Effects of Kaolin and Pinolene application on the physiological parameters and on the grape quality *of Vitis Vinifera* cv. Cabernet Sauvignon in South Italy

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Abstract

The study has been undertaken over two growing seasons (2011–2012) in the DOC area "Cirò", (South Italy), with t aim to investigate the effectiveness of two anti-transpirants, Kaolin and Pinolene, in reducing the accumulation of g soluble solids and in saving water in the variety Cabernet Sauvignon. The trial has been carried out in a 10 years irrigated, cordon trained vineyard, with a density of 5000 vines per hectare and N-S row orientation. The experimental is characterized by deep and loamy soil, with summer climate characterized by high temperatures and low rainfall.

A full randomized design has been adopted, with three replicates per treatment, comparing Pinolene, Kaolin and untreated control (Test). In 2011 and 2012 the anti-transpirants were applied twice on the canopy (at veraison and 1 days after veraison), using 2% (v/v) and 30 kg/ha doses respectively for the Pinolene and Kaolin. Measurements inclu canopy efficiency parameters, as water potential (stem) and gas-exchanges, the main production parameters, grape a must composition (soluble solids, acidity, flavonoids and total anthocyanins content) and wine sensory analysis.

The results showed lower yields for all treatments in 2011 compared to 2012; in 2011, as a consequence of a lower bun weight, the Pinolene and Kaolin treatments recorded lower yields, compared to the Test. The same effect was observed 2012. Similar sugar contents were obtained in the Pinolene and Test treatments (23.1° Brix and 23.3° Brix respective the Kaolin treatment presented slightly lower values. In 2012 the Pinolene and Kaolin treatments had a similar su content, lower than that of the control.

With regard to gas-exchange, data collected after the treatments in the two years of the study were decidedly differen all treatments the levels of photosynthesis resulted very low in 2011 (about 8 µmol CO2 m2s-1) and were considera lower in 2012, by reason of the higher temperatures reached in the leaves. The difference observed in the first two year the study are probably due to the different annual weather conditions. Thus, it can be inferred that anti-transpirants more effective in hot season and hot environments.

Introduction

Climate changes that we have been experiencing in the last thirty years are clearly influencing rain distribution and sea thermal values. These changes greatly affect grape phenological phases by bringing them forward in comparison to period before 1990 (Tomasi et al 2011). Increased temperatures and radiation, coupled with rising CO_2 concentration the atmosphere, have an effect on transpiration (Schultz, 2000) and also on those processes responsible for the gra qualitative components such as aromas, coloring substances, sugars, acids (Gladstones 1992; Jones 2005; Webb et a 2007). One of the consequences is the increase in sugar substances that might constitute a problem, given the actual w market demand. In fact, on the basis of recent studies (Seccia and Maggi 2011), consumers increasingly prefer wines l in alcohol, due to restrictive rules regulating alcoholic beverages consumption. Therefore, obtaining low alcohol win nowadays a main requirement that needs the implementation in vineyards of agronomical practices aimed at optim source/sink relations (correct canopy management in relation to the crop load) or cultural techniques (correct train systems, row spacing, rootstocks, clones) enabling to provide grapes with a good balanced between sugar and acids.

In this respect, one possible solution is the application during grape vegetative cycle of specific substances of natural o that, distributed on leaves walls can control transpiration and gas exchanges and consequently reduce the accumulatio soluble solids in the berries. Aim of present study is to evaluate the effect and the effectiveness of two natural substance the reduction of sugar accumulation in grapes; the first one Pinolene (Di-1-p-menthene) has an anti transpiring action. polymer creates an elastic film on the leaf surface that reduces gas exchanges and water loss through stomata. The sec product, Kaolin, is a clay of natural origin. It creates a protective/reflective layer on leaf surface that combine an transpiring action with a protective effect on the grapes from possible sunburns, since the obtained film reflects UV infrared rays (Rosati 2007). Thus, both products seems to be effective in limiting the photosynthetic activity with a pos consequent reduction of sugar accumulation in grapes.

