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**Evaluación del ambiente lumínico-térmico y su influencia
en la acumulación de terpenos en *Vitis vinífera* L. cv.
Moscatel de Alejandría, bajo sistema de conducción en
cabeza**

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GUILLERMO ALFONSO PASCUAL ABURTO
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Profesor Guía: Felipe Laurie Gleisner
Dpto. de Horticultura, Facultad de Ciencias Agrarias
Universidad de Talca

EVALUACIÓN DEL AMBIENTE LUMÍNICO-TÉRMICO Y SU INFLUENCIA EN LA ACUMULACIÓN DE TERPENOS EN *VITIS VINÍFERA* L. CV. MOSCATEL DE ALEJANDRÍA, BAJO SISTEMA DE CONDUCCIÓN EN CABEZA.

Aprobada por:

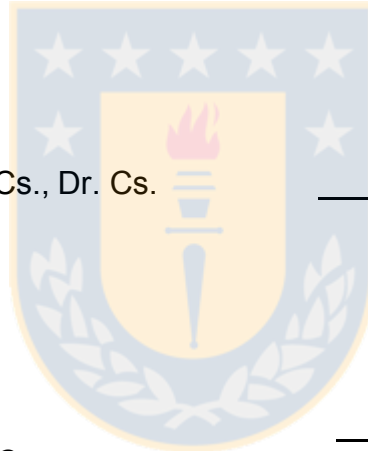
Felipe Laurie Gleisner
Ing. Agrónomo, MSc, PhD
Profesor Asociado

Profesor Guía

Ignacio Serra Stepke
Ing. Agrónomo, MSc, PhD
Profesor Asistente

Profesor Co-Guía

María Dolores López Belchí
Licenciada en Química, Mg. Cs., Dr. Cs.
Profesora Asistente



Evaluador Interno

Inés Figuera Cares
Ing. Agrónomo, Mg. Cs., Dr. Cs.
Profesor Asistente

Directora del Programa

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RESUMEN

En la actualidad, una de las alternativas empleadas para potenciar la producción de algunos metabolitos secundarios en plantas, es el uso de diferentes sistemas de manejo tales como la intervención del microclima de la planta. Uno de los principales metabolitos en las plantas es el linalol, monoterpeneo que aporta aromas florales y picantes, así como una variedad de efectos farmacológicos potenciales (ej. efectos antioxidantes, antimicrobianos, anti-inflamatorio y previene riesgo cardiovascular). De hecho, varias especies vegetales productoras de linalol son utilizadas en sistemas de medicina tradicional. El linalol tiene mucha importancia en el aroma de las uvas de la familia de las moscatel, este compuesto está presente en contenido de 5 a 10 veces superior a su umbral olfativo. En cuanto a la producción de vino, el aroma y el sabor del producto final depende fuertemente de los compuestos químicos presentes en las uvas; los cuales, generalmente sufren cambios en su estructura y concentración durante el proceso de maduración de la fruta. El objetivo de este estudio fue evaluar la acumulación de compuestos volátiles, con énfasis en linalol, en bayas de *Vitis vinifera* cv. Moscatel de Alejandría manejadas bajo diferentes exposiciones de los racimos a la luz. El estudio se realizó en dos viñedos conducidos bajo un sistema tradicional o gobelet en el Valle del Itata, (Pinihue y Cerro Verde), Región del Biobío, Chile. Los análisis de compuestos volátiles fueron realizados mediante microextracción en fase sólida sobre el espacio de cabeza de las muestras (HS-SPME), seguido por cromatografía de gases acoplada a un detector de ionización de llama (GC-FID). Los resultados del estudio indicaron que la concentración de linalol fue superior en los tratamientos con mayor grado de exposición a la luz. El tratamiento con defoliación severa presentó concentraciones mayores en un 40% respecto al tratamiento control, lo que indica un efecto positivo de la exposición a la luz sobre este carácter aromático de la fruta. En general, altos niveles de defoliación en vides conducidas en un sistema tradicional entregan un incremento en la concentración de compuestos volátiles, particularmente en relación a los monoterpenos. Estos resultados muestran diferencias importantes en el comportamiento de la biosíntesis o degradación de linalol en respuesta al deshoje, lo cual tiene implicancias en el potencial sensorial de las bayas y el efecto en la salud humana.

INTRODUCCIÓN GENERAL

El aroma es uno de los atributos de calidad más importantes del vino (Guasch, 1999; de la Calle García *et al.*, 1996), el que se encuentra constituido principalmente por compuestos volátiles, los que se clasifican en cinco grupos: Monoterpenos (típicamente descritos como aromas 'florales'), norisoprenoides, aromas bencénicos, aromas alifáticos, y metoxypyrazinas (Williams y Allen, 1996). Estos compuestos volátiles se sintetizan durante la etapa de maduración de las bayas (Battilana *et al.* 2011, Fenoll *et al.*, 2009) y su producción dependerá de varios factores, tales como la exposición a la luz, la temperatura de la fruta, el manejo del cultivo y la fecha de vendimia (Forde *et al.*, 2011).

Entre los compuestos aromáticos del vino, los terpenos tienen un papel importante dentro del perfil sensorial de cultivares blancos como Moscatel de Alejandría (Stevens, 1996). Estos compuestos volátiles presentan concentraciones que van desde ng L⁻¹ hasta mg L⁻¹ (Ebeler, 2001; Mateo y Jimenez, 2000), donde linalol, geraniol, nerol, α -terpineol, β -citronelol, hotrienol y limoneno a menudo exhiben las concentraciones más altas en las bayas (Guth, 1997). Entre los terpenos, linalol aporta aromas florales y especiados, así como propiedades anti-neurodegenerativas que podrían ofrecer efectos positivos en la salud humana relacionados con la uva y el consumo de vino (López y Campoy, 2015; López y Pascual-Villalobos, 2015).

El sabor y el aroma del vino pueden ser influenciados por variables en todo el proceso de producción, como temperatura de fermentación, concentración de oxígeno disuelto, madurez de la uva, entre otras. Si bien existen diversas técnicas de elaboración del vino que pueden modificar sus atributos sensoriales, el resultado de este tipo de intervenciones dependerá de la composición de la fruta, debido a la presencia de compuestos de sabor y aroma varietal que se transfiere de la uva al vino con ninguna o mínima conversión química durante el proceso fermentativo (Dunlevy *et al.* 2009, Ebeler y Thorngate 2009, Robinson *et al.* 2014). Los compuestos aromáticos libres, como los terpenos, pueden ser detectados directamente por el olfato en el vino, mientras que los glicosilados (unidos a un azúcar mediante un enlace glicosídico, y por lo tanto inodoros), se manifiestan

únicamente cuando son liberados a través de diversas reacciones químicas o enzimáticas (Canosa *et al.*, 2010).

