TEMPERATURE EFFECTS ON THE BIOSYNTHESIS OF AROMA COMPOUNDS IN GLERA GRAPES

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Abstract
This paper describes the first year results of a study that investigated the effects of altitude and related temperature parameters on the biosynthesis of aromas in the Italian cultivar Glera.
The trial was carried out in a commercial vineyard planted on a steep slope in the Valdobbiadene area (North-East of Italy). Three sites were selected at three different altitudes, ranging from 200 m a.s.l. to 380 m a.s.l. In each site air and berry temperature were constantly monitored during the ripening period. Starting from veraisón, grape samples were collected from each site approximately every 10 days, and then analyzed to determine the ripening level (soluble solids, acidity, pH), the amount of aroma volatile compounds and the expression of some key genes involved in the terpenoid biosynthesis.
Preliminary data collected in 2012 highlighted the strong influence that altitude exerts on both air temperature and fruit temperature during the ripening period. The lowest site recorded the lowest minimum night temperatures, about 2°C lower than the medium and high sites, and consequently the grape ripening in this site was notably delayed compared to the medium and high sites. A similar delay was not observed in the synthesis of aroma compounds. At harvesting, the three principal classes of compounds (terpenes, norisoprenoids and benzenoids) showed lower levels in the low site. However, comparing grape samples from the three sites at the same level of ripeness, the low one displayed significantly higher amounts for all the classes of aromas.
Preliminary results from gene expression analysis showed that the linalool synthase *VvPNLinNer1* was more expressed in samples collected from the medium site. This result correlated with the higher accumulation of linalool plus its derivatives in this site.

Keywords: aroma compounds, temperature, altitude, climate

1 INTRODUCTION

The impact of climate on grape's aroma is an important but not extensively researched area of investigation. A few studies reported that climatic parameters such as temperature, solar radiation, rainfall and water availability seem to be of special significance, influencing grape macro and micro-composition to a great extent.
In this regard, Marais et al. (1999) reported that high sun exposure and temperature promoted the synthesis of norisoprenoids and monoterpenes of Sauvignon blanc in South Africa. In the same region Reynolds and Wardle (1997) reported that cooler temperatures and high diurnal thermal ranges were favourable for terpene synthesis in Gewurztraminer. In general, in warmer regions, impressions linked to norisoprenoid compounds (ripe and tropical fruit) usually predominate (Sefton et al. 1994, Lee et al. 2007), the explanation being the more rapid degradation and transformation of carotenoids into norisoprenoids, under the influence of temperature (Marais et al. 1991, Rozungles et al. 1993). Grapes grown under cool conditions usually give wines with more evident vegetal notes due to higher methoxypyrazine levels (Lacey 1991, Falcao et al. 2007).
It appears clear that, along with soil and other parameters, climate defines a *terroir* and is capable of conferring a specific composition, style and quality to the grapes and to the wines produced in a certain area.
Furthermore, mesoclimate can differ between vineyards within a region, depending on factors such as slope, exposition, altitude and surrounding vegetation. Altitude, in particular, could exert an important effect on grape maturation and on the composition of grape and wines that is strictly related to local climate (Falcao et al. 2007, Tomasi et al. 2012, Tomasi et al. 2013). It is well known that temperature is strictly related to altitude and it drops approximately 0.8°C every 100 m elevation gains. Therefore, mean and extreme temperatures, at higher altitudes, are usually lower in comparison to those found at the bottom of the hills. Depending on the altitude and the position on the hillside, diurnal thermal range can record a significant difference too, due to the influence of cold air masses that descend the slopes at night causing a reduction of night temperatures in the valley floor.
The Prosecco DOCG wine growing area, located in the North-East of Italy, is characterized by a very complex hilly morphology that comprises a series of extended relieves arranged in a north-south direction in the southern part and east-west in the northern portion, and separated by deep valleys. Moreover, different lithologies present in the area determined different shapes of the relieves, ranging from steep inclinations to gentle slopes and with elevations varying from 200 to 450m asl.

This aspects are of great importance for Glera grapevine cultivation, as within the Prosecco DOCG area vineyards are located in very different orographic and altimetric situations with climatic conditions varying as a consequence.
The present study investigated the effects of temperature on the aroma compounds of Glera variety cultivated at different altitudes, using the 2012 vintage data. Air and berry temperatures recorded during the ripening period were recorded. Aroma compounds were quantified at different dates from veraison to harvest and the expression level of some key genes involved in the terpenoid biosynthesis was analyzed. The objective of this study was therefore to evaluate the influence of microclimate associated to different altitudes on the aroma composition of Glera grape, on aroma compound synthesis and on some aroma gene expression, and to use the results for a better comprehension and exploitation of the Prosecco *terroir*.