Materials and methods

The trial has been performed in the two-year period 2011-2012 in a 10 years old commercial vineyard located in the DC area "Cirò" in the Province of Crotone, South of Italy (39°11'18" N; 17°, 01' 15" E). The variety is Cabernet sauvign (*Vitis Vinifera* L.) grafted onto 1103 P and spur pruned to 6/8 buds per vine. Rows are oriented to a N – S direction and density of 5050 vines/ha (row spacing 2,2 x 0,9 m) secures on average a production of 130 ql/ha.
Soil is deep with a sandy clay texture, scarcely endowed with organic substance and active limestone, pH is sub-alkal The vineyard was irrigated twice in 2011 and once in 2012 with a water volume of 24 liters/plant for each irrigation. treatments were performed every year for Pinolene (2% doses V/V) and for Kaolin (30Kg/ha): the first application w done at the beginning of July (stage 75, BBCH) and the second in the first days of August. Starting from mid July and grape harvest, instrumental surveys were performed on a regular basis. Gas exchange of single leaves was measured u the portable measurement system CIRAS 2 (PPSYSTEMS Europe, Herts, UK). The readings were carried out by settir the air flow to 320 mL / min and the concentration of CO2 at 370 ppm, at ambient relative humidity and under conditi of saturating photosynthetically active radiation (PAR 1800 □ mol m2 s-1); the assimilation of CO2 (A), transpiration and stomatal conductance (gs) were obtained on the basis of the relative concentrations of CO2 (difference betwe incoming and outgoing) and H_2O, while efficiency intrinsic water use (WUEi) was obtained from the relationship bet A and gs, 12 primary leaves per treatment were chosen among those inserted at nodes 5 - 7 above the distal bunch on main shoot. The same leaves were measured at different dates, in the morning between 9:00 and 12:00. On the sa dates, the water status of the vines was monitored by measuring the stem water potential, using a pressure chamber such Scholander (Skye Instruments). Measurements were taken on 5 primary leaves per treatment, inserted at nodes 5 - above the distal bunch on a main shoot.
At harvest, 20 representative vines per treatment were chosen to measure the productive parameters (bunch weight, bur number and yield/vine. Soluble Solids (° Brix), tritatable acidity (g/L di HTH), pH, total and extractable conten anthocyanins and flavonoids (tab. 1) were determined on musts, following the Di Stefano method (Di Stefano et al 1994 Stefano et al. 1989), whereas anthocyanin profile was determined by HPLC. At the commercial maturity, 200 kg grapes per treatment were hand-harvested for the wine-making and the wines obtained were judged by trained tasters.

All the data were processed by one-way analysis of variance, using Statistica 8.0. The comparison betwe treatments were assessed using the Student-Newman-Keuls test with $P \leq 0.05$.

Results and discussion

Climate

Considering the data reported in Tab.1, 2011 was characterized by lower rainfalls (about < 100 mm) and higher maxim temperatures (> 1.8°C) than 2011; maximum temperature were also over the average of the last ten-year-period. In figu 1 and 2 the mean monthly temperature, rainfall and temperature trend (average and effective – GDD -) are reported a compared to the last ten-year-period .

Year	Maximum temperature (°C)	Minimum temperature (°C)	Average Temperature (°C)	Diurnal thermal range (°C)	Montly growing degree days (GDD)	Rainfall (mm)	Radiation (W/m^2)	Winds Hours/day
2003	27,9	16,0	22,3	14,7	2556	419	268,7	9,3
2004	28,1	13,8	21,2	14,3	2155	229	266,6	9,4
2005	27,2	13,5	20,4	13,6	2202	309	265,5	9,3
2006	30,3	15,3	22,9	14,9	2751	271	277,0	9,5
2007	29,0	15,1	22,3	12,2	2539	264	nd	9,7
2008	28,1	17,6	22,5	12,5	2360	691	nd	8,9
2009	28,2	14,6	21,5	13,4	2436	820	334,2	8,9
2010	28,0	14,0	21,2	13,8	2384	711	270,4	9,0
2011	28,4	14,3	21,6	14,1	2480	334	270,3	9,3
2012	30,2	14,6	22,7	15,6	2692	243	285,2	10,3
Average	**28,5**	**14,7**	**21,9**	**13,9**	**2456**	**429**	**280,0**	**9,4**

Tab. 1. Mean climate data of the period April-October (Data from Servizio di Agrometeorologia di Crotone).

