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Research Article

Particle film treatments on 'Assyrtiko' grapevines enhance physiology and grape attributes in Santorini Island

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Abstract

Like many viticultural regions of the Mediterranean region, Santorini is also affected by climate change. These changing climatic conditions pose challenges to adapt the island's traditional viticulture practices to the new climatic conditions of climate change, which will intensify in the future, with the use of short- and long-term solutions with a sustainable manner respecting the tradition and the island's landscape. Viticulture in this region copes with high temperatures, heat waves and drought which affect the maturity process, the technological maturity, as well as the physiology of the grapevine. The aim of this study was to investigate the effects of kaolin and calcium carbonate foliar application on water relations, photosynthesis and berry composition of vines of grape cultivar 'Assyrtiko', trained with the traditional training system 'Kouloura', under drought conditions and vertical shoot positioned training system in Santorini. The effects of foliar application with kaolin and $CaCO_3$ on vines of grape cultivar 'Assyrtiko' were evaluated, focusing on the impact on the vine's physiology attributes, grape quality, and environmental sustainability. Based on the results of the present study, there were statistically significant differences on vine water stress where the foliar application of inert films reduced water stress during the three studied years. Kaolin and $CaCO_3$ increased significantly the photosynthesis and stomatal conductance of the vines in comparison to the control. Furthermore, the foliar application with inert minerals has reduced sunburn and pest damage. The results of the present study confirm that the application of kaolin and $CaCO_3$ constitutes an effective and economical solution for the water saving of the vines in dry conditions, while at the same time, it can improve the physiology of the plant and preserve the qualitative and quantitative attributes of the grapes. The foliar use of inert particle films on Santorini's traditional vineyards could consist a sustainable and effective alternative to mitigate the effects of climate change which have intensified over the last decades in the region.

Keywords: adaptation strategies; calcium carbonate; Kaolin; Kouloura

Introduction

The viticulture of Santorini Island, in the Cyclades, is unique and has contributed to the remarkable breakthrough of Santorini's wine into the global quality wine market. The wine industry is a leading economic activity for the island with a significant added value in terms of GDP, investments, and revenues. Closely related

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to tourism, wine is linked to Santorini's brand name as one of the top world destinations. Hence Santorini remains one of the few places in Europe where traditional viticulture is still being practiced. Local varieties trained with the traditional training systems 'Kouloura' and 'Kladeftiko' have been cultivated for thousands of years on the island producing worldwide recognized high quality PDO wines (Xyrafis *et al*., 2021). Santorini has a Mediterranean climate characterized by high temperatures (an average of 3 days during the summer with max temperatures of > 35 °C was observed for the 2009-2019 period), heatwaves (which occur often during the summer), and long periods of drought. These climate challenges affect yield, berry development and composition, and the associated wine aromatic profiles, and are intensifying due to climate change in the Mediterranean region (Fraga *et al*., 2017; Alba *et al*., 2021; Xyrafis *et al*., 2022).

Wine grapes are one of the world's most valuable horticultural crops (Alston and Sambucci, 2019) and viticulture is facing massive challenges in many regions due to climate change (Jones and Goodrich, 2008; Tomasi *et al*., 2011; Petoumenou *et al*., 2019; Jones *et al*., 2022; Xyrafis *et al*., 2022). One of the chief concerns is that a combination of increased temperatures and decreased rainfall will increase the frequency and/or severity of droughts (IPCC, 2022). High temperatures in combination with decreased precipitation can bring about complete yield loss depending on the phenological stage. Even if yield itself is not affected, these conditions can lead to early technological maturation of the grapes with significant sugar increases and negative impacts on wine quality (acidity, aroma, color) (Costa *et al*., 2016; Pastore *et al*., 2017).

Adaptation measures must be planned and applied in order to maintain the sustainability of vineyards (Metzger and Rounsevell, 2011) and several adaptations have been reported for use in viticulture (Koundouras *et al*., 2008; Duchêne *et al*., 2012; Fraga *et al*., 2018; Dinis *et al*., 2020; Santos *et al*., 2020). These include a blend of long-term strategies such as the use of more suitable clones/rootstocks/varieties, decreasing planting densities, and/or modifying-changing training systems (Naulleau *et al*., 2021; Stavrakaki *et al*., 2023 Xyrafis *et al*., 2023). In hot and dry climates, where water stress is a problem for viticulture, several short-term practices have been applied to moderate the effects of excessive radiation or temperature in wine grapes, including canopy management, irrigation strategies and the use of shading nets (Keller, 2010; Santos *et al*., 2020; Cataldo *et al*., 2022). Recent studies have shown that particle film technology appears to be a valid tool to increase sustainability in the vineyard, and limit irrigation use (Brillante *et al*., 2016; Dinis *et al*., 2016; Frioni *et al*., 2019; Valentini *et al*., 2021). In particular, the use of this technology could provide complementary solutions to mitigate the effects of high temperatures, radiation, and water deficit. These practices have also been reported to reduce plant temperature and sunburn damage, soluble solids and anthocyanin accumulation, stomatal conductance and repel insects (Lobos *et al*., 2015; Frioni *et al*., 2019; Tacoli *et al*., 2019; Dinis *et al*., 2020; Biniari *et al*., 2023; Teker, 2023). As these practices can be labor-consuming, the use of alternatives such as reflective particle films might be interesting and effective. The most common material used for this purpose is kaolin, a white clay based on layered aluminum silicate, capable of leaving a thin deposit on the surface of the fruit (Yazici and Kaynak, 2006). It has been proved that kaolin reflects potentially damaging ultraviolet and infrared radiation, as well as allow the transmission of photosynthetically active radiation (Glenn and Puterka, 2005), reduce leaf temperature (Shellie and King, 2013) and sunburns, and increase leaf carbon assimilation (Glenn *et al*., 2001). Kaolin exogenous application mitigates the effect of adverse abiotic climatic stresses in grapevines while enhancing grape quality including phenolics, flavonoids and anthocyanins (Dinis et al., 2016; Biniari *et al*., 2023). Processed calcite particles are also used in foliar application to alleviate most of the adverse effects of water stress on grapevine and sweet potato photosynthesis, affecting some photosynthetic parameters (rate, efficiency, etc.) (Attia *et al*., 2014; Oliveira *et al*., 2022). These effects on photosynthesis are dependent on the recommended dose and they have been clearly observed in water-stressed plants (mainly increasing stomatal conductance throughout the day and in the entire plant canopy) as opposed to well-watered plants, whose photosynthetic parameters were not significantly affected (Attia *et al*., 2014). At the same time, it has been shown that foliar applications with calcium carbonate also contribute to increase in yield as well as in the accumulation of phenolic compounds in grapes (Maya-Meraz *et al*., 2020), while reducing stomatal aperture.

