



**UNIVERSITY OF TALCA
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Effect to use a product based on kaolinite (Nufresh) and shading nets on grapevines (*Vitis
vinifera* L.) cv. Cabernet Sauvignon.
Project Nutriprove S.A. and Nufarm.Chile Ltda

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RESUME

In order to evaluate two methods of control of sunburn in grapes, a study was conducted during the 2011-2012 season, in a trade headquarters cv. Company Cabernet Sauvignon "Viña San Pedro" located in Molina, Curico Valley, at Maule Region (35 ° 06 'S, 71 ° 20' LW WGS84, 230 m). The vineyard is located on a floor belonging to the series Condell, corresponding to the order Mollisol, established in 1994, with an orientation of north-south rows conducted on a vertical shoot.

Three treatments were established: i) application of Nufresh (kaolinite based product) at a dose of 7 to 12 kg per hectare of product, every 15 to 20 days (depending on growth stage) from the curd (berry diameter 5.22 mm) and up to 1 month before harvest (7 total applications), ii) installing a shading net (35% shade) black on bunches from post-to pre-harvest fruit set and iii) a control treatment. The trial was arranged in a completely randomized design with 3 treatments and 3 replications. During the course of the season, physiological variables was evaluated as plant gas exchange (stomatal conductance (gs)), transpiration (E) and assimilation (A)); measurements xylem water potential, temperature of the berry, fruit and leaf reflectance (350 to 2500 nm).

Prior to harvest, we measured the incidence and severity of berries/dried grapes, and harvest time, variables were evaluated chemical composition of the fruit as sugars, acids and phenolic compounds.

The results indicated that both, Nufresh application at the dose used in this study as the use of shading nets, no significant changes on plant physiological variables (gs, E and A) or xylem water potential (Ψ_x) at different growth stages of the grapevine. Regarding the energy reflectance and berry temperature, significant changes were observed depending on the date of evaluation, suggesting that both treatments, shading nets and Nufresh-produced a decrease of the temperature of the fruit, and in the Nufresh case, an increased ultraviolet light refection. For its part, the values of incidence of sunburn (any amount of damage) were 21%, 44% and 84% for treatments with mesh, Nufresh and control, respectively. Furthermore, the severity of damage to significant differences, were generally less affected clusters mesh corresponded to treatment, followed by treatment Nufresh and control.

Finally, there was no statistically significant difference in chemical composition variables assessed fruit, except for a tendency to a lower content of total phenolic compounds in the fruit of treatment with shading nets, which were not collected in the case of color measurement.

INTRODUCTION

In recent decades, the Chilean wine industry has made significant progress in terms of productivity and quality, the latter being one of the cornerstones of the national wine industry. The advances made have been obtained largely due to the incorporation of productive handling in the vineyard, among which are those performed to foliage (Lavin et al. 2001).

Among others, these changes have sought to address some of the problems that plague productive wine production, such as those derived from thermal stress or lighting.

As in other plant species, the combined effect of high temperatures and exposure to ultraviolet radiation produce burns on the cell tissues of the skins on the grapes, which can result in later stages, in dehydration of the fruit (Spayd et al. 2002). In the case of grapes for wine making, anecdotal evidence suggests that 5 to 15% of the grapes will be affected as a result of this problem (Greer et al. 2006), resulting in a reduction in the quality of the fruit, its prices, and possibly on the quality of the wines. In recent years, in central of Chile, where grow the most of the grapes for wine making, there has been an increase of 5-7% of the solar radiation incident on the period of maturation of the berries. Also, the average maximum temperatures during the ripening period of the grapes in years under the climatic effects of La Niña have been up to 1 ° C higher than in normal years (climate records from the University of Talca). In addition, we estimate that in 40 years, temperatures could rise by 4 ° C (Minetti et al. 2003; Santibañez and Santibañez, 2008).

Besides, canopy management in the case of vines for wine making trellising systems established vertical, has resorted to the use of shading nets, willing to level clusters to reduce the effects of sunburn, a situation that required a significant number of man days for installation and removal, resulting in increased production costs. In other fruit species, wherein the color development requires a greater level of exposure to direct radiation, as in the case of apples; the use of sunscreens on the basis of kaolinite (Suncrops or Fresh)) has been used successfully (Glenn et al. 2002) .

In the case of vines for wine production, there is little history of the use of this type of products in commercial production; only as scientific assessments (Ou et al., 2010, Glenn et al., 2010, Shellie and Glenn 2008, Tubajika et al . 2007).

Based on the above, the following objectives were defined for this study: i) To study the effectiveness of a product based on kaolinite (Nufresh) and a shading nets (Rashell type) located at the level of the clusters for controlling the incidence and severity of sunburn damage

in berries. ii) the effects of using mesh Nufresh and physiological variables on gas exchange (stomatal conductance, photosynthesis and transpiration) in different periods of fruit ripening. iii) To analyze the effects of using Nufresh and mesh on the water potential of the plant at different periods of ripening of the fruit. iv) To study the effect of temperature on the pulp and skin of the berry, and leaf and fruit reflectance (350 to 2500 nm). v) To examine the effects of the treatments described on some of the variables of major chemical composition of the fruit (eg sugars, acids and phenolic compounds).

LITERATURE REVIEW

CULTIVAR DESCRIPTION

The Cabernet Sauvignon is from the Bordeaux region in France, and it is believed that their parents would Cabernet Franc and Sauvignon Blanc. In Chile, this cultivar was introduced in the first half of the nineteenth century to the collection of varieties of the Quinta Normal, in the city of Santiago. In Chile, is also known by the synonym of Cabernet, but given its wide distribution, is also known also as Vidure, Petite Vidure, Sauvignon rouge and red Bordeaux (France) and Petit Cabernet (Morocco) (Gil and Pszczółkowski, 2007). Cabernet Sauvignon corresponds to a variety widely grown throughout the world. In Chile, by 2010, the National Register of vines for wine grapes produced by the Agriculture and Livestock Service (SAG), indicating the existence of a total of 38,425 hectares. That area is equivalent to 45% of total planted varieties in the country, which are distributed from the Atacama Region to the Bio Bio region, being the largest area in the regions of Maule and O'Higgins, with 14,784 and 16,079 hectares, respectively (SAG, 2010).

