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The Effect of Elicitors and Canopy Management in the Chemical Composition of *Vitis vinifera* Red Varieties in Warm and Hot Areas in Spain

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Abstract: Canopy management practices in vineyards, such as sprawling systems and shoot trimming, can change the accumulation of metabolites in grapes. The use of elicitors of biological origin on grapevines of *Vitis vinifera* red grape varieties may also modulate the chemical composition of the berries. These modifications are often observed in the accumulation of phenolic compounds, including pigments. Both technical approaches are alternatives involved in minimizing the effects of global climate change in warm areas. The increase of temperature related to climate change accelerates the accumulation of sugars, but produces unbalanced grapes. This work establishes the use of button sensors to monitor the climate changes occurring at grape cluster level. Together with climate monitoring, conventional instrumental analytical techniques are used to follow up the chemical composition and the phenolic fraction of grapes in four different production areas in Spain. The effect of either treatment seems variable and to be affected by external factors besides the treatment itself and the climate conditions. While there is a fine effect that correlates with the use of elicitors in varieties like Merlot and Tempranillo, there is minimal improvement observed in Tintilla de Rota. The total phenolic index increases were between 2.3% and 11.8% in the first two parcels. The same happened with the vineyard's canopy management systems, with increased pigment accumulation and the total phenolic index rising (37.7% to 68.7%) after applying intense shoot trimming, or a variation in sugar concentrations when using sprawl conduction. This study aims to provide viticulturists and oenologists in particular, and farmers in general, with data on the field regarding the use of alternative sustainable practices in the cultivation of grapes. The techniques used involved 100% natural products without adjuvants. The benefits obtained from applying some of these practices would be to produce technically mature grapes despite climate changes, and the elaboration of more balanced wines.

Keywords: polyphenols; shoot trimming; *Vitis vinifera*; microclimate; pigments; sprawling system; climate change; inactivated yeast

1. Introduction

Elicitors could be described as alternative products produced by plants, microorganisms, or from mineral origin, the result of which is specific to the accumulation of secondary metabolites in plants [1]. The plant resistance activated against pathogens, being one of the mechanisms used to increase the levels of the phenolic composition through the phenylpropanoids route [2,3], is enhanced with the use of elicitors. Accordingly, elicitors can be used to improve the phenolic maturation in grapes, as has been evaluated in other studies.

There are different types of elicitors. Chemical elicitors, such as chitosan or methyl jasmonate [4,5], physical elicitors, such as UV or gamma radiation [2], and elicitors from biological origins, such as yeasts derivatives [6]. Studies have demonstrated that the use of chemical elicitors, such as chitosan, have increased phenolic compound concentrations [7]. Moreover, the use of methyl jasmonate and benzothiadizole combined has improved anthocyanin concentrations in comparison to control grapes [3]. Yeast derivatives have compounds such as chitin or lipids coming from the cellular wall and plasmatic membrane, respectively, that activate the plant resistance [8] through the accumulation of phenolic compounds.

A second approach that viticulturists have introduced to promote a balanced maturation of grapes is the management of the vine's canopy. Canopy management has been used worldwide for many years. There are diverse strategies in vineyards to find a balance between all grape components [9]. Shoot trimming and sprawl systems are two canopy management strategies applied to grapevines production. Shoot trimming aims to eliminate branches to decrease the shadow that vineyards project on each other during setting. Shoot trimming modifies the grapevine's microclimate to benefit grape maturation and to ensure that all the nutrients are well distributed [10,11]. A sprawl system aims to minimize undesirable effects like chlorophyll degradation and hydric stress in plants. It is a non-positioned system where vegetation is aligned producing a multidirectional shoot. Therefore, when the sun position changes, some leaves are first shaded and then others, so the sun exposure time of the leaves decreases [12]. It has been demonstrated that the use of this technique is an alternative in supporting the sugar accumulation in grapes without affecting the phenolic compounds.

Viticulturists are evaluating the possibility of countering the negative effects that climate change has on grape maturation, using these two technical approaches. Agriculture may threaten itself to a certain degree since it contributes to global warming, with slightly less than a quarter of the global greenhouse gas (GHG) emissions, mainly CO_2, attributed to deforestation and peatland drainage [13]. The climate changes observed worldwide are endangering crops globally and it has become a major concern, invoking food security. Variations in maximum temperatures could directly affect the production of crops such as wheat, while rainfall pattern changes would be detrimental to most crops in vulnerable geographical regions [14]. Climate change has the potential to increase the average growing season temperature and to modify the daily temperature variation and the average annual rainfall of grapevine cultivars. According to the four climate groups established to determine whether a particular variety is likely to ripen [15], Spanish grapevines in Cádiz, La Mancha and Uclés are located in the so-called groups warm and hot, with historical average growing temperatures between 17 and 19 °C and 19 and 21 °C, respectively. Ribera del Duero is the exception located in the upper part of the intermediate group (15–17 °C).

The massive accumulation of sugars in grapes begins at *i* and continues over to the ripening phase. High temperatures and light tend to induce the growth onset of the berries sooner than those fruit clusters at lower air temperature or located in highly shaded canopies [16]. An accelerated accumulation of sugars will trigger cultivars to ripen early producing unbalanced wines due to the lack of acidity and aroma compounds [17]. The delay in harvesting to overcome some of these drawbacks and to improve the colour and the roundness of wines increases the concentration of sugars and, therefore, the concentration of ethanol in wines [18]. Another major group of compounds in wine grapes affected by climate change is that of the phenolic compounds. The concentration of these compounds, including flavonoids and non-flavonoids, depends directly on the variety of the grape, but it is also influenced by environmental conditions and viticulture practices [19]. Light and temperature also promote the synthesis of phenolic compounds in skins and seeds [20], although the speed at which these are produced is not parallel to that of sugar production.

