model simulation, based on RCP8.5 scenario. Rise of temperature will result in prolonged summer drought periods, which tends to reduce plant aboveground primary productivity. Unlike for temperature, where models show a general agreement about future regional changes, concerning water availability different models present the same region as becoming much wetter or much drier in a warming global environment.

Viticulture is also particularly sensitive to changes in climatic parameters [2], and the impact of climatic change in viticulture and native vegetation is expected to be more severe in the Mediterranean region [3,4]; the Mediterranean region is expected to have approximately 20% less precipitation by 2100 according to the RCP8.5 scenario. This change will possess a big impact on agriculture industry. Geographic locations in Greece will undoubtedly be influenced by oncoming weather conditions due to global warming, making some of the cultivated plants impossible to grow in such a hot climate. The increase of the temperature due to climate change has been proven to reduce the photosynthetic ability of the plants [5, 6]. Water stress linked to global warming is considered to be a major inhibitor of plants' development, affecting the main functional and structural characteristics of the leaves, which are exposed to the air conditions.

The grapevine cv. Assyrtiko is a native and well-known variety in Greece. *Vitis vinifera* cv. Assyrtiko is a white grape variety and one of the most important varieties found in the Mediterranean basin. The young leaves of *Vitis vinifera* cv. Assyrtiko are yellow-green with copper-colored areas, possessing smooth upper surface, and pubescent abaxial surface, while the mature leaves are wedge-shaped and symmetrical [7]. It is an early-ripening variety spread throughout Greece and is, in terms of quality, one of the most important indigenous varieties. It is mostly cultivated on Cyclades islands of the Aegean archipelago, but mainly in the volcanic soil of Santorini (Assyrtiko Santorini), which is its original terroir. Despite the observed trends of climatic factors and the importance of the wine sector in the Greek economy, to the best of our knowledge there has been a lack of studies on the impact of climatic change on Greek viticulture in general [8]. Also, there is little knowledge about the productivity response of indigenous grapevine varieties to fluctuations of climatic parameters [9,10].

2. Experiments

Numerous plants of cv. Assyrtiko grapevine, were obtained from canes (approximately 50 cm) each with 4–5 buds. They were grown in pots filled with a soil substrate composed of sand 50-60%, clay 20-30% and 10-20% sludge. The soil pH varied from 7.5 to 8.0 and 1.5% organic substance was included. The rootstock that was used was 1103P. The plants were placed in a growth chamber in the Botany Department of the National and Kapodistrian University of Athens (Greece). During the first fifteen days, all plants were exposed to a temperature of 14^{o}C, 15h photoperiod and approximately 70% relative humidity [11]. After two weeks that were left to be adjusted in the indoor environmental conditions, 4 groups of 20 plants were created (control, exp1, exp2 and exp3). The considered abiotic factors (temperature and irrigation) were changing according to the mean monthly values of temperature and precipitation of the last ten years taken from the nearest national weather station (37.933 N, 23.950 E). The control group includes well-watered plants. The 2nd group of plants (exp1) includes less watered plants than the control plants; in exp1 the soil water content is approximately 30% lower than that of the control plants. Soil water content was gravimetrically estimated on a mass volume basis. Besides the water deficit all the rest of the factors are set as the control plants. The 3rd group of plants (exp2) was translocated to another chamber with elevated ambient temperature of 2^{o}C according to the studying IPCC scenario. The plants were sufficiently watered without causing runoff and the rest of the ambient conditions were set as the control. The last group of plants (exp3) was also transferred to the second chamber with the elevated temperature and the plants were watered less than the control (soil water content is approximately 30% lower than that of the control plants).

2.1 Estimating specific leaf area and chlorophyll content

The leaves were collected and were rapidly scanned in a flatbed scanner, in order to calculate the fresh leaf area using ImageJ Pro, then they dried at 60^{o}C for 48 h to a constant mass and weighed to the nearest 0.001g. Specific leaf area (SLA) was calculated by the ratio of fresh leaf area per dry leaf mass (cm^2g^{-1}). The dried material was then powdered, using a MFC mill (Janke and Kunkel GMBH & Co, Germany) and stored in tightly sealed containers, in a cool dry and dark environment. The total chlorophyll (Chl) content was spectrophotometrically determined in leaf samples according to modified acetone method [12]. Chlorophyll concentration was extracted from dried, grounded leaf samples mixed, and homogenized with acetone (80% v/v) using china pestle and mortar, and filtered through Whatman #2 filter paper. The chlorophyll content was measured in aliquots of the leaf extracts using a spectrophotometer (Pharmacia Biotech Novaspec II) at A663.2, A646.8, A470 and the absorbance readings were applied to equations published by [13], in order to determine the chlorophyll content.