The aim of the present study was to investigate the effects of kaolin and calcium carbonate foliar application on water relations, photosynthesis and berry composition and quality attributes of vines of grape cultivar Assyrtiko, trained with the traditional training system 'Kouloura' and the vertical shoot position training system under the climatic conditions of Santorini.

Materials and Methods

Experimental design

The experiment took place in the cultivation seasons 2018-2019 and 2019-2020 and 2020-2021 on vines of white wine grape cultivar 'Assyrtiko' (A) (*Vitis vinifera* L.), in vineyards located in Oia, Santorini, Greece (36°28'22.5"N; 25°23'14.7"E) (Figure 1A). All vines were own rooted. For grape cultivar Assyrtiko, there were two vineyards: one with the traditional training system of Santorini 'Kouloura' (K) (Figure 1B) where the vines are cane-pruned to 4 canes of 6-8 nodes at 2.3 m × 2.3 m intervals, resulting in a vine density of 1900 plants/ha; and one vineyard N-S oriented where the vines are unilateral cordon-trained (unilateral Guyot)(V) and cane-pruned to 8-10 nodes canes at 1.9 m x 0.9 m intervals (double lines) (Figure 1C-D), resulting in a vine density of 6500 plants/ha. In addition, the vineyard was older than 70 years old for the vineyard trained with the 'Kouloura' training system and was not irrigated. VSP vineyard was established in 2008 and was irrigated. The weather conditions that prevailed during the studied period are mentioned in Table 1 (the meteorological data for the Fira region were obtained from the National Observatory of Athens), while rainfall distribution during the studied years is mentioned in Figure 2. Soil is characterized by a rocky-sandy texture and floor management was carried out as full tillage.

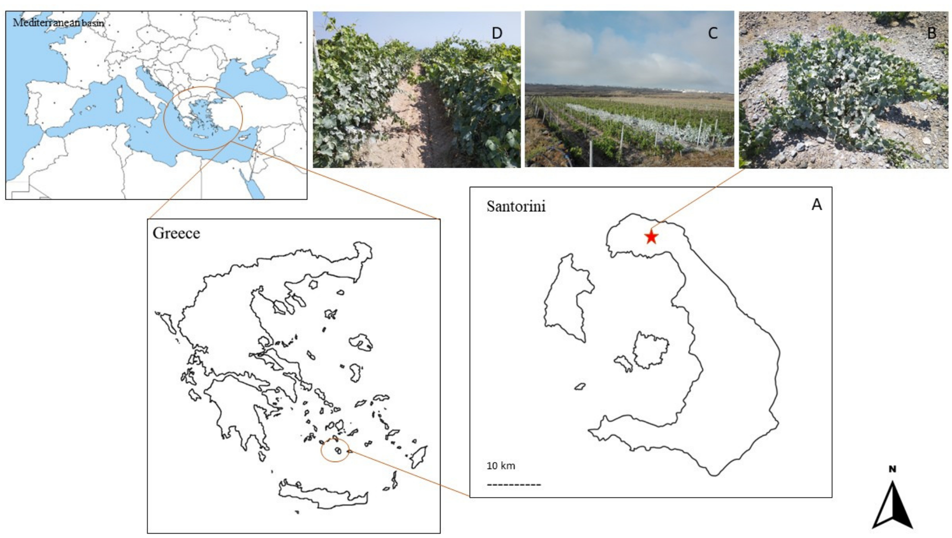

Figure 1. Map of Santorini Island and experimental plots in Oia village (A), cv 'Assyrtiko' trained with 'Kouloura' (B) and cv Assyrtiko trained with vertical shoot position training systems (C and D)

The experiment was designed and implemented following the principles of the Randomized Complete Block Design with four replications per treatment for each training system. Each group of 5 vines constituted one replication. Two treatments were evaluated as follows: a) Kaolin (K) (Surround WP®; Engelhard Corp., Iselin, NJ, USA) and b) $CaCO_3$ (Ca) (OmyaPro® Sun, Omya International AG, Switzerland), while there was also a control (C). Both products were prepared in an aqueous solution at the manufacturer recommended dosage of 5% (w/v), which was directly applied to leaves according to standard operating procedures adjusted for agricultural practices. The foliar applications were applied at 154 DOY in 2019, at 160 DOY in 2020 and 150 DOY in 2021.

Table 1. Regional weather data, Santorini, Greece

	Mean temperature (°C)			Min temperature (°C)			Max temperature (°C)			Number of days with temperatures >30 °C			Precipitation (mm) October-May
Month	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021	2018-2019
March	13.5	13.5	12.5	11.6	11.5	10.21	15.8	16.2	15.2	0	0	0	*563.6*
April	15.1	14.9	16.3	12.7	12.6	13.5	18.2	17.8	20.2	0	0	0	2019-2020
May	18.9	20.1	20.8	16.2	16.9	17.9	22.6	24	24.6	0	5	3	*388.8*
June	24.7	22.4	24	21.9	19.7	20.9	28.1	26.2	28	13	2	9	2020-2021
July	25.8	25.8	26.9	22.5	22.8	23.9	29.9	29.8	30.5	15	15	21	*178.4*
August	26.2	26.1	27.6	23.2	23.3	24.5	29.9	30.0	31.4	21	15	18	-
Mean	*20.7*	*20.5*	*21.4*	*18*	*17.8*	*18.5*	*24.1*	*24*	*25*	-	-	-	-

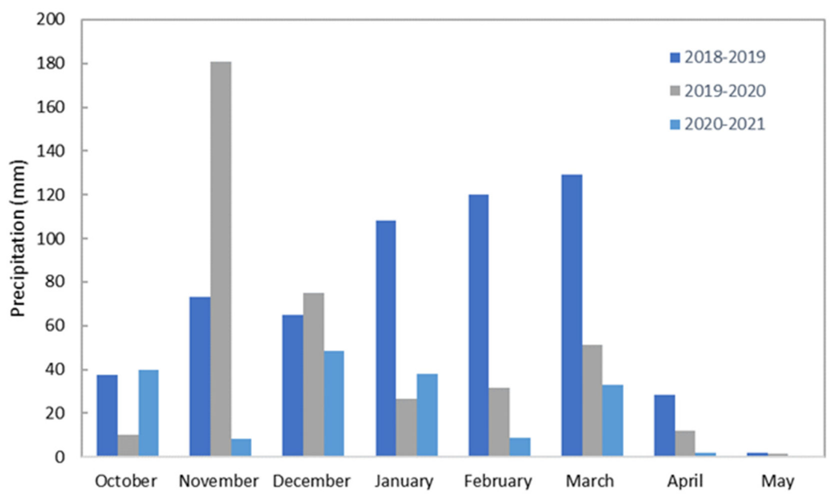

Figure 2. Rainfall distribution from October through May for 2018-2019, 2019-2020 and 2020-2021 periods