For physiological and biochemical characteristics, it is a late budding variety, which would help prevent frost damage. It is a vigorous growing shoots and erect. Their buds have fertility variable depending on weather conditions. Their clusters are cylindrical-conical, small to medium compaction winged and medium to high, showing a low incidence of the "corredura". Its berries are spherical, small to medium, black, very giving it a bluish bloom. It features a rich medium to high in sugar, good acidity, low pH medium and medium high phenolic concentration (Gil and Pszczółkowski, 2007).

GROWTH AND MATURATION OF THE BERRY

The development of wine grape berries can be divided in two main periods of growth separated by a lag phase and explained in graphic form through a double sigmoid curve (Coombe, 1976 cited in Dokoozlian and Kliewer, 2006). During stage I, there is a rapid growth of the pericarp of the berry, due to increased pericarp division of cells anticarp division mesocarp and epicarp cell, and subsequent elongation of the cell as a whole pericarp count of the second half of this phase (Harris et al. 1968; Gil and Pszczółkowski, 2007). It is at this stage where the accumulated berry organic acids, tannins, and generate some volatile compound type attributes "or herbaceous plant", remaining green and hard, while the seeds are close to their final size (Geny et al . 2003). Stage II is known as delayed development as berry growth slows, being barely noticeable occurs some softening and pigmentation acquisition berry product accumulation of anthocyanin, also

known as paint or veraison (Gil and Pszczółkowski, 2007) while the seed embryo develops and starts hardening of tissues outside of this (Geny et al. 2003). In stage III, the last stage of growth, there is a rapid reactivation berries growth as a result of cell elongation associated with the accumulation of water and carbohydrates (250 g/L). It is in the latter stage where the color rapidly increases and the concentration of sugars, while simultaneously decreasing the concentration of organic acids and aroma compounds responsible for the crops. Solar radiation and temperature during the ripening period are crucial in the composition and growth of berries during all these stages and may also cause damage to cell tissues, a situation known as sunburn (Bokoozlian and Kliewer, 2006; Gil and Pszczółkowski, 2007; Geny et al. 2003).

In the case of vines, the optimal growth temperature of the berries is in the range 25 to 30 ° C (Hale and Buttrose, 1974), although there are studies that suggest that temperature frequently exposed to sunlight berries exceed 30 ° C (Kliewer and Lider, 1968, Reynolds et al., 1986, cited in Bokoozlian and Kliewer, 2006). Also, the light incident on the fruit may also be essential for the regulation of carbon metabolism import or other assimilated in small fruits (Bokoozlian and Kliewer, 2006), so that those therapies that contribute to excessive fruit shading could be detrimental to their sensory quality.

CHEMICAL COMPOSITION OF THE BERRY

As discussed in the previous section, the development of berries follows a growth pattern of a double sigmoid curve, and in each period or growth stage differ considerably biochemical activity and composition of the berry (Geny et al. 2003). Many physical and chemical characteristics of the fruit of different cultivars of grapevines are correlated with the degree of maturity of the berries (Carroll and Marcy, 1982). Both the sensory quality of the wine and its stability rely heavily on chemical composition and concentration of berries (Liu et al. 2006). Sugars, primarily glucose and fructose, determine the content of alcohol in wine, hence the great interest generated by the known wine concentration. These sugars represent over 90% of the total final content of the berry. At the beginning of berry development is the predominant sugar glucose, while towards the end, both reach similar concentrations (Liu et al. 2006; Carroll and Marcy, 1982).

Other major chemical component of the berries, and whose concentration may vary depending on the temperature and radiation, is organic acids. Although these have a low concentration as compared with the sugars, their effects on fruit quality are important, helping to stabilize the color and impart sensations in the mouth when consuming the wine. The concentrations of malic acid and tartaric typically represent over 90% of the acidity, and the relationship between one cultivar and another cultivars, soil and climatic conditions of the place of production (Liu et al. 2006).

Thus, the production of malic acid is highly dependent on the temperature during the growing season and during phase I, which is the highest concentration present and predominates to about veraison condition, then its concentration decline until harvest. The concentration of malic acid to the maturation of the berries, as just mentioned, is temperature dependent, since it regulates the enzyme that has a respiratory coefficient which is dependent on this, whereas the tartaric acid is more difficult to metabolize and therefore is often more stable than before during the ripening of the grapes (Liu et al. 2006). Decreasing the concentration of tartaric acid as development progresses berries is the result of a dissolution due to cell expansion and growth of cells (Carroll and Marcy, 1982). A high acidity has a negative influence on the production of wine, and in terms of taste acid tartaric acid is stronger than malic acid (pKa: 3.04 vs 3.40) (Liu et al. 2006).

A third important component in the chemical composition of the berries is the concentration of phenols, especially anthocyanins, flavonols, catechins and other flavonoids. These compounds play an important role in the quality of wine, being those who endow sensory characteristics such as color and astringency (Mazza et al. 1999). Phenols, mainly tannins, are mostly in the seeds and skins of fruits, and its concentration decreases dramatically during berry maturation. In the case of anthocyanins and flavonoids, these are found in the skin of the fruit (Koyama et al. 2012; Obreque-Slier et al. 2010) of grapes. Both the concentration and composition of phenols in red cultivars varies with species, variety, season and management and environmental factors (climate, soil conditions, canopy management, crop load, irrigation management) (Jackson and Lombard, 1993). The level of solar radiation is an important parameter in the formation of phenols, which are responsible for the red coloration of grapes, as is the case of anthocyanins, it has been the subject of previous studies which have found significant differences in the concentration anthocyanins between clusters exposed to sunlight and shaded bunches (Crippen and Morrison, 1986, cited in Mazza et al. 1999). According to this, it is reasonable to think that treatments that promote wine temperature regulation and cluster-level radiation, could have an impact on the concentration of these important constituents, the potential step affecting wine grapes in question.

DAMAGE BY SUNBURN

The sunburn is a problem that affects many fruit species grown in central Chile, manifesting mostly in fruits that grow and develop in the south-west side of the plant (Yuri, 2001). One of the hypotheses that try to explain the problem is the increased ultraviolet (UV) radiation that penetrates through the planet's atmosphere. In apples, now there is another theory which refers to the above, but adds that the damage by sunburn be explained by excessive temperature in combination with high levels of UV radiation (Yuri, 2001; Flecetti and Schrader, 2009a) .