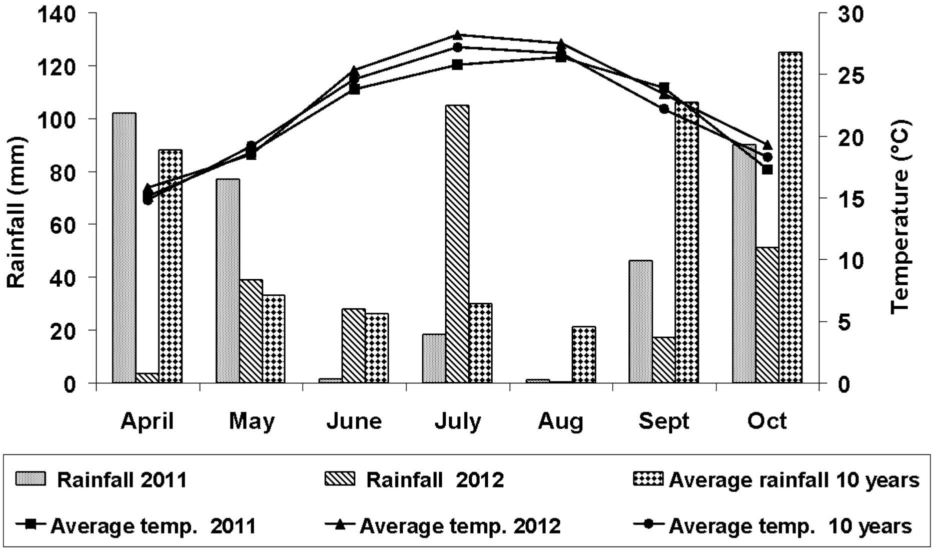


Fig. 1. Mean monthly temperature and rainfalls in 2011- 2012 and 10-year-average (2003 -2012).

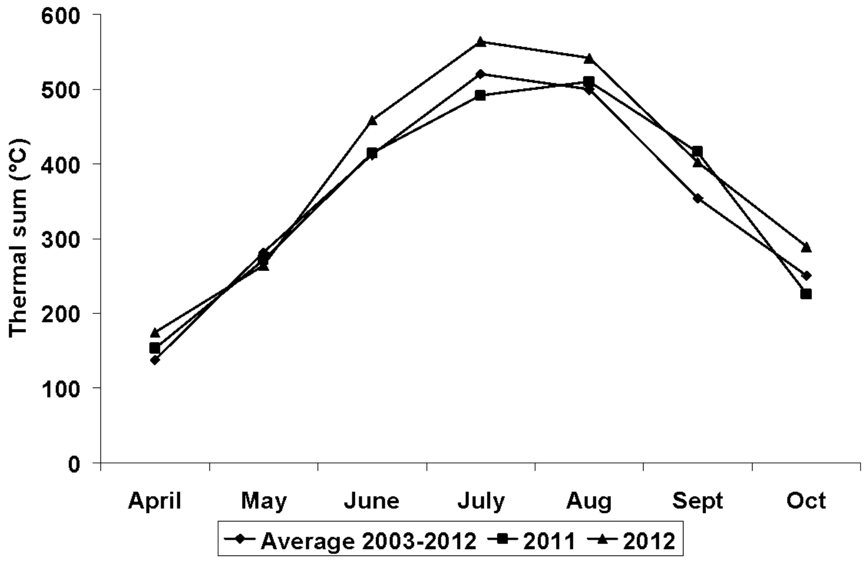


Fig. 2 Monthly growing degree days (GDD) in 2011-2012 and 10- year-average.

Year 2011

Yield, yield parameters and grape quality

In 2011 the yield and production parameters (bunch weight, number of bunches/vine, etc) in Kaolin and Pinol treatments did not record statistically relevant differences compared to the control (Tab. 2). Concerning the grape qual the grape macrocomposition (soluble solids, acidity and pH) was not affected by the treatments and only a tendency Kaolin to lower sugar concentration and Pinolene to an increase in anthocyanin content was observed.