Gas exchange, water potential, leaf-related measurements

During the season, the midday leaf water potential (Ψleaf) was measured at bunch closure, veraison and harvest stages using a pressure chamber. Measurements were taken at sun zenith for Ψleaf on five primary leaves per treatment, placed inside plastic bags and sampled from five random vines.

Assimilation rate (Pn, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}) and transpiration (E, mmol H_2O m^{-2} s^{-1}) were obtained by measurement of inlet and outlet CO_2 and H_2O relative concentration using a portable photosynthesis system (Li-6400XT, Li-Cor, Lincoln Nebraska, USA). Single leaf gas exchange measurements were taken in the midday hours (10:30-12:30) on five primary leaves, in the same day and on same vines of the water potential measurements, while chlorophyll concentration was measured on the same leaves using a SPAD 502 (Konica Minolta, Europe). Five primary leaves were measured among those inserted at nodes 4-6 above the distal bunch on a main shoot.

Leaves and grapes' temperatures were measured on five replications per treatment with an infrared thermal camera (HT-02D KKMOON, China).

Characteristics of the must, grape and berry mechanical properties, and damage record

At harvest, grapes were randomly selected from each treatment. Harvest was performed on 12 August (DOY 224) in 2019, 14 August (DOY 227) in 2020 and 8 August (DOY 220) in 2021 when the sugar accumulation on control vines reached 23 degrees Brix (°Bx). The weight of each one of the grapes was measured using a precision scale. The grapes' length and width were determined using calipers of an 0.01 mm accuracy. Three (3) random groups of fifty (50) berries were collected from each grape. Each group's weight was measured using a precision scale. It was then divided by the number of berries in order to calculate the mean berry weight per group. The length and width of each berry in all three (3) groups were measured using a Vernier caliper. Last, the mean value of each group's berry length and width was calculated. The number of grapes per vine was also recorded on 5 vines per treatment.

Soluble solids in must, pH and total titratable acidity were determined and measured according to Stavrakaki *et al*. (2018).

During 2019 and 2020 seasons, damages from sunburn, *Lobesia botrana* and *Plasmopara viticola* were recorded randomly from 20 grapes per treatment. The incidence was observed with the use of glass magnifier and expressed as the percentage (%) of affected berries.

Statistical analysis

All statistical analysis were obtained using the JMP v.16 statistical software (SAS Institute Inc., Cary, NC, USA). The significance of the results was tested by Analysis of Variance (ANOVA) and comparisons were analyzed using the Tukey's test for pairwise comparison with mean separation by $p < 0.05$. Principal component analysis was generated using JMP v16.

Results

Vine water status

There were statistically significant differences in vine water status between the treatments of cv. Assyrtiko depending on the developmental stage, and generally, kaolin and calcium carbonate always maintained a less stressed water status (i.e. less negative Ψ) for both training systems (Figure 3). Regarding cv. Assyrtiko trained with 'Kouloura' training system in 2019, at bunch closure and veraison, no statistically significant differences in Ψmidday were observed, although at harvest AKC was significantly more negative (~ 0.2 MPa difference) than AKK and AKCa (Figure 3A). In 2020, during veraison and harvest, AKK and AKCa again exhibited less negative Ψmidday than AKC (~ 0.15 MPa difference) (Figure 3). In 2021, there was a clear separation between foliar treatments (AKK and AKCa) and the control (AKC), where AKK presented the least negative values during the three developmental stages followed by the AKCa treatment.

Figure 3B shows the water potential of cv. Assyrtiko trained with vertical shoot position training system treated with kaolin and calcium carbonate for the three studies years. Globally, both treatments maintained a less stressed water status similar to the 'Kouloura' training system case. In 2019, during bunch closure AVCa was significantly higher than AVK and AVC while at harvest both treatments AVK and AVCa were higher than AVC (~0.15 MPa difference). In 2020, during the three developmental stages treated vines had higher Ψ leaf values than AVC while during bunch closure AVCa presented the highest values than AVK and AVC. In 2021, during veraison AVK was significantly higher than AVCa and AVC while AVCa was significantly higher than AVC. During harvest both treatments were significantly higher than AVC (~ 0.2 MPa difference).

