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Influence of the Microclimate Defined by the Training System on the Vineyard Behaviour and the Oenological Quality of Merlot Grapes

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Abstract

Temperature and plant water status are considered determinant for physiological processes and grape composition, and plant architecture contributes in a significant way in their regulation. The aim of this study was to evaluate the effects of micro-thermal conditions and water supply on the evolution and composition of berries on Merlot cultivar, influenced by plant architecture. The experiment was carried out in a commercial vineyard, in 2002 and 2003 with cv Merlot. The following parameters were measured in plants trellised to Vertical Shoot Positioning (VSP) and lyre: characterization of plant architecture (Point Quadrat), thermal conditions (canopy and air temperatures), water supply (pre-dawn leaf water potential), berry weight, and berry evolution and composition (sugar content, total acidity and pH and anthocyanins). Samples were taken weekly from veraison to harvest with two replicates. Quantity and evolution of berry compounds depended on plant architecture, and this affected the microclimate and the water status of plants. Lyre showed higher yields and higher sugars and anthocyanins values. Acidity was higher in VSP. The maximal temperature of the canopy was higher than the environmental maximal temperature, as well as the thermal amplitude. VSP registered thermal peaks and a broader thermal amplitude, whereas lyre showed more hours within the thermal ranges favorable to photosynthesis and anthocyanins synthesis. The greater leaf surface of the lyre caused water deficit situations.

1. Introduction

The micro-meteorological variables and the vine water status affect the evolution and composition of berries. Plant architecture contributes in a significant way to the enological potential of wine grapes. It is also one of the main factors that determine the plant's microclimate: spatial distribution of leaves, leaf area and bunch exposure. Temperature has the most noticeable effect on the development and metabolism of plant and berry, to an extent that both processes are sensitive to its magnitude, its diurnal, nocturnal and daily oscillation, and to its distribution along the day [1-7].

The temperature within the canopy compared to the ambient could increase from +6°C

to +10°C, and it fluctuates along the day, from values between 18.4°C to 32.3°C. Also, the range of favorable temperatures for the different chemical and physiological processes [1, 3, 8-10]. Glen *et al.* [11] demonstrated vines under deficient irrigation increase leaf and canopy temperature and reduce the capacity for transpirational cooling.

The effect of temperature on sugar content, on acidity degradation and on the synthesis-degradation of anthocyanins in the berry is explained by its influence on the photosynthesis; mainly on the respiration of malic acid and on the expression of genes associated to the accumulation of anthocyanins [3, 10]. Berries grown in the shadow during the initial stage of development, have lower weight and less sugar accumulation, as well as higher malic acid and anthocyanins content than those exposed to light [1, 3, 12, 13]. Generally, the optimal temperature for all these processes is of 25-30°C for diurnal temperatures and 16-25°C night/day temperatures [7,14]. At 37°C sugar accumulation is inhibited and, from 35 upwards the anthocyanin synthesis and values were lower than control samples subjected to 25°C. The acidity reduction was higher at 30°C than at 20°C [7, 15, 16].

Guidoni *et al.* [17] demonstrated that anthocyanins accumulate in two phases: the first one with fast growth and correlated with accumulation of sugars, and then in a second in which these two compounds are independent from each other. The first phase is associated with favorable conditions for photosynthesis and the second one with climatic conditions influencing anthocyanins balance.

The vine water status is another factor influencing composition of grapes and plant response. It is related to the leaf area of the system and to the atmospheric supply and demand [4, 18-20]. A gradual and moderate water deficit, imposed before veraison, boosts the buildup of sugar and anthocyanins, diminishes acidity, regulates vegetative and fruit growth and canopy microclimate. [6, 21-23]

During the first stage or growth of the berry, three weeks after the start of veraison, there are changes in accumulation and transportation of sucrose and water, an increase of sugar and anthocyanin concentration, and a reduction of the acidity through the respiration of malic acid. During the second stage of growth, there is a deceleration in the kinetic of ripening compared to the three previous weeks. In the last growth stage, water deficit or thermal stress, cause a reduction of berry size. Under this situation, and due to water losses in the fruit, the concentration of sugars and anthocyanins increases [3, 6, 15, 24, 25].

Tandonnet *et al.* [2], comparing lyre trellis and Vertical shoot positioning (VSP), showed that this last trellis system results in longer shoots but smaller leaf areas and the spatial distribution of leaf shows more layers of leaf and higher canopy density. The effect of shading causes an unfavorable microclimate for grape ripening and composition, generating an unbalanced and incomplete berry ripening, due to insufficient carbohydrates and higher contents of malic acid in berries [1, 5].

The aim of this study was to evaluate the effects of

micro-thermal conditions and water supply on the evolution and the composition of the berries on the Merlot variety by the influence of two plant architectures (lyre and VSP).

2. Materials and Methods

2.1. Experimental Design and Plant Material

The experiment was established in a seven year-old commercial vineyard (*Vitis vinifera* L. cv. Merlot), located in the south of Uruguay (34°35'12.43" S; 56°15'2.26" W). The vines were grafted on SO4 rootstock and planted at 2.5 m between rows and 1.25 m between plants, in the case of VSP trellis system and at 3.0 m between rows and 1.0 m between plants, in the case of lyre system. The surface area allocated to each vine of each treatment was reasonably similar (i.e. 3.13 m² for VSP and 3.0 m² for lyre). The row orientation is N-S. The trellising systems consisted of a bi-lateral permanent spur cordon at 0.70 m above the ground with four pairs of surmounting catch wires for a canopy wall extending about 1.5–1.6 m above the cordon, and the total height was 2.20-2.30 m. In both training systems, an average of 18 buds per vine was retained and systems shoots were upwards positioned and summer trimming was carried out. Standard commercial management practices for the region were applied to the field trial.

The climate of the region is classified, according to the Multicriteria Climatic Classification System [22, 26] as Temperate, Temperate nights, Moderately dry, with sea influence. The maximum and minimum average temperatures in summer are 28.9°C and 16.4°C, respectively.

The soil of the experimental site (Argiudol, USDA) is characterized by the typical profile: A (0-15 cm), Bt (15- 85 cm) and C (> 85cm) with the presence of fluctuating water table, 33-40 cm in budbreak and within the reach of the roots at floraison. The average textural composition of the upper horizon is: 19.7% sand, 44.7% silt and 39.7% clay. The readily available water (RAW) was 141.6 mm. The trials took place during two years (2002 and 2003) in two different sites in adjacent vineyards. Thirty vines were selected from the three rows per each trellising systems, considering each individual vine as unit, so there were ten repetitions per treatment.

2.2. Climate and Microclimate in the Canopy

General climatic data of the years 2002 and 2003 were collected from the weather station "INIA Las Brujas" that is built and operates under the technical norms of the WMO (34°40'S y 56°20'W; 32 m above sea level). It is located at a distance of about 5 km from the test plots. Maximum, minimum and mean temperatures (°C) as well as, average thermal amplitude (°C) were calculated.

Microclimatic data at canopy level for the same years were registered using termo-par data loggers +/- 0.1°C (SolTec, Argentina) located near the clusters. Temperature was recorded at 15-minute intervals, from flowering until harvest, using four sensors for each system. Based on the obtained data,

the following parameters were estimated on a daily basis for each system over the ripening period: maximum and minimum temperatures (absolute and average), average thermal amplitude (temperature range), percentage of hours in four temperature ranges ($\leq 25^{\circ}\text{C}$, $25\text{-}30^{\circ}\text{C}$, $30\text{-}35^{\circ}\text{C}$ y $\geq 35^{\circ}\text{C}$). Temperature evolution and hourly increases in canopy, in relation to air temperature, were calculated for the hottest day of the period.

2.3. Canopy Structure

Estimation of spatial distribution of leaf and cluster position was determined using the Point Quadrat method (PQ), proposed by Smart and Robinson [1]. Twenty random punctures were made in the three rows for each training system (ten on the Eastern side, ten on the Western side). PQ was positioned perpendicularly to the canopy surface at the height of the fruit zone and measured at the veraison (35 state of Eichhorn and Lorenz [27]), when leaf area was at its maximum.

Shoot length was measured weekly on 20 shoots starting at flowering, for each architecture. In order to reduce heterogeneity, shoots were picked from spurs located in the central part of the cordon and with bunches.

The potential exposure leaf area (ELAp) was estimated following the method proposed by Carbonneau [28], which considers ten points for each architecture (five on the east side, five on the west side). Pruning weight was assessed by single measures in 30 plants for each architecture, using a Moretti RS – 232 - C scale (10 kg +/- 0.1 Moretti, Argentina).

The total annual dry matter for each plant architecture was estimated based on 20 representative vine shoots with bunches. The shoots were heated in a stove at 60°C until a constant weight was achieved. Weight was taken for different plant organs (leaves, bunches, shoots), using an Ohaus Scouts +/- 0.1 g. scale (Ohaus Corporation, EE.UU).