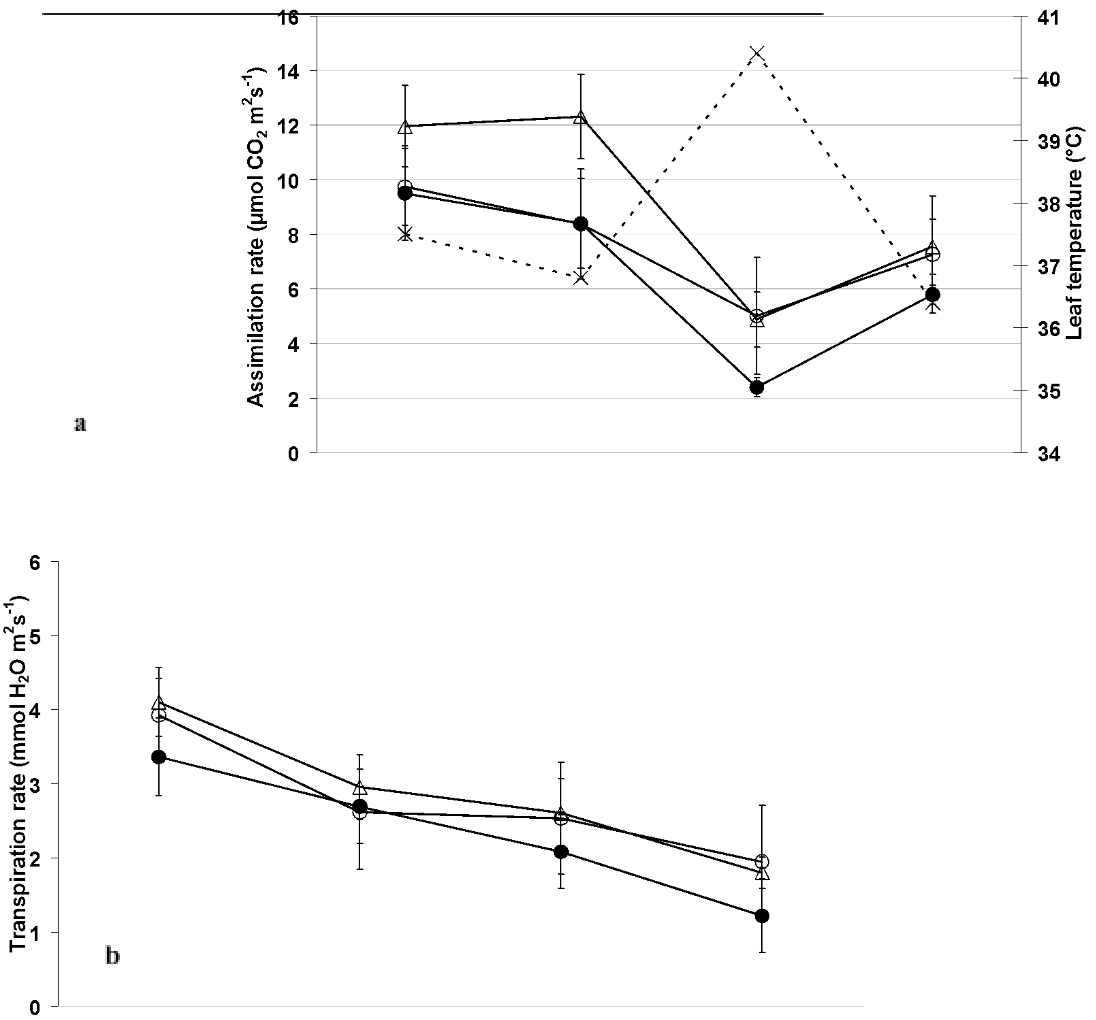
2011	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)	Anthocyanins (mg/kg grape)	Flavonoids (mg/kg grape)	Bunch number/vine	Yield/vine (Kg)	Bunch weight (g)	Berry weight (g)
Test	23,2 a	3,93 a	3,0 a	414 a	1420 a	16 a	2,3 a	149 a	1,2 a
Kaolin	22,7 a	3,99 a	3,1 a	391 a	1400 a	15 a	2,0 a	135 a	1,1 a
Pinolene	23,1 a	4,03 a	2,6 a	535 a	1414 a	14 a	1,9 a	136 a	1,2 a

Tab. 2 - Grape composition, yield and production parameters.
Small letters indicate significant differences between treatments at P≤0.05, Student-Newman- Keuls test