La calidad y rendimiento de las vides dependen del correcto equilibrio entre la carga frutal y el área foliar, la que debe estar iluminada adecuadamente, para la obtención de una composición aromática adecuada (Cañon *et al.*, 2014).

Uno de los trabajos publicado por Shaulis *et al.* (1982), reconoció tempranamente la profunda influencia de la formación y conducción de la vid en el ambiente lumínico dentro de los viñedos, y los efectos de la arquitectura del dosel en la productividad de la vid y la composición de la fruta.

Gracias al desarrollo de la investigación en las prácticas culturales como la poda, el sistema de formación, arreglo de árboles, y el diseño del huerto, dirigidas a mejorar la "cantidad de luz" (es decir, la cantidad de radiación fotosintéticamente activa, PAR) interceptada y distribuido por los huertos (Bastias y Corelli-Grappadelli, 2012), se ha logrado optimizar la intercepción de luz en algunos cultivos.

En vides, es bien sabido que la fruta bien expuesta a la luz presenta mostos con mayores concentraciones de azúcar, antocianinas y fenoles totales, así como niveles más bajos de ácido málico, potasio y pH (Kliwer y Smart, 1989). Sin embargo, el grado de exposición de la fruta contribuye a la producción de una gran variedad de compuestos químicos, lo que estimula diferentes respuestas en la composición del aroma en las bayas y la calidad de la fruta. Por ejemplo, la fruta y la calidad del vino de variedades de uva Moscatel, ampliamente reconocidos por sus propiedades aromáticas intensas, pueden mejorar mediante el aumento de la penetración de la luz en la zona de los racimos (Macaulay y Morris, 1993). Por otro lado, la exposición excesiva a la luz puede reducir el contenido de terpeno en las bayas (Belancic *et al.*, 1997). Así, mientras que el efecto positivo de la penetración de la luz en la calidad de la fruta de uva Moscatel está directamente relacionada con la síntesis de compuestos volátiles (Boss, 2014), la influencia negativa de la sobre exposición es probablemente debido al efecto de las altas temperaturas influenciando en la concentración de terpenos, ácidos, y otros compuestos de importancia enológica (Belancic *et al.*, 1997).

En una escala más amplia, diferentes zonas vitícolas con diferentes condiciones climáticas pueden presentar variaciones importantes en el perfil aromático (Heymann y Noble 1987; Lund *et al.* 2009). Por ejemplo, se ha informado que la oscilación térmica entre el día y la noche puede influir en la interacción entre los compuestos aromáticos no volátiles y volátiles, como ocurre con los polifenoles (Heymann y Noble 1987; Lund *et al.* 2009).

Agosin *et al.* (2000) compararon los niveles de terpenos de Moscatel de Alejandría de la zona de Pisco con los reportados en Francia por Gunata (1985), concluyendo que la composición terpénica de la Moscatel de Alejandría de la zona pisquera es marcadamente diferente de su homólogo francés, pudiendo deberse a las diferencias climáticas, así como el hecho de que las vides chilenas no han sido injertadas, aunque tales consideraciones aún no se han estudiado formalmente.

Otras investigaciones en el extranjero, realizadas en variedades de alto contenido terpénico como Moscatel de Alejandría, Riesling y Gewürztraminer, son congruentes en señalar la importancia del clima y la tipicidad del lugar donde se cultiva. Así, resultados reportados en Canadá por Reynolds y Wardle (1997), señalan que la exposición de los racimos, la manipulación del dosel, las prácticas de manejo, la prefermentación y sitio del viñedo pueden influir en el contenido de monoterpenos de las bayas y mostos de varios cultivares de *Vitis vinífera* L.

En cuanto a la vitivinicultura chilena, la situación actual de la Región del Biobío es distinta a la del centro y norte de Chile. La gran cantidad de pequeños agricultores, los tipos de cultivares que se producen (ej. cv. tradicionales como Moscatel de Alejandría, el 95 % de la existencia país, se encuentra en la región del Biobío la cual se emplea principalmente para vinificación de vinos a granel (SAG, 2014), las condiciones edafoclimáticas, la falta de tecnología en las bodegas, entre otros factores, han dificultado un desarrollo vitivinícola en la región. Favorablemente, ciertos cultivares tradicionales, como Moscatel de Alejandría, y algunas zonas productivas dentro de la región del Biobío están presentando mejoras en el manejo agronómico, por lo que ha vuelto más atractiva la producción de vinos de calidad, situación que ofrece un nuevo potencial para la vitivinicultura de la región. Una característica particular de Moscatel de Alejandría en la región del Bio Bio,

Chile, es el uso del sistema de formación en *gobelet* (o arbolito), que no tiene una estructura permanente que permita la disposición de los brotes para mejorar la intercepción de luz y rendimiento en las vides. En general, estos tipos de viñedos se caracterizan por bajas tasas de crecimiento vegetativo, pero contrariamente presentan follajes densos (Lacoste, 2010). La ventaja de este sistema es que las vides están cerca del suelo, lo que favorece la emisión de radiación desde el suelo a las bayas, lo que puede acelerar la maduración en climas fríos. Por el contrario, la proximidad al suelo en climas cálidos puede ser una desventaja, debido al impacto de las altas temperaturas sobre la composición de las bayas (Coomby y Dry, 2006) y su posible deshidratación (Dai, 2013).

Considerando todo lo anterior, en este estudio analiza el perfil aromático de Moscatel de Alejandría conducidas en un sistema tradicional o *gobelet*, mediante diferentes tratamientos de defoliación en el envero, con un enfoque particular en los terpenos.

HIPÓTESIS

1. La arquitectura del follaje en vides cv. Moscatel de Alejandría, bajo un sistema de conducción en cabeza, determina que exista una alta variabilidad en los niveles de radiación y temperatura a nivel de racimos.
2. El deshoje parcial de brotes post-pinta determina un aumento de la radiación y temperatura a nivel de racimos, así como un aumento en la concentración de terpenos en bayas de vides cv. Moscatel de Alejandría bajo sistema de conducción en cabeza.