This type of cell damage to the epidermis, produces changes in the pigmentation of the skin of the fruit, showing yellow, orange and brown, which varies according to the degree of severity of the damage and the skin color of the fruit, and can be linked in some species, including fruit dehydration problems. In some cases, such as apples, it has been shown that the color change is associated with degradation of chlorophyll and carotenoids increased (β -Carotene, antheraxanthin and violaxanthin) (Flecetti and Schrader, 2009b; Merzlyack et al. 2002).

SUNBURN IN THE FRUIT, OF THE VINE

Solar radiation is essential for maturation and coloration of the berries, as well as being carried out, other important biochemical processes (photosynthesis, the accumulation of sugars, synthesis of secondary metabolites, etc..) (Chorti et al. 2010). As in other plant species, the combined effect of exposure to high temperatures and high ultraviolet radiation causes burns on the cellular tissues of the grape skins (Spayd et al. 2002). In the case of grapes for wine making, anecdotal evidence suggests that 5 to 15% of the grapes will be affected as a result of this problem (Greer et al. 2006), resulting in a reduction in the quality of the fruit, its prices, and possibly on the quality of the wines. In the case of black grapes, the effect produced by over-exposure to UV radiation of the berries is increasing the temperature of these, which generates a favorable microclimate for anthocyanin biosynthesis (Chorti et al. 2010). The effect is explained by the decrease in concentration or degradation of abscisic acid (ABA), a hormone associated with the accumulation of anthocyanins during ripening of berries to excessive temperatures (above 30 ° C). The low levels of endogenous ABA *VvmybA1* affect expression (Yamane et al. 2006), the gene encoding a transcription factor that induces the biosynthesis of anthocyanins in the skin of grape berries (Jeong et al., 2006).

Moreover, the incidence of photosynthetically active radiation (PAR) in moderate amounts, not more than 100 mol m⁻² sec⁻¹, increasing the total soluble solids content and phenols, while simultaneously decreasing the acidity, malic acid content and pH of the juice (Bergqvist et al. 2001; Bokoozlian and Kliewer, 2006). Exceeding this amount of PAR radiation, there is a gradual decrease of the total mass of the berries, causing a decrease in caliper and an increase in the rate of transpiration of the fruit and subsequent dehydration of berries (Bergqvist et al. 2001), which in extreme cases can result in cell lysis. Some researchers like Bokoozlian and Kliewer (2006) claim that the solar radiation received during development clusters, is directly related to the weight and diameter of berries at harvest time, and also influences the total sugar content. At the same time report that the stages (stages I, II and III) in which the light receiving berries has an effect on the weight and diameter of these, as well as some chemical components such as phenols, anthocyanins and tartrates.

The increases of temperature and radiation are observed in different parts of the world, including central of Chile, for this reason that the viticulture techniques of temperature regulation and radiation from the foliage are becoming increasingly relevant.

PREVENTATIVE TO SUNBURN DAMAGE ON FRUIT

There are a number of measures and / or treatments that are currently applied to prevent sunburn on fruit in various crops and grapevines are no exception. As main options are: i) the evaporative cooling method, which is based on spraying water on plants and fruits during the hour of greatest risk, ii) use of shades and mesh materials, iii) coverage of fruits with special wrappers or paper bags, iv) application of reflective materials such as kaolinite, v) chemicals (Sunshield, Vitamin E, Vapogard) (Yuri et al., 2002, quoted in Yazici et al. 2009). The shading nets are located at the level of the fruit zone. For this method to prevent sunburn some authors claim that the use of such a delay in the maturation of the grapes, but also does not affect yield components to reach harvest (Chorti et al. 2010) , making it a viable option for early crop cultivars, but not for late cultivars.

Another method used increasingly prevalent and is the application of reflective materials based on kaolinite, also known as method of application particle technology (Rosati et al 2006), which has some degree of preference over other methods because the ease of application and treatment acceptable cost. The most important property which these materials have is white in color and reflects the light capacity (Yazici et al. 2009). There are some crop management practices, such as defoliation, trellis and pruning, which can improve the amount of scattered light reaching the fruit production area of the plant. Although, there is a method that directly helps prevent sunburn damage on fruit, it is important to mention these practices, as they are made frequently and any excess exposure to sunlight will cause damage to the fruit. It should be mentioned that exposure of the fruit to sunlight can also be improved by considering the driving system and the orientation of the rows (Bergqvist et al. 2001), thus avoiding exposure to excessive clusters radiation solar. The decision of whether or not a defoliation will depend exclusively on the cultivar, taking into consideration the time or phenological stage of the crop that can do the job. Hunter et al. (1991) state that a partial defoliation (33% of the leaves of fruit zone) from the second stage of development of the berry (paint) significantly increases the concentration of anthocyanins not affect the concentration of phenols and significantly improves wine quality. Such crop management practices must not exceed the fruit exposure to sunlight.

USE OF THE KAOLINITE IN AGRICULTURE

kaolinite, $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$, chemical formula, belongs to the category of aluminum silicate minerals. is a hydrated aluminum silicate formed by the decomposition of feldspar and other

aluminum silicates clay and corresponds to a white color. It has a lot of uses in ceramics, plastics, pharmaceuticals, cosmetics, construction, electrical, chemical and agrochemical formulation. It is in this latter application that kaolinite has boomed, initially being used to control the pest attack., its use has been studied in pest control of some fruit as *Ceratitis capitata* in citrus, olive fly (*Bactrocera oleae*), olive black Conchuela (*Saissetia oleae*) and olive Cotton (*Euphyllura olivine*) (Lo Green et al. 2011 , Romero et al. 2006; Pascual et al. 2010) and pests of cereal grains during storage as the maize weevil (*Sitophilus zeamais*) (Nguenmtchouin et al. 2010), all directed in most cases to organic agriculture. As an example for explanation of the operating mechanism in the control of pests, in the case of *Ceratitis capitata* on citrus, this reduces the female oviposition, masking leaves, stems and fruits, making it difficult to recognize normal organs for oviposition distance, since in the case of females of *C. capitata* white is the least attractive for oviposition. Furthermore, kaolinite layer causes the surface of the fruit is harder and less suitable for oviposition (The Green et al. 2011). Similarly, this product has been used successfully in controlling sunburn on apples, to create a protective barrier against UV radiation in the tissue on which it is deposited.