Gas exchanges and water potential

Fig. 3 shows how CO_2 assimilation had an almost constant trend among the three treatments in the first two measureme July 14 and 28 (Doy 195 e 209) with Kaolin treatment recording slightly higher values than the other two treatments. In following date, August 18 (Doy 230), there was a sharp drop of assimilation in all the treatments (reduction of about 7 59%, and 48% respectively for Pinolene, Kaolin and control sample). This performance should be attributed to wa stress increase that in that day reached the highest values of the period (Fig. 4). As a consequence, a reduction transpiration was observed in all treatments (fig 3b): in this phase stomatal conductance values (data not reported) resu the lowest of the whole period. In the last survey, on August 31 (Doy 243) there was a good recover of the assimilation all the treatments (explainable by the effect caused by irrigation occurred on 23 August), however assimilation values lower than those recorded at the season beginning as a consequence of leaves decay.

The Kaolin and control treatments showed always slightly higher CO_2 assimilation values in comparison to the Pin treatment, with the exception of the survey of August 28. The Pinolene treatment was also the less performing in relati water use efficiency, with exclusion of the last phase of vegetative growth cycle (fig. 3c).



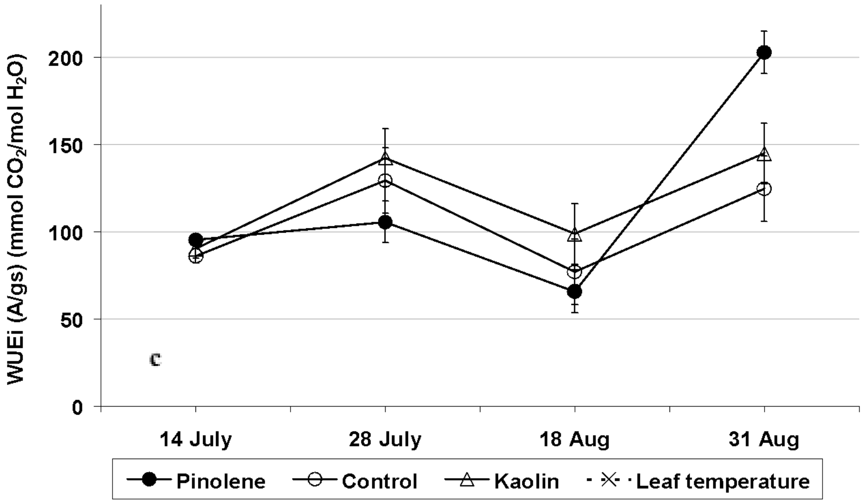


Fig. 3 - Gas exchange in 2011 and standard error: assimilation rate (a), transpiration rate (b) and intrinsic water use efficiency (c). WUEi calculated as assimilation/stomatal conductance ratio.

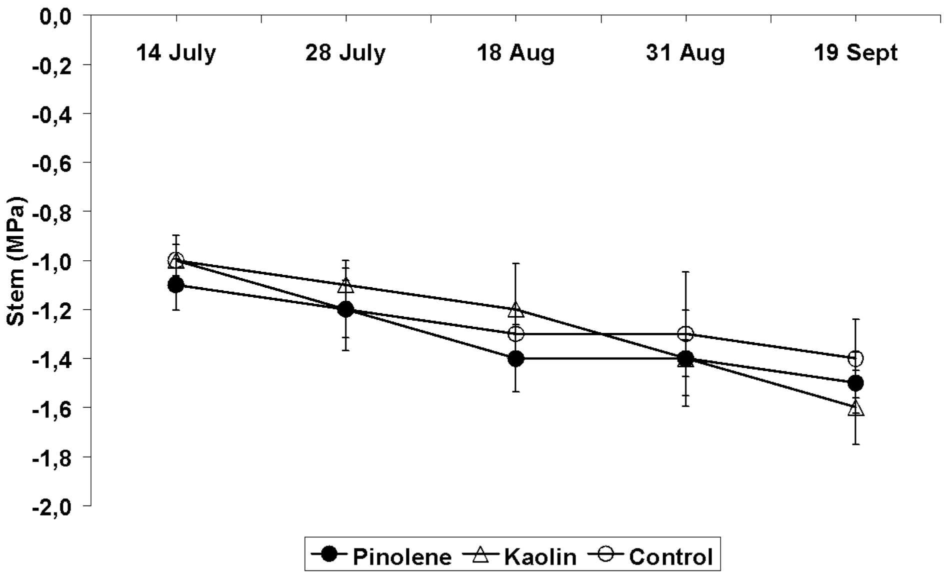


Fig. 4 - Stem Water Potential in 2011 and standard deviation.