The technique of the pressure chamber [29] was used (Soil moisture equipment mod. 3005-1412) for the determination of pre-dawn leaf water potential (Ψ_{PD}). These measurements were made before dawn at veraison (E-L 35) and at harvest, in 20 adult, healthy and fresh leaves per trellis system (10 on the east side of the canopy and 10 on the west side). Threshold values for Ψ_{PD} have been proposed by Carbonneau [30], which makes it possible to evaluate the level of water deficit (WD) experienced by the plant. (Values: $\Psi_{\text{PD}} \geq -2$ bar no WD, $-2 \text{ b} > \Psi_{\text{PD}} \geq -4$ b mild to moderate WD, $-4 \text{ b} > \Psi_{\text{PD}} \geq -6$ b moderate to severe WD, < -6 b severe to high WD).

2.4. Grape Samples

Samples of 250 berry were taken weekly from veraison (E-L 35) to harvest, with two replicates, taken from 30 plants of the three rows of vineyard for each system and using the method proposed by Carbonneau [31], consisting of collecting three to five berries from clusters located on shoots in the middle of vine. Berries were randomly selected from the upper and lower parts of the clusters.

At harvest, individual yield of 30 plants was weighted using

a Moretti RS – 232 –C scale (10 kg +/- 0.1). The decision to harvest was taken according to the trellis systems and the characteristics of the year. The criteria considered to harvest were the maximal sugar contents (g/L), total acidity values (g/L H_2SO_4) in the range: 3.4 – 4.5, and pH values in ranges between 3.40 – 3.60. Moreover, the evolution of the weight of the berries (g) was taken into account, in order to avoid dehydration. The harvesting date for 2002, was February 22nd for lyre and February 27th for VSP. In 2003 the harvesting date was February 14th for lyre and February 20th for VSP

2.5. Basic Analysis of Berry

Half of the berries from each sample were used to determine basic parameters and berry weight. The weight of the berry was determined with an Ohaus Scout scale (Ohaus Corp., USA). Basic composition was determined by using classical analyses (sugar content, total acidity and pH), performed on the juice obtained from the berries manually destemmed and the crushing of the pulp with a juice extractor Phillips HR2290 (Phillips, Netherlands). Analyses were carried out according to O.I.V. [32], using an Atago N1 refractometer (Atago, Japan) and a pH meter Hanna HI8521 (Hanna instruments, Italy).

2.6. Phenolic Potential of the Grapes

Half of the berries from each sample were analyzed according to Glories and Augustin [33], in order to determine the total potential in anthocyanins (ApH1). After grinding the grapes with a blender, model solutions at pH 1.0 were added, homogenized and macerated four hours. The extracts were filtered and centrifuged for 3 min at 3500 rpm before analysis, using a MSE Mistral 2000 centrifuge (Sanyo-Gallenkamp, UK). Anthocyanins contents at pH 1.0 were measured according to Ribéreau-Gayon and Stonestreet [34]. The measurements were carried out in duplicate with a Shimadzu UV-1240 Mini (Shimadzu, Japan) spectrophotometer, using glass (for the anthocyanins analyses) and quartz (for the absorbance at 280 nm analyses) cells with 1 cm path length. The indexes were calculated considering the respective dilution of the grape extracts, according to González-Neves [35].

2.7. Statistical Analysis

An analysis of variance (ANOVA) was used for assessing differences between trellis systems and year on the variables considered in this study. Mean separation was carried out using the test of t Student ($\alpha=0.05$) or Duncan test ($\alpha=0.05$, $\alpha=0.10$). Moreover, a correlation analysis was performed using Pearson's correlation coefficient. All the statistical analyses were carried out using the Info Stat (1998) software.

3. Results

3.1. Thermal and Hydric Characterization of Canopies – Relationship with the Environment During Grape Ripening

Maximum daily air temperature inside the canopy was

significantly higher than air temperature for both architectures and years. Average temperature related to the environment was higher in the VSP trellis for both years (+ 5.5°C and + 5.2°C, in 2002 and 2003 respectively). Canopy temperatures stayed above the environmental air temperature 72.5 % of the days in 2002 and 59 % in 2003. The largest temperature gap between the plant architectures and the environment was 8.9 ° C and 4.6 ° C considering lyre, 10.8 ° C, and 5.9 ° C considering VSP in 2002 and 2003, respectively. Some days, maximum temperatures inside the canopy were lower than the maximum environmental temperatures for both architectures (2002 and 2003 were - 3.8°C and -7.3°C in lyre and -3.9°C and -7.0°C in VSP). The temperature differences found between both architectures are also related to the

phenological stage during which the maximum temperatures were registered. The lyre showed the highest temperatures at the beginning of the ripening period (peaks between 40.5°C and 37.3°C at around 16:00), while in VSP those peaks were found at the end of the cycle (peak values between 41.9°C and 39.7°C; at around 14:00). In 2002, the maximum temperature of VSP in several dates was significantly higher than lyre. In general, air temperatures within canopy, equal the minimum temperatures for both architectures. Average temperature amplitude values in canopy, compared to air temperature values, were significantly higher. Differences between trellis systems depend mainly on the variation of maximum temperatures (Figs 1 and 2, Table 1).

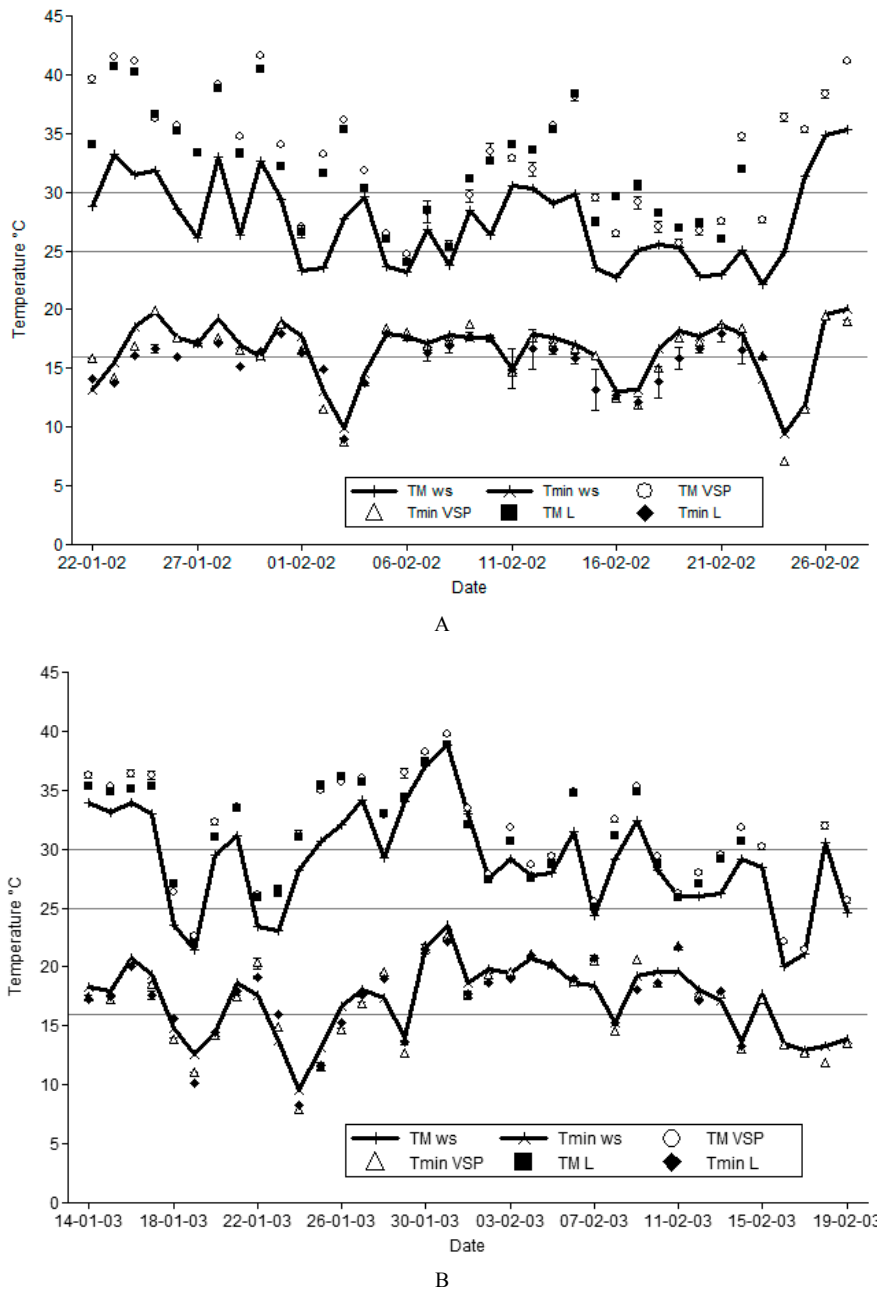


Fig. 1. Evolution of maximum and minimum temperature inside the canopy (lyre (L), VSP), and in the weather station (WS). Standard errors are indicated. A year 2002, B year 2003.

