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Effect of foliar applications on the qualitative and quantitative characters of cv. Assyrtiko and cv. Mavrotragano in the island of Santorini, under vineyard conditions

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Abstract. The Mediterranean basin is regarded as one of the world's most affected regions by climate changes. Traditionally, viticulture in this region has been coping with high temperatures, heat waves and drought. Such particularly extreme conditions, which induce severe abiotic stress on plants, are expected to intensify due to the predicted climate changes in the future. Santorini Island seems not to be an exception. The increase of temperature and solar radiation, in combination with the low availability of water, made necessary the development and the application of methods in order to cope with the abiotic stresses of the grapevine. This study examined the foliar applications and their effect on the qualitative and quantitative characteristics of the grapes by spraying application of the two indigenous varieties of the vineyard of Santorini (Greece), Assyrtiko, and Mavrotragano. The experiment took place in the cultivation season 2019-2020. Specifically, treatments with kaolin and calcium carbonate, two inert materials capable of reflecting radiation were evaluated. In the case of Assyrtiko, the effect of kaolin and calcium carbonate was examined on two different training systems, on the traditional training system of Santorini 'kouloura', and on a single Guyot training system. In the case of Mavrotragano, the effect of kaolin and calcium carbonate was examined on the vines which are trained in double Guyot training system. Mechanical analyses of the grapes and berries were performed, as well as measurements of the characters of the must during the stage of technological maturity. The content of the phenolic compounds was determined in the skins and seeds and the antioxidant capacity of the samples was measured by using different methods, FRAP and DPPH, by the use of spectrophotometry. At the same time, the analysis of High-Pressure Liquid Chromatography (HPLC) indicated the concentrations of the main individual sugars and acids in the must as well as the measurements of the skin individual anthocyanins for cv Mavrotragano. The results of the treatments showed that the analyses related to weight, length, and width of the grapes and berries and the measurements of pH and total titratable acidity for both training systems and both varieties did not seem to be affected. Also, compared to grapes from control treatments, the levels of most phenolic compounds and anthocyanins of the sprayed samples mostly increased, and as a result, this leads to grapes of better quality, thus better wine quality, since most measurements conducted in the current experiment are also directly correlated with the organoleptic properties of the wine. Therefore, the use of kaolin and calcium carbonate through foliar applications constitutes an important means of adaptation of the vines which are grown under conditions of drought, in terms of economic and environmental sustainability reasons, while improving the quality of grapes.

1 Introduction

The productivity of a vineyard is inextricably linked to the climate, therefore the increase in atmospheric temperature due to climate change affects both the yield and composition of the grape as well as the organoleptic properties of the wine [1]. Among all climatic parameters, air temperature is considered the most important for the growth and maturation of vines and their grapes, when the plant's requirements for water, radiation and nutrients are met [2].

Water is one of the world's critical, non-renewable resources, and the largest global consumption of fresh water is consumed in agriculture, with 48% in Europe. About 60% of the vines are grown in semi-arid conditions and therefore in many wine-growing regions the water consumption per vine (300–700 mm) is often higher than the average annual rainfall. A moderate water stress has been found beneficial for grape quality in several studies, but severe water stress can cause ripening delays, low yields, and reduced grape color [3].

Santorini is characterized by periods of heat and drought conditions. During the summer months, heat stress conditions have been observed at high frequencies, between June and September, reaching a maximum in the month of July, followed by the month of August, June, and finally September. The frequencies of strong and very strong heat stress (temperatures higher than 32°C) exceed 4.4% during the summer months, presenting a maximum of 7.6% in July. In addition, heat stress conditions in

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Santorini show a statistically significant increasing trend at a rate of 28.2 hours/decade during the period 1982-2019, which is consistent with ongoing global warming, as the Mediterranean region has been identified as one of the most sensitive regions to climate change worldwide in relation to increased thermal risk [4]. However, in the Mediterranean basin, not only a radical increase in air temperature is observed but also a change in the distribution of precipitation during the season [5].