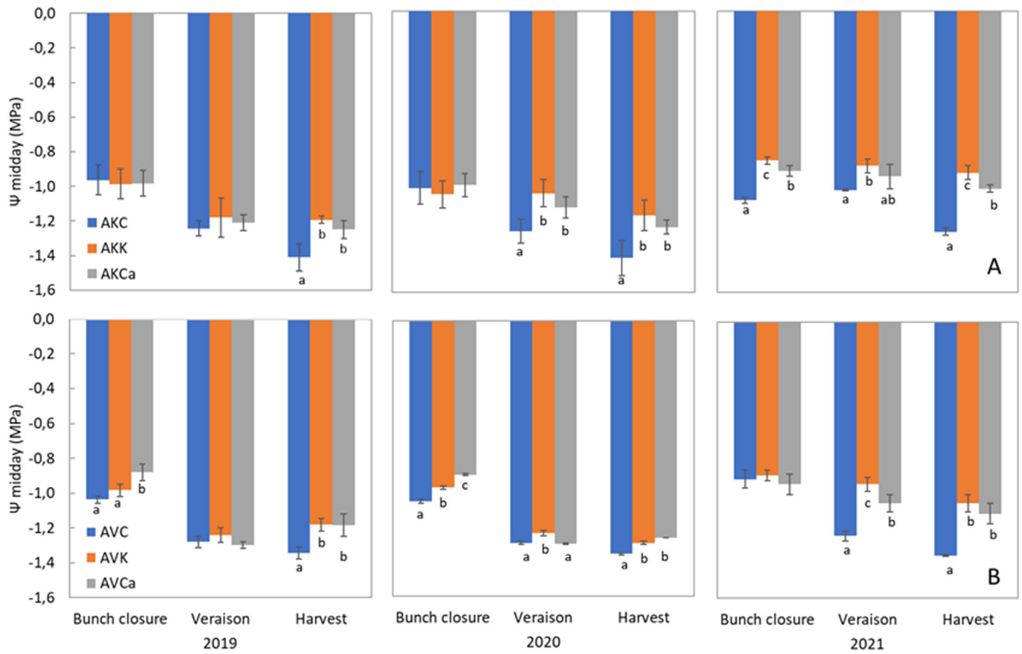

Figure 3. *Ψ* midday of cv. Assyrtiko trained with 'Kouloura' [A] and vertical shoot position [B] training systems treated with Kaolin (AKK and AVC), $CaCO_3$ (AKCa and AVCa) and a control (AKC and AVC) at three growth stages (bunch closure, veraison and harvest) during the 2019, 2020 and 2021 seasons Different lowercase letters represent statistically significant differences between the treatments ($p<0.05$).

Photosynthesis, gas exchange, leaf and grape temperature

Leaf assimilation rate, leaf stomatal conductance and the leaf transpiration of Assyrtiko vines trained with 'Kouloura' training system exhibited statistically significant differences at the three growth stages (bunch closure, veraison and harvest) during the 2019, 2020 and 2021 seasons (Table 2). Photosynthesis in 2019 and 2021 at bunch closure presented no statistically significant differences, while in 2020 AKK and AKCa treatments were significantly higher from C (~2 μmol m^{-2} s^{-1} difference). During veraison in 2019 and 2020, photosynthesis of AKK and AKCa were higher from AKC. At harvest, again both foliar applications showed significantly higher photosynthesis for the three studied years, where in 2020 Ca treatment presented the highest value. Regarding the stomatal conductance, in 2019 and 2021 at bunch closure and veraison, no statistically significant differences have been recorded between the treatments. At harvest, stomatal conductance of AKK and AKCa has increased and presented higher values than AKC (~0.07 mol m^{-2} s^{-1} difference). In 2020, during veraison and harvest, AKK and AKCa treatments were characterized by higher stomatal conductance than C (~0.06 mol m^{-2} s^{-1} difference). At bunch closure, no statistically significant differences were recorded. Leaf transpiration values in 2019 did not exhibit statistically significant differences. In 2020 and 2021, leaf transpiration was higher in AKK and AKCa during bunch closure (~1 mmol H_2O m^{-2} s^{-1}). At veraison of 2020 and harvest of 2021, AKK presented the highest transpiration in comparison to AKCa and AKC. While at harvest, the AKC was significantly higher than the foliar application treatments.

Table 2. Leaf assimilation rate, leaf stomatal conductance, and the leaf transpiration of 'Assyrtiko' vines trained with 'Kouloura' training system treated with Kaolin (AKK), CaCO3 (AKCa) and a control (AKC) at three growth stages (bunch closure, veraison and harvest) during the 2019, 2020 and 2021 seasons

Developmental stage	Treatment	P_n (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})
2019				
Bunch closure	AKC	12.3 ± 1.1	0.24 ± 0.03	3.2 ± 0.41
	AKK	13 ± 0.67	0.24 ± 0.05	4.3 ± 0.2
	AKCa	13.21 ± 0.42	0.22 ± 0.01	4 ± 0.19
	Significance	ns	ns	ns
Veraison	AKC	11.7 ± 0.33 b	0.15 ± 0.01	1.33 ± 0.37
	AKK	12.8 ± 0.14 a	0.12 ± 0.02	1.17 ± 0.22
	AKCa	12.55 ± 0.09 a	0.13 ± 0.01	1.42 ± 0.3
	Significance	*	ns	ns
Harvest	AKC	8.2 ± 0.21 b	0.08 ± 0.02 b	0.98 ± 0.13
	AKK	10.07 ± 0.17 a	0.15 ± 0.00 a	1.14 ± 0.20
	AKCa	9.85 ± 0.10 a	0.14 ± 0.03 a	1.3 ± 0.28
	Significance	*	*	ns
2020				
Bunch closure	AKC	10.33 ± 0.19 b	0.22 ± 0.02	3.27 ± 0.16 b
	AKK	12.3 ± 1.21 a	0.25 ± 0.02	4.10 ± 0.58 a
	AKCa	12 ± 1.59 a	0.27 ± 0.03	4.21 ± 0.87 a
	Significance	*	ns	*
Veraison	AKC	14.01 ± 0.34 a	0.14 ± 0.01 b	3.03 ± 0.4 c
	AKK	12.31 ± 1.41 b	0.23 ± 0.04 a	4.5 ± 0.18 a
	AKCa	12.52 ± 0.47 b	0.25 ± 0.02 a	3.65 ± 0.28 b
	Significance	*	*	*
Harvest	AKC	8.20 ± 1.03 c	0.19 ± 0.08	3.94 ± 0.22 a
	AKK	11.45 ± 1.05 b	0.12 ± 0.02	2.47 ± 0.11 b
	AKCa	11.71 ± 0.48 a	0.13 ± 0.03	3.13 ± 0.05 b
	Significance	*	ns	*
2021				
Bunch closure	AKC	7.37 ± 0.55	0.06 ± 0.003	2.57 ± 0.1 b
	AKK	7.6 ± 0.56	0.07 ± 0.005	3.66 ± 0.05 a
	AKCa	7.56 ± 0.43	0.07 ± 0.006	3.5 ± 0.07 a
	Significance	ns	ns	*
Veraison	AKC	15.66 ± 0.52	0.22 ± 0.01	4.26 ± 0.03
	AKK	14.71 ± 0.66	0.22 ± 0.02	4.04 ± 0.22
	AKCa	15 ± 0.53	0.22 ± 0.03	4.11 ± 0.11
	Significance	ns	ns	ns
Harvest	AKC	6.3 ± 0.41b	0.13 ± 0.09 b	2.55 ± 0.08 c
	AKK	8.55 ± 0.71a	0.16 ± 0.02 a	3.71 ± 0.12 a
	AKCa	8.13 ± 0.33a	0.17 ± 0.01 a	3 ± 0.21 b
	Significance	*	*	*
Year × Treatment		ns	ns	ns

The values are averages ± SD. Different lowercase letters represent significant differences between the treatments ($p<0.05$). Year x Treatment followed by * represents significant differences between years within the same treatment ($p < 0.05$).