For kaolinite using either pest control or prevention of sunburn damage, it must be diluted in water and sprayed on plants. Once dry, kaolinite forms a thin white film (typically <3 microns) on the surface of fruit and leaves. In previous studies, it was found that this film does not reduce photosynthesis and plant growth but does reduce water stress (Glenn et al. 1999; Kerns and Wright, 2000, cited in The Green et al. 2011) and photo-inhibition caused by intense solar radiation and high vapor pressure difference produced in warmer areas (Jifon and Silversten, 2003, cited in Lo Green et al. 2011). Despite this, some authors argue that under normal temperature and stress, the application of kaolinite reduces the rate of photosynthesis of the leaves covered individually. This decrease in photosynthesis in the leaves would be related to the reduction of the light that is capable of reaching the photo systems, since the reflection increases, or put another way, the absorption decreases by 20 to 40%, except situations in which plants are under stress conditions. At the same time, an alteration of the distribution of light within the canopy, especially increasing the radiation incident to the leaves that are found within this, which produces a compensation PAR reduction absorbed from leaves covered by kaolinite, overshadowing the decrease in the rate of photosynthesis of these, in some cases to result in increased yields (Rosati et al. 2006). The above highlights the importance of proper selection of time and rate of application of the product.

For the application of kaolinite based products for the prevention of damage by sunburn, this should be applied as far as possible, only the fruits or fruit production areas most at risk of developing the problem. The effect produced is kaolinite increases the reflection of the light, reflecting the ultraviolet radiation (UV) and infrared (IR) from the surface of the fruit. At the same

time, the fruit or body in general (eg leaf) reduce their temperature and in the case of apple species and stomatal conductance increases the treated leaf (Glenn et al. 2010). Currently, there is at least one other product besides Nufresh also formulated based on kaolinite, used for crop protection, trade name Surround WP™ (95% kaolinite, Engelhard Corporation, Iselin NJ, USA). For this use, the manufacturer recommends a dose of 5 kg of product in 100 L of water for applications in citrus (Lo Green et al. 2011), although some studies have made applications with a dose of 3 kg of product in 100 L of water (Pascual et al. 2010) for the same purpose in olive orchards. In the case studies of experiences in different applications in vineyards under water stress have used a dose of 60 g L⁻¹ with a wetting of 950 L ha⁻¹ (Shellie and Glenn, 2008).

MATERIALS AND METHODS DESCRIPTION OF EXPERIMENTAL DEVICE

In this study, we compared the effects of using Nufresh (based product made kaolinite) versus treatment with shading net and a control in vines for winemaking, commercially, in a vineyard of the company "Viña San Pedro". Nufresh applications were made during the fruit ripening period, and assessed their effects on physiological variables (eg stomatal conductance), incidence and severity of sunburn on the fruit, and chemical composition of the berries.

TEST OVERVIEW

The study was conducted during the 2011/2012 season on a vineyard adult, and full production in grape (*Vitis vinifera* L.) cultivar Cabernet Sauvignon, belonging to the Viña San Pedro, whose production is intended for the production of quality wine "Castillo de Molina". This vineyard is located in the town of Molina, Curico Valley, Maule Region, Chile (35 ° 06 'S, 71 ° 20' LW WGS84, 230 m). The headquarters was established in 1994, at that date, the plants are older than 18. The vines were established with a row orientation in a North-South and plantation frame 3x1,5 m. The drive system is on a vertical shoot, cordon pruning system and drip irrigation emitters 4 L h⁻¹ (a drip per plant.)

FEATURES EDAPHOCLIMATIC

The climate of this area is Mediterranean, with long dry season, characterized by having a thermal regime that varies on average from a high of 29.4 ° C and a minimum of 3.3 ° C during the summer. In addition, this area has a dry period of 6 months. The average annual rainfall in the region amounted to 676 mm, which falls during the winter months. The summer period is usually hot and dry, with 2.2% of rainfall a year, while spring is wet with 16% of the year's total rainfall.

The soil in which the test is one of a series Condell, corresponding to the order Mollisol, sedimentary soil, moderately deep, dark brown almost throughout the profile, flat and saturated under the 90cm deep (CIREN-CORFO, 1997). This soil has a surface texture ranging from sandy loam and loamy clay-silt, has a current depth of root growth of 60 cm.

EXPERIMENTAL DESIGN

The trial was conducted with a completely randomized design (DCA) of 3 treatments with 3 replicates each (3x3), where each replicate consisted of 4 plants (Figure 1). These treatments were arranged on rows of vines with NS orientation, approximately 50 m long.

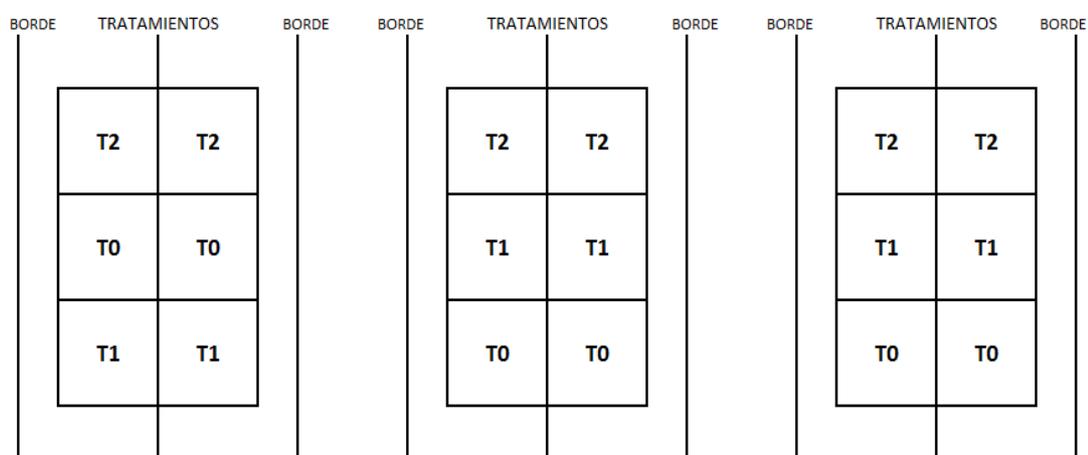


Figure 1 Distribution of the trial treatments in the barracks.