As the interest of recent studies has been to preserve varietal character, to enhance freshness and to promote roundness in wines providing a product with superior quality, the aims of this work include: measuring the accumulation of secondary metabolites in grapes, assessing the accumulation of phenolic compounds in berries, and comparing the effectiveness of the use of elicitors and canopy management systems. This evaluation has included the use of biological elicitors, shoot trimming, and sprawl conduction. The experimental setup monitored the microclimate of grapevines with button sensors and analysed the effect that such treatments had with analytical techniques (HPLC-DAD, FTIR, enzymatic analyser). The study was carried out in four wine-producing regions in Spain which experienced climate change extremes over the 2019 vintage. The results obtained should provide viticulturists and oenologists with field data regarding the use of alternative sustainable practices in the cultivation of grapes to optimize the harvest conditions.

2. Materials and Methods

2.1. Sampling and Vine Monitoring

The study included vineyards over the 2019 season in the quality wine regions of La Mancha (lat. 39°08′11.0′′ N; long. 3°03′43.0′′ W; elevation 670 m above sea level), Tierra de Cádiz (lat. 36°40′41.1′′ N; long. 5°47′27.0′′ W; elevation 115 m above sea level), Ribera del Duero (lat. 41°38′17.0′′ N; long. 4°06′23.3′′ W; elevation 801 m above sea level), and Uclés (lat. 39°50′00.8′′ N; long. 3°09′48.6′′ W; elevation 746 m above sea level). All of the vineyards are located in Spain in what are currently considered warm and hot viticulture areas. Parcels of the *Vitis vinifera* L. *cv*. Merlot, *cv*. Syrah, *cv*. Tintilla de Rota (Graciano) and *cv*. Tempranillo were selected to perform the study. The composition of the soil and the altitude of each parcel was homogeneous for the blocks tested. The parcels were divided into different blocks of 50 vines each from four neighbouring rows to obtain representative data from each treatment.

Each of the blocks had 10 vines selected for berry sampling and out of those, five vines were selected for microclimate monitoring. Electronic button sensors for temperature monitoring DS1921H-F5# and temperature and relative humidity tracking DS1923-F5# (Embedded Data Systems, Lawrenceburg, KY, USA) were placed at fruit cluster height, recording data once every 1800 s during the final maturation phase of the grapes. The stored data was downloaded to a PC with the use of a dual interface cable and processed with wire viewer software (Embedded Data Systems, Lawrenceburg, KY, USA). The data presented for microclimate monitoring is the average of 5 sensors per block.

Berry sampling was done every third day during the final 15 days before the estimated harvest date. A single sample comprised 50 berries collected from the clusters of the 10 selected vines in each block. The analytical results presented here are the average value of all the blocks belonging to a treatment per day.

2.2. Treatments

This experimental approach evaluated the use of elicitors on the one hand, and the canopy management on the other. The elicitor tested in this study was inactivated yeast derivate spray LalVigne® Mature (Lallemand Bio, Barcelona, Spain) in foliar application to promote technologic and phenolic maturation. The formulation of LalVigne® Mature is 100% natural and is produced from inactivated wine yeast metabolites. The yeast species used in the formulation is *Saccharomyces cerevisiae* and is designed using patented technology already available in several countries. Two doses were applied according to the producer's recommendation (1 kg/Ha of the product diluted in water with no adjuvants), and applied with motorized backpack sprayers: the first application at *veraison* and the second 7–14 days later. The canopy management followed in some of the vineyards comprised shoot trimming and a sprawl system. The shoot trimming involved soft and heavy trimming leaving a control with no vegetation manipulation. Soft trimming corresponded to top canopy trimming and minimal lateral distortion; heavy trimming corresponded to top canopy and lateral vegetation trimming. Both approaches were conducted by the viticulturists before the beginning of the microclimate monitoring with sensors and the collection of berries. Table 1 summarises the experimental set up, showing the grape variety, the treatment followed (elicitor or canopy management), and the number of blocks established per cultivar in the various studied vineyards.

Table 1. Summary of vineyards under study: wine DOP (protected denomination of origin in Spanish), variety, treatment and number of blocks involved.

DOP	Variety	Elevation (m)	Temp H/S (°C)	Treatment	No. Blocks	ID
Test a—Elicitors						
Vino de la Tierra de Cádiz	Tintilla de Rota	118	20.6/21.7	Control Elicitors	4 4	a1.1 a1.2
La Mancha	Merlot	670	19.5/21.7	Control Elicitors	3 3	a2.1 a2.2
Ribera del Duero	Tempranillo	801	16.3/16.9	Control Elicitors	3 3	a3.1 a3.2
Test b—Canopy management						
Vino de la Tierra de Cádiz	Syrah	110	20.6/21.7	Control Shoot trimmin [1] Shoot trimming [2]	3 3 3	b1.1 b1.2 b1.3
Uclés	Tempranillo	746	18.2/19.2	Control Sprawl system	3 3	b2.1 b2.2

H = Historical average season temperature from 1 April to 30 October. (1989–2019); S = Average season temperature from 1 April to 30 October 2019 retrieved for each province [21]. Average temperature groups: cool (13–15 °C), intermediate (15–17 °C), warm (17–19 °C), hot (19–21 °C). [1] Soft shoot trimming; [2] Heavy shoot trimming.

2.3. Must Composition

The pulp was crushed once the skin and seeds were removed. Then, 5 mL of must of each block per day was poured in 5 mL vials and centrifuged at 6500 rpm at 4 °C for 3 min. Every measurement was done with 1 mL of supernatant. The composition of the musts was determined using OenoFoss™ (FOSS Iberia, Barcelona, Spain) with Fourier transform infrared spectroscopy (FTIR). The oenological parameters identified included organic acids, glucosides, α-amino and pH values [22].