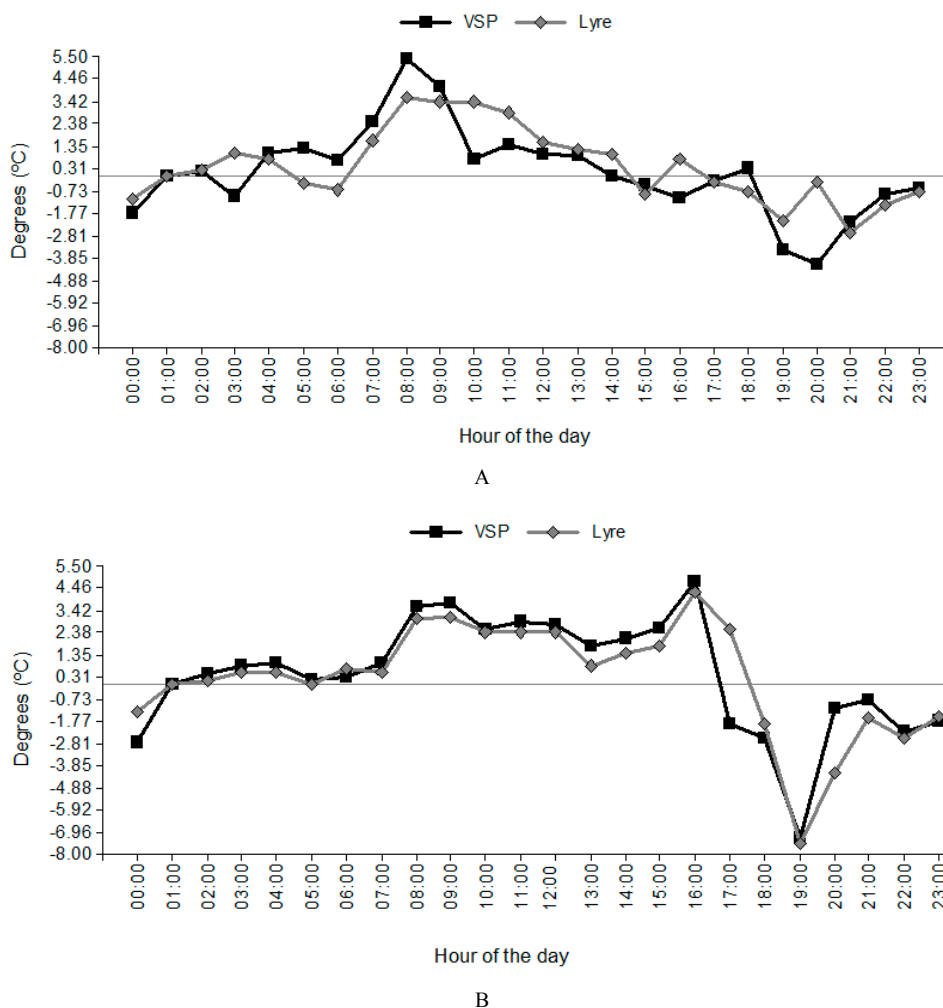


Fig. 2. Dynamics and evolution of air temperature inside of canopy daily temperature in relation to environmental temperature (0) of the warmest day of ripening (the same days in both architectures: 30/01/2002 and 31/01/2003). A year 2002, B year 2003.

Table 1. Thermal characterization of the canopy (n°=4) and the environment during the ripening period* for each trellis system.

	2002			2003		
	Weather station	Lyre	VSP	Weather station	Lyre	VSP
Temperature maximum average (°C)	28.6 b	31.8 a	33.12 a	27.6 b	31.6 a	32.8a
Temperature minimum average (°C)	16.3 a	16.2 a	16.1 a	16.8 a	16.4 a	16.5 a
Temperature maximum(°C)	31.1	40.5 (±0.1)	41.9 (±0.3)	30.7	37.3 (±0.4)	39.7 (±0.1)
Thermal amplitude average (°C)	12.5 b	15.6 a	17.0 a	10.8 b	15.2 a	16.3 a

*2002 = Lyre January 22nd at February 22nd, VSP January 22nd at February 27th, 2003 = Lyre January 14th at February 14th, VSP January 14th at February 20th Duncan 10%

The daily thermal dynamic was different according to plant architecture and year in relation to environmental temperature. The hourly temperature increase in the hottest day during ripening started at 07:00 and went on until 16:00, when it reached its maximum value in both architectures. In 2003, the temperature increased until 14:00, when a thermal maximum was registered. From 10:00 onwards, the VSP presented higher values than lyre. In VSP, temperatures ranged between 20°C and 30°C between were registered from 08:00 to 11:00 and after 19:00. In lyre, that temperature range was registered

between 08:00 and 13:00 and then after 17:00 (Fig. 2).

The percentage of hours with temperatures under 25°C was bigger for VSP (60.5 % and 65.6 % in the years 2002 and 2003) than for lyre (53.4 % and 56.4 %, 2002 and 2003). Temperatures >35°C were reached 4.2 % - 3.5 % of the time in lyre and 16.5 % - 14.9 % in VSP, in 2002 and 2003 respectively. In lyre, the temperature range between 25°C and 30°C – the second in importance considering percentage of hours – was 22.8 % and 26.4 % ; in VSP it was 12.6 % and 10.1 % , in 2002 and 2003, respectively (Fig. 3).

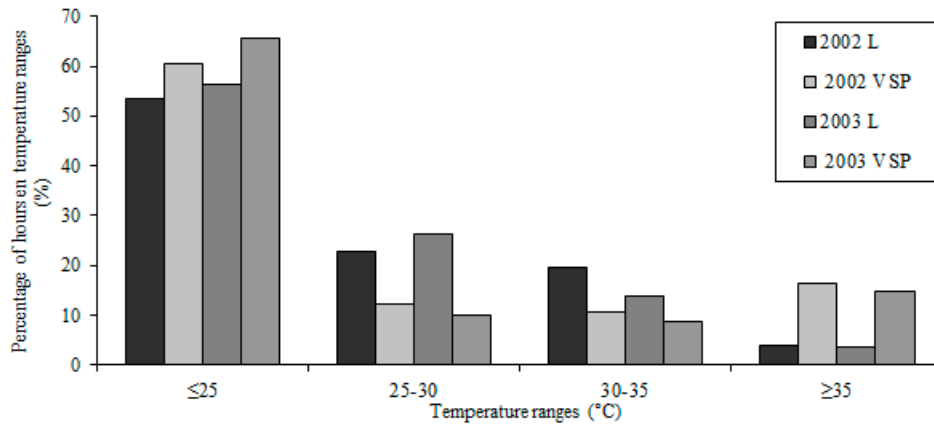


Fig. 3. Percentage of hours in temperature ranges according year and trellis system registered during the ripening period: 2002 = Lyre (L) January 22nd at February 22nd, VSP January 22nd at February 27th, 2003 = Lyre January 14th at February 14th, VSP January 14th at February 20th.

The year 2002 was warmer and during ripening, water availability kept the plants with values, according to the Ψ_{PD} range, of mild to moderate (VSP) or of moderate to severe water stress (lyre). In 2003, a fresher year with larger water availability and values for plants of Ψ_{PD} range were mild to moderate stress (lyre) or well-watered (VSP). The conditions of the year were reflected in the water status of both architectures; the pre-dawn leaf water potential (Ψ_{PD}) values were, in both years, significantly more positives in VSP than in lyre (Tables 1 and 2)

3.2. Characterization of Plant Architecture

The plant architectures showed differences in the spatial

distribution of leaves and bunches, in the vegetative expression and grape production (Table 2). The leaf area measured in lyre was significantly larger than in VSP; 101.5% larger in 2002 and 70.6% in 2003. The PQ showed, that area the area in lyre had, in average, 78.5% of external leaves, while in VSP that value was 20.0%. The VSP showed a larger leaf superposition due to the larger number of leaf layers (7.1 as average), while in lyre that average was 3.6 layers. The average percentages of exposed bunches were 48.8% in lyre and 24.4% in VSP. The vegetative expression: pruning weight, length and number of growth days for shoots and lateral shoots were significantly larger in VSP for the two years tested.

Table 2. Canopy structural indices, vegetative growth, yield components and water status grapevine to the trellis system.

	2002		2003	
	Lyre	VSP	Lyre	VSP
ELAp (m ² /he) †	8280 aA	4110 bB	8100aA	4747bB
External leaves (%)	80.2aA	31.3bC	77.0aA	49.1bB
Leaf layers	3.50bB	7.30aA	3.80bB	6.90aA
External clusters (%)	52.12aA	16.73bC	45.51aA	32.10bB
Pruning weight /vine(kg)	0.78bB	1.07aA	0.60bB	0.87aB
Shoots length (m)	67.62aC	95.32bB	105.21bB	159.90aA
Days shoots growth	51.38bB	70.91aA	59.94bB	78.47aA
Lateral shoots length (m)	13.81bD	36.13aC	64.58bB	102.59aA
Yield/vine (kg)	8.48aA	3.78bC	5.95aB	3.94bC
Clusters/vine	35.81aA	25.73bB	28.75aB	18.51bC
Berry wt (g)	1.81aB	1.50bC	1.98aA	1.77bB
$\Psi_{PDV} \ddagger$ (bars)	-5.78aA	-3.17bB	-2.72aC	-0.72bD
$\Psi_{PDH} \S$ (bars)	-1.92aA	-1.41bB	-1.43aB	-0.91bC

Potential exposed leaf area, † pre-dawn leaf water potential at veraison (n=20), ‡ pre-dawn leaf water potential at harvest (n=20) Means followed by the same letter are not different by t Students test (p=0.05) lower-case letters between architectures, capital letters between years

Yield, number of bunches, and weight of berries were significantly larger in lyre in the two years considered.