T0 corresponds to the control treatment (no application or coverage Nufresh mesh)

T1 corresponds to Nufresh treatment application

T2 corresponds to the shaded with mesh treatment level for the fruit.

For T1; 7 applications were made during the season Nufresh fruit ripening. According recommendations of NUTRIPROVE S.A representative, Agronomic engineer, Mr. Mario Guerrero and vineyard Ricardo Pereira, the first application was made on December 14, 2011, the second at 21 days after the first, and the rest in 12 days (Table 1). The dosage used Nufresh varied between 7-12 kg of product in 800 L of water, according to plant phenological period (range recommended by Mr. Guerrero). These doses are in the range of concentrations recommended by the distributor in Chile. The product was sprayed with a nebulizer, turbo, at constant speed and equivalent applications normally made by the producer, Viña San Pedro (Figure 2).



Figure 2. Photographs of the application and level of coverage of the product.

Table 1 Calendar Application and Nufresh dose during the test.

N° Aplicación	FECHA	DOSIS CAOLINITA
1	14-12-2011	7 kg/800 L agua
2	04-01-2012	12 kg/800 L agua
3	16-01-2012	12 kg/800 L agua
4	30-01-2012	12 kg/800 L agua
5	10-02-2012	12 kg/800 L agua
6	21-02-2012	12 kg/800 L agua
7	05-03-2012	12 kg/800 L agua

In the case of T2 shading net used a black color Raschell 35% shade, available in the local market, which was located just to the west side of the line, from fruit set to harvest state, covering mainly the lower area of the back, where there are clusters, and trying to produce the lowest possible shading to silver foliage (Figure 3).



Figure 3. Treatment photographs used shading

EVALUATIONS

Evaluation of plant water status

For the physiological response of the plant to each of the treatments, was performed to characterize the water status of plants in the different treatments. For this, we used a camera Scholander type pressure (PMS Instrument Co., model 600, Corvallis, Oregon, USA), which measured the xylem water potential (Ψ_x) at noon.

For each of the selected two sheets repetitions adult, healthy and fully exposed to solar radiation, from the middle third of the canopy on both sides of the row. The selected sheets were covered with a plastic sheet, so as to prevent transpiration of the sheet, and then, above the latter, the leaves were covered with foil, so as to avoid heating of the sheet.

This procedure was carried out two hours before the measurements in order to achieve a balance between leaf water potential (Ψ_h) deck and the water potential of the plant stem or xylem (Ψ_x). The measurement was done enter 12:00 and 14:00 hours (Avalo Henriquez, 2010; Araya Alman, 2010), making a total of four measurements during the period of study (Figure 4).



Figure 4. Photography and measuring leaf isolated xylem water potential Scholander bomb (PMS Instrument Co., model 600, Corvallis, Oregon, USA).

EVALUATION OF GAS EXCHANGE

The following variables related ecophysiological gas exchange was measured in the test during the season: stomatal conductance (gs), transpiration (E) and assimilation (A). These measurements were made with an infrared gas analyzer (Li-6400 Li-Cor, Inc., Lincoln. NE) at ambient conditions of light saturation (about 800 mmol m⁻² s⁻¹ PAR). Gas exchange measurements were made between 12:00 and 14:00 hours. For these measurements, both T0 and T1 treatment were selected leaves from the middle third of the canopy, mature, healthy and completely exposed (Figure 5). In the case of T3 (mesh shading) the selection criterion sheets was the same as that of the other treatments, but leaves selecting be under conditions shadow produced by the mesh.



Figure 5 Shooting infrared gas analyzer (Li-6400 Li-Cor, Inc., Lincoln. NE).

EVALUATION OF CELLULAR TISSUE DAMAGE OF BERRIES

The incidence and severity (intensity) of sunburn on fruit was evaluated in the state of pre-harvest. Visual assessment was performed on the outer surface of all clusters on the west side of the rows of each repetition, the three treatments, where the incidence was obtained from the number of clusters that had some type of cell damage, in total each repeat clusters (Figure 6). Moreover, the severity of damage was obtained from the sum of the clusters with some degree of sunburn damage in each cluster, clusters in total each repetition.

$$Incidencia(\%) = \frac{N^{\circ} Racimos\ Afectados}{N^{\circ} Racimos\ Totales} \times 100 \quad (1)$$

$$Severidad(\%) = \frac{\sum N^{\circ} Racimos_con_Nivel(x)_de_daño}{N^{\circ} Racimos\ Totales} \times 100 \quad (2)^*$$

*Severidad calculada por nivel según la escala de la Esquema 1

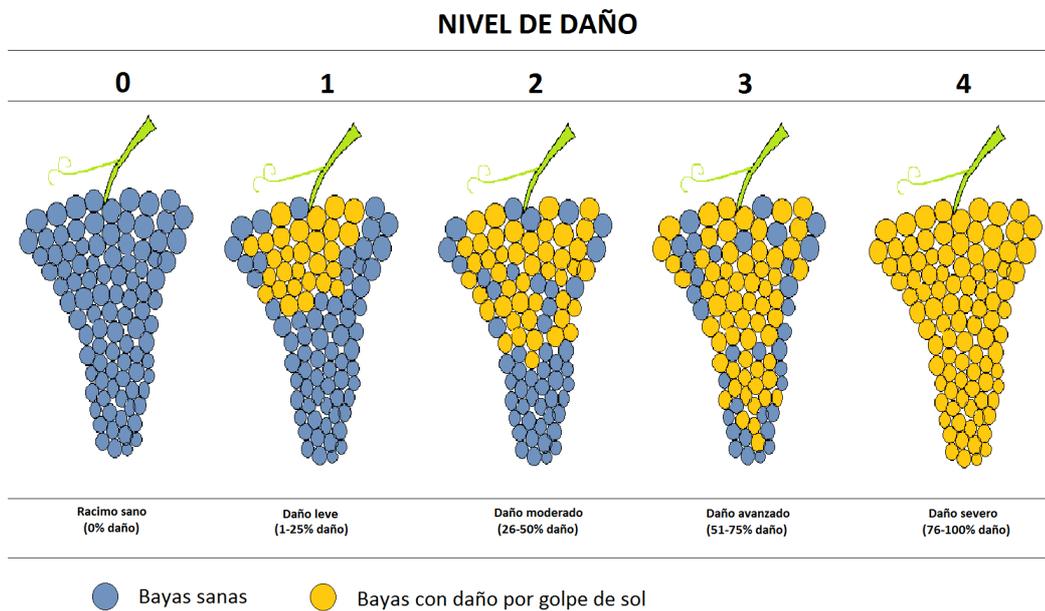


Diagram 1: Scale of the different levels of severity of fruit, used like nivel of damage