2 MATERIALS AND METHODS

Experimental site. The trial was carried out in a commercial vineyard of Glera variety, planted on a steep slope in the Valdobbiadene area (North-East of Italy). Three sites were selected within the vineyard, at three different altitudes, named high (380 m a.s.l), medium (290 m a.s.l.) and low (200 m a.s.l.) site, respectively. In each site 40 vines were selected for agronomic measurements and for grape sampling. Environmental and agronomic factors (soil type, exposition, variety, clone, rootstock, vineyard characteristics, vine management) are reported in Tab.1, and were the same in the three sites, with the exception for temperature, that varied with altitude.
Climate data. Air temperature was constantly monitored from veraison to harvest with a meteorological station situated in close proximity to each site. Berry temperature was recorded by a WatchDog 1000 datalogger (Spectrum Inc), provided with 4 sensors placed inside the berries. The data consisted of daily observations of maximum, minimum, mean temperatures, rainfall and thermal amplitude.
Sempling and harvesting of the grape
Starting from veraison, grape samples were collected at random from each site approximately every 10-15 days until harvest. Three replicates of 30 berries were collected for macro-constituent analysis (soluble solids, acidity, pH). Three replicates of 100 berries were collected for the aroma compound analysis. A sample of 80 berries was collected for the molecular analysis. As the grape ripening was delayed in the low site, grapes were harvested on September 12 in the medium and high sites, and on September 18 in the low one, when total soluble solids was > 16°brix
Aroma component analisys
The free volatile compounds were extracted and detected by solid phase extraction (SPE) procedure coupled to gas chromatography-mass spectrometry as described in D'Onofrio et al. (2012).
Molecular analysis
RNA extraction, cDNA production and gene expression analyses by RT-qPCR were performed as described in Matarese et al. (2013).
Statistical analysis. Analisys of variance (ANOVA) and Duncan's test were carried out using Statistics 7.0 (StatSoft Inc).

3 RESULTS AND DISCUSSION

Climate. The ripening period in 2012 was characterized by temperatures above the average of the area and by long periods without rainfall (data not shown). August had an average temperature of 24.4°C and only 69 mm of rainfall. Similar weather conditions characterized the first decade of September too, while lower temperatures and abundant rainfall were recorded in the second and third decades.
Fig. 1 and Tab. 2 show that altitude exerted an important effect on temperature. Consistent differences were found between the sites for both air and berry temperature variables. Considering in particular the extreme temperatures, the most consistent differences between sites were observed for minimum temperature, that showed the lowest values in the low site, both in the air and in the berry.
Despite air maximum temperature were similar among sites, berry maximum temperature resulted higher in the medium one. This difference could be imputable to a less evident wind-effect in the middle part of the hill. Ventilation might contribute in cooling the berry in the hottest hours of the day, diminishing the difference between air and berry temperature. Taking as example the period August 11-28 (Tab.2), the middle site recorded 33 hours with temperature above 35°C, while the low and the high ones had 22 and 17 hours, respectively. These data are particularly important considering that above this threshold, temperature becomes too high for an optimal vine physiology.
Considering the thermal range, it decreased with altitude, displaying a berry temperature difference of about 2,5°C between the high and the low site.
Berry ripening. As effect of the lowest mean temperatures, berry maturation (in terms of total soluble solids and acid content) was considerably delayed in the low site (fig.2). Grapes from this site were harvested on September 18, with a soluble solid content of 16.2° brix, similar to that found in the grapes from the medium and high sites sampled on August 22. Moreover, the lowest temperatures in the low site resulted in the maintenance of a higher

acidity along the whole ripening period. Titratable acidity at harvest was 6.1 g/l in the low site, against 5.1 and 5.5 in the medium and high sites, respectively.

Grape aroma compounds and relation with temperature. Different climatic conditions manifested differently in aroma component concentration. Curves of aroma accumulation displayed a similar trend in the three sites, but the amounts of grape aromas were significantly different among sites in different dates. At harvest the low site showed the lowest amounts for norisoprenoid and benzenoid aroma classes (data not shown) Medium and low sites had similar amounts, with slightly higher values of monoterpenes and norisoprenoids in the medium site. Considering the whole ripening period, data seems to confirm that warmer conditions are more favourable for the synthesis of monoterpene, norisoprenoids and benzenoids too.