2.4. Pigments

The skins removed from the berry were kept at −18 °C until freeze-dried with an Edwards Modulyo freeze-drier (Crawley, UK) for 48 h. Samples of 0.5 g were mixed with 0.5 g sterile sea sand and ground. The ground sample was extracted with 15 mL methanol solution (methanol/water/formic acid with ratio 0.5:0.49:0.01 *v*/*v*/*v*). The extraction took place over 15 min and continuous mixing. The extract was transferred to a 25 mL volumetric flask and dilute with ultrapure water. The sample was centrifuged for 10 min at 4000 rpm and 4 °C. Samples were kept at 4 °C until the chromatographic analysis. HPLC vials with caps with 2 mL of extract filtered using methylcellulose 0.45 µm (Teknochroma, Barcelona, Spain) membranes were used for the determination of anthocyanins.

The chromatographic procedure that was followed is a modification of a previously reported method [23] on the eluents' gradient. HPLC-DAD was an Agilent Technologies series 1200 (Agilent Technologies, Palo Alto, CA, USA) chromatograph with a column RP Kinetex C18 (100 mm × 4.6 mm; 2.6 µm) (Phenomenex, Torrance, CA, USA). Two solvents were used: solvent A (water/formic acid 95:5 *v*/*v*) and solvent B (methanol/formic acid 95:5 *v*/*v*) with the following gradient of solvent B (1 mL/min): 25% to 50% from time 0 to 5 min; 50% from time 5 to 12 min; and 50% to 25% from time 12 to 15 min, until a steady state was reached. The injection volume was 4 µL. The quantification of pigments was based on an external standard of malvidin-3-O-glucoside (M3G), while the identification of pigments was based on the maximum wavelength observed for each peak and according to experimental data [24].

2.5. Total Polyphenols Index

The total polyphenols index (TPI) of the trials was determined with a UV-visible spectrophotometer 8453 from Agilent Technologies (Agilent Technologies, Palo Alto, CA, USA) with a photodiode array detector and 1 mm path-length quartz cuvettes [25]. The absorption at wavelength 280 nm to determine TPI was obtained from the samples using the sample procedure defined for pigment extraction.

2.6. Statistical Analysis

Determination of mean values, standard deviation, analysis of variance (ANOVA), and principal component analysis (PCA) were determined with Statgraphics v.5 software (Graphics Software Systems, Rockville, MD, USA). The LSD test was used to detect significant differences between means. Significance was set at $p < 0.05$.

3. Results

3.1. Variations in the Microclimate of Grape Clusters

The first observation regarding temperature changes can be observed in Table 1. The monthly average temperature is higher than the historic average registered for each geographical area. The results of the microclimate monitoring varied according to the function of the treatment used and the geographic location of the vineyard. When considering the results in each vineyard, there were slight differences in daily temperatures associated with the different vine treatments and, as seen in the case of the vineyard located in Cádiz, differences in the pattern of the maximum temperature of the air over a longer period of time observed in distributions with wider summits (Figure 1(a1)).

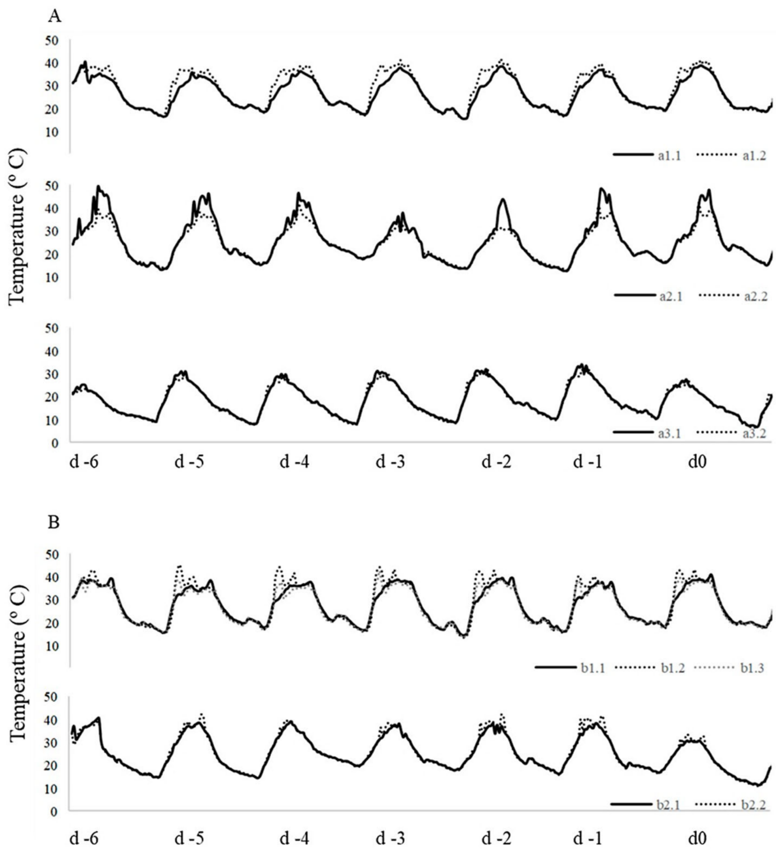

Figure 1. Average daily temperature (°C) over the last six days before harvest for each variety and treatment. Continuous lines correspond to control cultivars while dotted lines represent treatments. The image shows (**A**) the use of elicitors and (**B**) canopy management systems.

The effect of the climate in this region may be tangible regardless of the treatments with higher average maximum temperatures at grape clusters (Figure 1(a1)) and, simultaneously, relatively higher humidity all day long (Figure 2(b1)). Nonetheless, some of these changes seemed to be at odds with other studies [26], where the changes are greater in relative humidity on leaves and clusters than the fluctuations observed in temperature, particularly in the region of La Mancha, in central Spain, with significant differences in daytime temperatures (Figure 1(a2)).