Therefore, vines are affected by a combination of many abiotic stresses that lead to physiological and biochemical changes during their development and fruit composition [5].

Prolonged periods with temperatures greater than 30°C can cause problems during the ripening phase, such as in the movement of photosynthesis products from the leaves to the grapes, thus affecting the sugar and acid concentrations in the grapes as well as the secondary metabolism of the vine [6]. Moreover, temperatures above 30°C after the onset of ripening may inhibit the biosynthesis of anthocyanins [7]. High temperatures during the ripening phase are very likely to contribute to the increase of sugar levels and the reduction of the acid concentration of the grape at harvest, while higher temperatures during the night can damage the composition and favor the breakdown of secondary metabolites, such as aroma and color compounds [1].

Warmer conditions due to climate change are also associated with shorter phenolic stage intervals and earlier harvest [8]. In addition, when temperatures during the growing season exceed 22-24ºC they often lead to excessive heat stress of the vines, which is often associated with severe water stress in dry climates [2]. High heat waves during ripening may also cause an increase in the accumulation of soluble solids combined with a faster organic acid depletion, an increase in pH and the formation of atypical aromatic compounds. Therefore, the resulting wines do not have increased aging potential, they have poor color and altered aromatic profiles [5].

In addition, the growth and ripening of grapes in warmer periods can cause their shrinkage, a phenomenon of weight loss of grapes from dehydration, and can occur at various developmental stages, either before flowering or after flowering [9]. The opening of the stomata is reduced and from the increased transpiration the leaves can absorb water from the berries and lead to shrinkage of the berries [7]. Therefore, the weight loss comes from the change in the available water of the vine, that is, when the transpiration and return of water to the plant exceeds the input of water to it. Shrinkage of grapes can have a significant economic impact as it can reduce production by 25% and can bring about significant effects on the structure of the grapes which will affect the final wine [9]. Wines from shrunken berries are observed to be higher in alcohol, more astringent, and highly oxidatively aged [10]. Water loss in grapes is also a common feature in other types of shrinkage, such as bunch stem necrosis, sugar accumulation disorder as well as sunburn in various cultivars [10].

Significant solutions to the above-mentioned problems can be given by using certain plant substances (pinolin) and minerals (kaolin, calcium carbonate) [3] for the protection against biotic (pests and diseases) and abiotic (water shortage and high temperatures) conditions which can eventually lead to a sustainable management of the vineyard, by improving water use efficiency of the vines, while maintaining or improving the qualitative and quantitative characteristics of the grapes and berries.

The aim of the present study was to investigate the effects of kaolin and calcium carbonate foliar applications on berry composition of grape cultivars Assyrtiko and Mavrotragano, under drought conditions in Santorini and with two different training systems.

2 Material and methods

2.1 Experimental design

The experiment took place in the cultivation season 2019-2020 on vines of grape cultivars Assyrtiko and Mavrotragano (*Vitis vinifera* L.), in commercial vineyards located in Oia, Santorini, Greece (36°28'22.5"N; 25°23'14.7"E) (Fig. 1). All vines were own rooted. Regarding white grape cultivar Assyrtiko, there were two vineyards: one with the traditional training system of Santorini 'kouloura' where the vines are cane-pruned to 4-6 canes of 8-10 nodes at 2m × 2m intervals, resulting in a vine density of 2000 plants/ha; and one vineyard N-S oriented where the vines are unilateral cordon-trained (unilateral Guyot) and cane-pruned to 8-10 nodes canes at 2m × 0.9m × 0.9m intervals, resulting in a vine density of 6200 plants/ha. Both vineyards were not irrigated. Soil is characterized by a clay-sandy texture and floor management was carried out as full tillage. Regarding red grape cultivar Mavrotragano, the vines are bilateral cordon-trained (double Guyot) and cane-pruned to 8-10 nodes canes at 2m × 0.9m intervals, resulting in a vine density of 5500 plants/ha.

Figure 1. Location of vineyards, Oia, Santorini.