Leaf assimilation rate of 'Assyrtiko' vines trained with vertical shoot positioned training system, presented significant differences at the three growth stages (bunch closure, veraison and harvest) during the 2019, 2020 and 2021 seasons similar to those of the 'Kouloura' training system (Table 3). Photosynthesis for the three studied years during harvest was higher for AVK and AVCa than the control (~2 μmol m^{-2} s^{-1} difference). In 2019 and 2020 at bunch closure presented no statistically significant differences, while in 2021 AVK and AVCa treatments were significantly higher from C (~2.5 μmol m^{-2} s^{-1} difference). During veraison in 2020, photosynthesis of AVK and AVCa were higher from AVC. Table 3 shows that the stomatal conductance transpiration in 2019 at bunch closure both treatments presented significantly lower values. In 2020 during veraison both treatments exhibited higher stomatal conductance (~0.13 mol m^{-2} s^{-1} difference) and transpiration (~1 mmol H_2O m^{-2} s^{-1}) than the control. At harvest in 2021, stomatal conductance of AVK and AVCa has increased and presented higher values than AVC (~0.03 mol m^{-2} s^{-1} difference) and leaf transpiration was again higher for both treatment (~0.8 mmol H_2O m^{-2} s^{-1}) than the control.

Table 3. Leaf assimilation rate, leaf stomatal conductance and the leaf transpiration of cv. 'Assyrtiko' trained with VSP training system treated with Kaolin (AVK), $CaCO_3$ (AVCa) and a control (AVC) at three growth stages (bunch closure, veraison and harvest) during the 2019, 2020 and 2021 seasons

Developmental stage	Treatment	P_n (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})
		2019		
Bunch closure	AVC	15.36 ± 0.25	0.27 ± 0.02a	3.89 ± 0.30 a
	AVK	13.87 ± 2.28	0.16 ± 0.05b	2.71 ± 0.41 b
	AVCa	13.90 ± 1.1	0.18 ± 0.03b	3.00 ± 0.29 b
	Significance	ns	*	*
Veraison	AVC	7.84 ± 0.78	0.08 ± 0.01	0.87 ± 0.15
	AVK	6.86 ± 0.82	0.09 ± 0.02	1.06 ± 0.08
	AVCa	7.11 ± 0.55	0.09 ± 0.01	0.90 ± 0.08
	Significance	ns	ns	ns
Harvest	AVC	8.74 ± 0.21 b	0.22 ± 0.03	3.58 ± 0.06
	AVK	10.07 ± 0.17 a	0.23 ± 0.05	3.29 ± 0.16
	AVCa	10.85 ± 0.10 a	0.21 ± 0.03	3.33 ± 0.23
	Significance	*	ns	ns
		2020		
Bunch closure	AVC	13.44 ± 0.20	0.20 ± 0.03	3.57 ± 0.16
	AVK	13.21 ± 0.09	0.25 ± 0.03	3.82 ± 0.28
	AVCa	13.78 ± 0.35	0.25 ± 0.03	3.88 ± 0.31
	Significance	ns	ns	ns
Veraison	AVC	5.44 ± 1.26 b	0.10 ± 0.04 b	1.37 ± 0.32 b
	AVK	6.88 ± 1.05 a	0.24 ± 0.05 a	2.29 ± 0.26 a
	AVCa	6.75 ± 1.68 a	0.23 ± 0.04 a	2.25 ± 0.32 a
	Significance	*	*	*
Harvest	AVC	5.75 ± 0.21 b	0.09 ± 0.05	1.96 ± 0.12
	AVK	7.58 ± 0.44 a	0.11 ± 0.04	2.17 ± 0.11
	AVCa	7.60 ± 0.19 a	0.15 ± 0.06	2.33 ± 0.21
	Significance	*	ns	ns
		2021		
Bunch closure	AVC	7.31 ± 1.14 b	0.12 ± 0.02	3.92 ± 0.41 a
	AVK	10.8 ± 1.42 a	0.07 ± 0.02	2.46 ± 0.72 b
	AVCa	10.11 ± 1.20 a	0.08 ± 0.03	2.71 ± 0.33 b
	Significance	*	ns	*
Veraison	AVC	11.21 ± 0.65	0.11 ± 0.01	2.43 ± 0.17

	AVK	11.67 ± 2.02	0.13 ± 0.03	2.54 ± 0.56
	AVCa	12 ± 0.53	0.11 ± 0.03	2.58 ± 0.22
	Significance	ns	ns	ns
Harvest	AVC	5.27 ± 0.39 c	0.07 ± 0.01 b	2.22 ± 0.03 b
	AVK	7.11 ± 0.50b	0.10 ± 0.01 a	3.11 ± 0.17 a
	AVCa	8.09 ± 0.33a	0.11 ± 0.01 a	3.00 ± 0.11 a
	Significance	*	*	*
Year × Treatment		ns	*	ns

The values are averages ± SD. Different lowercase letters represent significant differences between the treatments ($p<0.05$). Year x Treatment followed by * represents significant differences between years within the same treatment ($p < 0.05$).

There were statistically significant differences in chlorophyll content, leaf and grape temperature between the treatments for cv. Assyrtiko trained with the 'Kouloura' training system. Globally, foliar treatments (AKK and AKCa) exhibited higher chlorophyll content and lower leaf and grape temperatures than the control (AKC) (Table 4). Chlorophyll content for AKK and AKCa was significantly higher than AKC for the three studied years. According to Table 4 leaf temperature of treated vines AKK and AKCa was found lower than AKC and exhibited similar differences from the AKC for the three studied years (~1-2 °C difference). At midday during harvest in 2019 grape temperature was lower for AKK and AKCa treatments than AKC (~2 °C difference). Concerning grape temperature in 2020 and 2021 no significant differences were observed.

Table 5 shows the chlorophyll content, leaf and grape temperature of the treated vines and the control for cv. 'Assyrtiko' trained with the vertical shoot position training system. Chlorophyll content presented no significant differences between treatments and control during the three studied years. Foliar treatments (AKK and AKCa) exhibited a clear separation from the control (AKC) with lower leaf temperatures (~2 °C difference), while grape temperature was statistically significantly lower than AKC during 2020.