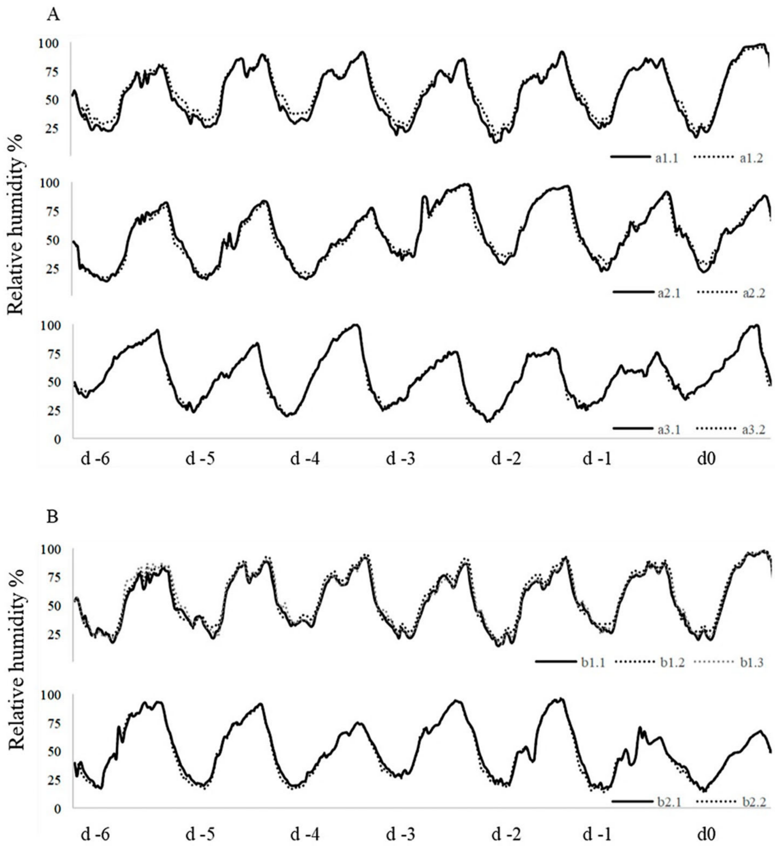

Figure 2. Average daily relative humidity (%) over the last six days before harvest for each variety and treatment. Continuous lines correspond to control cultivars while dotted lines represent treatments. The image shows (**A**) the use of elicitors) and (**B**) canopy management systems.

Another factor that may influence the microclimate of grape clusters is the ability of the vineyard soil to retain and release heat during the day. The temperature of the vineyard soil can reach over 50 °C during daytime (Figure 3), and thus keep irradiating heat during the night.

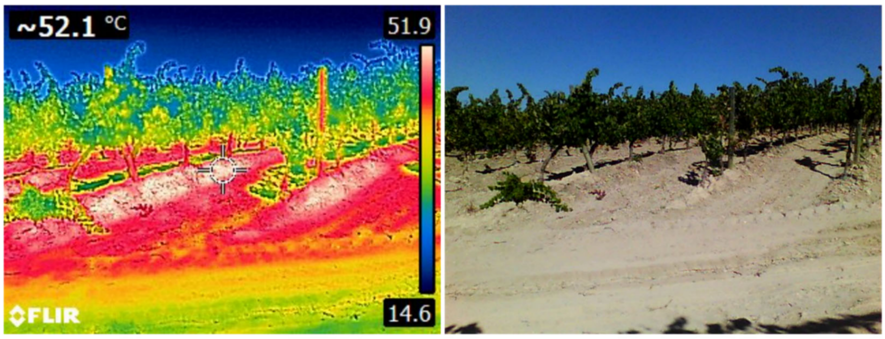

Figure 3. Differences in temperature reached by the soil, the canopy and the shadows cast by the canopy in a vineyard in Cádiz, Southwest Spain, at midday.

3.2. Chemical Composition

In test A, for the case of Tintilla de Rota, one of the least evaluated varieties in Spain, the use of elicitors showed differences between the control and the treated cultivars (Figure 4A). There were two clusters formed in a PCA with 76% of the variability of the test explained, and these clusters were grouped according to the concentration of organic acids and reducing sugars. The concentration of reducing sugars was higher in the control cultivar (1.8%) (Table 2), with statistical differences, as was the case for the concentration of malic acid, tartaric acid and ammonia. On the other hand, parameters such as pH, α-amino, gluconic acid and volatile acidity exhibited no statistical differences between the control and the treated cultivars. However, in this variety, the use of elicitors did not show an improvement in the chemical composition of the grapes.