OBJETIVO GENERAL

Caracterizar el ambiente lumínico-térmico y determinar la influencia del manejo de follaje en la composición de terpenos de las bayas de Moscatel de Alejandría en dos viñedos conducidos en cabeza en el valle del Itata.

OBJETIVOS ESPECÍFICOS

1. Caracterizar el ambiente lumínico (intensidad de radiación) y la temperatura

a nivel de racimos desde cuaja a madurez de bayas de vid cv. Moscatel de Alejandría conducidas en cabeza.

2. Determinar el efecto de tres niveles de exposición a la luz solar en la producción de terpenos en bayas del cv. Moscatel de Alejandría.

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CAPÍTULO I

CHANGES IN LINALOOL CONCENTRATION IN RESPONSE TO DEFOLIATION OF MUSCAT OF ALEXANDRIA GRAPEVINES GROWN UNDER A TRADITIONAL FARMING SYSTEM

Guillermo A. Pascual^{1*}, Ignacio Serra¹, Arturo Calderón-Orellana¹, V. Felipe Laurie² and María Dolores López.¹

1) Department of Vegetal Production, Faculty of Agronomy, Universidad de Concepción. Av. Vicente Méndez 595, Chillán, Chile

2) Department of Horticulture, Faculty of Agricultural Sciences, Universidad de Talca. Campus Lircay s/n, Talca, Chile.

*Corresponding author: e-mail: gpascual@udec.cl

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ABSTRACT

BACKGROUND: One of the current approaches to enhance the production of secondary plant metabolites is the use of different crop management and environmental systems. Linalool is a monoterpene, which exhibits floral and spicy aromas as well as a variety of pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory and cardiovascular. In fact, several linalool-producing species are used in traditional medical systems. In terms of wine production, flavor of the final product is highly dependent on the chemical compounds present in the grape, which changes as fruit ripens. The objective of this study was to evaluate the accumulation of volatile compounds, especially linalool, in grapes from *Vitis vinifera* cv. Muscat of Alexandria grown under different cluster light exposure by using headspace solid-phase microextraction (HS-SPME), followed by gas chromatography coupled to a flame ionization detector (GC-FID). The study was conducted in two different locations of the Itata Valley in Chile (Pinihue and Cerro Verde), with a solar radiation of 25.42 MJ/m².

RESULTS: Linalool concentration was more prevalent in treatments with higher cluster exposure. The treatment with the highest defoliation showed a linalool

concentration 40% higher than the control treatment, which indicates a positive effect of light exposure on aroma.

CONCLUSIONS: Higher levels of defoliation in grapevines grown under a traditional farming system result in an increased concentration of volatiles compounds, particularly monoterpenes. These results revealed important differences in the behavior of the synthesis of linalool. This compound has been identified as relevant to sensorial properties and health-related issues.

Keywords: Aroma, radiation, monoterpene fraction, wine, grape.

INTRODUCTION

Aroma is one of the most important quality attributes of wine^[1,2]. It consists mainly of volatile compounds that are classified into five groups: monoterpenes (typical of the so-called 'floral' grapes), norisoprenoids, benzenic aromas, aliphatic aromas, and methoxypyrazines.^[3] These aroma compounds are synthesized during the ripening stage^[4,5] and their production depends on several factors, such as light exposure, fruit temperature and crop management.^[6]

Among the aroma compounds of wine grapes, terpenes have a very important role in the flavor profile of white varieties like Muscat of Alexandria.^[7] These wine compounds have concentrations that range from ng L⁻¹ to mg L⁻¹,^[8,9] in which linalool, geraniol, nerol, α -terpineol, β -citronellol, hotrienol and limonene often exhibit the highest concentrations in grape berries.^[10] Among the terpenes, linalool contributes floral and spicy aromas as well as anti-neurodegenerative properties that form part of the health effects related to grape and wine consumption.^[11,12]

Both aroma and taste (i.e., flavor) of wine can be influenced by a wide range of variables, including agricultural management,^[13,14,15] vineyard location, and harvest time. Therefore, fruit quality and yield of wine grapes depend on achieving an adequate balance between fruit load and a properly illuminated leaf area, which constitutes an essential requisite for obtaining a suitable aroma composition.^[16]

It is well known that fruit exposed to UV-light generally exhibits higher concentrations of sugar, anthocyanins, and total phenols, but lower levels of malic acid, potassium, and pH than shaded fruit.^[17] However, the degree of fruit exposure

contributing to the production of a great variety of chemical compounds may induce different responses in berry aroma composition and fruit quality. For example, fruit and wine quality from Muscat grape varieties, which are widely recognized for their strong aromatic properties, can be improved by increasing light penetration at fruit zone.^[18] On the other hand, excessive fruit exposure may reduce terpene content of berries.^[19] Whereas the positive effect of light penetration on fruit quality of Muscat grapes is directly related to the synthesis of volatile compounds,^[20] the negative influence of fruit exposure is probably due to the effect of high fruit temperatures on the concentration of terpenes, acids, and other compounds of oenological importance.

In a broader scale, different viticultural areas with varying weather conditions may present important variations in the aroma profile of berries. For instance, thermal oscillation between day and night has been reported to influence the interaction between non-volatile and volatile aroma compounds, as occurs with polyphenols.^[21,22]

A particular feature of Muscat of Alexandria in the Itata Valley (Bio Bio Region, Chile) is the use of the *gobelet* (or *bush vines*) training system, which does not have a permanent structure to allow the arrangement of shoots to improve light interception and grape yield. In general, these types of vineyards are characterized by low vegetative growth rates, but dense canopies.^[23] The advantage of this system is that vines are close to the ground, favoring the emission of longwave radiation from the soil to the fruit, which may accelerate ripening in cool climates. In contrast, proximity to the soil in warm climates may be a disadvantage due to the impact of high temperatures on fruit composition ^[24] and berry water budget (dehydration).^[25]

This study analyzes the aroma profile of Muscat of Alexandria berries produced in gobelet-trained grapevines subjected to different defoliation treatments at *veraison*, with a particular focus on terpenes.

MATERIALS AND METHODS

Chemicals and reagents

Linalool ($\geq 97\%$), nerol ($\geq 97\%$), geraniol (98%), β -citronellol (95%), α -terpineol (90%), benzaldehyde (99.5%), benzyl alcohol (99.8%), hexanal (98%), *trans*-2-hexenal (98%), 1-hexanol ($\geq 98.5\%$) were used as standards to identify the main aromas present in grapes as a result of light and temperature conditions. All these reagents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, United State).