In all vineyards, there were vines that underwent kaolin application, vines that underwent calcium carbonate treatment and there were control vines. The trial was conducted using a randomized block design, with three blocks composed of 20 vines each selected on three different rows. The three selected rows were separated by two untreated rows in order to limit drift effects. Three treatments were evaluated as: a) untreated control, b) kaolin application (Surround® WP, 95% kaolin, 5% inert ingredients, AgNova Technologies Pty Ltd., Australia), and c) experimental calcium carbonate (Omya AG, 5%). Treatments were made in the morning and in absence of wind. Kaolin and calcium carbonate were applied at bunch closure and veraison.

2.2 Grape sampling

At harvest, grapes were randomly selected from each of the three blocks for all treatments. The sampling process, described in a previous study [11] involved the random selection of three (3) grapes from different vines of each treatment and three (3) sampling processes, whereas each sampling was considered as one (1) replication.

2.3 Polyphenolic analysis

For the evaluation of the phenolic content and antioxidant capacity of the samples studied, in terms of the qualitative and quantitative characters of grapes, berries, and must, the following were measured: (i) mechanical analyses of grapes and berries, (ii) analyses on the must (pH, soluble solids content, total titratable acidity), (iii) determination of total phenols (in skins and seeds), (iv) determination of total and individual anthocyanins (in skins), (v) determination of total flavonoid content (in skins and seeds), (vi) determination of total flavanols (in skins and seeds), (vii) determination of flavone and flavonol content (in skins and seeds), (viii) antioxidant activity with FRAP and DPPH methods (in skins and seeds), (ix) determination of individual organic acids and individual sugars (in must).

All reagents and chemicals as well as all procedures regarding the determination of mechanical analyses of grapes and berries, the analyses on the must, and the preparation of samples for spectrophotometric and HPLC analyses have been performed by following protocols described in a previous study [12].

2.4 Statistical analysis

All statistical analyses and correlations were obtained using the JMP v.10 statistical software (SAS Institute Inc., Cary, NC, USA). The significance of the results was tested by Analysis of Variance (ANOVA) and the means of the values were compared by Tukey's range test at $P \leq 0.05$. Letters in columns denote statistically differences (Tukey-HSD, $P \leq 0.05$).

3 Results and Discussion

As mentioned above, during the technological maturity of the grapes, measurements related to the qualitative and quantitative characters of the grapes, berries, and must were performed, in order to examine whether there were differences between the treatments, the training systems and the grape cultivars.

The results (mean value and standard error) of each parameter measured and for each treatment are shown in Tables 1-12. Three separate statistical analyses were performed: i) Assyrtiko traditional training system, ii) Assyrtiko Guyot training system, and iii) Mavrotragano Guyot training system. The statistically significant differences found between the treatments and for each parameter measured have been highlighted and flagged with discrete letter.

3.1 Mechanical properties of grapes and berries, and characters of the must

In the case of Assyrtiko and both training systems, there are no statistically significant differences observed in the grape width and weight, with control treatment recording the higher value in the traditional system and kaolin treatment in the Guyot system (Table 1).

Mavrotragano recorded the highest values in grape width and weight in the calcium carbonate treatment, but with no statistically significant difference, compared to control. Regarding grape length, control treatment scored the highest values for both cultivars and both training systems.

Table 1. Mechanical properties of the grapes.