2.2 In situ measurements of optical properties of fresh leaves

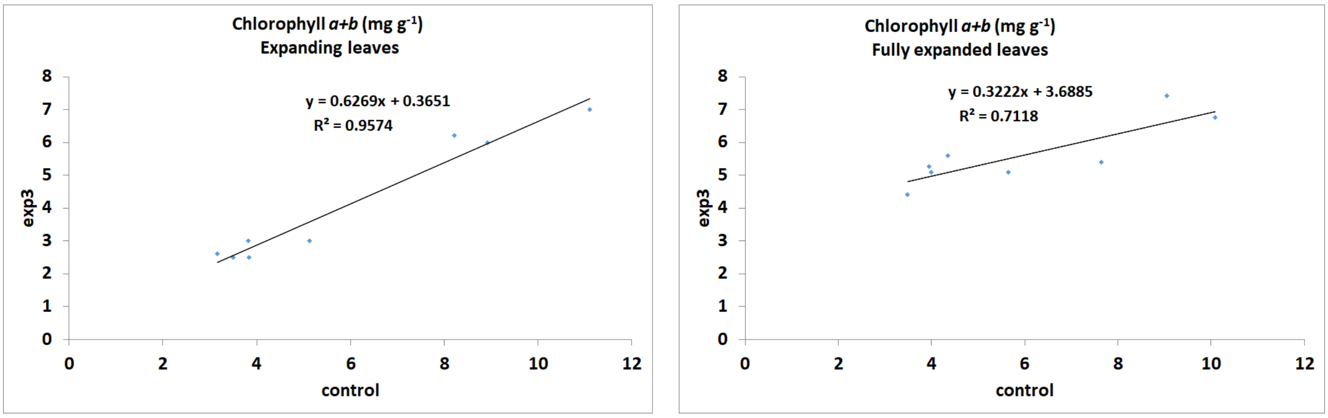
Leaf reflectance (R) and transmittance (T), for both adaxial and abaxial fresh leaf surfaces of grapevine cv. Assyrtiko, was measured *in situ* on attached to the plants leaves, between 250 nm and 2500 nm wavelength (bandwidth 2nm), using a UV/VIS spectrophotometer (Perkin Elmer Lambda-950), equipped with an integrating sphere and glassfibre tubes [14, 15]. The calculated leaf absorbance (pigments, water, dry matter) at a range of wavelengths from 250 nm to 2500 nm [A = 100 – (R + T)] was used to assess the different environmental treatments of *Vitis vinifera* cv. Assyrtiko.

3. Results

3.1 Chlorophyll content

A significant reduction of the concentration of chlorophyll *a+b*, was observed between the group of control and exp3 vines. The data are linearly correlated (Figure 1), concerning the expanding leaves (r^2=0.957) and the fully expanded leaves (r^2=0.712), between exp3 plants and control plants.

Figure 1. Relationship of chlorophyll *a+b* accumulation between control and combined treatments during a four-month period (June -September).



3.2 Leaf absorbance

The leaf absorbance (A) was calculated [A = 100 – (R + T)] for the four groups of vines by measuring transmitance (T) and reflectance (R) using a UV/VIS spectrophotometer (Perkin Elmer Lambda-950), in the range between 250 nm and 2500 nm assessing pigments concentration, water content, dry matter etc. It is likely that there is a slight difference of the absorption in the visible wavelength range (400-700 nm) and the leaf absorption