Interesting observations can be made comparing grape samples from three sites at the same ripening stage (between 16-17 °brix- doy 22/8 for the medium and high sites, doy 18/9 for the low site), (Tab.3). In this case, benzenoids were higher in the low site and their synthesis seemed to be influenced by diurnal thermal range (dtr), with high dtr being associated to higher values of this class of compounds. This confirmed what was found by Tomasi et al. (2012, 2013) in previous studies.

On opposite to what observed at harvest, also norisoprenoids and monoterpene classes showed higher values in the low site. As reported before, in fact, the synthesis of these aromas is usually favoured by warm temperatures. However, in particularly hot weather conditions, like those occurred during august 2012, high temperatures can lead to the degradation and volatilization of these compounds. Comparing the maximum berry temperatures recorded in the three sites along an exemplificative period of two weeks in August (Fig.1, Tab.2.), temperatures in the medium sites were more frequently higher than 35°C compared to the other two sites. This imposed sub-optimal conditions for the vine physiology an for the synthesis and preservation of several secondary and aromatic compounds (Kliever, 1973; Belancic, 1991).

Therefore, it can be assumed that norisoprenoids and terpenes were higher in the low site, because on one hand this site had temperatures warmer than the high one, but not so high to cause excessive aroma degradation as might be occurred in the medium site. It must be added that in the low site a longer ripening period might have favoured a higher synthesis of aromas.

Analyzing the C_{13}-norisoprenoid class, β-damascenone, 3-oxo-α-ionol and vomifoliol are among the most important compounds that contribute to the Prosecco wine typical aroma; their concentration was higher in the low site and similar in the medium and high site.

Among the monoterpenes, linalool, geraniol, terpineol and their derivates were the highest in the low site, but displayed some differences in their response to temperature. Linalool concentration was three times higher in the low site than in the other two, that showed very similar values. Geraniol and terpineol showed smaller difference between sites, suggesting that these compounds might be less affected by temperature than linalool and its derivates. A positive effect of high thermal amplitude on linalool synthesis might be also suggested by the data collected in this first year of the trial.

***Aroma gene expression*.** Preliminary gene expression analysis revealed mixed results. We analysed the expression of two linalool synthases, *VvPNLinNer1* and *VvPNLinNer2*, that in a previous work (Matarese et al., 2013) were found more expressed in green and ripening berries, respectively, and of a geraniol synthase *VvPNGer*. Between the two linalool synthases, *VvPNLinNer1* showed higher levels of expression than *VvPNLinNer2* in all samples confirming previously results on ripening berries of Moscato bianco (Matarese et al., 2013). Concerning *VvPNLinNer1*, we found a peak of expression in the first sample at the low site. In addition in all samples collected on the other dates, this gene showed its highest level of expression at the medium site. This result correlated with the highest accumulation of linalool plus its derivatives in this site. On the contrary the expression of *VvPNGer* showed a peak of expression in the first samples (the highest at low site), when the synthesis of geraniol started. Then the expression rate of this gene was similar in the three sites. Further analysis will clarify the results achieved so far.

4 CONCLUSIONS

Microclimate conditions associated to different altitudes showed a consistent effect on the aroma composition of Glera grapes. The role of extreme temperatures in the synthesis and maintenance of aroma compounds appear to be of particular importance. From preliminary data collected in 2012 it can be stated that warm conditions are favourable for the terpenoid, norisoprenoid and benzenoid development in this variety. However, a principal role is exerted by maximum temperatures, as when they becomes too high a negative effect is observed on the aroma synthesis. Among the studied aromas, benzenoids and some terpenes, like linalool, showed a positive correlation with thermal amplitude.

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Table 1 – Characteristics of the experimental sites used in the study

Site	Altitude (m)	Soil type	Exposition	Variety	Clone	Rootstock	Conduction system	Rows x vine spaces (m)	Age (years)
High Medium Low	380 290 200	Sandy Loam	South	Glera	ISV10	1103P	Sylvoz	3,5 x 1	20

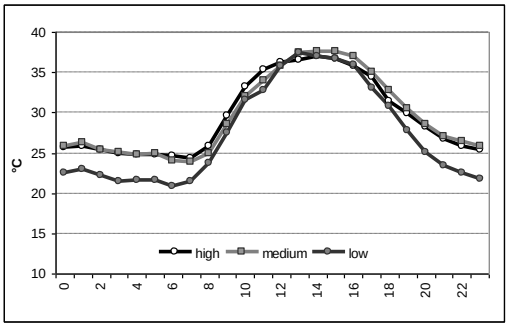

Fig.1 – Berry hourly temperature recoded in the three sites on August 21, 2012.

Table 2 – Air and berry temperatures of the three sites for the exemplifying period August 11-28, 2012.