Year 2012

Yield, yield parameters and grape quality

Values reported in tab. 3 show that the treatments have influenced significantly the content of sugar and the pH. In Ka and Pinolene treatments the sugar content in the berries was about 10% lower than in the control. An interesting data also the anthocyanin and flavonoid content. Both components had a remarkable increase in respect to the previous y and this might be explained by the higher temperatures and radiation recorded in 2012 (Fig.1). It has been demonstrated fact, that high thermal and light availability determine an increase of the phenylalanine ammonia lyase activity, that le raise of anthocyanins in the skins (Roubelakis-Angelakis et Kliewer, 1986; Kliewer et al. 1977; Bergqvist 200

Moreover, the Kaolin treatment showed the highest amount of anthocyans and flavonoids. This confirms the results of c recent researches conducted in hot-arid climates on Cabernet sauvignon and Malbec (Krista C. et al., 2013).

2012	Total soluble solids (°Brix)	pH	Total acidity (g/L)	Anthocyanins (mg/kg grape)	Flavonoids (mg/kg grape)	Bunch number/vine	Yield/vine (Kg)	Bunch weight (g)	Berry weight (g)
Test	23,8 a	4,35 a	2,6 a	751 a	2205 a	17 a	3,3 a	182 a	1,0 a
Caolino	21,6 b	3,72 b	2,4 a	972 a	2830 a	19 a	2,8 a	143 a	0,8 a
Pinolene	21,5 b	4,27 a	2,5 a	727 a	2125 a	20 a	3,5 a	177 a	1,0 a

Tab. 3. Grape composition, yield and production parameters.
Small letters indicate significant differences between treatments at P≤0.05, Student-Newman- Keuls test

Gas exchanges and water potential

Data demonstrated consistent differences in gas exchanges compared to the previous year (fig. 5).
The higher temperature in 2012 have driven leaves temperatures until values next to 47°C (values recorded on July 3 16, between 10 and 10.40 a.m). As a consequence (Carbonneau et al, 2011), in 2012 the photosynthetic activity wa lower than in 2011, with assimilation rates ranging from 9,2; 6,5 and 7,6 µmol CO_2 m^2 s^{-1} in 2011 for Kaolin, Pinolene control respectively and 2,9; 4,1 and 5,2 µmol CO_2 m^2 s^{-1} for the same treatments in 2012 (fig. 6). Under hot clima conditions, the Kaolin treatment presented lowest assimilation (except in first survey, on July 3) and transpiration (Fig. b) levels, associated with more negative stem water potential values (fig.7).
The Pinolene treatment had, on the other side, a less regular photosynthetic activity, with lower values compared to control. The assimilation rate increased after the irrigations, until the end of August, when the effect of leaves decay an increasing stress reduced the photosynthetic activity. As already noted, high temperatures and low water potential val that in certain moments determined extreme stress conditions by reaching almost -2 MPa, induced to a dramatic reduct of gas exchanges, with stomatal conductance that showed extremely low values (data not reported).
In both treatments the net photosynthesis has been noticeably reduced in comparison to the control ((-44% and - 20 respectively for Kaolin and Pinolene), and this explains the lower sugar concentration in grapes, compared to that of control (tab. 2).
There are no significant differences in water stem potential, since these are in line with those of 2011 and their ave values in 2012 are -1,4, -1,2 and -1,3 MPa respectively for Kaolin, Pinolene and control sample.