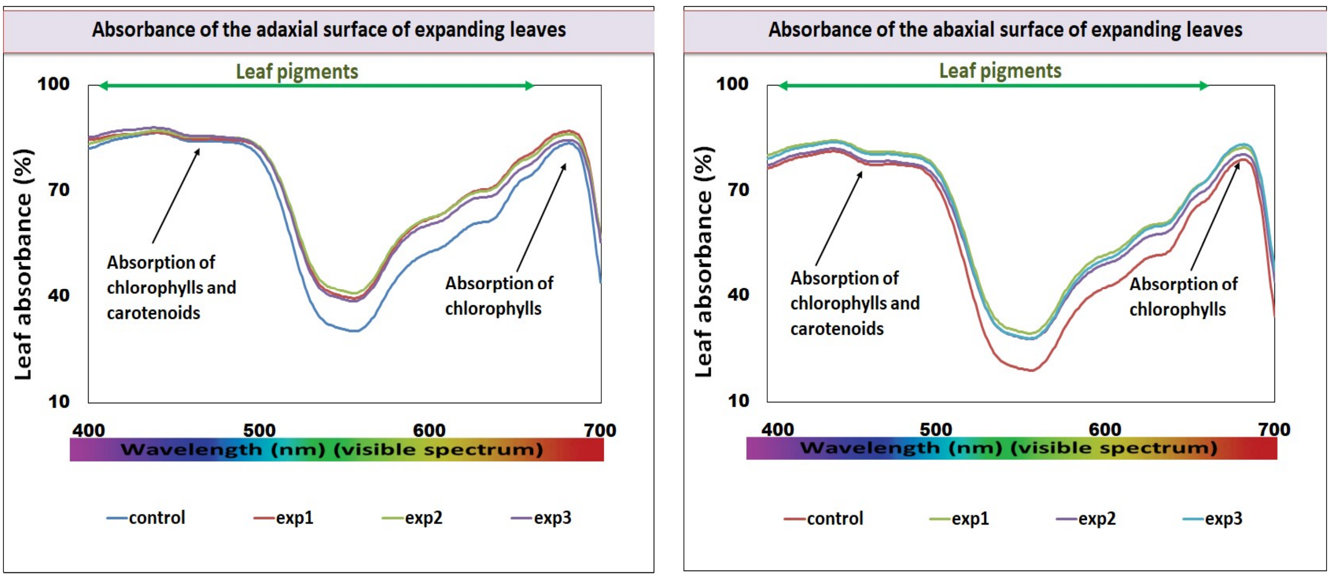


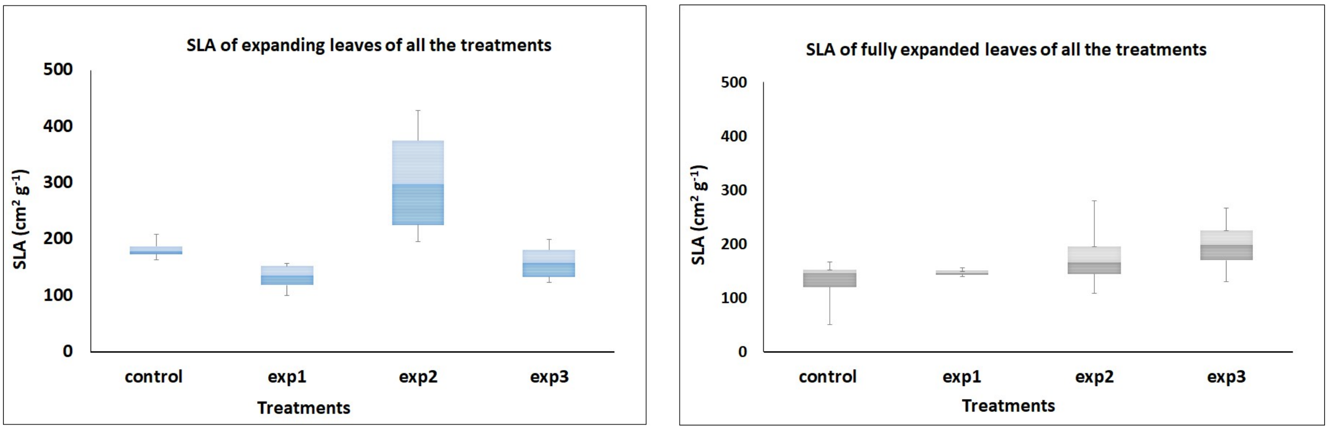
Figure 2. Absorbance of the abaxial and the adaxial surface of expanding leaves of *Vitis vinifera* cv. Assyrtiko.

peaked in 470 nm, 647 nm and and 664 nm (Figure 2); slighty higher values were detected in the adaxial leaf surface.

3.3 Specific Leaf Area (SLA)

The specific leaf area was also measured between June to September. A significant difference was found between the control and the treatments exp1,exp2 and exp3 (Table 1, p-value<0.05). Prolonged water stress reduced significantly the total plant leaf area of vines exposed to drought (exp1), as well as the group of plants exposed to the combined treatments, affecting mostly the expanding leaves (Figure 3). Furthermore, the increase of temperature (exp2) resulted to the increase of the specific leaf area of the expanding leaves during the growing season, varying from 195 cm^2 g^{-1} to 428 cm^2 g^{-1}. The SLA for the fully expanded leaves was not significantly changed among the considered groups of plants during the summer months. The water-stressed plants and plants exposed to the combined treatments possessed lower SLA than the control plants.

Figure 3. Measurements of SLA during a four-month period (June - September) of expanding and fully expanded leaves of *Vitis vinifera* cv. Assyrtiko, exposed to control, water deficit (exp1), elevated temperature (exp2) and combined water deficit



with elevated temperature (exp3) conditions.