Treatments	Grape length (cm)	Grape width (cm)	Grape weight (g)
Assyrtiko – traditional - control	20.67 ± 0.67 a	9.67 ± 1.17 a	399.34 ± 37.92 a
Assyrtiko – traditional - calcium	14.33 ± 0.88 b	7.67 ± 1.20 a	203.67 ± 32.09 b
Assyrtiko – traditional - kaolin	18.17 ± 0.60 a	8.50 ± 0.76 a	348.34 ± 37.36 ab
Assyrtiko – Guyot - control	13.67 ± 0.88 a	7.00 ± 0.00 b	128.00 ± 5.19 b
Assyrtiko – Guyot - calcium	13.50 ± 0.28 a	6.34 ± 0.33 b	199.67 ± 4.33 a
Assyrtiko – Guyot - kaolin	14.33 ± 0.88 a	9.67 ± 0.33 a	208.67 ± 2.42 a
Mavrotragano – Guyot - control	17.83 ± 0.33 a	7.34 ± 0.33 a	295.67 ± 7.22 a
Mavrotragano – Guyot - calcium	16.50 ± 0.29 b	8.00 ± 0.58 a	315.00 ± 7.00 a
Mavrotragano – Guyot - kaolin	16.00 ± 0.29 b	6.34 ± 0.33 a	217.00 ± 6.66 b

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–b) are significantly different at significance level $p \leq 0.05$.

Regarding the mechanical properties of the berries, the treatments for both cultivars and training systems did not exhibit statistically significant differences (Table 2).

The calcium carbonate treatment for the traditional training system and Guyot system of Assyrtiko recorded the higher values in berry length and width, while for

Mavrotragano, the higher value was recorded in control treatment.

No statistically significant differences were recorded in the weight of 50 berries for both cultivars and training systems, with control treatment exhibiting the higher values.

Table 2. Mechanical properties of the berries.

Treatments	Berry length (mm)	Berry width (mm)	Weight of 50 berries (g)
Assyrtiko – traditional - control	16.83 ± 0.06 a	15.65 ± 0.26 a	124.34 ± 4.81 a
Assyrtiko – traditional - calcium	17.18 ± 0.39 a	16.15 ± 0.27 a	117 ± 3.32 ab
Assyrtiko – traditional - kaolin	16.99 ± 0.01 a	15.94 ± 0.24 a	122.34 ± 4.33 a
Assyrtiko – Guyot - control	14.89 ± 0.55 a	13.33 ± 0.31 a	101.67 ± 3.84 a
Assyrtiko – Guyot - calcium	14.92 ± 0.15 a	13.68 ± 0.35 a	84 ± 3.05 b
Assyrtiko – Guyot - kaolin	14.04 ± 0.36 a	12.62 ± 0.44 a	92.34 ± 2.51 ab
Mavrotragano – Guyot - control	13.8 ± 0.27 a	13.68 ± 0.32 a	90.34 ± 0.88 a
Mavrotragano – Guyot - calcium	13.78 ± 0.13 a	13.67 ± 0.12 a	80.00 ± 2.30 b
Mavrotragano – Guyot - kaolin	12.36 ± 0.34 b	12.55 ± 0.34 a	85.67 ± 0.44 ab

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–b) are significantly different at significance level $p \leq 0.05$.

The higher pH values were recorded in kaolin treatment for Mavrotragano, while the higher total titratable acidity values were recorded in control for Guyot training system of Assyrtiko, and in control treatment for Mavrotragano (Table 3).

In the case of the traditional training system in Assyrtiko, total soluble solids concentration was higher in the kaolin treatment compared to control and calcium carbonate treatments. Therefore, kaolin delayed the accumulation of sugars and consequently the maturation of the grapes in the case of the traditional training system.

Table 3. Characters of the must.

Treatments	TSS (Brix°)	pH	Total titratable acidity (g/L)
Assyrtiko – traditional - control	25.20 ± 0.00 b	3.43 ± 0.02 a	5.25 ± 0.03 a
Assyrtiko – traditional - calcium	24.67 ± 0.13c	3.24 ± 0.00 c	5.25 ± 0.21 a
Assyrtiko – traditional - kaolin	26.20 ± 0.00 a	3.30 ± 0.12 b	5.25 ± 0.66 a
Assyrtiko – Guyot - control	23.40 ± 0.00 a	3.23 ± 0.00 c	6.38 ± 0.57 a
Assyrtiko – Guyot - calcium	20.00 ± 0.20 c	3.30 ± 0.00 b	6.00 ± 0.22 a
Assyrtiko – Guyot - kaolin	22.27 ± 0.06 b	3.46 ± 0.00 a	6.25 ± 0.50 a
Mavrotragano – Guyot - control	23.13 ± 0.06 b	3.34 ± 0.02 c	5.88 ± 0.38 a
Mavrotragano – Guyot - calcium	25.20 ± 0.00 a	3.48 ± 0.02 b	5.50 ± 0.87 a
Mavrotragano – Guyot - kaolin	25.40 ± 0.00 a	3.74 ± 0.01 a	5.13 ± 0.66 a