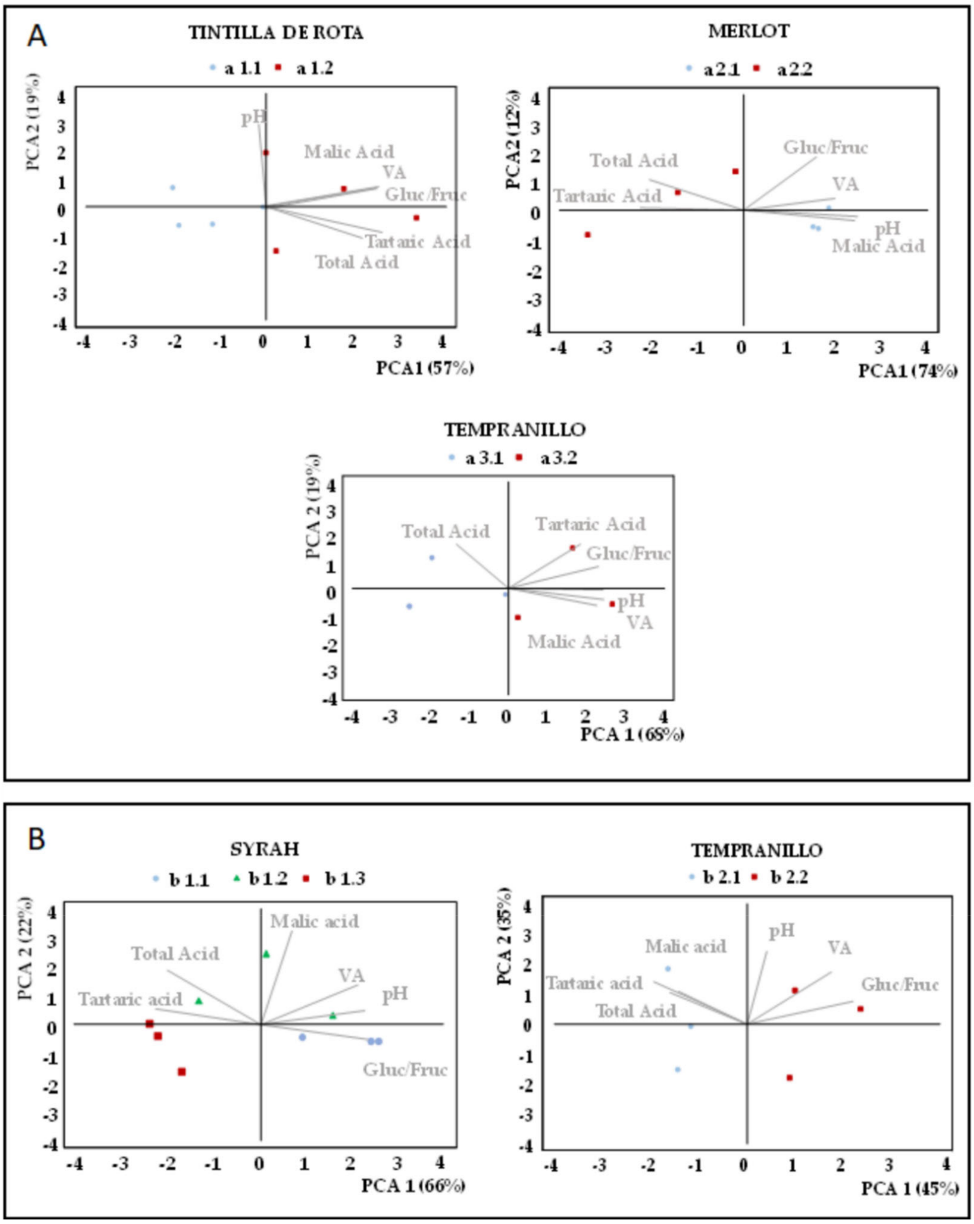

Figure 4. Principal Component Analysis (PCA) of the chemical composition in different grape varieties. (**A**) the use of elicitors and (**B**) canopy management systems.

Table 2. Oenological parameters determined with FTIR. Comparison of blocks with elicitors and canopy management systems against controls. Average and standard deviation. Different letters indicate statistical differences ($p < 0.05$) for each treatment (e.g., between a1.1 and a1.2).

ID	Sugars	pH	Malic Acid	Tartaric Acid	α Amino	Ammonia	Gluconic Acid	Volatile Acidity
	g/L		g/L	g/L	g/L	g/L	g/L	g/L
				Test a—Elicitors				
a 1.1	238.4 ± 1.6 a	3.4 ± 0.0 a	1.3 ± 0.1 a	5.7 ± 0.9 a	123.2 ± 12.6 a	66.1 ± 2.0 a	0.9 ± 0.1 a	0.1 ± 0.0 a
a 1.2	234.1 ± 2.2 b	3.4 ± 0.0 a	1.1 ± 0.1 b	4.2 ± 0.8 b	113.7 ± 5.8 a	39.6 ± 9.4 b	0.7 ± 0.2 a	0.1 ± 0.0 a
a 2.1	268.2 ± 2.4 a	4.2 ± 0.0 a	1.7 ± 0.0 a	4.5 ± 0.1 a	192.3 ± 12.0 a	81.2 ± 13.3 a	1.7 ± 0.3 a	0.2 ± 0.0 a
a 2.2	266.9 ± 4.3 a	4.1 ± 0.0 b	1.5 ± 0.1 b	4.7 ± 0.1 a	179.7 ± 7.8 a	78.2 ± 4.2 a	1.6 ± 0.2 a	0.2 ± 0.0 a
a 3.1	221.3 ± 7.9 a	4.1 ± 0.1 a	2.6 ± 0.2 b	6.0 ± 0.5 a	232.2 ± 57.1 a	114.9 ± 11.7 a	0.2 ± 0.4 a	0.1 ± 0.0 b
a 3.2	232.5 ± 3.6 a	4.2 ± 0.1 a	3.4 ± 0.2 a	6.4 ± 0.5 a	303.6 ± 91.0 a	161.5 ± 44.0 a	0.5 ± 0.2 a	0.1 ± 0.0 a
				Test b—Canopy management				
b 1.1	262.0 ± 6.2 a	3.7 ± 0.1 a	1.9 ± 0.1 a b	2.7 ± 0.2 c	142.6 ± 11.5 a	69.6 ± 3.1 a	1.0 ± 0.4 a	0.1 ± 0.0 a
b 1.2	236.9 ± 10.4 b	3.7 ± 0.1 a	2.3 ± 0.4 a	5.6 ± 0.5 b	149.5 ± 5.1 a	70.6 ± 2.6 a	0.6 ± 0.1 a	0.1 ± 0.0 a
b 1.3	228.3 ± 3.2 b	3.6 ± 0.4 b	1.7 ± 0.1 b	7.2 ± 1.1 a	141.5 ± 27.2 a	76.8 ± 35.5 a	1.0 ± 0.1 a	0.1 ± 0.0 a
b 2.1	237.4 ± 5.1 b	4.2 ± 0.1 a	2.8 ± 0.2 a	4.7 ± 0.6 a	201.6 ± 18.8 a	69.73 ± 7.5 a	1.3 ± 0.3 a	0.2 ± 0.0 a
b 2.2	256.4 ± 9.3 a	4.2 ± 0.1 a	2.6 ± 0.2 a	3.7 ± 0.3 a	215.1 ± 24.5 a	77.3 ± 30.9 a	1.7 ± 0.3 a	0.2 ± 0.0 a