3.3. Micro - Thermal Conditions and Grape Ripening Evolution

Initial ripening date indicated by veraison,(E-L-35), had an 8-day difference between years, but not between plant architectures. In 2002, veraison (E-L 35) was registered on January 22nd, whereas in 2003 that stage was reached on

January 14th. The harvesting date for 2002, was February 22nd for lyre and February 27th for VSP. In 2003 the harvesting date was February 14th for lyre and February 20th for VSP. Thus, the duration of veraison - harvest was of 32 days in 2002 in the lyre and 37 days in the VSP. In 2003, the duration was of 29 days in the lyre and 36 days in the VSP. Values of sugars (+10%), anthocyanins (+42%) pH (+6.6%) and berry weight (+12%), at the beginning of the ripening process, were larger in lyre, while values for acids were higher

in VSP (+22.2%). These significant differences were maintained during the whole ripening period (Figs. 4 to 7).

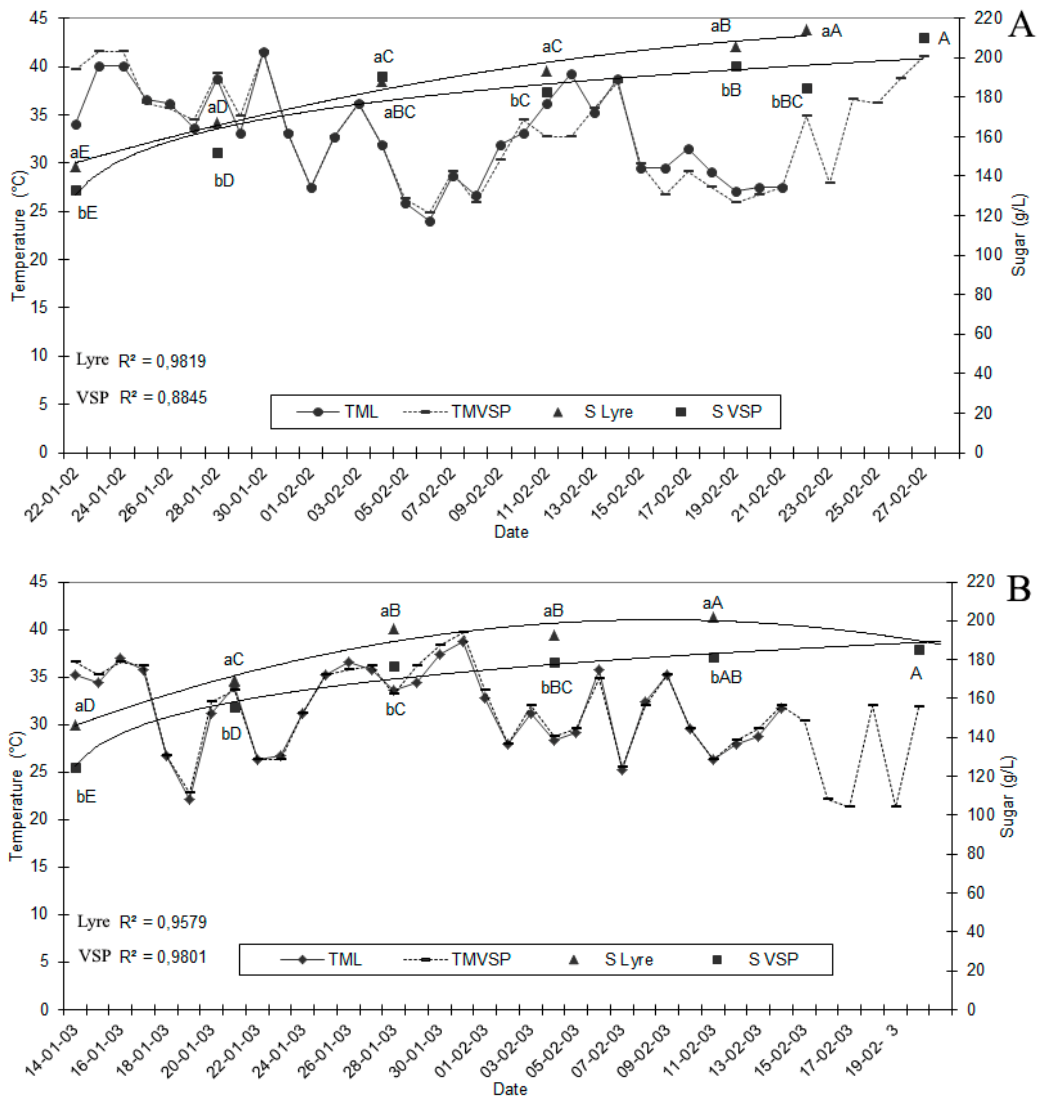
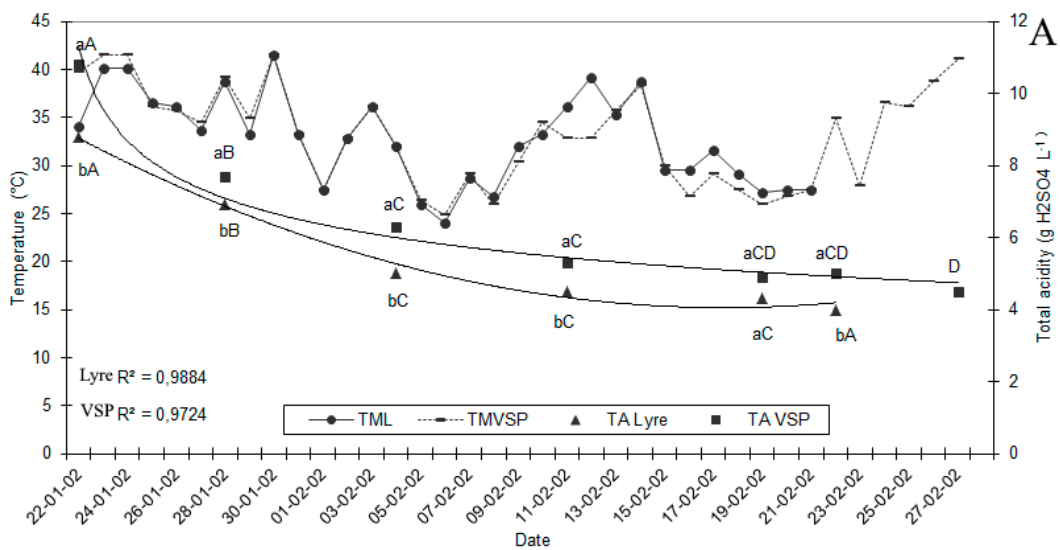


Fig. 4. Evolution of concentration of sugar of the grape according year and architecture in function to the maximum temperatures of the canopies Means followed by the same lower-case letter are not different by t-student test ($p = 0.05$) and capital letter (treatment x time) by Duncan ($p=0.05$). A year 2002 B year 2003