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

3.2 Polyphenolic compounds

3.2.1 Total phenolics in seeds and skins

The results showed that the highest concentration of total phenolics in grape cultivar Assyrtiko and the traditional training system was recorded in the kaolin treatment (Table 4), both in the seeds and skins, with a statistically significant difference compared to the other treatments. The calcium carbonate and kaolin treatment had a positive effect in the concentration of total phenolics, particularly those of the skins and in the case of Guyot training system.

In the case of grape cultivar Mavrotragano, the calcium carbonate treatment exhibited the higher values in skins and seeds total phenolics (Table 4).

The above results agree with the results of previous studies [5, 13], where the foliar application with calcium carbonate and kaolin increased the concentration of total phenolic compounds.

Table 4. Total phenolics in seeds and skins.

Treatments	Total phenolics seeds (mg gallic acid/g FW)	Total phenolics skins (mg gallic acid/g FW)
Assyrtiko – traditional - control	32.48 ± 0.61 b	3.58 ± 0.19 b
Assyrtiko – traditional - calcium	30.73 ± 0.28 b	3.20 ± 0.01 b
Assyrtiko – traditional - kaolin	45.08 ± 0.56 a	4.53 ± 0.01 a
Assyrtiko – Guyot - control	32.66 ± 0.10 a	3.41 ± 0.24 b
Assyrtiko – Guyot - calcium	29.93 ± 1.39 a	4.91 ± 0.01 a
Assyrtiko – Guyot - kaolin	24.05 ± 3.18 a	4.56 ± 0.16 a
Mavrotragano – Guyot - control	38.18 ± 2.12 a	9.79 ± 0.17 b
Mavrotragano – Guyot - calcium	39.65 ± 1.64 a	11.35 ± 0.13 a
Mavrotragano – Guyot - kaolin	29.40 ± 0.20 b	10.04 ± 0.14 b

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–b) are significantly different at significance level $p \leq 0.05$.

3.2.2 Total flavanols in seeds and skins

The highest concentration in seed total flavanols was recorded in control vines of grape cultivar Mavrotragano, whereas in the case of skin total flavanols, the highest concentration was recorded in kaolin treatment of grape cultivar Assyrtiko and the traditional training system (Table 5). For Guyot training system in grape cultivar Assyrtiko, kaolin treatment recorded the lowest concentration.

Table 5. Total flavanols in seeds and skins.

Treatments	Total flavanols seeds (mg catechin/g FW)	Total flavanols skins (mg catechin/g FW)
Assyrtiko – traditional - control	43.32 ± 0.10 b	5.42 ± 0.21 b
Assyrtiko – traditional - calcium	42.20 ± 0.49 b	6.49 ± 0.14 a
Assyrtiko – traditional - kaolin	54.37 ± 1.12 a	6.17 ± 0.13 a
Assyrtiko – Guyot - control	40.23 ± 0.63 a	6.08 ± 0.02 b
Assyrtiko – Guyot - calcium	36.49 ± 0.66 a	7.21 ± 0.11 a
Assyrtiko – Guyot - kaolin	26.99 ± 2.65 b	6.89 ± 0.06 a
Mavrotragano – Guyot - control	37.85 ± 0.55 a	8.54 ± 0.10 a
Mavrotragano – Guyot - calcium	38.56 ± 0.71 a	8.53 ± 0.27 a
Mavrotragano – Guyot - kaolin	33.77 ± 1.23 b	7.05 ± 0.01 b

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–b) are significantly different at significance level $p \leq 0.05$.