The Merlot variety showed few statistical variations between samples despite the cluster-formation observed in the PCA (Figure 4A). The two components explained the 86% variability of the test for this cultivar. Regarding chemical composition, the only parameters with statistical differences ($p < 0.05$) between control and treated cultivars were the malic acid concentration and the pH values (Table 2). Both parameters had slightly higher values for the control cultivar. The remaining oenological parameters exhibited no statistical differences between cultivars. The minimal differences observed in the Merlot cultivars, made the assessment of the elicitors in the improvement of the chemical composition of grapes of this variety difficult.

Finally, the use of elicitors in *cv*. Tempranillo revealed lower malic acid concentration with statistical differences in the control cultivar (Table 2). Here, the cultivar treated with elicitors had a higher concentration of malic acid despite the results observed in other studies. In this study, lower malic acid concentrations were observed in berries treated with elicitors compared with the control samples [16], which could be expected for grapes with a more advanced ripening stage. All the other parameters, tartaric acid, α-amino, ammonia, and gluconic acid, can be considered statistically similar in both cultivars. The concentration of sugars increased by 11.2% in the treated cultivar. The PCA, with 87% of the variability explained, grouped the treated and untreated cultivars into two separate clusters, even though the differences due to the use of elicitors were minimal.

In the case of the canopy management evaluation, test B, it can be seen that for the Syrah variety, the more intense the shoot trimming treatment was, the lower the concentration of reducing sugars produced (Table 2). Statistically, there were no differences between soft and heavy shoot trimming concerning the concentration of reducing sugars in this experiment. These results are the opposite of other studies, where more reducing sugar with heavy canopy management (12.97%) was obtained. Despite this, differences between the control and canopy management cultivars can be observed [27]. Other statistical differences between trimming trials can be seen in the concentrations of malic acid and tartaric acid. The former was more concentrated in grapes of cultivars with soft trimming, while the latter was more accumulated in grapes of cultivars with intense trimming. This effect could be associated with more ripped grapes as malic acid decreases towards the end of the maturation process and the proportion of tartaric acid increases. The PCA done for this cultivar showed two well-differentiated clusters for the control and the intense shoot trimming treatment. The soft shoot trimming cultivar exhibited a transitional behavior, with values falling between the other two (Figure 4B).

Finally, the Tempranillo cultivar showed statistical differences between the control and the cultivar with sprawl regarding the concentration of reducing sugars (Table 2). The sprawling system accumulated a larger concentration of reducing sugars (8%). In other studies, contrary to what has been observed, the minimal modification of the canopy has led to a slowdown in the accumulation of sugars as a consequence of the photosynthetic response of the vine [27]. In this case, the microclimate, besides the CO_2 exchange and the berries' exposure, may have had an impact on the chemical composition of the berries. The variability observed in the accumulation of sugar and other metabolites may be related to a high plasticity in the accumulation of these metabolites when evaluating single berries from different vines [28]. In addition, in contrast to what was observed in the shoot trimming test, the use of sprawl conduction did not modify the acidic profile of the berries. Accordingly, no statistical differences were observed in the remaining oenological parameters measured in the berries.

3.3. Pigments and Phenolic Compounds Accumulation

Starting with *cv.* Tintilla de Rota, test A, the use of elicitors did not increase the pigments and the phenolic compounds in these cultivars (Table 3). The concentration of total anthocyanins, including the acylated fraction, was statistically similar in the control and the treated cultivars. The blocks from the control and the treated cultivars were distributed in the PCA without being clustered by any component (Figure 5A), as previously occurred with the chemical composition. There were also no statistical differences between samples in the phenolic composition (TPI) (Table 3).

Table 3. Total phenolic compounds and colour parameters (UV-Vis) and main anthocyanins (HPLC-DAD). Comparison of blocks with elicitors and canopy management systems against controls. Average and standard deviation. Different letters indicate statistical differences ($p < 0.05$) for each treatment (e.g., between a1.1 and a1.2).

ID	Total Pigments	Acylated Monomeric Pigments	IPT	pH
		Test a—Elicitors		
a 1.1	198.7 ± 10.2 a	25.6 ± 1.8 a	12.8 ± 0.8 a	3.4 ± 0.0 a
a 1.2	191.8 ± 21.0 a	25.1 ± 2.0 a	13.1 ± 0.6 a	3.4 ± 0.0 a
a 2.1	233.4 ± 28.8 a	111.4 ± 17.3 a	8.9 ± 1.5 a	4.1 ± 0.0 a
a 2.2	201.5 ± 27.3 a	82.5 ± 21.8 a	7.0 ± 1.9 a	4.2 ± 0.1 a
a 3.1	491.2 ± 14.1 a	91.8 ± 4.6 a	25.4 ± 0.8 a	4.1 ± 0.1 a
a 3.2	582.4 ± 86.4 a	108.6 ± 20.8 a	28.4 ± 2.4 a	4.2 ± 0.1 a
		Test b—Canopy management		
b 1.1	179.4 ± 13.0 ab	87.1 ± 1.6 a	12.2 ± 0.9 b	3.7 ± 0.1 a
b 1.2	160.6 ± 6.7 b	84.3 ± 9.9 a	11.0 ± 1.6 b	3.7 ± 0.0 a
b 1.3	205.8 ± 22.1 a	95.8 ± 13.2 a	16.8 ± 1.3 a	3.6 ± 0.1 a
b 2.1	248.7 ± 15.5 a	69.8 ± 4.7 a	8.0 ± 0.7 b	4.2 ± 0.1 a
b 2.2	245.3 ± 36.2 a	74.1 ± 9.8 a	13.5 ± 1.1 a	4,.2 ± 0.1 a