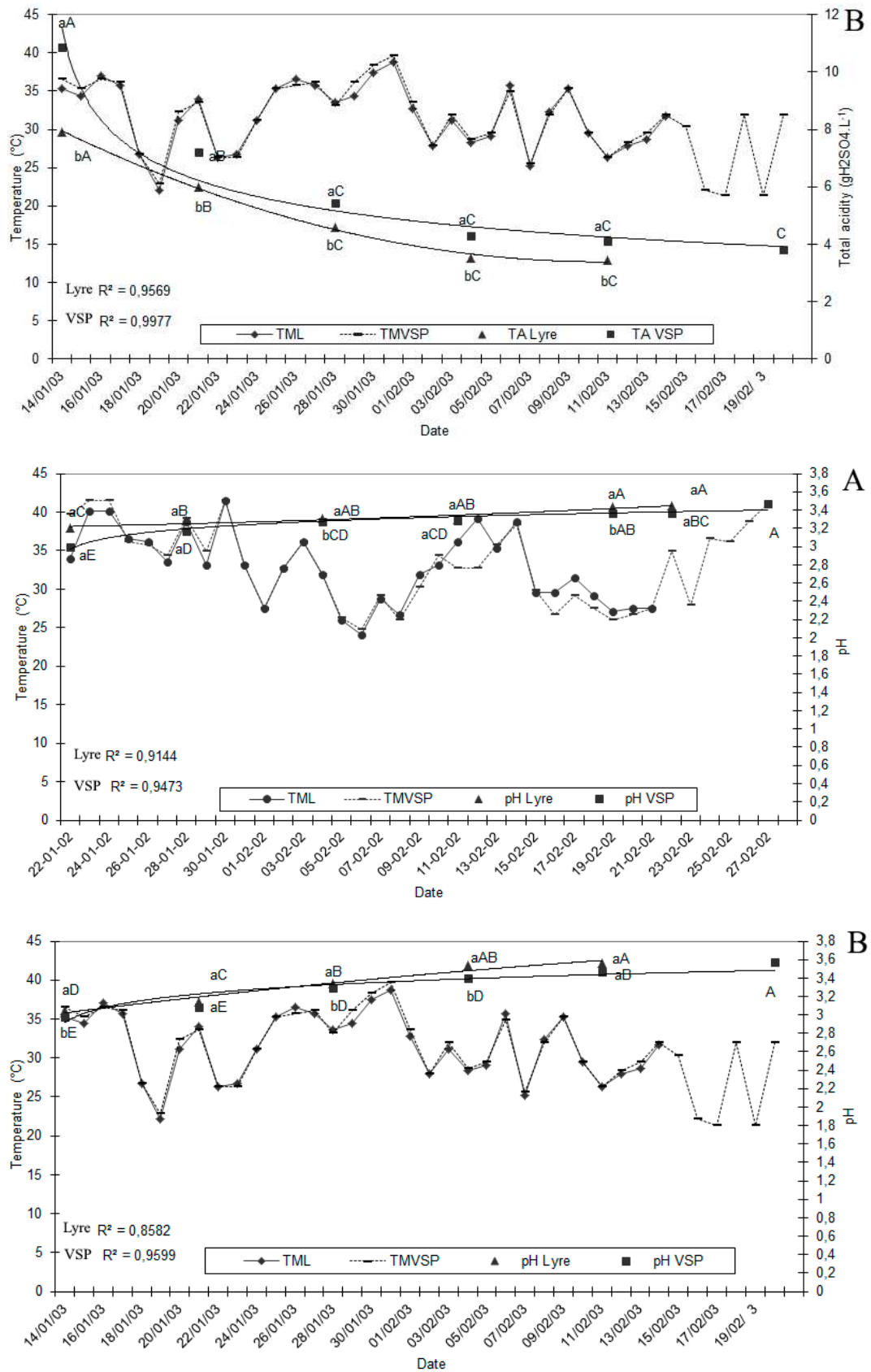


Fig. 5. Evolution of total acidity and pH of the grape according year and architecture in function to the maximum temperatures of the canopies Means followed by the same lower-case letter are not different by *t*-student test ($p = 0.05$) and capital letter (treatment x time) by Duncan ($p=0.05$). A year 2002 B year 2003.

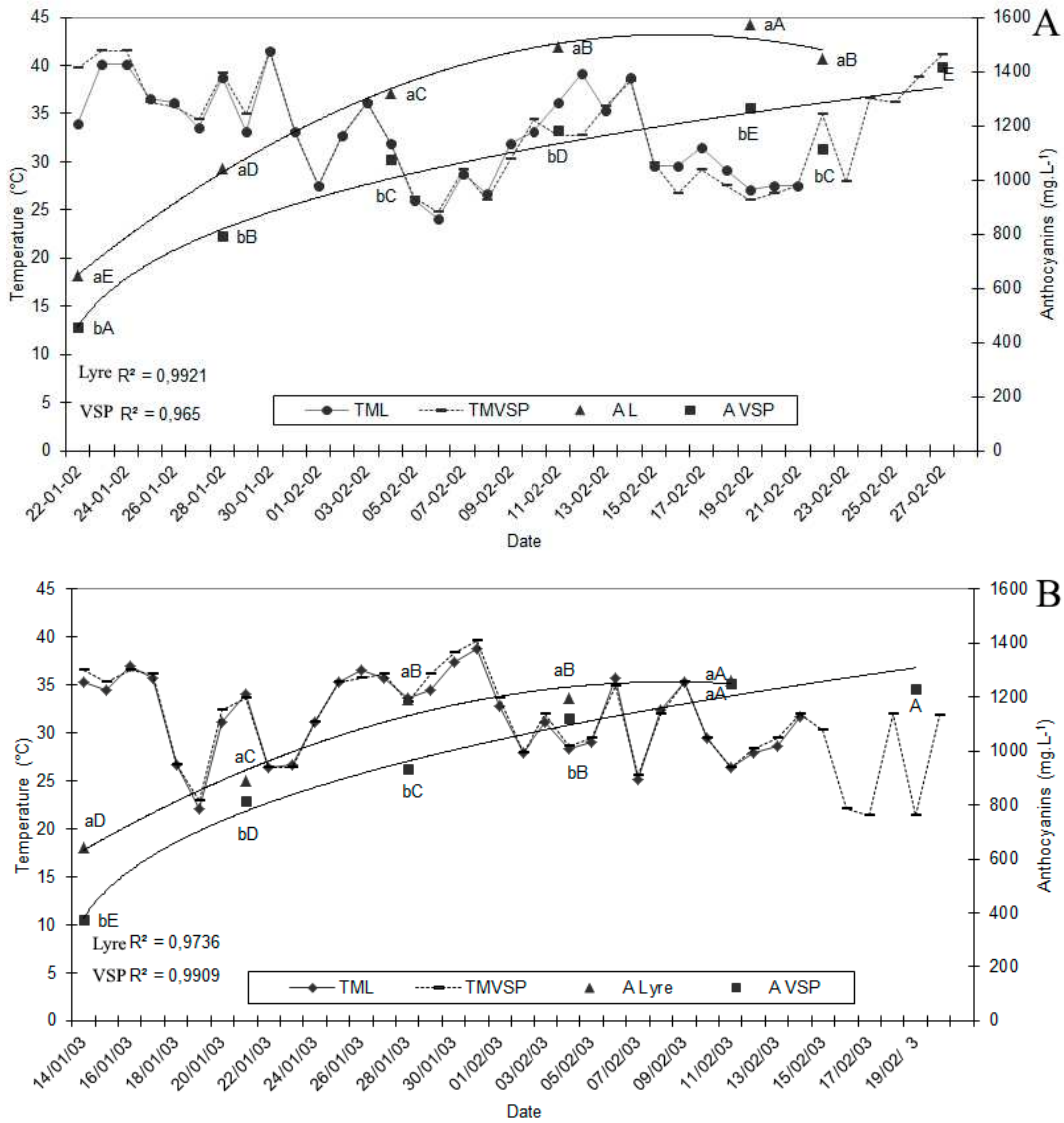
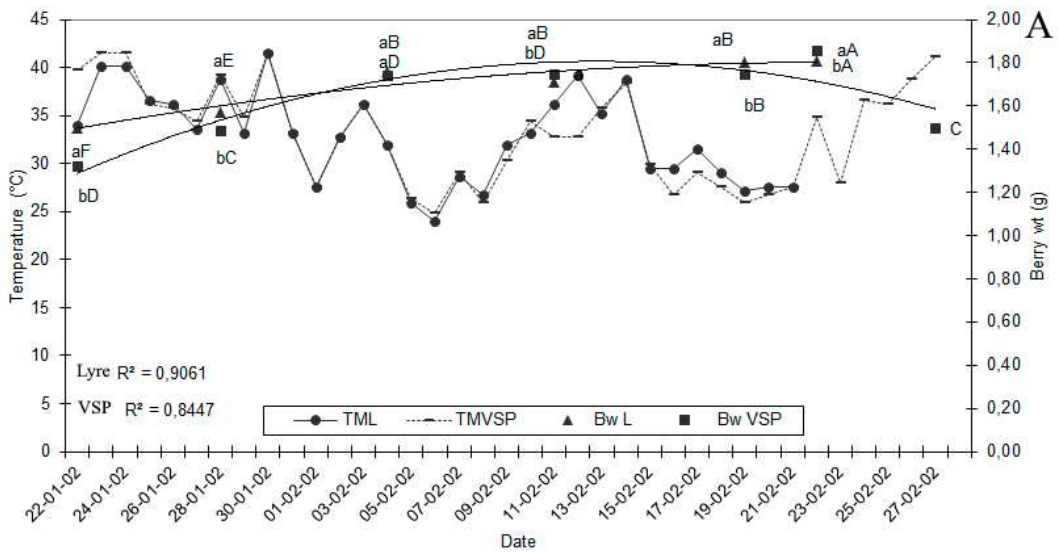


Fig. 6. Evolution of anthocyanins of the grape according year and architecture in function to the maximum temperatures of the canopies Means followed by the same lowercase letter are not different by t-student test ($p = 0.05$) and capital letter (treatment x time) by Duncan ($p=0.05$). A year 2002 B year 2003.