Location

This study was carried out in two commercial gobelet-trained vineyards of Muscat of Alexandria. These vineyards were located in two contrasting viticultural areas that represent different edaphoclimatic conditions of the Itata Valley, Bío Bío Region, Chile. One vineyard was located in Cerro Verde (36° 44' S, 72° 27' W), Ránquil and the other was located in Pinihue (36°36' S 72°44'W), Coelemu, near the coast of the Itata Valley. Both vineyards were 25 years old, and planted with a row and vine spacing of 1.5 m x 1.5 m, respectively. Grapevines were on a hillside facing northeast, were not irrigated and trained as bush vines with trunks ranging between 30 and 70 cm height.

Soil samples were taken from both sites. The results of their chemical composition are shown in Table 1. Weather conditions were also measured during the study and data obtained are summarized in Table 2.

Treatments

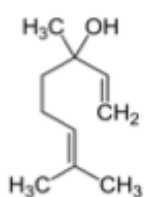
Four defoliation treatments were imposed at *veraison* in order to determine the effect of canopy management on the concentrations of monoterpene and volatile compounds: Intense defoliation (T1): defoliation until light at fruit zone reached between 60% and 80% of the outside radiation; Partial defoliation (T2): bunches defoliated until light at fruit zone reached between 40% and 60% of outside radiation; Semi-shaded clusters (T3): cluster with 20-40% incident light; and a control treatment with non-intervention during season 2014/2015.

Incident light at fruit zone was measured at midday (12:00 to 3:00 pm) using a

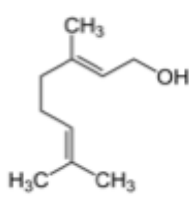
portable quantum sensor (LI-191, LI-COR Bioscience, Lincoln, NE, USA).

The experiment used a completely randomized block design, in which each defoliation treatment was replicated three times in 100 m² plots. Each block-treatment combination consisted of four representative plants, with two buffer grapevines between each plot.

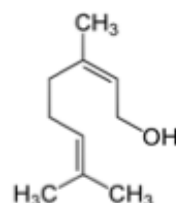
Monoterpenes



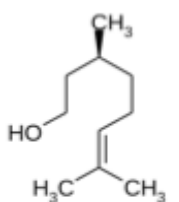
Linalool



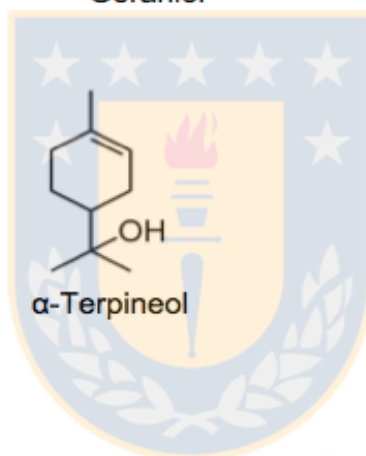
Geraniol



Nerol

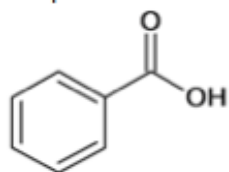


β -Citronellol

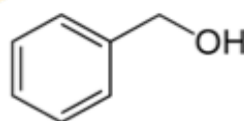


α -Terpineol

Benzenic compounds

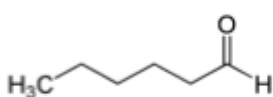


Benzaldehyde

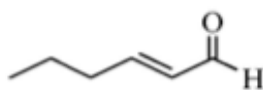


Benzyl alcohol

Aliphatic compounds



Hexanal



trans 2-Hexenal



1-Hexanol

Figure 1. Main volatile compounds of *Vitis Vinifera* cv. Muscat of Alexandria. source: prepared by autor.

Table 1. Soil chemical composition in the experimental sites.

Paramenter		Cerro verde		Pinihue	
			level		Level
pH in water		5,46	LOW	5,86	LOW
Organic Matter	%	0,89	LOW	2,05	LOW
Nitrates (N-NO3)	mg/Kg	1	LOW	1,3	LOW
Ammonium (N-NH4)	mg/Kg	5,3	LOW	5,4	LOW
Nitrogen available	mg/Kg	6,3	LOW	6,7	LOW
Olsen Phosphorus	mg/Kg	3,1	LOW	5,7	LOW
K available	mg/Kg	67,9	LOW	288,5	HIGH
K interchangeable	cmol/Kg	0,17	LOW	0,74	HIGH
Ca Interchangeable	cmol/Kg	3,52	LOW	2,72	LOW
Mg interchangeable	cmol/Kg	1,43	HIGH	0,65	MEDIUM
Na interchangeable	cmol/Kg	0,09	LOW	0,04	LOW
Sum of the Bases	cmol/Kg	5,21	MEDIUM	4,16	LOW
Al interchangeable	cmol/Kg	0,99	HIGH	0,15	MEDIUM
CICE	cmol/Kg	6,2	MEDIUM	4,31	LOW
AL saturation	%	15,98	HIGH	3,55	HIGH
K saturation	%	2,81	LOW	17,16	HIGH
Ca saturation	%	56,68	LOW	63,22	LOW
Mg saturation	%	23,06	HIGH	15,06	HIGH
S available	%	67,9	HIGH	21,6	MEDIUM
Fe	mg/Kg	4,8	HIGH	7,8	HIGH
Mn	mg/Kg	2,4	LOW	20,4	MEDIUM
Zn	mg/Kg	0,2	LOW	0,1	LOW
Cu	mg/Kg	0,2	LOW	0,9	HIGH
B	mg/Kg	0,9	MEDIUM	3,3	HIGH

Source: soils Laboratory, University of Concepción.

Table 2. Means of Relative humidity (%), Temperature (°C), Soil temperature 10cm (°C) and Radiation (MJ/m²) recorded in Itata Valley, Chile from December to March 2014-2015.

		HR (%)	Temperature (°C)	Soil Temperature 10cm (°C)	Radiation (MJ.m ⁻²)
	Dec	53.0	19.4	29.5	27.9
Itata	Jan	48.1	22.6	31.1	29.7
Valley	Feb	47.4	21.7	27.5	25.1
	Mar	54.3	20.3	23.1	19.0

source: prepared by autor with own data.