Table 4. Chlorophyll, leaf temperature and grape temperature of cv. 'Assyrtiko' trained with the 'Kouloura' training system treated with Kaolin (AKK), $CaCO_3$ (AKCa) treatments and a control (AKC) at midday in harvest period during 2019, 2020 and 2021 seasons

Year	Treatment	Chlorophyll (SPAD)	Leaf temperature (°C)	Grape temperature (°C)
2019	AKC	35 ± 0.30 b	36.27 ± 1.00 a	37 ± 0.52
	AKK	39.9 ± 1.76 a	34.24 ± 0.38 b	35.1 ± 0.59
	AKCa	36.33 ± 1.00 a	35.35 ± 1.33 ab	35.29 ± 0.29
	Significance	*	*	*
2020	AKC	36.63 ± 1.50 b	37.7 ± 0.34 a	35.4 ± 1.67
	AKK	39.60 ± 2.30 a	36.1 ± 0.22 b	34.8 ± 2.33
	AKCa	41.53 ± 2.36 a	36.37 ± 0.55 b	35 ± 1.98
	Significance	*	*	ns
2021	AKC	38.7 ± 2.41 b	32.3 ± 0.47 a	34.29 ± 1.67
	AKK	44.33 ± 0.95 a	29.67 ± 1.41 b	33.22 ± 1.51
	AKCa	39.11 ± 1.13 b	29.3 ± 1.35 b	34 ± 1
	Significance	*	*	ns
Year × Treatment		ns	ns	ns

The values are averages ± SD. Different lowercase letters represent significant differences between the treatments ($p<0.05$). Year x Treatment followed by * represents significant differences between years within the same treatment ($p < 0.05$).

Table 5. Chlorophyll, leaf temperature and grape temperature of cv. 'Assyrtiko' trained with the vertical shoot positioned training system treated with Kaolin (AVK), $CaCO_3$ (AVCa) treatments and a control (AVC) at midday in harvest period during 2019, 2020 and 2021 seasons

Year	Treatment	Chlorophyll (SPAD)	Leaf temperature (°C)	Grape temperature (°C)
2019	AVC	43.73 ± 0.94	35.30 ± 2.42 a	36.93 ± 2.35
	AVK	43.3 ± 1.40	32.03 ± 1.27 b	35.63 ± 0.38
	AVCa	39.53 ± 3.59	35.06 ± 1.37 a	37.5 ± 1.39
	Significance	ns	*	ns
2020	AVC	41.38 ± 0.92	35.67 ± 0.85 a	36.21 ± 0.30 a
	AVK	42.16 ± 2.09	32.7 ± 1.41 b	34.01 ± 0.75 b
	AVCa	41.00 ± 1.55	32.49 ± 1.05 b	35.13 ± 0.66 b
	Significance	ns	*	*
2021	AVC	41.96 ± 0.85	35.6 ± 0.61 a	34.25 ± 0.96
	AVK	43.1 ± 1.04	33.61 ± 0.66 b	33.92 ± 1.36
	AVCa	43 ± 0.96	34 ± 1.1 ab	34.37 ± 1.43
	Significance	ns	*	ns
Year × Treatment		ns	ns	ns

The values are averages ± SD. Different lowercase letters represent significant differences between the treatments ($p<0.05$). Year x Treatment followed by * represents significant differences between years within the same treatment ($p < 0.05$).

Characteristics of the must, grape and berry mechanical properties and damage record

Table 6 shows the must parameters (soluble solids, pH and acidity) and the grape and berry attributes of cv. 'Assyrtiko' trained with the 'Kouloura' training system in response to exogenous application of kaolin and calcium carbonate for the three studied years. Sugar content presented no clear separation between the foliar treatment and the control. In 2020, AKCa was significantly higher than AKK and AKC while in 2020 both AKK and AKCa had lower sugar content than AKC. Total acidity was higher in the AKK treatment during the three studied year. The major difference for AKK was observed in 2020. Regarding the pH, in 2019 no significant difference was mentioned while in 2020 both treatment AKK and AKCa were lower than the control. In 2021 we found that AKC has lower pH than AKK. Regarding berry's parameters there was no clear separation between treatments. Similar was the image for grape's parameters, except bunch weight where in 2021, AKK treatment presented a higher grape weight than AKC.

Table 6. Grape composition (soluble solids, pH and acidity) and mechanical properties of cv. 'Assyrtiko' trained with the 'Kouloura' training system treated with Kaolin (AKK), $CaCO_3$ (AKCa) treatments and a control (AKC) in 2019, 2020 and 2021 seasons

	Treatment	Soluble solids (°Brix)	pH	Titratable acidity (g L^{-1})	Bunch number per vine	Bunch weight (g)	Berry weight (g)	Berry length (mm)	Berry width (mm)
2019	AKC	22.8 ± 0.07 a	2.98 ± 0.01	7.1 ± 0.13	6.5 ± 1.3	211 ± 19	2.37 ± 0.07	16.6 ± 0.6	14.19 ± 0.43
	AKK	21.81 ± 0.27 b	3.1 ± 0.05	7.42 ± 0.24	5.8 ± 0.8	239 ± 6	2.55 ± 0.23	15.92 ± 0.33	14.9 ± 0.12
	AKCa	21.33 ± 0.51 b	3.15 ± 0.03	7.39 ± 0.20	6 ± 2.2	230 ± 8	2.57 ± 0.39	15.75 ± 0.51	14.27 ± 0.9
	Significance	*	ns	ns	ns	ns	ns	ns	ns
2020	AKC	23.5 ± 0.41 a	3.06 ± 0.03	6.9 ± 0.08	8.2 ± 1.1	220 ± 8 b	2.42 ± 0.05 b	16.83 ± 0.11	15.64 ± 0.45
	AKK	21.75 ± 0.56 b	3.05 ±0.01	7.2 ± 0.52	7.9 ± 1.3	247 ± 11 a	2.59 ± 0.04 a	16.99 ± 0.12	15.94 ± 0.24
	AKCa	21.9 0.22 b	3.08 ± 0.03	7.31 ± 0.19	9.1 ± 1.3	252 ± 14 a	2.63 ± 0.04 a	17.18 ± 0.68	16.15 ± 0.48
	Significance	*	ns	ns	ns	*	*	ns	ns
2021	AKC	23.01 ± 0.39 b	3.21 ± 0.01 b	5.50 ± 0.02 b	7.2 ± 2	223 ± 5 a	1.93 ± 0.13 b	15.58 ± 0.76	14.31 ± 0.67
	AKK	24.05 ± 0.18 a	3.32 ± 0.02 a	5.85 ± 0.05 a	8.1 ± 1.9	242 ± 8 b	2.40 ± 0.12 a	15.89 ± 0.21	14.05 ± 0.16
	AKCa	-	-	-	-	-	-	-	-
	Significance	*	*	*	ns	*	*	ns	ns
Year x Treatment		ns	ns	ns	*	ns	ns	*	ns

The values are averages ± SD. Different lowercase letters represent significant differences between the treatments ($p<0.05$). Year x Treatment followed by * represents significant differences between years within the same treatment ($p < 0.05$).