3.2.3 Total flavonoids in seeds and skins

Regarding the skin total flavonoids, in the case of Mavrotragano, the calcium carbonate treatment recorded the highest concentration, with no statistically significant difference compared to kaolin treatment, but both treatments had a statistically significant difference compared to control (Table 6). Regarding the seeds total flavonoids, control scored the highest concentration.

In the case of Assyrtiko and skin total flavonoids, the calcium carbonate treatment exhibits the highest values both in the traditional training system and in the Guyot training system.

Table 6. Total flavonoids in seeds and skins.

Treatments	Total flavonoids seeds (mg catechin/g FW)	Total flavonoids skins (mg catechin/g FW)
Assyrtiko – traditional - control	86.45 ± 0.12 b	22.32 ± 0.66 b
Assyrtiko – traditional - calcium	83.48 ± 0.95 c	33.69 ± 0.33 a
Assyrtiko – traditional - kaolin	117.78 ± 0.34 a	19.35 ± 0.78 c
Assyrtiko – Guyot - control	86.98 ± 0.10 a	25.2 ± 0.03 b
Assyrtiko – Guyot - calcium	85.09± 2.24 a	30.42 ± 0.21 a
Assyrtiko – Guyot - kaolin	62.41 ± 2.81 b	29.52 ± 0.69 a

Mavrotragano – Guyot - control	86.00 ± 2.00 a	21.09 ± 0.40 b
Mavrotragano – Guyot - calcium	81.38 ± 2.04 ab	28.17 ± 1.51 a
Mavrotragano – Guyot - kaolin	73.92 ± 1.45 b	27.06 ± 0.45 a

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

3.2.4 Total flavone and flavonol content in seeds and skins

Regarding the total flavone and flavonol content, the concentrations of the skins were positively affected in calcium carbonate and kaolin treatments, both in the case of Assyrtiko and Mavrotragano, with statistically significant differences compared to control (Table 7). In the analyses that took place in the seeds, control exhibited the higher concentrations for both cultivars.

Table 7. Total flavones and flavonols in seeds and skins.

Treatments	Total flavones and flavonols seeds (mg rutin/g FW)	Total flavones and flavonols skins (mg rutin/g FW)
Assyrtiko – traditional - control	0.77 ± 0.00 a	0.42 ± 0.00 b
Assyrtiko – traditional - calcium	0.67 ± 0.01 b	0.51 ± 0.03 a
Assyrtiko – traditional - kaolin	0.47 ± 0.02 c	0.45 ± 0.01 ab
Assyrtiko – Guyot - control	0.79 ± 0.00 a	0.53 ± 0.00 c
Assyrtiko – Guyot - calcium	0.71 ± 0.00 a	0.73 ± 0.02 b
Assyrtiko – Guyot - kaolin	0.75 ± 0.04 a	0.88 ± 0.00 a
Mavrotragano – Guyot - control	0.53 ± 0.00 a	1.56 ± 0.00 c
Mavrotragano – Guyot - calcium	0.50 ± 0.01 a	2.01 ± 0.00 a
Mavrotragano – Guyot - kaolin	0.38 ± 0.03 b	1.94 ± 0.00 b

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

3.2.5 Antioxidant activity in seeds and skins

Regarding the antioxidant activity, as was determined both with the FRAP and DPPH methods, the results showed that the treatments with foliar applications exhibited the highest values both in seeds and in skins for both cultivars and for both training systems, when compared to control vines (Table 8).

Antioxidants are important for wine since they affect its color, they offer antioxidant and antimicrobial protection, participate in the formation of certain characteristics and play an important role in aging. Particularly encouraging are the positive effects of the increase in antioxidants, since Assyrtiko is an eoxidous variety with great aging potential.

Table 8. Antioxidant activity in seeds and skins.