Similar to the *cv.* Tintilla de Rota results, the *cv.* Merlot grapes exhibited no statistical differences in the concentration of pigments, including the acylated fraction, and the values of TPI obtained from the phenolic fraction. Nonetheless, there was a perceptibly higher concentration of acylated pigments in the control cultivar (Table 3), however, due to their dispersion, they were not significant. This difference was also reflected in the slightly higher concentration of total pigments observed in the control cultivar (2.3%). It is also noticeable that the level of pigments extracted from the skin of the Merlot cultivar is similar to the levels obtained from the Tintilla de Rota cultivar. The PCA has clustered the cultivars along the PC1 in the horizontal axis (Figure 5A). The differences observed were not large enough to be attributed to the use of elicitors.

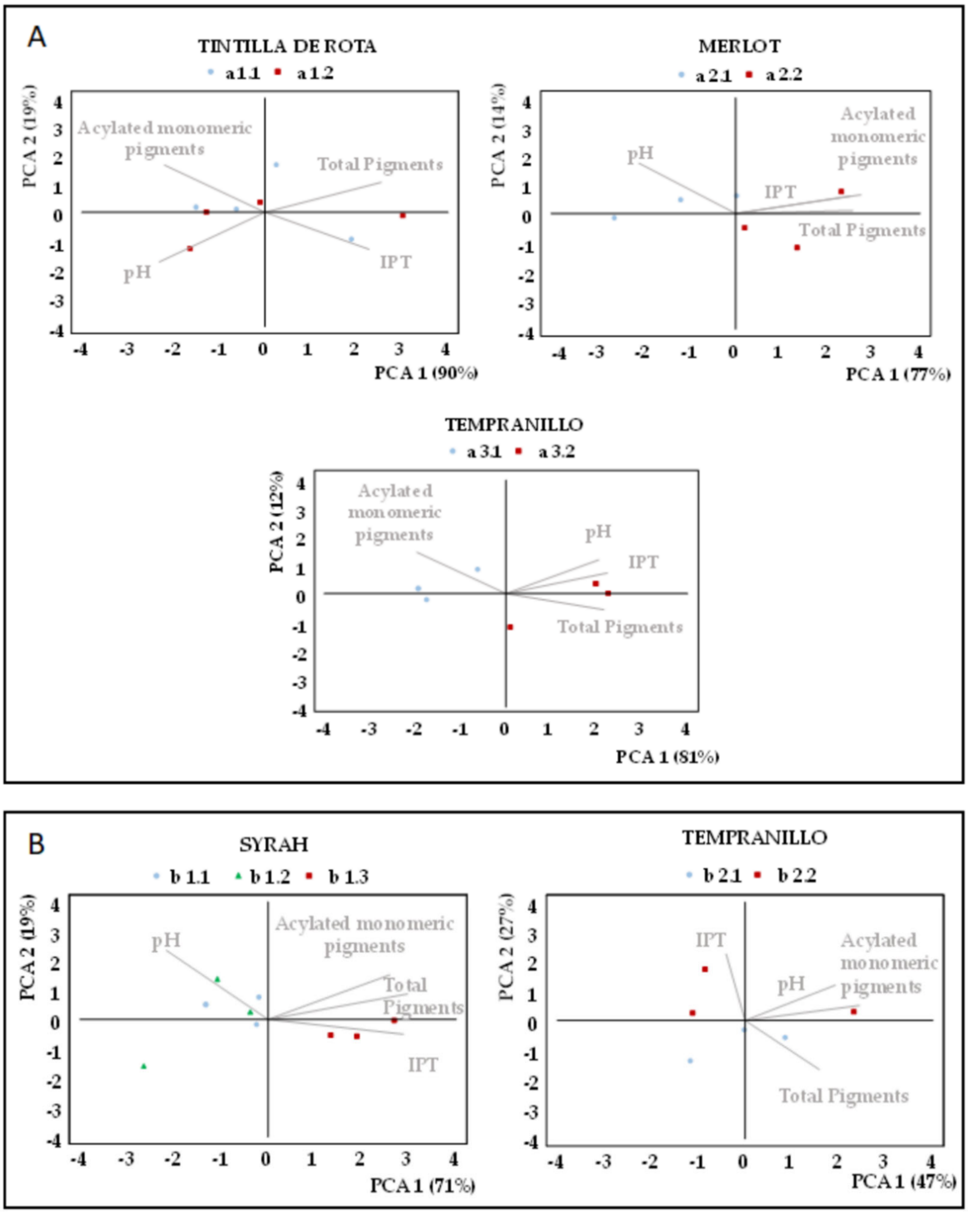

Figure 5. Principal Component Analysis for total and acylated monomeric pigments, total polyphenols index and pH in different varieties of grapes. The image shows (**A**) the use of elicitors and (**B**) canopy management systems.

In the case of *cv*. Tempranillo, the use of elicitors stimulates the pigment accumulation. The difference between the control and treated cultivars was not statistically significant mainly due to the high variability observed in the treated cultivar (Table 3). Portu et al. [16] did not find significant differences in pigment concentration with respect to control samples either, nevertheless, the increased concentration of acylated monomeric pigments and, thus, the total concentration of pigments, grazed significance ($p < 0.05$). The same results were obtained for the TPI, where the values obtained were not significant, although the difference was 11.8%.