Table 7 shows the must parameters (soluble solids, pH and acidity) and the mechanical properties of cv. Assyrtiko trained with the VSP training system in response to exogenous application of kaolin and calcium carbonate for the three studied years. Regarding sugar content in 2019 no significant differences were mentioned between the treatment and the control. In 2020 AVK and AVCa exhibited lower sugar content than AVC while in 2021 AVK was significantly higher than AVC. Total acidity in 2019 was lower for AVK and AVCa than the control. Further, in 2020 no significant differences were found. However, in 2021 AVK presented lower total acidity than AVC. Concerning pH measurements, in 2019 AVC was significantly lower than both treatments. Meanwhile in 2020 and 2021 no significant differences were observed. Regarding berry's parameters there was no clear separation between treatments. In 2019, AVCa presented lower berry width and length than AVK and AVC. Similar was the image for grape's parameters, where no significant difference was mentioned.

Table 7. Grape composition (soluble solids, pH and acidity) and mechanical properties of cv. 'Assyrtiko' trained with the VSP training system treated with Kaolin (AVK), $CaCO_3$ (AVCa) treatments and a control (AVC) in 2019, 2020 and 2021 seasons

Year	Treatment	Soluble solids (°Brix)	pH	Titratable acidity (g L^{-1})	Bunch number per vine	Bunch weight (g)	Berry weight (g)	Berry length (mm)	Berry width (mm)
2019	AVC	23.20 ± 0.09	3.17 ± 0.01 a	6.75 ± 0.18a	3.2 ± 1.2	210 ± 12	2.52 ± 0.2	17.40 ± 0.29 a	15.49 ± 0.23
	AVK	23 ± 0.22	3.30 ± 0.0 b	5.88 ± 0.12 b	3.5 ±0.5	229 ± 4	2.73 ± 0.06	17.10 ± 0.34 a	15.28 ± 0.06
	AVCa	23.27 ± 0.07	3.31 ± 0.01 c	5.63 ± 0.2 b	3 ± 0.3	224 ± 8	2.54 ± 0.13	16.21 ± 0.19 b	14.72 ± 0.17
	Significance	ns	*	*	ns	ns	ns	*	*
2020	AVC	23.4 ± 0.04 a	3.21 ± 0.01	6.21 ± 0.4	4.1 ± 2.3	128 ± 19 b	2.03 ± 0.2 b	14.89 ± 0.95	13.33 ± 0.53
	AVK	22.27 ± 0.07 b	3.23 ± 0.02	6.25 ± 0.52	4 ± 1.8	208 ± 84 a	1.85 ± 0.13 a	14.04 ± 0.63	12.62 ± 0.76
	AVCa	22 ± 0.2 b	3.23 ± 0.03	5.96 ± 0.22	3.9 ± 1.5	199 ± 54 a	1.68 ± 0.14 a	14.92 ± 0.26	13.68 ± 0.61
	Significance	*	ns	ns	ns	ns	ns	ns	ns
2021	AVC	24.03 ± 0.4 b	3.18 ± 0.22	5.93 ± 0.09 b	2.9 ± 0.7	214 ± 5	2.27 ± 0.4	16.55 ± 0.46	15.20 ± 0.19
	AVK	25.01 ± 0.27 a	3.32 ± 0.19	5.23 ± 0.1 a	3 ± 0.4	221± 9	2.40 ± 0.08	15.18 ± 1	14.79 ± 0.9
	AVCa	-	-	-	-	-	-	-	-
	Significance	*	ns	*	ns	ns	ns	ns	ns
Year x Treatment		ns	ns	ns	*	ns	ns	*	ns

The values are averages ± SD. Different lowercase letters represent significant differences between the treatments ($p<0.05$). Year x Treatment followed by * represents significant differences between years within the same treatment ($p < 0.05$).

Foliar particle films have decreased sunburn negative effects and pest infestation. Specifically, both AKK and AKCa have reduced grape shriveling (~13% difference from AKC) caused by the sunburn during a heatwave that took place at 2019's harvest (Figure 4). In 2020 no sunburn damages were observed. In 2019 and 2020 during harvest, AKK and AKCa presented a lower % of damaged by *Lobesia botrana* bunches than the AKC (3% and 7% difference 2019 and 2020, respectively). While in 2020, *Plasmopara viticola* infection was reduced in the case of both AKK and AKCa treatments (4% difference).

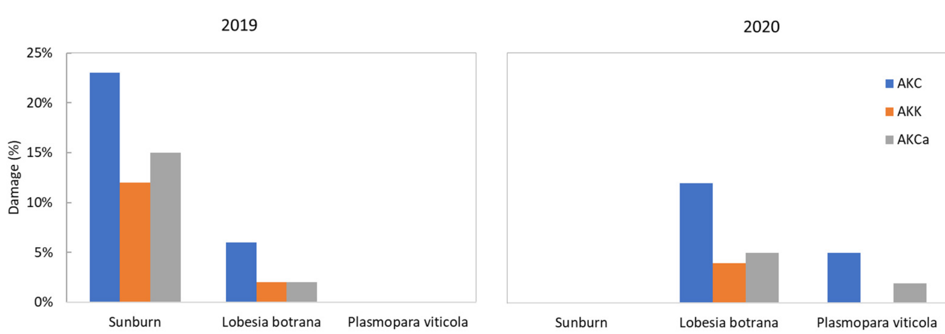

Figure 4. Damage estimation (%) of sunburn, *Lobesia botrana* and *Plasmopara viticola* pest of AKC, AKK and AKCa treatments in 2019 and 2020 seasons

Discussion

In this study, the effects of foliar application with kaolin and calcium carbonate on vines of the white grape cultivar 'Assyrtiko' trained with the traditional training system and VSP training system in Santorini Island, were evaluated, focusing on the impact on the vine's physiology attributes, grape quality, and environmental sustainability. Based on the results of the present study, there were statistically significant differences on vine water stress in which the foliar application of inert films reduced water stress during the three studied years for both cultivars. Both treatments significantly increased the photosynthesis, chlorophyll content, and stomatal conductance of the vines in comparison to the control, while they decreased leaf and grape temperature. Furthermore, the foliar application with inert minerals has reduced sunburn and pest damage.