Table 1. P-values of SLA for expanding and fully expanded leaves of *Vitis vinifera* L. cv. Assyrtiko

SLA	Treatment	P- value
Expanding leaves	control – exp1	0.00002
	control – exp2	0.0002
	control – exp3	0.0025
Fully expanded leaves	control – exp1	0.1286
	control – exp2	0.0273
	control – exp3	0.0012

4. Conclusions

Drought and moderate increase of temperature due to climatic change are a complex syndrome affecting several leaf biophysical properties that subsequently influence leaf reflectance spectra and morphological characteristics. In the present study, we examined the reliability of water absorption and small increase of temperature in order to assess changes in chlorophyll concentration, leaf absorbance and specific leaf area during water-stress and elevated temperature. It seems likely that the combined treatment (increase of temperature and water deficiency) affects the chlorophyll content in the expanding leaves. It is noteworthy though that there is no significant difference of leaf absorption for all the groups of treated vines (measured from 400 to 700 nm). Finally, there was a statistically significant difference of SLA between the control and the three treatments (exp1, exp2 and exp3) more in the expanding than in the fully expanded leaves. Combining the above mentioned findings, it is assumed that the leaves of grapevine cv. Assyrtiko were affected by water stress and increase of temperature, and mostly by the combination of these abiotic factors. It is expected that elevated temperature and water deficiency in the Mediterranean Basin will affect leaf properties of the cultivation of grapevine cv. Assyrtiko, in Greece, by altering its physiological response.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Stocker T.F.; Qin D.; Plattner G.K.; Tignor M.; Allen S.K. Intergovernmental Panel on Climate Change. *Climate change 2013: the physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, 2014.
2. Santos J.A.; Fraga H.; Malheiro A.C. et al. A review of the potential climate change impacts and adaptation options for European viticulture. *Applied Sciences* **2020**, 10(9), 3092, doi: 10.3390/app10093092.
3. Gauquelin T.; Michon G.; Joffre R., et al. Mediterranean forests, land use and climate change: a social-ecological perspective. *Regional Environmental Change* **2018**, 18, 623-636.
4. Del Pozo A.; Brunel-Saldias N.; Engler A., et al. Climate change impacts and adaptation strategies of agriculture in Mediterranean-climate regions (MCRs). *Sustainability* **2019**, 11(10), 2769, doi: 10.3390/su11102769.
5. Allen D.J.; Ort D.R. Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends in Plant Science* **2001**, 6(1), 36-42.
6. Vico G.; Way D.A.; Hurry V.; Manzoni S. Can leaf net photosynthesis acclimate to rising and more variable temperatures? *Plant, Cell & Environment* **2019**, 42(6), 1913-1928.
7. Liakopoulos G.; Nikolopoulos D.; Klouvatou A., et al. The photoprotective role of epidermal anthocyanins and surface pubescence in young leaves of grapevine (*Vitis vinifera*). *Annals of Botany* **2006**, 98(1), 257-265.
8. Koufos G.; Mavromatis T.; Koundouras S.; Fyllas N.M.; Jones G.V. Viticulture–climate relationships in Greece: the impacts of recent climate trends on harvest date variation. *International Journal of Climatology* **2014**, 34(5), 1445-1459.
9. Myles S.; Boyko A.R.; Owens C.L.; Brown P.J.; Grassi F. Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences* **2011**, 108(9), 3530-3535.
10. Ponti L.; Gutierrez A.P.; Boggia A.; Neteler M. Analysis of grape production in the face of climate change. *Climate* **2018,** 6(2), 20, doi: 10.3390/cli6020020.
11. Bertamini M.; Zulini L.; Muthuchelian K.; Nedunchezhian N. Effect of water deficit on photosynthetic and other physiological responses in grapevine (*Vitis vinifera* L. cv. Riesling) plants. *Photosynthetica* **2006**, 44(1), 151-154.
12. Meletiou-Christou M. S.; Rhizopoulou, S. Leaf functional traits of four evergreen species growing in Mediterranean environmental conditions. *Acta Physiologiae Plantarum* **2017**, 39(1), 34, doi: 10.1007/s11738-016-2330-4.
13. Lichtenthaler H.K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* **1987**, 148, 350-382.
14. Stratakis E.; Ranella A.; Farsari M.; Fotakis C. Laser-based micro/nanoengineering for biological applications. *Progress in Quantum Electronics* **2009**, 33(5), 127-163.
15. de Jong S. M.; Addink E. A.; Doelman, J. C. Detecting leaf-water content in Mediterranean trees using high-resolution spectrometry. *International Journal of Applied Earth Observation and Geoinformation* **2014**, 27, 128-136.