Microclimate conditions

Air temperature was analyzed in both study sites using two data loggers (TinyTag model TALK2 TK-4023). One of the data loggers was placed above plant level to record canopy temperature of the plots. The other data logger was installed at the center of the canopy in T4 to measure air temperature in each site. Temperature was recorded on an hourly basis from December 2014 to March 2015.

Values obtained during berry ripening (December 2014 to March 2015) were divided into day (average readings between 8:00 a.m. to 6:00 p.m.) and night (average readings between 7:00 p.m. to 07:00 a.m.), and separated by temperature intervals. Ranges used for daytime temperatures were $<20^{\circ}\text{C}$; $\geq 20^{\circ}\text{C} < 25^{\circ}\text{C}$; $\geq 25^{\circ}\text{C} < 30^{\circ}\text{C}$; $\geq 30^{\circ}\text{C} < 35^{\circ}\text{C}$; $\geq 35^{\circ}\text{C}$, while night intervals corresponded to $\leq 12^{\circ}\text{C}$; $> 12^{\circ}\text{C} \leq 14^{\circ}\text{C}$; $> 14^{\circ}\text{C} \leq 18^{\circ}\text{C}$; $> 18^{\circ}\text{C}$.

Light Exposure

Regarding photosynthetically active radiation (PAR), values were recorded within the canopy at heights of 30 cm and 15 cm above ground and at ground level, using a portable Quantum Sensor LI-191sa connected to a data logger LI-1400 (LICOR, Lincoln, NE, USA). Light was recorded three times a day (10 a.m., 12 noon and 2 p.m.).

Data collection started when clusters had 5% to 15% soft berries and were changing color (*veraison*) and continued until one week before harvest.

Photosynthetic active radiation (PAR $\mu\text{mol}^{-2} \text{s}^{-1}$) throughout the season was obtained using five measurements in the control treatment for each site.

Berry sampling

All clusters from the four plants selected for each treatment were harvested. This was carried out using a ripening level between 22–23°B. Two hundred berries were removed from the clusters for each replicate corresponding to the four treatments. They were crushed in order to analyze titratable acidity, soluble solids, and volatile compounds. Titratable acidity was measured by titration with NaOH and was expressed as g tartaric acid L^{-1} ; soluble solids were analyzed using a digital refractometer (Pocket PAL-1, Atago, Japan). For aroma analyses, samples were placed inside hermetic sealed bags, frozen and kept at -80°C until chemical analyses were performed.

Aroma analysis

Analyses were conducted using solid phase micro-extraction, SPME, followed by gas chromatography coupled with a flame ionization detector, FID. Sample pre-treatment for volatile analyses was performed as follows: 90 g of pulp was mixed with 30 mL of NaCl solution (20%) using a homogenizer model Wids MSH-20A for 10 min. The mixture was centrifuged at 1.826 g and the supernatant was placed in a 15 mL vial. Then, samples were heated at 40°C for 15 min and SPME fiber was exposed to the headspace during 30 min. Desorption was carried out for two minutes in the injection port of a gas chromatograph (GC, Varian 3900) coupled with FID. The chromatographic separation was performed on a CP-Wax 52 CB column under the following conditions: Injector T° 260°C; oven T° started at 40°C for one min, increasing at a rate of 5°C min⁻¹ until 200°C and then at a rate of 2°C min⁻¹ up to a temperature of 230°C, which was maintained for 10 min. Hydrogen was used as the gas carrier at a flow rate of 0.8 mL min⁻¹. As indicated before, identification of volatile compounds was conducted using pure chemical standards (Fig 1).

Statistics

All variables studied were statistically analyzed by one-way ANOVA using Infostat software (Infostat Group, Argentina). Mean values were compared using Tukey's test when all the assumptions of the ANOVA were met.

RESULTS AND DISCUSSION

Temperatures

In general, Pinihue exhibited colder nighttime and warmer daytime temperatures above the canopy compared to those recorded in Cerro Verde (Fig 2). Temperatures inside the canopy showed important differences between day and night. Pinihue had 45% of days with temperatures above 25°C and up to 35°C inside the canopy. Similarly, 57.5% of the days had the same temperatures in Cerro Verde. In Pinihue, January temperatures above the canopy level reached 37.5% for the range $\geq 30^\circ\text{C} < 35^\circ\text{C}$ and 9.7% for $\geq 35^\circ\text{C}$. Cerro Verde had fewer days with temperatures above 30°C compared to Pinihue.

Differences in nighttime temperatures during the season were observed mainly below 12°C, reaching 27% in Pinihue and 12% in Cerro Verde. Similarly, Pinihue reached

23.8% within the range $< 12^{\circ}\text{C}$, while only 8.7% was recorded in Cerro Verde.

Light exposure

The results showed values close to $2,000 \mu\text{mol}^{-2} \text{s}^{-1}$ during December and January in both experimental sites, and values close to 1,500 and $1,700 \mu\text{mol}^{-2} \text{s}^{-1}$ during February and March in Pinihue and Cerro Verde, respectively. These values were recorded at a height of 30 cm above ground level and the canopy (Fig 3).

As expected, incident light inside the plant decreased as measurements were made closer to the ground in both experimental sites. Values obtained at 15 cm height ranged between 1,000 and $800 \mu\text{mol}^{-2} \text{s}^{-1}$, while those obtained at ground level fell below $100 \mu\text{mol}^{-2} \text{s}^{-1}$ during the season.

Temperature of berries in the field is usually regulated by the radiation flux density absorbed and convection heat loss. Moreover, berry temperature increases linearly with incident radiation.^[26]

It is important to notice that some values showed irregular patterns in both sites. This may be due to free training systems, in which foliage does not present reinforcement to prevent the movement produced by wind and light variations that may occur. These variables could produce changes in light interception.

Incident light values recorded at midday for all levels were higher compared to those recorded at 10:00 a.m. and 2:00 p.m., except at 30 cm with higher values at 2:00 p.m. in both sites.

Fruit maturity

In general, severe defoliation caused small changes in fruit maturity. However, the effect of the treatment was not the same in both study sites. Whereas T1 exhibited slightly higher Brix and TA than the control treatment in Pinihue, no effects were observed in Cerro Verde (Table 3). Higher PAR and daytime temperatures in Pinihue may explain higher Brix values of severely defoliated vines.^[27] However, no known reason can explain why T1 showed higher TA than the control treatment since changes in radiation and temperature are not generally related to increases in TA.^[28]

Concentration of volatile compounds

The content of volatile compounds identified in both experimental sites through SPME-GC/FID are shown in Table 4.