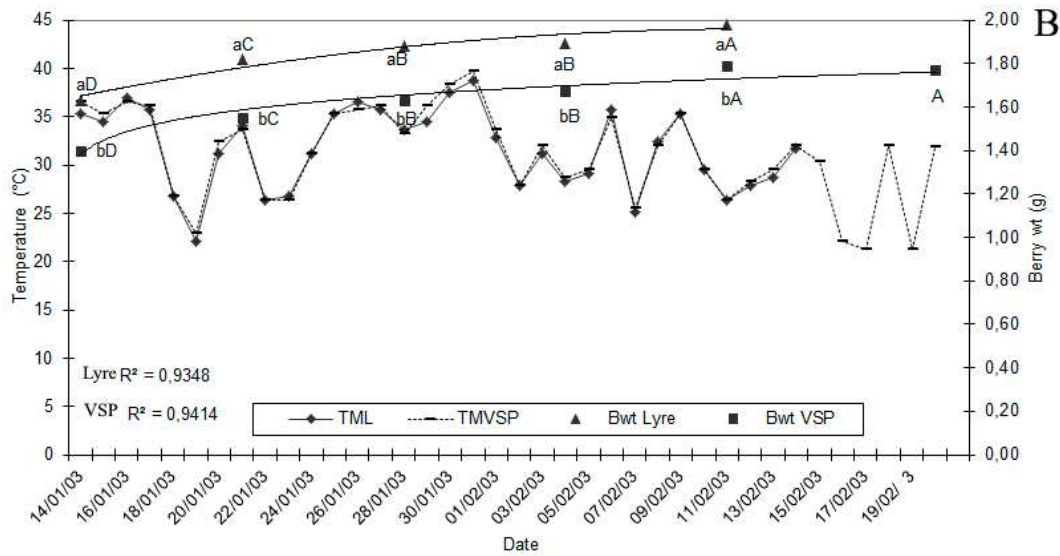


Fig. 7. Evolution of berry weight according year and architecture in function to the maximum temperatures of the canopies Means followed by the same lowercase letter are not different by t-student test ($p = 0.05$) and capital letter (treatment x time) by Duncan ($p=0.05$) . A year 2002 B year 2003.

Table 3. Composition of the grapes at harvest as a function of the trellis system and year:

	2002		2003	
	Lyre	VSP	Lyre	VSP
Sugars (g/L)	213ns	215	202a	186b
Sugars (g/berry)	0.31ns	0.30	0.36a	0.30b
Anthocyanins (mg/L)	1444ns	1417	1248ns	1232
Anthocyanins (mg/berry)	2.12a	1.98b	2.22a	2.00b
Total acidity (g/L H ₂ SO ₄)	3.90b	4.50a	3.40b	3.80a
pH	3.42ns	3.47	3.55ns	3.56

Means followed by the same letter are not different by t Student's test ($p=0.05$), ns = not significant

At harvest, sugar concentration and content (g/berry) was not significantly different between trellis systems in 2002, but, was superior for lyre in 2003. Differences in anthocyanin potential and pH were not significant in both years. However, the total anthocyanin potential (mg/berry) was higher in lyre

Table 4. Percent of accumulation or decrease of berry's metabolic compounds, in the 3 first weeks and 4th week (Lyre) or 4th and 5th (VSP) after veraison.

	2002				2003			
	Lyre		VSP		Lyre		VSP	
%	3 th	4 th	3 th	4-5 th	3 th	4 th	3 th	4-5 th
Sugars (g/L)	89.2	90.1	90.3	93.3	96.7	95.3	95.2	98.0
Total acidity (g/L H ₂ SO ₄)	78.5	86.7	71.4	91.2	73.9	97.1	70.2	92.7
Anthocyanins (pH1,mg/L)	91.4	103.0	75.8	89.4	95.3	95.6	75.6	101.5
Berry wt (g)	97.1	97.5	93.5	94.5	94.9	96.5	81.1	85.6

In the second stage, during the 4th and 5th weeks after the beginning of ripening in VSP and during the 4th week in lyre, a slowing in the kinetics of ripening compared with the three previous weeks was observed. The weekly increase or decrease (treatment x time) was not significantly different for the composition variables: sugars concentration, total acidity, pH and berry weight, while anthocyanins maintained a significant increase. Taking the values of the first three weeks as a reference, the maximum increases were: sugars 3%, anthocyanins 13.6%, berry weight 4.5% and decrease of acids

in both years. Total acidity was significantly higher for VSP in both years. Berry weight was significantly major in lyre for the two years (Table 3).

The evolution of the metabolic compounds of the grape, in which three stages were identified, adjusts to a polynomial trend in lyre and a potential trend in VSP. The first stage corresponds to the first three weeks following the start of veraison. During this period, a fast increase of sugars, anthocyanins, pH and berry weight and the decrease of acids were registered for both plant architectures and in both years. The weekly increase or decrease (treatment x time) was significantly different for the composition variables and berry weight. Taking the values at harvest as a reference and according to architecture and year, the minimum and maximum values were: sugar 89.2% and 96.7%, the anthocyanins 75.6% and 95.3%, the decrease of acids 70.4% and 78.0% and the berry weight 80.2% and 97.2%. (Figs.4 to 7, Table 4).

23.2% (Figs. 4 to 7, Table 4). During this stage, and independently from system and year, the highest level of anthocyanins was recorded. Average canopy temperatures during this phase were 39.1°C/17.1°C day/night in the VSP and 37.8°C/16.4°C in lyre during 2002. In 2003, those values were 34.8°C/18.7°C day/night in VSP and 33.9°C/17.4°C in lyre. The temperature amplitude recorded higher average values (Fig. 1).

In the third stage, during the last week of ripening, lyre reached its maturity before VSP in both years (5 days earlier in

2002 and 6 days in 2003). Sugars concentration increased significantly in both architectures and in both years, being that value, the largest in the lyre 2002, with 10.9% of the value at harvest (Fig 4). Acids decreased in both years and in both architectures, being the highest values in 2002 in lyre 13.3% (Fig 5). The evolution of anthocyanins showed the largest differences between years and plant architectures. In 2002, and taking the week previous to harvest of lyre as a reference period, those chemical compounds decreased significantly in both systems: 134 mg/L in lyre and 154 mg/L in VSP (Fig 6). Temperature conditions in canopy, registered during this period were of 32.0°C of maximum average temperatures in VSP and 34.8°C in lyre. The minimum average temperatures was 17.5°C in both systems, with temperature amplitude of 17.3°C in lyre and 14.8°C in VSP. The delay of the harvest in VSP under different micro-thermal conditions, allowed the vines under this system to recover anthocyanin potential up to 304 mg/L, amount that represented 21.5% of the value at harvest. In 2003, and taking the week previous to harvest of lyre as a reference period, anthocyanin potential increased significantly in both systems. In lyre, the increase was 53.5 mg/L and 125 mg/L in VSP. Thermal conditions registered by both architectures, compared with the same period of 2002 were: decreased temperature amplitude (12.1°C in lyre and 11.9°C in VSP), lower maximum temperatures (28.4°C in lyre and 29.0°C in VSP) and lower night temperatures (16.9°C in VSP and 16.5°C in lyre). Similar to the previous year, VSP reached its maturity a few days later, under different thermal conditions. Maximum temperature was drastically lower (21.3°C) and night temperatures registered minimal values of 11.5°C. Anthocyanin potential dropped in 19.5 mg/L.

4. Discussion

4.1. Thermal Characterization of the Plant Architectures and Its Relationship with the Environment

The most important modification on the thermal regime was the increase of maximum temperature of the canopy in relation to the environmental. This had consequences on the temperature amplitude differences between the systems and with the environment, according the ranges reported in the literature [1, 3, 8, 10]. Temperatures higher than the environmental ones, recorded at canopy level are due to the fact that the canopy functions as an enclosure and according to Smart and Robinson [1], and Millar [8] in absence of air exchanges, internal temperature within the canopy increases more than the surrounding air's temperature, reaching higher values, as the ones recorded in our tests. Maximum temperatures lower than the environmental ones, recorded in some days could also be explained by a lower radiation in rainy and cloudy days. Minimum night temperatures, equal to or lower than the environmental ones, could correspond to radiation losses during the night, as communicated by Smart and Robinson [1]. In accordance with several authors [4, 11, 36], one would expect the Lyre to have higher leaf temperature

than VSP, based on the lowest values of Ψ_{PD} and highest values of leaf area, registered in the lyre. (Table 2). This situation was recorded at the beginning of the ripening cycle, but it reverted quickly due to the spatial disposition of leaves and the larger percentage of bunches located outside the canopy. This characteristic of plant architecture increased the ventilation flow of the system, causing a lower vapor pressure deficit at the outer leaf layer and, as a consequence, a temperature lowering [3, 8]. Therefore, lower air circulation in VSP would explain higher values of maximum average temperatures, hourly thermal increases and the larger average thermal amplitude recorded for this system.

4.2. Plant Architectures and Grape Ripening Evolution

Ripening start – harvesting. Acidity and pH values were especially considered to obtain ripen grapes. Similar to what many other authors express, there is a negative relation between total acidity and pH with an $r = -0.94$ ($p < 0.0001$) was observed. The significantly higher total acidity and lower pH values in VSP, compared to lyre, were a consequence of the negative effect on micro-climate, due to a larger foliage superposition in this system, lower percentage of fruit exposure and greater vigor [5, 12, 13]. These characteristics of the VSP create shaded conditions that block the degradation of the malic acid [1-3, 7, 10, 20, 36].