Year	Site	Temperature (°C)								
		Mean		Max		Min		Diurnal thermal range		n° hours >35°C
		Air	Berry	Air	Berry	Air	Berry	Air	Berry	Berry
2012	High	25.3	25.6	31.4	32.9	20.3	19.4	11.1	13.1	17
	Medium	25.4	26.2	31.0	34.7	20.2	20.1	10.8	14.6	33
	Low	24.0	24.2	31.3	33.5	17.7	17.0	13.5	15.8	22

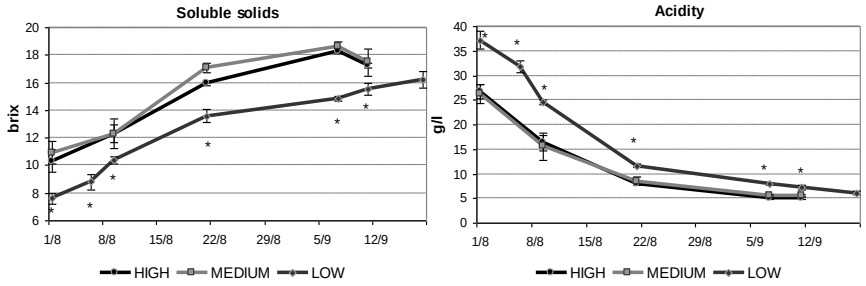

Fig.2 – Ripening curves for the grapes from sites at three different altitude (data 2012)

Table 3 – Benzenoid, norisoprenoid and monoterpene content in Glera grapes from sites at three different altitudes, at the same ripening stage (between 16-17 °brix- doy 22/8 for the medium and high sites, doy 18/9 for the low site, data 2012)

Benzenoids (ng/berry)	Low	Medium	High
Benzaldehyd	4.2 ± 1.7	3.0 ± 0.1	3.4 ± 0.4
Methyl benzoate	58.8 ± 21.3	5.8 ± 2.0	4.2 ± 2.3
Acetophenone	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
Ethyl benzoate	3.1 ± 0.3	2.8 ± 0.1	2.7 ± 0.1
Methyl salicylate	27.6 ± 12.5	18.3 ± 3.3	75.0 ± 17.3
Benzaldehyde, 2,5-dimethyl-	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
1-Fenylethanol	3.5 ± 0.1	3.8 ± 0.1	2.7 ± 0.3
Benzyl Alcohol	211.9 ± 8.0	191.7 ± 14.9	215.9 ± 10.1
2 Feniletanol	345.9 ± 5.2	388.0 ± 20.2	403.3 ± 43.9
Benzenepropanol	1.8 ± 0.1	0.6 ± 0.0	0.4 ± 0.0
p-Cymen-7-ol	1.7 ± 0.1	1.8 ± 0.2	1.6 ± 0.1
2-(4-Methoxyphenyl)ethanol	26.9 ± 3.1	13.5 ± 2.7	15.7 ± 1.4
6-Methoxy-3-methylbenzofuran	0.5 ± 0.1	0.3 ± 0.0	0.5 ± 0.2
Benzoic acid	1665.8 ± 100.3	1432.6 ± 100.3	1231.9 ± 247.2
3',5'-Dimethoxyacetophenone	28.8 ± 3.1	31.8 ± 2.7	19.8 ± 12.1
3,4-Dimethoxybenzyl alcohol	0.4 ± 0.0	14.5 ± 1.1	29.5 ± 48.8
Cinnamic acid	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.1
2,3,4-Trimethoxybenzyl alcohol	12.2 ± 1.5	13.0 ± 0.1	15.7 ± 0.5
total	**2394.1 ± 142.0**	**2122.4 ± 87.2**	**2022.7 ± 203.5**
Norisoprenoids (ng/berry)			