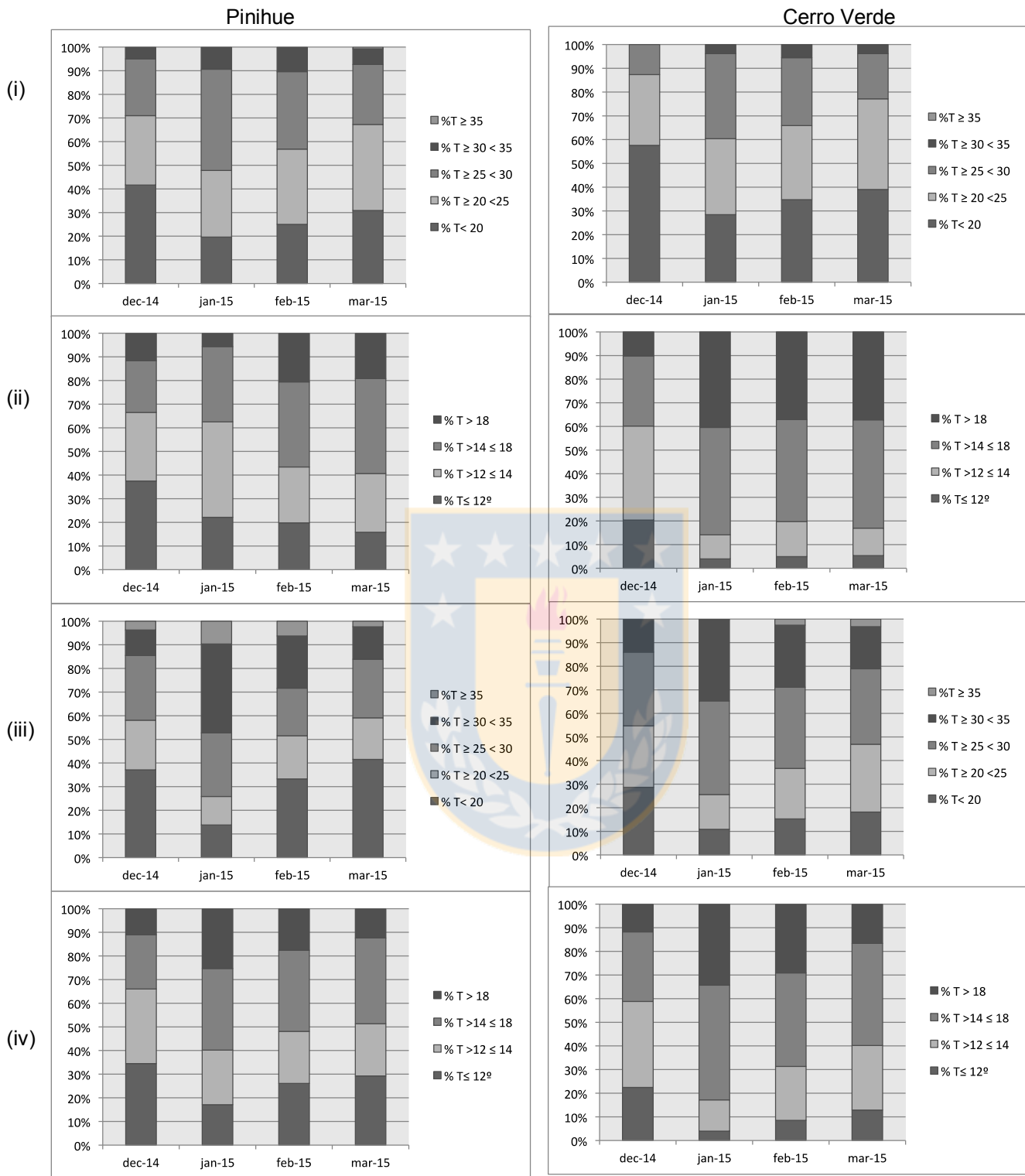


Figure 2. Day and night temperatures recorded during the period December 2014 - March 2015 in both experimental sites: (i) above canopy - day; (ii) above canopy - night; (iii) within the canopy - day; (iv) within the canopy - night. (source: prepared by autor with own data)