Generally, grapevine water status values were higher due to the hot climate of the experimental site in all treatments. According to van Leeuwen *et al*. (2010), the values measured in this study were classified as follows: no water stress (>−0.6 MPa), moderate to severe water stress (between −1.1 to −1.4 MPa), and very severe water stress (−1.4 MPa). During the three growing seasons (2019, 2020 and 2021), the Ψ midday values of control grapevines reached close to −1.4 MPa at harvest for both studied cases. The lowest Ψ midday values were recorded in control vines during the three seasons. In the present study, foliar kaolin and calcium carbonate treatments had positive effect on plant water status when compared to the control on all measurement days. In the case of the cultivar 'Assyrtiko' trained with VSP, foliar treatments' efficiency was greater for each developmental stage than the case of Assyrtiko trained with 'Kouloura' training system. This happens probably due to the early water stress condition of the VSP case which induce the function of the foliar coatings (kaolin and calcium carbonate). Other studies (Ou *et al*., 2010; Teker, 2023) stated that no difference in leaf Ψ midday was observed between vines with and without kaolin treatment, under different irrigation conditions. Nevertheless, studies on the use of kaolin have shown that it can improve water availability and abiotic stress tolerance of vines under extreme environmental conditions (Brillante *et al*., 2016; Dinis *et al*., 2018). Based on the data collected in the three studied seasons for both training systems of cv Assyrtiko, foliar applications have increased Ψ midday of treated vines about 0.15 to 0.2 MPa. In literature, there are many references reporting notable increases in midday leaf water potential (up to +40.7% in 2013 in the Douro region according to Dinis *et al*. (2018) after kaolin application, and no effects or a markedly reduced stem water potential in Cabernet Sauvignon kaolin treated plants (Brillante et al., 2016). Studies have shown that the rate of water uptake from the soil during drought as well as the dynamic of decreasing stem water potential were not affected by kaolin sprays (Shellie and King, 2013; Frioni *et al*., 2019). Regarding the effects of Ca on Ψ measurements, the literature is scarce.

The kaolin and calcium carbonate applications presented a significant increase of gs and Pn, when compared with the untreated plants. The leaves sprayed with kaolin and calcium carbonate remain photosynthetically active for a longer period of time than the control especially in the case of Assyrtiko cultivar. These interventions protect the photosynthetic functions and help the vines to store reserves and enhance the ripening process. Similar studies reported that kaolin particularly around midday throughout the growing season enhanced leaf net photosynthetic rate (Dinis *et al*., 2018; Bernardo *et al*., 2021). The lower values of Pn and gs in the control plants suggested that the decline in Pn could be related to stomatal limitations which are closely associated with lower leaf water potential and high leaf temperatures. The regulation of stomatal aperture limits the CO_2 entry into leaves and consequently leads to a decrease of Pn as a result of the reduced CO_2 availability (Centritto *et al*., 2005). In addition, the E was also lower in the control plants, which is in close association with the variation in gs, as reported by Poni *et al*. (2009). The kaolin and calcium carbonate coatings work by significantly reducing leaf and grape temperatures in comparison to untreated vines, with an average of about − 2 °C and up to −4 °C for both training systems for all studied seasons. Concerning the chlorophyll content, both foliar treatments maintained a higher chlorophyll content for all the studied cases with an average of 2-3 SPAD units from the control. In summary, this study showed that kaolin foliar spray reduced leaf and grape temperature and increased leaf water potential, while they maintained high the photosynthetic activity preventing irreversible photoinhibition phenomena, whereas untreated vines exhibit a marked physiological damage with chlorotic and necrotic leaves, dehydrated berries and sunburn damages.

Our results have shown that from three studied years the ones of 2020 and 2021, which were the drier ones, in the case of 'Assyrtiko' cultivar and both training systems, both foliar applications enhanced grapevine's physiology and water status at a higher rate than 2019.

Concerning grape and berry attributes our study revealed that no clear separation was mentioned between the treatments and the control for both training systems during the three studied years.

Harvest parameters (soluble solids, pH and acidity) were quantified, and presented. Overall, no clear separation was observed in soluble solids, pH, acidity of berries with and without kaolin application on both studied varieties and both training systems. Our results strengthen the findings of several works in *Vitis vinifera* L. (Shellie and Glenn, 2008; Glenn *et al*., 2010; Shellie and King, 2013; Lobos *et al*., 2015; Shellie, 2015; Brillante *et al*., 2016; Frioni *et al*., 2019b; Luzio *et al*., 2021) which indicate that the reflective particle film had no detectable influence on berry soluble solids, pH, and titratable acidity of berries.

Recent studies have shown that kaolin protects grapevines from downy mildew as well as from *Lobesia botrana* and sunburn damages. Wang *et al*. (2022) showed that the efficacy of kaolin against grape downy mildew was demonstrated by in vitro inoculation assays. Kaolin acted both as a barrier and a resistance inducer to protect leaf tissues, and it can also directly act on sporangia by limiting the release of zoospores. Tacoli *et al*. (2019) found that kaolin reduced the infestation of *Lobesia botrana* with good efficacy. Teker (2023) showed that kaolin treatment was more resistant to sunburn damages than the control, 46.25% and 27.27% of damaged clusters exhibited varying severity in 2020 and 2021, respectively. In the present study, it was shown that K and Ca proved to be more resistant against *Plasmopara viticola, Lobesia botrana* and sunburn damages than the control.

Conclusions

In conclusion, this work details the physiological response and grape quality attributes of vines of grape cultivar 'Assyrtiko' after foliar application with kaolin and carbonate calcium. Kaolin has revealed to be particularly effective on grapevine's water status at the arid conditions of Santorini while both kaolin and carbonate calcium enhanced the physiology condition of treated vines. The present study also showed that the inert particle film technology in the climatic conditions of Santorini Island can reduce heat stress on vine physiology, and leaf and berry temperatures, extending the ripening process while it can shield grape berries from sunburn damage in hot climates. The foliar use of inert particle films on Santorini's traditional vineyards could consist a sustainable and effective alternative to mitigate the effects of climate change which have intensified over the last decades in the region.

Authors' Contributions

Conceptualization X.E.G and B.K; Data curation X.E.G; Investigation X.E.G, K.B and S.M; Methodology X.E.G, B.K and S.M; Supervision B.K and S.M; Writing - review and editing X.E.G, B.K and S.M.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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