Measurements	FRAP (mg Trolox/g FW)		DPPH (mg Trolox/g FW)	
	Seeds	Skins	Seeds	Skins
Assyrtiko – traditional - control	253.27 ± 10.30 b	21.56 ± 1.05 a	138.38 ± 0.02 b	34.46 ± 0.13 b
Assyrtiko – traditional - calcium	330.27 ± 0.97 a	19.24 ± 0.62 a	127.75 ± 0.50 c	42.68 ± 1.52 a
Assyrtiko – traditional - kaolin	276.01 ± 6.31 b	21.63 ± 0.09 a	153.038 ± 2.78 a	25.54 ± 1.04 c
Assyrtiko – Guyot - control	232.50 ± 0.83 a	18.74 ± 1.05 b	146.96 ± 1.69 a	37.44 ± 0.14 c
Assyrtiko – Guyot - calcium	224.29 ± 0.03 a	27.77 ± 0.23 a	125.61 ± 2.50 b	44.33 ± 0.00 a
Assyrtiko – Guyot - kaolin	251.42 ± 3.67 a	27.32 ± 0.92 a	168.88 ± 7.63 a	41.16 ± 0.86 b
Mavrotragano – Guyot - control	205.55 ± 2.10 b	38.21 ± 0.24 a	140.48 ± 2.20 c	40.78 ± 0.38 b
Mavrotragano – Guyot - calcium	243.79 ± 5.81 a	42.60 ± 0.94 a	187.16 ± 4.92 a	47.68 ± 0.25 a
Mavrotragano – Guyot - kaolin	226.72 ± 5.64 a	31.23 ± 1.48 b	164.33 ± 1.26 b	40.96 ± 0.24 b

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

3.3 Total and individual anthocyanins

In the case of grape cultivar Mavrotragano, total and individual anthocyanins in skins were determined for all treatments (Table 9). The treatments with calcium carbonate and kaolin record the highest concentrations in total anthocyanins with statistically significant differences compared to control. These results are also in agreement with previous studies [5, 13], which also confirm the increase in the anthocyanin concentration.

Similar results were observed in the case of individual anthocyanins, except for the coumaric ester of malvidin, where no statistically significant differences were recorded between calcium carbonate treatment and control.

Table 9. Total and individual anthocyanins.

Treatments	Mavrotragano – Guyot - control	Mavrotragano – Guyot - calcium	Mavrotragano – Guyot - kaolin
Total anthocyanins (mg malvidin / g FW)	7.56 ± 0.24 c	10.87 ± 0.15 a	9.48 ± 0.23 b
Delphinidin (mg / g FW)	0.45 ± 0.00 c	1.26 ± 0.00 a	0.94 ± 0.02 b
Cyanidin (mg / g FW)	0.093 ± 0.00 c	0.24 ± 0.11 b	0.43 ± 0.01 a
Petunidin (mg / g FW)	0.53 ± 0.00 c	1.25 ± 0.00 a	0.93 ± 0.02 b
Peonidin (mg / g FW)	0.21 ± 0.00 c	0.46 ± 0.01 b	0.66 ± 0.01 a
Malvidin (mg / g FW)	2.88 ± 0.02 c	4.54 ± 0.18 a	3.58 ± 0.10 b
Acetic ester of malvidin (mg / g FW)	1.17 ± 0.00 a	1.31 ± 0.03 a	1.29 ± 0.05 a
Coumaric ester of malvidin (mg / g FW)	2.99 ± 0.02 a	3.02 ± 0.05 a	2.59 ± 0.06 b

Values are the means of triplicates (±SE). Values on the same row carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

3.4 Individual sugars and organic acids in must

The results regarding the individual sugars showed that the concentration of glucose was higher than the one of fructose in grape cultivar Assyrtiko, whereas in Mavrotragano, the opposite was recorded (Table 10). The concentration of sugars exhibits statistically significant differences between control and the treatments with calcium carbonate-kaolin, and the highest value in glucose is recorded in the kaolin treatment for grape cultivar Assyrtiko and the traditional training system, and the highest value in fructose is recorded in the calcium carbonate treatment for grape cultivar Mavrotragano and the Guyot training system.

Table 10. Individual sugars in must.