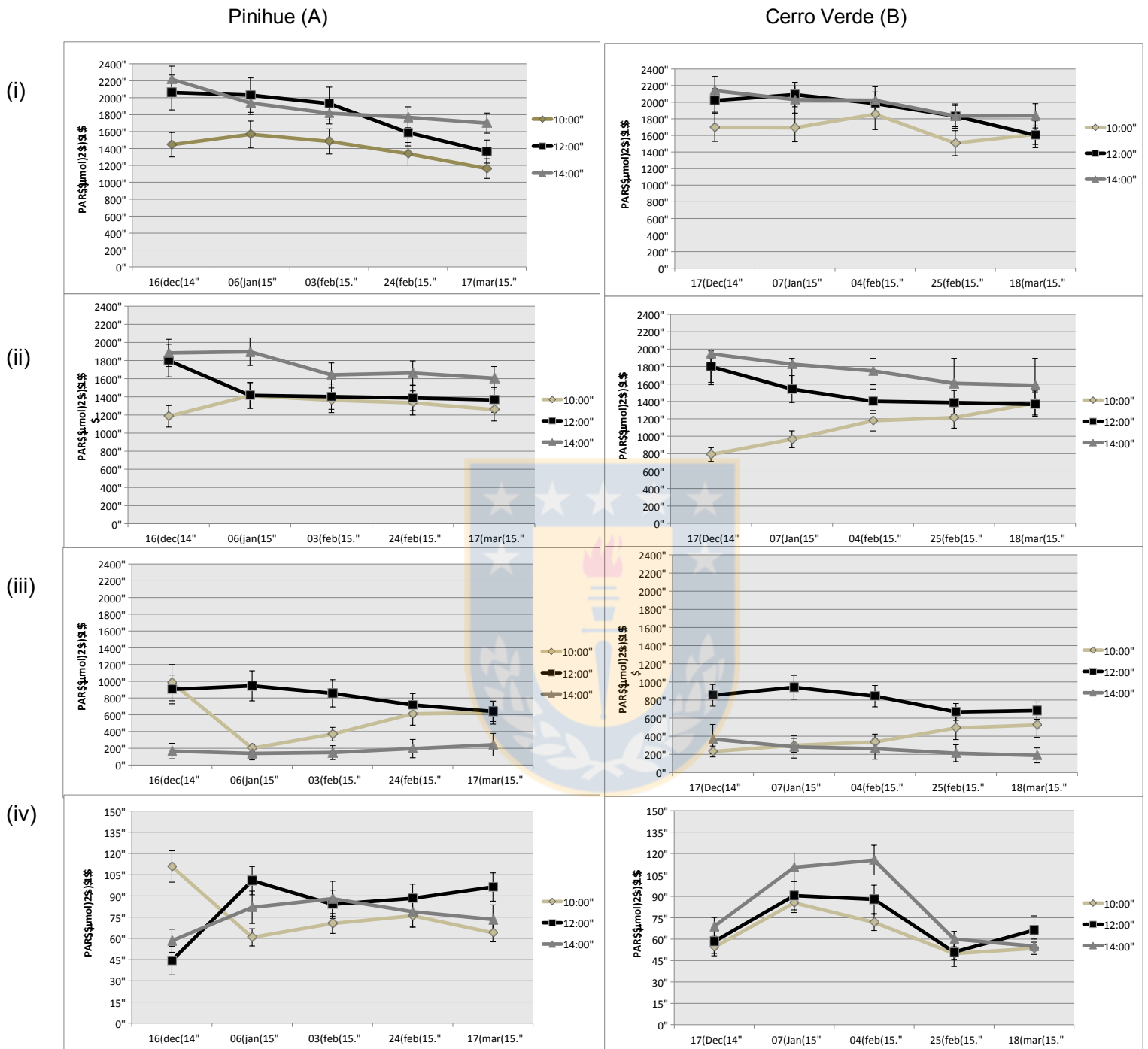


Figure 3. Photosynthetically active radiation (PAR, $\mu\text{mol}^{-2} \text{s}^{-1}$) for both experimental sites (A) Pinihue; (B) Cerro Verde. (i) Measurements above canopy; (ii) 30 cm; (iii) 15 cm; and (iv) ground level. Measurements were taken between December 2014 and March 2015: Measurements were made at 10:00 am, 12:00, and 2:00 pm. Error bars represent standard error from mean (n=6). (source: prepared by autor with own data)

Intense defoliation (T1) resulted in higher concentrations of linalool compared to the control treatment in both sites, regardless of differences in climatic conditions and viticultural management. On the other hand, geraniol, nerol and α -terpineol, were significantly higher only in Cerro Verde. These results highlight the importance of canopy management in the profile aroma of Muscat of Alexandria since microclimate conditions seem to be more relevant to determine linalool concentration in berries than differences in mesoclimatic characteristics.

Table 3. Measurement of Brix and total acidity in *Vitis vinifera* cv. Muscat of Alexandria at harvest in both experimental sites (Pinihue and Cerro Verde).

Treatment	Pinihue		Cerro Verde	
	Brix	Titrateable acidity g/L H ₂ SO ₄	Brix	Titrateable acidity g/L H ₂ SO ₄
T1	23.1 a	3.5 a	22.6 NS	3.1 NS
T2	22.6 ab	3.4 a	22.5	3.0
T3	22.3 bc	3.3 a	22.4	3.0
Control	21.7 c	2.9 b	21.2	2.7

Values with different letters in the same column indicate significant differences (P<0,05)

NS.: Not significant.

source: prepared by autor with own data.

Conversely, other important monoterpenes, such as α -terpineol, geraniol, and nerol, were more sensitive to mesoclimatic differences and the effect of defoliation was not consistent. The influence of mesoclimatic conditions on the effect of canopy management on monoterpene accumulation has been reported by Skinkis *et al.*,^[29] in Gewürztraminer vines, in which defoliated vines showed no changes in monoterpene concentrations when vineyards were established in warm areas. Canopy temperatures during the day suggest that vines from Pinihue have more open canopies than those from Cerro Verde. Therefore, vines from Pinihue may be more exposed to the effect of sunlight on berry temperature, which could be related to the lack of consistency in terms of the effects observed on α -terpineol, geraniol and nerol.

The results of the present study showed that α -Terpineol was the most abundant monoterpene in berries from both experimental sites, which is not in agreement with previous studies. Materase *et al.*,^[30] reported that linalool was the main monoterpene in grape berries, while another study conducted by Del Caro *et al.*^[31] reported higher

concentrations of geranic acid than linalool, which is widely regarded as the most abundant terpene in white wines.^[32]

Severely defoliated vines exhibited also higher concentrations of benzyl alcohol than the control treatment in both sites. Benzyl alcohol is a varietal compound that occurs in berry skins in the free state.^[33] In general, benzoic compounds accumulate from veraison through to the middle of the ripening stage. Thereafter these concentrations progressively decrease.^[34] This may explain the small concentrations of volatiles studied, mainly in terms of monoterpenes and particularly in Pinihue. Summer pruning, which is a method of canopy management, can be crucial in obtaining higher concentrations of volatile compounds and, therefore, improving fruit aroma potential. It should be highlighted that Linalool contributes to the very characteristic and rather strong aroma of Muscat wines, being the most abundant monoterpene found in white wines.^[35] The results obtained in this study can help improve the understanding of the relationship between canopy management and concentration of the main aroma compounds in berries and wines as well as the beneficial health effects related to moderate wine consumption.

Table 4. Content of the main volatile compounds of *Vitis vinifera* cv. Muscat of Alexandria analyzed by SPME / GC -FID in both experimental sites (Cerro Verde and Pinihue).

Compounds	n° Peack	T1		T2		T3		Control	
		µg/L		µg/L		µg/L		µg/L	
		Cerro Verde	Pinihue	Cerro Verde	Pinihue	Cerro Verde	Pinihue	Cerro Verde	Pinihue
<i>Monoterpenes</i>									
Linalool	1	21.7 a	19.1 A	15.9 b	12.6 B	13.8 b	16.0 B	13.2 b	12.6 B
Geraniol	2	10.5 a	ND	4.0 b	ND	3.4 b	ND	3.3 b	ND
Nerol	3	5.0 a	40.5*	2.8 b	ND	2.7 b	ND	2.1 b	ND
b-Citronellol	4	3.1*	ND	ND	ND	1.9*	ND	1.9*	ND
a-Terpineol	5	30.7 a	41.2*	27.5 a	50.7*	25.2 ab	32.6*	20.6 b	49.6*
<i>Benzenic</i>									
Benzaldehyde	6	2.2 a	1.8*	1.2 a	1.4*	1.0 ab	1.3*	1.0 b	3.2*
Benzyl Alcohol	7	245.4 a	105.0 A	195.8 b	183.3 BC	194.1 b	165.3 B	186.2 b	232.6 C
<i>Aliphatic</i>									
Hexanal	8	54.7 a	49.3 AB	19.1 b	8.8 C	19.1 b	45.6 A	71.7 c	53.1 B
trans-2-hexenal	9	18.0 a	22.9*	15.9 b	20.4*	15.5 b	21.1*	21.4 c	28.3*
1-Hexanol	10	12.5*	2.8*	ND	ND	17.0*	9.4*	9.5*	10.6*

ND: not detected

Volatile compounds concentrations are expressed as relative concentration, using a calibration factor of 10.