Figure 6. Photographs of the type of tissue damage of the berry analyzed

SPECTRORADIOMETRIC AND THERMOGRAPHIC ASSESSMENTS

Reflection and temperature measurements at the level of cluster and foliage, were made with a portable analyzer spectroradiometric (ASD Fieldspec Jr. 3 inc., USA) and a thermal imager (FLIR i-40) (Flir system inc. Wilsonville, Oregon. USA) (Figure 7), on the same dates in which assessments were made of plant water status.

The absolute reflectance spectral measurements (350-2500 nm) were made at a distance of 1.5 m and 0.2 m foliage cluster. To avoid errors associated with the angle of incidence of the sun, was calibrated on a white panel (Spectralon, ASD inc., USA) every 15 minutes. The data obtained was performed summation in each of the four regions of the spectrum: (1) ultraviolet (UV) = 350-399 nm, (2) photosynthetically active radiation (PAR) = 400-700 nm, (3) near infrared (NIR) = 701-1400 nm and (4) infrared (IR) = 1401 to 2500 nm (the wavelengths associated with CO₂ absorption points and atmospheric water was removed from the analysis).

On the same plants evaluated spectroradiometry was measured cluster temperature at 40 cm distance using thermal imaging. Temperature data were processed with the Flir Quick Report (Flir system inc. Wilsonville, Oregon. USA). In the measurement of treatment under meshes, these were lifted so that the foliage or fruits continue shaded but not measuring.

Moreover, since the second measurement date, was recorded with a pulp temperature precision thermocouple thermometer (TR di Turoni & C., Forlì, Italy). As always measurements were made on the west side of the plants, took advantage of measuring the temperature of the low pulp in the eastern face of the canopy, as a way to contrast these results.



Figure 7. Photographs spectroradiometric portable analyzer (The FieldSpec ®) and thermal imager (FLIR i-Series) used.

EVALUATION OF THE CHEMICAL COMPOSITION OF THE BERRIES

We performed a chemical analysis of the main constituents of the berries. These were determined juice density (g / L), the concentration of soluble solids, pH, titratable acidity (g L⁻¹ of sulfuric acid), the assimilable nitrogen content (mg / L), and Total polyphenol index (mg / L eq. gallic acid) (Bordeu and Scarpa 2000). For this, two baskets of harvested fruit, about 11-13 kg / gamela, for each repetition (Figure 8). Once harvested, the bunches were uniformly mixed and divided into two groups for analysis fruit above. In the case of the analysis of juice press proceeded to approximately 15 kg of fruit for each repetition, laboratory press using a stainless steel. Of total juice obtained, approximately 1 L was separated, which was filtered to perform the above composition analysis.



Figure 8. Picture of the fruit harvested for chemical analysis of the fruit.

For analysis of phenolic compounds from the fruit, extractions were performed according to a protocol (Venencie et al., 1997) which includes some of the following steps: (a) Separation and berry weight (b) extraction of phenolic compounds in alcohol solution with pH adjustment (c) purification of the extract and (d) chemical analysis (Figure 2).

After separating 100 berries, these peel are separate of the skins are careful not to pulp remains. After separating the skins, these are weighed to obtain the phenol concentration in the skins.

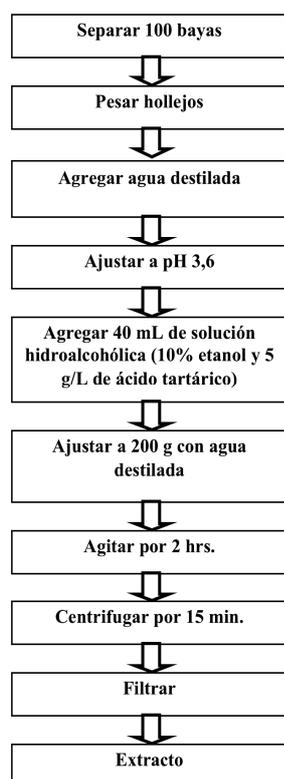


Figure 2: Flowchart of the process of extraction of fruit

STATISTICAL ANALYSIS OF INFORMATION

The analysis of the different variables measured: xylem water potential (Ψ_x), stomatal conductance (gs), assimilation (A) and transpiration (E), the incidence and severity of damage by sunburn, reflectance and temperature of clusters and plants, and the physical-chemical variables of the fruit were analyzed by analysis of variance, where we used the Tukey HSD test to determine significant differences between treatments. All statistical analyzes and tests were considered significant for a probability value $p \leq 0.05$, using the Statgraphics Centurion XV.I (StatPoint Inc., Virginia, USA). In the case of the incidence and severity values, the values are expressed in percentages, these were previously processed using the following transformation:

$$\text{ArcSin}\sqrt{\frac{\%}{100}} \quad (3)$$

RESULTS AND DISCUSSION

PLANT PHYSIOLOGICAL PARAMETERS

STOMATAL CONDUCTANCE

No significant differences ($p < 0.05$) are between treatments (Table 2). From the results described, it can be inferred that both the application and the use Nufresh mesh no alterations in stomatal conductance (gs) of leaves. According to the above by Encrypt (2005) the results obtained in this study show values within the normal range of plants with mild water stress ($gs > 0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) for the first two dates measurement (30-Jan-12 to 21-Feb-12), while in the third round (27-Mar-12) the values would be in the range of plants with moderate stress ($0.15 > gs > 0, 05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$).

Moreover, the results obtained in this study where gs values are similar among the three treatments do not agree with the statement made by Medina et al. (2002); who in orange plants, found that increased shading nets the gs. This difference in results may be because the study by Medina et al.(2002) was conducted under greenhouse covered with shading net (50% shade) as opposed to complete this study was that only cover the fruit zone (mesh 35% shade) and carried out under field conditions (outdoor).