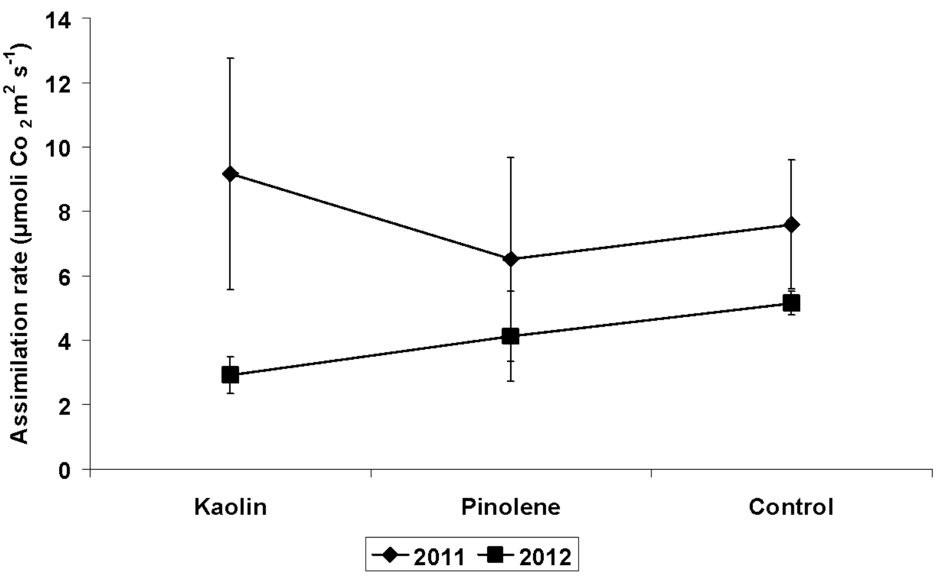


Fig. 5 – Photosynthesis (Pn) and standard deviation in 2011 and 2012.