vitispirane	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
TDN	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Damascenone	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Actinidol A	0.8 ± 0.0	0.8 ± 0.1	0.6 ± 0.0
Actinidol B (2-Propenal, 3-(2,6,6-trim	1.4 ± 0.0	1.6 ± 0.2	1.2 ± 0.1
3,4-diidro-3-oxo-a-ionol (I)	26.8 ± 2.2	21.4 ± 1.5	16.5 ± 2.8
3,4-diidro-3-oxo-a-ionol (II)	24.1 ± 1.9	18.4 ± 1.5	16.2 ± 0.5
3,4-diidro-3-oxo-a-ionol (III)	28.5 ± 1.8	23.4 ± 1.5	21.1 ± 0.6
3 OH Beta Damascone	1.5 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
3-oxo-a-ionol	279.2 ± 21.6	203.9 ± 8.6	189.8 ± 11.4
2,3-dehydro-4-oxo-7,8-dihydro-beta-ionone	9.0 ± 0.9	7.3 ± 0.9	7.3 ± 1.1
Epimanool	2.7 ± 0.8	2.8 ± 0.3	5.0 ± 3.8
Methyl-beta-ionone	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3,9 diidrossi megastigma-5-ene	3.3 ± 0.1	3.6 ± 0.3	3.2 ± 0.3
Blumenol C	9.4 ± 8.1	15.0 ± 0.7	12.4 ± 1.5
3-Hydroxy-7,8-dihydro-beta-ionol	3.3 ± 1.3	2.8 ± 0.5	3.3 ± 0.1
Vomifoliol	826.8 ± 107.8	560.5 ± 85.8	546.2 ± 136.9
7,8 dihydrovomifoliol	12.8 ± 1.5	10.7 ± 2.1	12.2 ± 4.3
total	**1230.5 ± 128.9**	**873.7 ± 86.5**	**836.7 ± 448.5**
Monoterpenes (ng/berry)			
Oxide A[a]	12.6 ± 1.1	4.6 ± 0.3	3.3 ± 0.5
Oxide B[a]	4.7 ± 0.4	3.4 ± 0.2	3.2 ± 0.5
Linalool[a]	88.5 ± 8.1	10.8 ± 1.2	3.3 ± 1.0
Oxide C (Epoxylinalool) [a]	8.0 ± 0.9	3.1 ± 0.1	1.8 ± 0.4
Oxide D (Epoxylinalool) [a]	1.3 ± 0.2	1.2 ± 0.1	0.6 ± 0.1
Diol 1[a]	2.4 ± 0.3	0.5 ± 0.1	0.4 ± 0.1
endiol (6,7-diidro-7OH-linalool) [a]	10.4 ± 0.8	7.5 ± 1.6	9.4 ± 1.1
Diol 2[a]	3.8 ± 0.6	0.5 ± 0.1	0.3 ± 0.1
T 8-OH Linalool[a]	26.3 ± 3.9	7.0 ± 0.6	4.7 ± 0.6
Cis 8-OH Linalool[a]	154.2 ± 19.7	55.6 ± 4.4	44.1 ± 7.4
4-Terpineol[b]	0.8 ± 0.3	0.9 ± 0.1	0.8 ± 0.1
a-Terpineol[b]	9.1 ± 0.1	9.1 ± 0.5	11.3 ± 1.2
Nerol[a]	14.2 ± 0.3	10.4 ± 0.7	9.2 ± 1.1
p-Menth-8-en-3-ol[b]	52.2 ± 3.9	28.0 ± 7.5	34.8 ± 2.3
7OH a-terpineol [b]	29.5 ± 1.7	22.6 ± 7.4	44.3 ± 6.1
Hotrienol [c]	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
Citronellol [c]	4.2 ± 0.3	4.2 ± 0.5	4.2 ± 0.4
Isogeraniol [c]	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Geraniol [c]	191.4 ± 7.6	161.1 ± 10.8	161.0 ± 13.0
OH-citronellol [c]	26.5 ± 0.7	16.5 ± 6.2	22.4 ± 2.2
Geranic acid [c]	153.5 ± 6.1	130.1 ± 9.5	117.8 ± 23.6
Myrcenol	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.2
Ocimenol 1	1.3 ± 0.1	3.4 ± 3.4	1.3 ± 0.0
Lilac alcohol A	2.8 ± 0.3	2.1 ± 1.7	0.8 ± 0.1
Myrtenol	0.3 ± 0.3	0.0 ± 0.0	0.4 ± 0.0
Lilac alcohol B	0.5 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
exo-2-Hydroxycineole	9.3 ± 0.8	9.8 ± 0.8	8.2 ± 0.9
p-Mentha-1,8-dien-6-ol	0.7 ± 0.1	1.2 ± 0.2	0.9 ± 0.3
2,3-Pinanediol	3.0 ± 0.2	2.8 ± 0.3	2.5 ± 0.1
4-Octene-2,7-diol, 2,7-dimethyl-, Z	3.9 ± 0.3	3.5 ± 1.2	6.2 ± 1.4
Linalool + deravates [a]	312.3 ± 35.5	94.2 ± 7.3	71.2 ± 10.9
Terpineol + derivates [b]	105.8 ± 4.8	71.1 ± 15.9	100.4 ± 5.2
Geraniol + derivates [c]	376.5 ± 11.3	312.4 ± 26.0	305.9 ± 36.2
total	**816.3 ± 51.6**	**500.7 ± 49.18**	**498.1 ± 43.8**