Regarding the canopy management systems, there were significant differences ($p < 0.05$) between the soft and the intense shoot trimming in the *cv*. Syrah cultivar (Table 3). The control cultivar had intermediate values, thus, it was not statistically different from either trimming test. Despite the statistical observations, the intense trimming stands out among the tests because of the larger amount of total pigments and the TPI values reached (37.7% higher). In line with these observations, Brillante et al. [29] also observed higher pigment concentration with heavier canopy treatments. Comparing those results with the pH values measured in these cultivars, the slightly more acidic berries were produced with the more intense trimming treatment as well. These results could be due to the variation in the accumulation of metabolites in single berries already discussed [28], as a lower concentration of organic acids is expected in the berries at harvest, especially from berries more exposed to solar irradiance and temperature. The accumulation of malate and other metabolites such as aspartate and maleate, tends to be reduced in berries more exposed to sunlight [30].

For *cv*. Tempranillo, there were no significant differences ($p < 0.05$) regarding the concentration of pigments including the acylated fraction. Nonetheless, the TPI was significantly higher for the cultivar with the sprawling system (Table 3). For some reason, the accumulation of phenolic compounds, other than anthocyanins, was larger in the cultivar with the canopy management system (68.7%). Another perceptible, yet not significant, difference was the larger amount of acylated monoglucoside anthocyanins. This difference did not modify the total anthocyanins content, but it reflects the difference in the nature of the pigments when having an impact on the colour of the wine, and the wine's stability during winemaking and throughout the ageing process [31,32]. The PCA has clustered both cultivars with respect to the vertical component, the use of the sprawling system being better explained by the accumulation of acylated monoglucosides (Figure 5B).

4. Discussion

One of the main purposes of the use of elicitors is to reduce, or avoid, the use of pesticides to control grapevine diseases [33]. The protection of grapevines with sustainable approaches involves promoting the accumulation of phenolic compounds to boost the grapevine's natural self-protection [7]. Such is the case in other studies with BTH (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester), a salicylic acid analog that triggers the response for plants resistance against pathogens [34], and the case of jasmonic acid in the production of phenolic compounds and other metabolites [4,8,35]. The use of inactivated yeasts as elicitors on the vine foliar canopy in this experiment is expected to have an impact on the accumulation of primary and secondary metabolites. The changes are observed as differences in titratable acidity, in the concentration of phenolic compounds, including anthocyanins, or the extractability of the pigments. In this experiment, it has been observed that after the treatment with inactivated yeast metabolites, two out of three varieties reported an increase in the concentration of total phenolic compounds of between 2.3% and 11.8%. The use of canopy management systems has also induced an increment in the accumulation of phenolic compounds of 37.7% in the case of intensive shoot trimming, and 68.7% in the case of the sprawling system. This suggests an alteration of the biosynthesis pathways of shikimic and chorismic acids [36]. These two acids are precursors in the synthesis of phenolic compounds. Nonetheless, the difference observed with respect to the control cultivars is not significant in some cases due to the plasticity observed in the results. Other studies have noted the stimulation of the phenylpropanoid pathway genes, and the accumulation of phenolic compounds, such as the rosmarinic acid, in cell cultures [37]. The phenylpropanoid pathway uses the amino acid L-phenylalanine through the deamination of the L-phenylalanine by the enzyme L-phenylalanine ammonia lyase [38]. The phenylpropanoid pathway is responsible for the accumulation of hydroxycinnamic acids, as well as pigments and condensed tannins.

The variations observed in the concentrations of pigments and phenolic compounds may also be due to a modification in the extractability of the tannins [39] and pigments from the grape berries. The interaction that tannins and anthocyanins have with cellular structures would change the rates at which these molecules are extracted into wines and, in this case, during the extraction process using methanol and water. In this regard, the variations observed in this experiment can also be explained by this phenomenon.

In the case of reducing sugars, the accumulation is mediated via transporters that locate the sugars, mainly sucrose and other hexoses (fructose and glucose) in the various tissues of the grapevine including the berries [40]. This uptake of sugars in the vacuoles is increased on the onset of ripening and continues until harvest. According to results obtained in the different cultivars, the accumulation of sugars seemed higher in the untreated vineyards and the cultivars subjected to the sprawling system. In this case, the use of elicitors seemed to slow down the sugar accumulation at the same time that the phenolic compounds increased. These grapes are expected to have more balanced berries approaching technological maturation. An exception was observed in the vineyards with shoot trimming treatments with lower sugar accumulation, despite having more exposure to sunlight.

The use of elicitors and canopy management systems has been demonstrated to have an impact on the accumulation of phenolic compounds and sugars following the onset of the maturation of the berries. The effect is statistically not significant in some cases, and this may be because there are other factors involved that have not yet been considered.

5. Conclusions

This study presented the evaluation of techniques used to optimize the harvest conditions of *Vitis vinifera* grape varieties. The use of elicitors from inactivated wine yeasts, on the one hand, presents minimal differences between grape varieties in the plant response in terms of chemical composition. High variability in the values was observed for single berries, which could be considered to be high plasticity in the accumulation of metabolites. This was observed in the stimulation of sugar concentration in some cultivars, while in others the effect was observed in the accumulation of pigments and phenolic compounds. On the other hand, canopy management treatments, such as shoot trimming and the sprawling system, represent an alternative to use in warm areas for achieving an increment of pigments, as well as for a better control of the accumulation of reducing sugars. The increment in phenolic compounds due to the use of elicitors, or the management of the canopy, is expected to produce more balanced wines in hot winemaking areas. Any synergy between elicitors and canopy management systems towards the accumulation of phenolic compounds and their effects on winemaking is to be assessed in future studies.

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