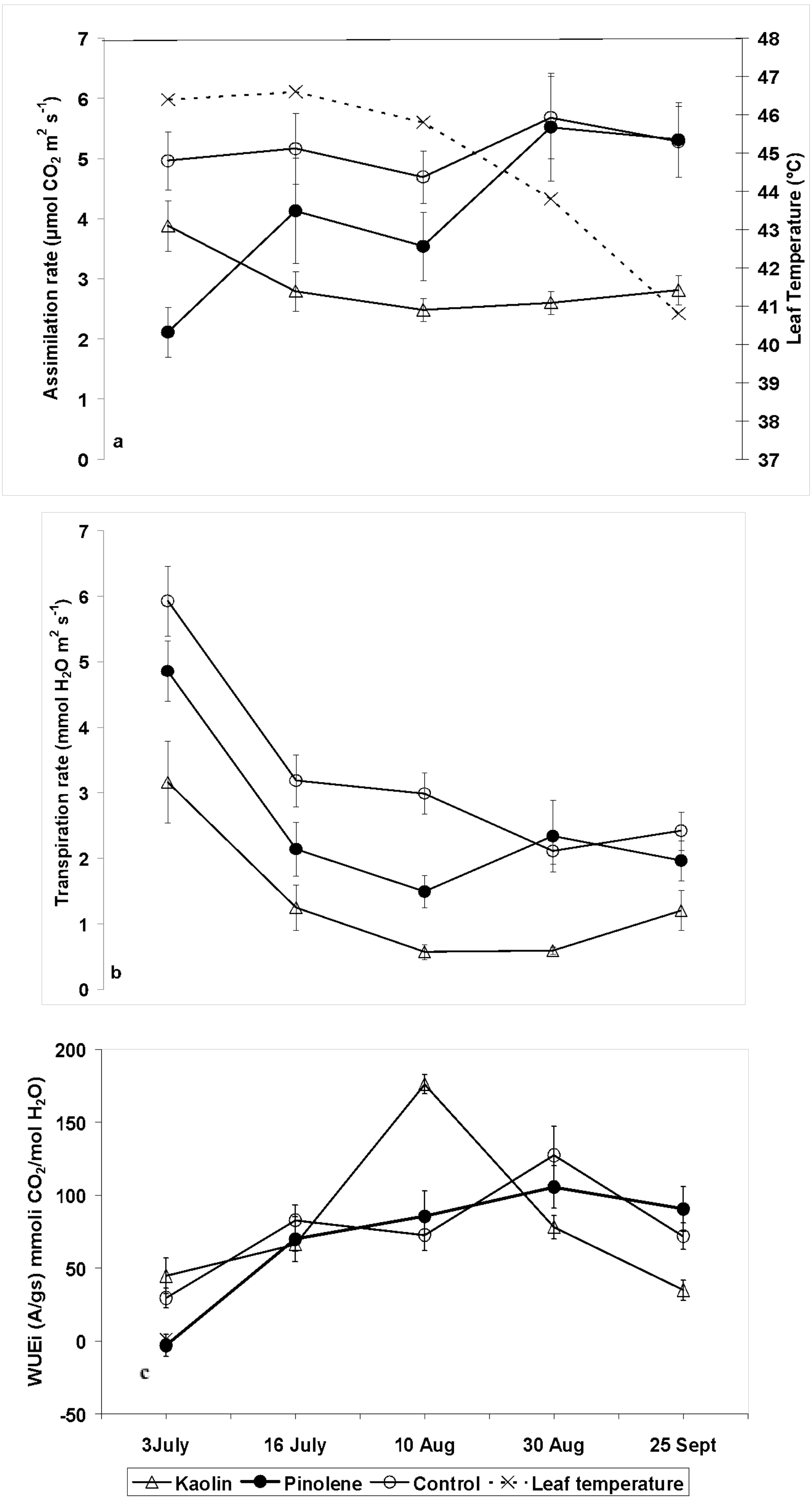


Fig. 6 - Gas exchanges in 2012 and standard error: assimilation rate (a), transpiration rate (b) and intrinsic water use efficiency (c).

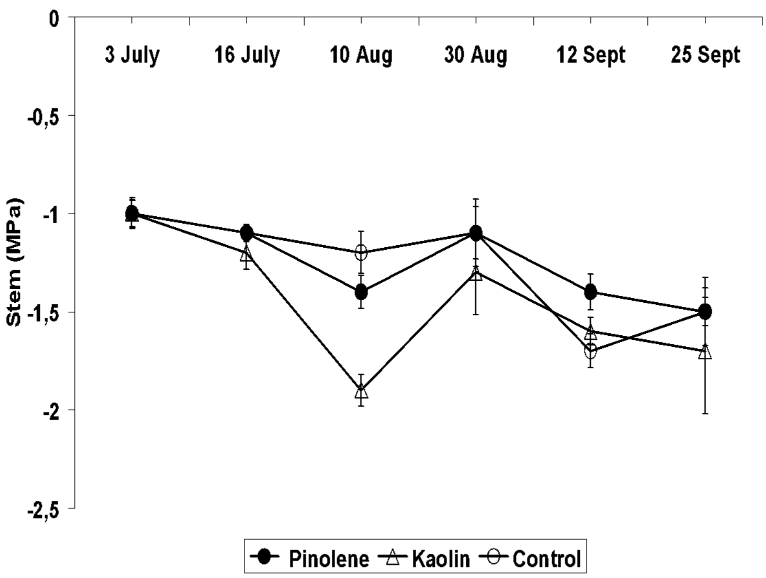


Fig. 7 - Stem Water Potential and standard deviation in 2012.

Finally, we wanted to verify the treatment effect on wine sensory profile. Sensorial analysis highlighted that only in there was a slight influence by kaolin treatment, which enhanced the visual and olfactory qualities. The wines o treatment presented superior color and olfactory intensity, marked fruit notes and elegant flower nuances.

Conclusions

Tests conducted on Cabernet sauvignon confirmed the results obtained in precedent experiences (Palliotti et al 20 2010) which reported that Pinolene is effective in reducing the sugar content in the grapes. We can affirm that in the cli and environmental conditions where it has been operated, the two products tested have induced a clear effect, limi carbohydrates synthesis. Considering that all the agronomical variables (bud load, summer pruning, irrigations, management, fertilizations, number and period of the treatments) were the same in the two years of the trial, it can als affirmed that product effectiveness seems to be enhanced by the higher temperatures experienced in 2012. The analysi variance highlighted the year effect, and this confirms that product effectiveness is linked to the climatic conditions.

In 2012 the Pinolene treatment and the control had very similar productions per unit (Tab. 2), but Pinolene ha significantly lower content of sugar in the musts. The Kaolin treatment, even recording the lowest productivity rates, l sugar content of 2,3 ° Brix lower than the control. Therefore it seems to be the most promising product for the aims of experimentation.

Even if Pinolene and Kaolin treatments can not entirely substitute the traditional agronomical practices, they ca implemented in the vineyard management to naturally control the sugar content and the wine alcohol degree.

Further investigation will continue in the course of 2013, to confirm if these substances might represent a suitable to face climate change and new consumer's requests.

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