Data followed by different letters within each compound are significantly different at $P < 0.05$, small letters for Cerro Verde and capital letters for Pinihue.

*: Means do not present significant differences

source: prepared by autor with own data.

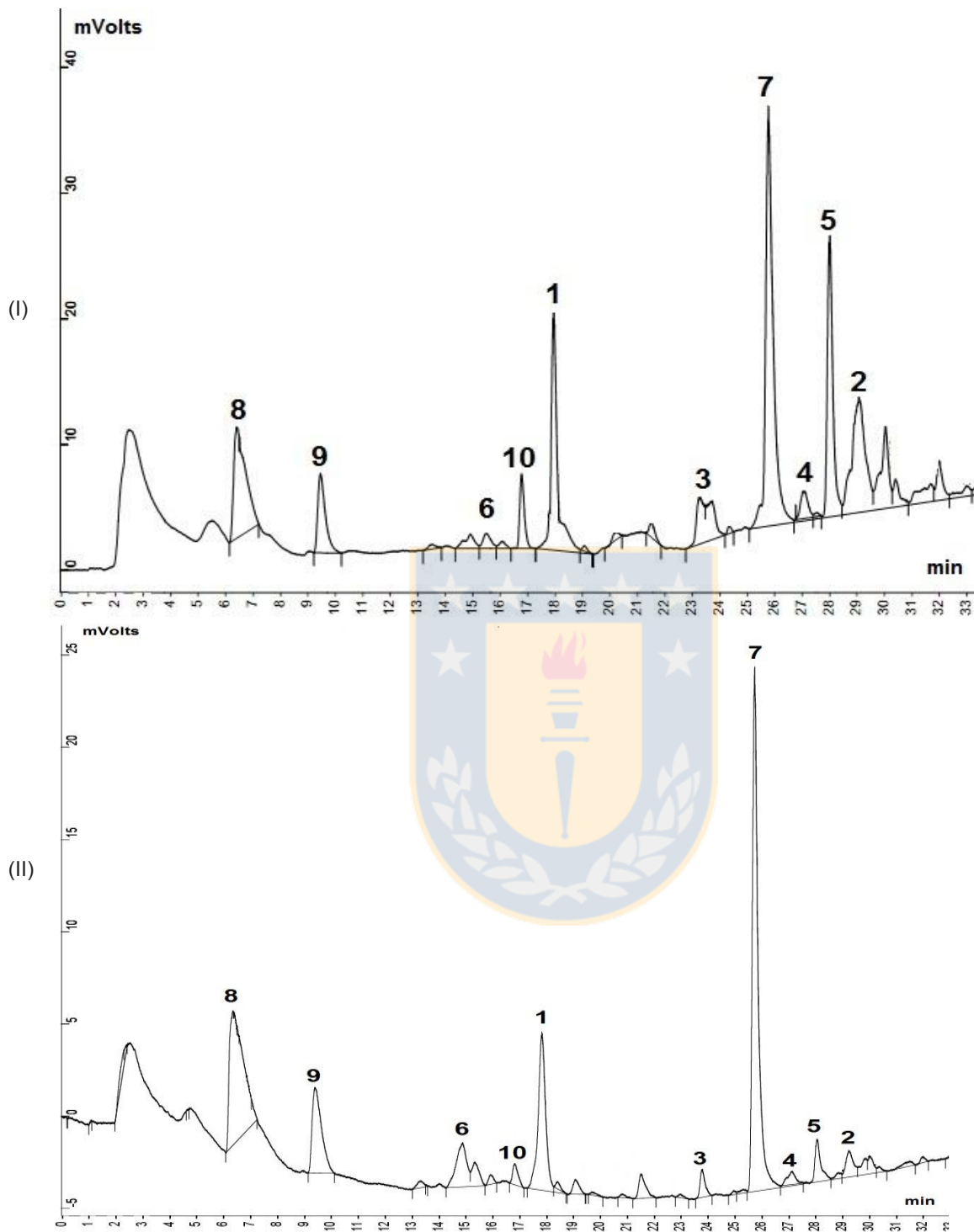


Figure 4. Chromatogram SPME/GC-FID of volatiles compounds treatment T1, obtained from Muscat of Alexandria grapes in both experimental sites; (I) Cerro Verde; (II) Pinihue. (source: prepared by autor with own data)

CONCLUSIONS

Light interception at fruit zone is determined to a large extent by the training system used in vineyards. In the present study, the use of the gobelet system seems to limit the accumulation of monoterpenes in berries due to low exposure to sunlight. In this context, more severe defoliation (60-80% of fruit light exposure) of gobelet-trained vines increased concentrations of volatiles compounds, particularly monoterpenes. Although linalool was not the most important monoterpene found in berries, it was the only monoterpene that showed a consistent response to defoliation. This study highlighted the importance of canopy management during the ripening stage to determine the profile aroma of Muscat of Alexandria. These results provide useful information for sustainability of traditional Chilean systems and production of high quality must with beneficial health effects.

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CONCLUSIONES GENERALES

1. La intercepción de luz en la zona de la fruta presenta una gran variabilidad en vides del cv. Moscatel de Alejandría conducidas en cabeza sin un sistema de conducción que permita acomodar los brotes durante el periodo de maduración.
2. Una defoliación severa aumenta la concentración de monoterpenos en las bayas de Moscatel de Alejandría, especialmente linalol, en comparación a el tratamiento testigo sin intervención.
3. Los tratamientos con defoliación parcial no se asocian a una mejora en la concentración de compuestos volátiles en vides cultivadas bajo un sistema tradicional de cultivo.
4. La variabilidad climática presente en el valle del Itata, influiría de manera significativa en las características distintivas de las uvas.