Table 2. Stomatal conductance values (gs) measured by the infrared gas analyzer (Li-6400 Li-Cor, Inc., Lincoln. NE) for the various treatments in vine cv. Cabernet Sauvignon at three points of the season.

Conductancia Estomática (mol H₂O m⁻² s⁻¹)			
Tratamiento	30-ene-12	21-feb-12	27-mar-12
Control	0,17	0,18	0,13
Caolinita	0,15	0,14	0,11
Malla	0,18	0,17	0,10
Significancia	n.s.	n.s.	n.s.

In each measurement date: n.s. = Not significant, with $p < 0.05$ as determined

ASSIMILATION AND TRANSPIRATION

For measurements of Assimilation and Transpiration, no significant differences ($p < 0.05$) between treatments (Tables 3 and 4). These results are consistent, for the case of absorption, with the exposed by Glenn et al. (1999) and Kerns and Wright (2000), who argue that the application of kaolinite in lemon not reduce photosynthesis.

Although no significant differences between treatments for uptake ($p < 0.05$), however a gradient can be observed in the measured absolute values of absorption, with the control treatment that has values greater followed by treatment with Nufresh and finally that mesh.

Table 3. Absorption (A) measured with infrared gas analyzer (Li-6400 Li-Cor, Inc., Lincoln, NE) for the various treatments in vine cv. Cabernet Sauvignon at three points of the season.

Tratamiento	Asimilación ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		
	30-ene-12	21-feb-12	27-mar-12
Control	6,29	9,57	5,59
Caolinita	5,19	8,55	5,14
Malla	4,30	7,70	4,35
Significancia	n.s.	n.s.	n.s.

En cada fecha de medición: n.s. = no significativo, con value $p < 0,05$

Transpiration of leaves behaved similarly to the measurements of stomatal conductance and assimilation, in which there was very slight variations, showing no significant differences ($p < 0.05$). In this case, despite the above, it is noteworthy that treatment with mesh lower record values for this parameter (Table 4), followed by treatment with Nufresh and then the control treatment. This partly agrees and verifies that shown in studies by Al-Abdel-Ghany and Hedai, (2011), who mentioned that the shading nets produce a decrease in crop transpiration. In turn, the effect of reduction of transpiration caused by Nufresh regarding matches control treatment mentioned by Glenn et al. (1999) who disclose that the application of kaolinite reduce water stress achieved by lowering the temperature and perspiration of the leaves.

Table 4. Perspiration (E) measured by the infrared gas analyzer (Li-6400 Li-Cor, Inc., Lincoln, NE) for the various treatments in vine cv. Cabernet Sauvignon at three points of the season.

Transpiración (mmol H ₂ O m ⁻² s ⁻¹)			
Tratamiento	30-ene-12	21-feb-12	27-mar-12
Control	6,78	5,03	4,94
Caolinita	6,28	4,30	4,14
Malla	4,94	4,19	3,62
Significancia	n.s.	n.s.	n.s.

En cada fecha de medición: n.s. = no significativo, con value $p < 0,05$

The above results indicate that the application of treatments (Nufresh and mesh) in the manner and time periods used in this study no effect or alterations in the behavior of transpiration and photosynthate assimilation in the plant.

CONDITION ASSESSMENT

PLANT WATER

For the case of xylem water potential (Ψ_x), two of the measurements show no significant difference ($p < 0.05$) between treatments (04-Jan-12 and 27-Mar-12), while the other two (30-Jan-12 to 21-Feb-12) did show statistical differences ($p < 0.05$) among treatments. In the latter case, both dates, the control treatment showed slightly lower values (-0.62 MPa to -0.67 MPa) than treatment with application of Nufresh (-0.69 MPa to -0.75 MPa) while this also showed values slightly below the mesh treatment (-0.74 MPa and -0.78 MPa) (Table 5). By making a more general analysis of the values of Ψ_x all values in all measurement dates made, are in a range from -0.59 MPa to -0.78 MPa, values that are within the range proposed by Sibille et al. (2007) for grape plants with a level of light to moderate water stress (-0.5 MPa to -0.8 MPa), so they can be applied to interpret both treatments (Nufresh and mesh) produce no effect or significantly alter the water status of the plant. Similarly, measurements that was no statistical difference ($p < 0.05$, Tukey HSD test) can be attributed to soil conditions where the repeats were distributed, since as shown in Figure 1 repeats mesh treatment were placed randomly on the lower end of the experimental device

Table 5. Xylem water potential (Ψ_x) measured at midday with a Scholander type pressure chamber (PMS Instrument Co., model 600, Corvallis, Oregon, USA) for the different treatments.

POTENCIALES DE XILEMA Y HOJA (MPa)				
Tratamiento	04-ene-12	30-ene-12	21-feb-12	27-mar-12
Control	-0,59	-0,62a	-0,67a	-0,68
Caolinita	-0,60	-0,69ab	-0,75ab	-0,68
Malla	-0,67	-0,74b	-0,78b	-0,68
Significancia	ns	*	*	ns

Valores seguidos de distintas letras en una misma columna indican una diferencia significativa (*), n.s. : no significativo, con value $p < 0,05$

SPECTRORADIOMETRIC ASSESSMENTS AND THERMOGRAPHIC

In general, for each of the measurement dates, and for different spectral regions evaluated (analysis in each column of Table 6), the berries under shading nets had lower absolute reflectance compared to control and that treated fruit with Nufresh (Table 6). The region with the highest reflectance corresponded to the NIR, where treatment with Nufresh reflect achievement only more energy in the last measurement date, which could be indicative of the need for higher concentrations or frequency of product application, especially in stages initial development of the fruit. When comparing data between different dates (analysis in each row) shows that all treatment tends to increase the reflection towards the last two dates evaluated (Table 6). For foliage (Table 7) the results are similar to the above, both between treatments and between dates, but with greater magnitudes of reflectance

For UV region (more related to sunburn), one can see differences in the pattern of reflection between fruit and foliage: In the case of foliage treatment Nufresh always reflected more energy than the control treatment, while in fruits, this behavior was alternated between dates (the second and last dates differed between the two treatments). This confirms the statement about the need to use a higher concentration or frequency of application of Nufresh, in early stages of fruit development, to achieve a good level of coverage of the berries with the product.