Treatments	Glucose (g/L must)	Fructose (g/L must)
Assyrtiko – traditional - control	177.67 ± 0.15 a	166.19 ± 0.05 b
Assyrtiko – traditional - calcium	174.02 ± 0.53 b	162.91 ± 0.42 c
Assyrtiko – traditional - kaolin	178.40 ± 1.08 a	170.83 ± 1.14 a
Assyrtiko – Guyot - control	161.73 ± 0.69 a	151.18 ± 0.94 a
Assyrtiko – Guyot - calcium	136.55 ± 3.51 c	120.06 ± 2.74 b
Assyrtiko – Guyot - kaolin	151.54 ± 0.18 b	145.62 ± 0.59 a
Mavrotragano – Guyot - control	153.13 ± 0.02 b	162.52 ± 0.43 c
Mavrotragano – Guyot - calcium	167.59 ± 1.85 a	175.21 ± 0.28 a
Mavrotragano – Guyot - kaolin	168.73 ± 0.53 a	172.76 ± 0.07 b

Values are the means of triplicates (±SE). Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

The individual organic acid with the higher concentration was tartaric acid, followed by malic acid (expressed as mg/mg must, as opposed to ascorbic acid which is expressed as μg/mg must) (Table 11). In the case of tartaric acid, the calcium carbonate treatment exhibited the higher concentration for the Guyot training system of Assyrtiko, with statistically significant differences, while in the case of unilateral Guyot and in the case of Mavrotragano, the calcium carbonate treatment recorded the higher concentration with statistically significant differences.

Consequently, it seems that the most important organic acid was affected by the foliar applications, as opposed to malic acid which recorded the higher concentrations in the control treatment for both cultivars.

The high concentration of tartaric acid, especially for grape cultivar Assyrtiko, which is a variety that can be aged for a long time, are significant and results in a lower pH which acts as a natural antioxidant for the wine.

Table 11. Individual organic acids in must.

Treatments	Tartaric acid (mg/mg must)	Malic acid (mg/mg must)	Ascorbic acid (μg/mg must)
Assyrtiko – traditional - control	6.55 ± 0.09 c	1.98 ± 0.00 a	83.66 ± 0.27 b
Assyrtiko – traditional - calcium	6.94 ± 0.00 b	1.31 ± 0.00 c	63.74 ± 018 c
Assyrtiko – traditional - kaolin	7.96 ± 0.02 a	1.55 ± 0.01 b	88.44 ± 0.21 a
Assyrtiko – Guyot - control	7.44 ± 0.15 b	1.23 ± 0.01a	63.38 ± 0.15 b
Assyrtiko – Guyot - calcium	9.57 ± 0.04 a	0.76 ± 0.00 b	54.60 ± 0.32 c
Assyrtiko – Guyot - kaolin	6.42 ± 0.02 c	1.22 ± 0.01a	74.31 ± 0.79 a
Mavrotragano – Guyot - control	4.26 ± 0.13 c	1.27 ± 0.02 a	22.33 ± 1.77 a
Mavrotragano – Guyot - calcium	6.22 ± 0.02 a	1.00 ± 0.03 c	17.30 ± 0.02 b
Mavrotragano – Guyot - kaolin	5.50 ± 0.13 b	1.15 ± 0.02 b	16.54 ± 0.06 b

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$.

4 Conclusions

The strong influence of climate change, with high temperatures and a combination of many abiotic stresses during the cultivation seasons, has worsened both the growth and yield of the vineyard of Santorini. The results of the present study showed that the foliar applications of kaolin and calcium carbonate brought about changes in the composition of the grapes and berries, with differences being observed both between the training systems and between the cultivars.

In particular, the positive effects of the treatments are enhanced since the phenolic compounds' concentration increased, including total phenolics and total anthocyanins. Moreover, they can constitute an effective and economical solution for the water saving of the vines in dry conditions, while at the same time they can improve the physiology of the plant and preserve the qualitative and quantitative characters of the grapes.

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