The evaporative demand and water status of the plants, condition the evolution of the ripening process, measured through acidity decline and sugar accumulation [20, 36]. VSP was in mild water stress or well-watered, that according to Etchebarne et al. [23], results in higher acidity values. On the other hand, significant higher leaf area in lyre at the beginning of the vegetative cycle, favors the generation of young leaves, a preferential place for the production of tartaric acid [37, 38]. This acid is less sensitive to degradation due to temperature; even content increases have been reported. Under such conditions, in order to reach the expected acidity and pH values, VSP required more days evolving, therefore harvest was delayed compared to the lyre [3, 20].

Furthermore, the larger leaf area in the lyre caused water stress situations, which caused a suspended shoot growth at the beginning of ripening and a smaller vegetative growth. This elements condition the source – sink relationships, in agreement with reports from several authors [2, 4, 6, 18, 23, 36]. This foliage area had a larger percentage of external leaves in lyre (78.5%) and created a more efficient carbohydrate balance system, because 80% and 90% of the photosynthesis takes place in those external leaves [1, 36, 39]. Furthermore, production and distribution of photosynthates was modified by the trellis system. Two year's average showed that lyre produced the largest amount of dry matter and favored its accumulation in bunches (43.7%) more than in VSP (33.8%). According to the results of Orlandini et al. [40], confronting VSP with lyre of the Sangiovese variety and Bota et al. [41] in the Tempranillo variety.

Regarding sugar and anthocyanins, the significantly higher initial values during ripening in lyre can be explained by a

larger surface of leaves and the higher percentage of exposed bunches. These characteristics, gathered all the optimal values needed to obtain favorable micro-thermal conditions, for the photosynthetic process, positive balance of anthocyanins and higher yield and oenological performance compared to VSP [1, 2, 5, 18, 22]. In terms of temperature, the lyre showed the highest percentage of hours exposed to the range of 25 to 30°C in canopy, in average for the two years; more than double registered for VSP. On the contrary, with temperatures above 35°C, which according to several authors [3, 9, 36], are negative for both processes, VSP registers, in average of the two years, 15.7% (Fig. 2). From the analysis of the hourly thermal dynamic it can be concluded that during the day, the lyre has at least 3 more hours of exposure to temperatures within favorable range for accumulation processes than VSP.

Evolution of chemical compounds of the grapes The evolution of grape metabolic compounds adjusts to a polynomial curve in lyre and to a potential one in VSP, which can be explained by the numerous parameters that control, directly or indirectly, such metabolism. In that sense, coincident with the differences found between architectures in our work, Reynolds and Van Heuvel [36] and Kappel [42] mentioned as parameters that modify grape metabolism: vegetative vigor, the source – sink relationship, the thermal indices, and the vine water status, among others. Trellis system also contributes to differences in kinetics and in values reached by the metabolites (Figs.4 to 6). Regarding the evolution of chemical compounds in the grapes, and according to reports of various authors, three stages were identified for both architectures and years [15, 16, 18, 22, 24, 43]. The stage of accumulation- degradation depends of plant architecture or year. Differences between architectures are explained by Maximum temperature and thermal dynamics recorded inside canopies [3, 7, 15, 16, 20]. Anthocyanins were mainly defined by plant architecture. Sugar contents were more dependent of year conditions, being those, higher accumulated percentages for both architectures in 2003. Acid degradation and berry weight showed a strong influence of the system and year. Micro-thermal values registered in VSP were higher than in lyre, which is coincident with results reported by Matese *et al.* [9]. The lyre, as a more ventilated system, adjusted thermal peaks and followed the values of ambient temperature, maintaining favorable thermal conditions for the photosynthesis and anthocyanins synthesis during longer periods of the day. This agrees with the reports of several authors [3, 4, 7, 15]. On the other hand, this stage was characterized by a maximum thermal amplitude at the canopy, with values close to 20°C, which is mentioned in the literature as favorable to anthocyanins synthesis. In VSP, this thermal amplitude was a consequence of the increase of the maximal temperature (37.8°C), adverse for the anthocyanin synthesis [15]. The most negative values of Ψ_{PDV} registered in lyre at this stage, would have a positive influence on the accumulation - degradation of the grape compounds and explain differences found with VSP [18, 21]. The year 2002 was warmer and the water stress in VSP was moderate and strong in lyre at the beginning of the ripening stage. 2003 was

cooler and the level of water stress was moderate in lyre and mild in VSP at the beginning of ripening. This created conditions that favored accumulation of sugar and anthocyanins in lyre and a slower degradation of acids (Table 4).

During the second stage, where a slowdown is recorded in the accumulation of sugars and anthocyanins, the decrease in acids and berry growth and the compound values reached would depend on the canopy micro-thermal conditions [17]. Thermal peaks and the registered increase of the maximum temperature within the canopy, created thermal stress and provoked several modifications in the berry composition [15,16]. The kinetics of berry weight increase was also slower; the VSP limited the amount of sugars and anthocyanins in the berry [12]. A lower sugar accumulation rate in this stage determined also a lower rate for anthocyanins, due to the close correlation established between both compounds ($r = 0.95$, $p < 0.0001$) in accordance to Guidoni *et al.* [17]. However, the thermal and water availability of the year influence the composition of the grape. There were significantly lower values in acids and anthocyanins in both architectures in 2003 compared with 2002. This could be explained because in this stage, in 2003 the high night temperatures recorded in three consecutive days were 20.9°C, 20.2°C and 20.6°C, above the optimal [3,7,14]. The thermal amplitude in that year was also narrower than in 2002, due to higher night temperatures. In 2003, was a larger water availability and the range values of Ψ_{PD} of the plants were mild to moderate stress (lyre) or well-watered (VSP).

The third stage, corresponding with the last week of ripening, took place under different thermal conditions for each architecture. The compounds, products of the primary metabolism, were less affected by the thermal conditions. Independently from the year considered and the plant architecture, sugars increased and acids decreased. The increase of sugars in VSP in 2002 could have been - as also Kappel [42] indicates - the consequence of a concentration process due to dehydration of the berries following the high temperatures registered in that canopy (above 39.0°C) and characterized by a sharp decrease of berry weight (from 1.86 g to 1.50 g). A result that confirms the negative relation established between berry weight and the maximum temperature in the canopy ($r = - 0.89$ $p < 0.0001$). Anthocyanins contents were dependent on micro-thermal conditions. In 2002, in both plant architectures a reduction of anthocyanins took place. Maximum temperatures in both canopies exceeded 30°C and minimal ones were above 17°C. In such situations, according to the literature, a negative balance of anthocyanins took place [3, 15]. The thermal amplitude, which was maximum, did not seem to create a positive balance for the accumulation of these compounds, because the thermal peaks that cause such value should remain within acceptable ranges for this process. In the VSP, that took one more week to evolve, anthocyanins values recovered, probably as a consequence of favorable changes in thermal conditions (lowering of maximum temperature to 26.6°C and a thermal amplitude of 10.0°C). However, in 2003, taking the week before the harvest

as a reference, anthocyanin potential increased in both systems due to maximum and minimum temperatures and thermal amplitude in the canopy, which were within the ranges considered favorable to the positive balance of such compounds [3, 15]. VSP registered a decrease of anthocyanins in the following week and previous to harvest, probably due to the influence of the night temperature, which was lower than 16°C quoted as positive in the literature [7,14].

5. Conclusions

The contents and evolution of berry compounds were influenced by the Plant architecture due to the modification of the micro-thermal conditions and the water status of the grapevines in relation to the environment.

The maximum temperature within the canopy, which was higher in VSP, caused a larger thermal amplitude. This temperature was not necessarily within the values favorable for a balanced grape composition.

Lyre, with larger ventilation capacity, moderated thermal peaks and generated temperatures within ranges favorable to photosynthesis and synthesis of anthocyanins for longer time periods. Larger leaf area determined more negative water potentials in this system. This water stress conditions stopped shoot growth at the beginning of the ripening period and caused also a lower vegetative growth, both elements affecting the source-sink relationship. As a consequence, sugars contents and anthocyanin potential were higher in lyre, even experiencing larger total yields. Total acidity was higher in VSP following the negative effects on microclimate, due to a larger leaf superposition, lower percentage of exposed bunches and larger vigor in well – watered plants.

The highest percentages of compound accumulation and berry weight, in relation to the final harvest values, were registered during the first of the three ripening stages identified in this study.

References

- [1] Smart, R.E. and Robinson, M. (1991). Sunlight into wine. A handbook for winegrape canopy management. (Ministry of Agriculture and Fisheries, New Zealand) 88pp
- [2] Tandonnet J, Samie B, Ollat N (1996). Etude des interactions entre trois systèmes de conduite et le mode d'entretien du sol. Proceedings of the IX.GESCO 21–23 August Budapest - Hungary (University of Horticulture and Food Industries) pp 61-68
- [3] Spayd SE, Tarara JM, Mee DL, Ferguson JC (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv Merlot berries. *Am. J. Enol. Vitic.* 53:3 171-182
- [4] Carbonneau A (2003) Rappel des principaux effets des composantes du système de conduite. *Progrès Agricole Viticole.* 120 (13-14) 293-294.
- [5] Gladstone E, Dokoozlian N (2003) Influence of leaf area density and trellis/training system on the light microclimate within grapevine canopies. *Vitis* 42 (3), 123–131
- [6] Dai Z, Ollat N, Gomes E, Decroocq S, Tandonnet J P, Bordenave L, Pieri P, Hilbert G, Kappel Ch, van Leeuwen C, Vivin P, Delrot S (2001) Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: A review *Am. J. Enol. Vitic.* 62:4 413- 424.
- [7] Hunter J, Bonnardot V (2011) Suitability of Some Climatic Parameters for Grapevine Cultivation in South Africa, with Focus on Key Physiological Processes. *S. Afric. J. Enol Vitic Vol.* 32, No. 1 137-154
- [8] Millar AA (1972) Thermal Regime of Grapevines. *Am. J. Enol. Vitic .Vol.* 23 no. 4 173-176
- [9] Matese A, Genesio L, Crisci A, Di Gennaro F, Fiorillo E, Primicerio J, Di Blasi S, Pedò S, Zorer R, Vaccari F. (2011) Impatto delle pratiche di gestione della chioma sulla dinamica microclimatica e relazioni con la qualità delle uve. Proceedings of the 7ma.Enoforum 3-5 May Arezzo-Italy (Societa Italiana de Viticultura ed Enologia)
- [10] Sweetman C, Sadras VO, Hancock RD, Soole KL, Ford C M (2014) Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit *Journal of Experimental Botany* http://jxb.oxfordjournals.org/open_access.html
- [11] Glen M, Cooley N, Walker R, Clingeffer P, Shellie K (2010) Impact of Kaolin Particle Film and Water Deficit on Wine Grape Water Use Efficiency and Plant Water Relations. *HortScience* 45(8):1178–1187
- [12] Rojas – Lara BA, Morrison JC (1989). Differential effects of shading fruit or foliage on the development and composition of grape berries. *Vitis* 28 199-208
- [13] Ristic R., Downey M., Iland P, Bindon K, Francis I, Herderich M, Robinson S (2007) Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties *Australian Journal of Grape and Wine Research* 13,53–65.
- [14] Tonietto J, Carbonneau A (2002) Régime thermique en période de maturation du raisin dans le géoclimat viticole. Indice de fraîcheur des nuits – IF et amplitude thermique Proceedings of the IV Symposium International sur le zonage vitivinicole 17 - 20 June Avignon-France pp 279 – 289.
- [15] Mori K, Goto-yamamoto N, Kitayama M, Hashizume, K (2007) Loss of anthocyanins in red-wine grape under high Temperature. *Journal of Experimental Botany* 1-11
- [16] Poudel PR, Mochioka R, Beppu K, Kataoka I (2009) Influence of Temperature on Berry Composition of Interspecific Hybrid Wine Grape 'Kadainou R-1' (*Vitis ficifolia* var. ganebu × *Vitis vinifera* 'Muscat of Alexandria'). *J. Japan. Soc. Hort. Sci.* 78 (2): 169–174
- [17] Guidoni S, Ferrandino A, Novello V (2008). Effects of seasonal and agronomical practices on skin anthocyanin profile of cv. Nebbiolo grapes. *Am. J. Enol. Vitic* 53(3):224-226.
- [18] González-Neves G, Ferrer M (2008) Efectos del sistema de conducción y del raleo de racimos en la composición de uvas Merlot *Agrociencia Vol XII N° 2* pág. 10 – 18
- [19] Chaves M, Zarrouk O, Francisco R, Costa J, Santos T, Regalado A, Rodrigues L, Lopes C (2010) Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105: 661–676.
- [20] Sadras VO, Petrie PR, Moran MA (2013) Effects of elevated temperature in grapevine. II juice pH, titratable acidity and wine sensory attributes. *Australian Journal of Grape and Wine Research* 19,107–115

- [21] Keller M (2007). *Grapevine Anatomy and Physiology 200p* Printed at Washington State University
- [22] Ferrer M (2007) Etude du climat des régions viticoles de l'Uruguay des variations climatiques et de l'interaction apportée par le microclimat et l'écophysologie des systèmes de conduite Espalier et Lyre sur Merlot. PhD Thesis, Ecole Nationale Supérieure Agronomique – Université de Montpellier II - France 360pp. <http://www.fagro.edu.uy/~viticultura/Docencia>
- [23] Etchebarne F, Ojeda H, Hunter JJ (2010) Leaf: Fruit Ratio and Vine Water Status Effects on Grenache Noir (*Vitis vinifera* L.) Berry Composition: Water, Sugar, Organic Acids and Cations. *S. Afric. J. Enol Vitic.* Vol. 31, No. 2, 106-115
- [24] Famiani F, Walker R, Técsi L, Chen ZH, Proietti P, Leegood R (2000) An immunohistochemical study of the compartmentation of metabolism during the development of grape (*Vitis vinifera* L.) berries. *J. Exp. Bot.* Vol. 51, No. 345, pp. 675–683
- [25] Kuhn N, Guan L, Dai ZW, Wu BH, Lauvergeat V, Gomès E, Li S.H, Godoy F, Arce-Johnson P., Delrot S (2013) Berry ripening: recently heard through the grapevine. *Journal of Experimental Botany* pp 1-17
- [26] Tonietto J, Carbonneau A (2004) A multicriteria climatic classification system for grape-growing regions worldwide. *Agric. Fort. Meto.* 124, 81-97
- [27] Eichhorn KW, Lorenz DH (1977) Phonologische entwicklungsstadien der rebe *Nachrichtenbl. Dtsch. Pflanzenschutzd (Braunschweig)* 29: 119-120
- [28] Carbonneau A (1995) La surface foliaire exposée – guide pour sa mesure. *Progrès Agricole et Viticole* 9. 204-212
- [29] Scholander P, Hammel H, Branssreer E, Hammingsen E (1965). Sap pressure in vascular plant. *Siences* 148, 339-346
- [30] Carbonneau A (1998). Irrigation, vignoble et produits de la vigne. In: Lavoisier (ed) *Traité d'irrigation, Aspects qualitatifs.* pp. 257–276
- [31] Carbonneau A, Moueix A, Leclair N, Renoux J (1991) Proposition d'une méthode de prélèvement de raisin à partir de l'analyse de l'hétérogénéité de maturation sur un cep. *Bulletin de l'OIV 727/728* : 679 – 690.
- [32] O.I.V (1999). *Recueil des méthodes internationales d'analyse des vins et des moûts.* Paris : Office International de la Vigne et du Vin. 368p
- [33] Glories Y, Augustin M (1995). Maturité phénolique du raisin, conséquences technologiques: application aux millésimes 1991 et 1992. *Proceedings of the Journée Technique. CIVB, Bordeaux – France (Conseil interprofessionnel du vin de Bordeaux)* pp 56 – 61
- [34] Ribéreau-Gayon P, Stonestreet E. (1965). Le dosage des anthocyanes dans le vin rouge. *Bulletin de la Société Chimique.* 9: 2649 -2652
- [35] González-Neves G, Charamelo D, Balado J, Barreiro L, Bochicchio R, Gatto G, Gil G, Tessore A, Carbonneau A, Moutounet M (2004) Phenolic potential of Tannat, Cabernet-Sauvignon and Merlot grapes and their correspondence with wine composition. *Analytica Chimica Acta* 513 (1): 191-196
- [36] Reynolds A, Van Heuvel J (2009) Influence of grapevine training systems on vine growth and fruit composition. *A review Am. J. Enol. Vitic* 60:3 251-268
- [37] Kriedemann PE (1977) Vine leaf photosynthesis. In: *Proc. Int. Symp. on the Quality of the Vintage, 14-21 Feb. 1977, Cape Town.* pp. 67-87.
- [38] Hunter J, de Villiers OT, Watts JE (1991) The Effect of Partial Defoliation on Quality Characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon Grapes 1. Sugars, Acids and pH. *S. Afr. J. Enol. Vitic.* Vol. 12, No. 1
- [39] Vanden Heuvel J, Leonardos E, Proctor J, Fisher H, Sullivan J (2002) Translocation and partitioning patterns of ¹⁴C photoassimilate from light-and shade-adapted shoots in greenhouse- grown Chardonnay grapevines (*Vitis vinifera* L.) *J. Am Soc Hortic Sci* 127 (6):912-918
- [40] Orlandini S, Dalla Marta A, Mattii G B (2008) Analysis and agrometeorological modelling of grapevine responses to different trellising systems. *Vitis* 47 (2), 89–96
- [41] Bota J, Stasyk O, Flexas J, Medrano H (2004) Effect of water stress on partitioning of ¹⁴C-labelled photosynthates in *Vitis vinifera*. *Functional Plant Biology* 31,697–708.
- [42] Kappel C D (2010) *Biologie integrative du métabolisme de la baie de raisin.* PhD Thèses Université Victor Segalen Bordeaux 2 177pp <http://www.theses.fr/>
- [43] Deluc L, Grimplet J, Wheatley M, Tillett R, Quilici D, Osborne C, Schooley D, Schlauch K, Cushman J, Cramer G. Transcriptomic and metabolite analyses of Cabernet Sauvignon grapeberry development *BMC Genomics.* <http://www.biomedcentral.com/1471-2164/8/429>