Figure 6. Reflect (Fieldspec Jr. 3 ASD inc., USA) as fruit at noon for the different treatments.

Región del espectro	04-ene	30-ene	21-feb	26-mar	Value-p
UV^w					
Nufresh ^z	1,73 b ^x A ^y	1,52 c A	2,9 b B	6,1 c C	0,0001
Control	1,79 b B	1,26 b A	2,8 b C	5,0 b D	0,0001
Mesh	0,85 a AB	0,67 a A	1,0 a B	2,0 a C	0,0001
Value-p	0,0001	0,0001	0,0001	0,0001	
PAR					
Nufresh	17,3 b B	10,4 b A	20,0 c C	45,4 c D	0,0001
Control	18,3 b C	10,4 b A	15,5 b B	28,8 b D	0,0001
Mesh	9,2 a C	6,3 a A	5,4 a A	12,1 a D	0,0001
Value-p	0,0001	0,0001	0,0001	0,0001	
NIR					
Nufresh	212,8 b C	123,3 b A	142,7 b B	238,7 c D	0,0001
Control	219,5 b B	136,7 b A	133,7 b A	213,5 b B	0,0001
Mesh	141,9 a B	91,6 a A	74,8 a A	127,3 a B	0,0001
Value-p	0,0001	0,0001	0,0001	0,0001	
IR					

Nufresh	35,8 b B	23,3 a A	24,2 b A	59,9 c C	0,0001
Control	31,6 b B	42,2 b C	19,3 b A	36,5 b BC	0,0001
Mesh	12,6 a B	19,6 a C	6,3 a A	24,4 a D	0,0001
Value-p	0,0001	0,0001	0,0001	0,0001	

wRegion spectrum: UV = 350-399 nm, PAR = 400-700 nm, NIR = 701-1400 nm, IR = 1401 to 2500 nm.
x Reflectance in the same column followed by the same lowercase letter does not show statistically significant difference. and reflectance in a row followed by the same capital letter do not show statistically significant differences

z First application on December 14, 2011.

Table 7. Absolute reflectance (ASD Fieldspec Jr. 3 inc., USA) measured in foliage at noon for the various treatments.

Region of spectrum	04-ene	30-ene	21-feb	26-mar	Value-p
UV^w					
Nufresh ^z	1,21 b ^x B ^y	0,87 c A	1,44 c C	3,09 c D	0,0001
Control	1,10 a C	0,69 b A	0,92 b B	1,21 b D	0,0001
Mesh	1,30 b D	0,47 a A	0,64 a B	0,85 a C	0,0001
Value-p	0,0007	0,0001	0,0001	0,0001	
PAR					
Nufresh	15,4 C	9,7 c A	12,9 c B	29,6 c D	0,0001
Control	14,3 C	8,3 b A	9,9 b B	18,0 b D	0,0001
Mesh	15,3 D	5,7 a A	7,3 a B	11,6 a C	0,0001
Value-p	n.s	0,0001	0,0001	0,0001	
NIR					
Nufresh	411,1 C	253,1 b A	287,8 b B	486,0 b D	0,0001
Control	397,3 C	260,3 b A	288,4 b B	469,4 b D	0,0001
Mesh	377,2 D	179,4 a A	225,5 a B	326,4 a C	0,0001
Value-p	n.s	0,0001	0,0001	0,0001	
IR					
Nufresh	159,0 C	91,0 b A	111,2 b B	179,7 b D	0,0001
Control	148,0 C	93,8 b A	110,6 b B	176,3 b D	0,0001
Mesh	160,4 D	67,5 a A	88,0 a B	127,1 a C	0,0001
Value-p	n.s	0,0001	0,0001	0,0001	

wRegion spectrum: UV = 350-399 nm, PAR = 400-700 nm, NIR = 701-1400 nm, IR = 1401 to 2500 nm.
x Reflectance in the same column followed by the same lowercase letter does not show statistically significant difference. and reflectance in a row followed by the same capital letter do not show statistically significant differences

z.First application on December 14, 2011.

In relation to skin temperature in the berry (Table 8), measured by infrared thermography, it is possible to distinguish that the shading nets generated conditions for the lower temperatures during the four measurement dates with values ranging between 34, 4 and 37.8 ° C. The treated fruit and fruit Nufresh control, except for the second measurement date, no significant statistical differences for this variable.

Table 8. Berry temperature measured by infrared photography (Flir system inc. Wilsonville, Oregon. USA) measured at midday for the different treatments.

Treatment	04-ene	30-ene	21-feb	26-mar
Control	41,67 b	41,3 c	40,8 b	41,7 b
Nufresh^z	41,15 b	38,9 b	40,1 b	40,9 b
Mesh	37,43 a ^y	37,8 a	37,2 a	34,4 a
Value-p	0,0001	0,0001	0,0001	0,0001

Temperature berries taken by infrared thermography. Temperatures and the same column followed by the same letter are not statistically significant differences.^z First application on December 14, 2011. As the temperature in the berry pulp (Table 9), during the four evaluation dates, the lowest temperature was observed in pulp berries located on the east side (shaded) of the back, with temperatures ranging between 24 and 29.9 ° C during the season, followed by fruit in clusters covered by shading net, with temperatures between 31 and 34.4 ° C. The berries with Nufresh applications presented, in general, slightly lower temperatures berries and bunch without application directly exposed to sunlight. As the behavior of the reflectance in the UV region, in the second and fourth evaluation date, the application on the bunches of Nufresh showed a positive effect on the temperature control of the setting regarding the control pulp.

Table 9. Temperature berry pulp measured by a precision thermocouple thermometer (TR di Turoni & C., Forli, Italy). As at noon for the various treatments.

Treatment	30 de January	21 de february	06 de March	26 de March
Control east	29,9 a ^y	24,0 a	28,0 a	24,6 a
Mesh	34,5 b	33,9 b	33,3 b	31,0 b
Nufresh^z	36,3 c	35,5 c	36,8 c	33,0 c
Control West	36,9 c	39,5 d	37,0 c	35,1 d
Value-p	0,0000	0,0000	0,0000	0,0000

Temperature taken by infrared thermography berries.

Temperatures and the same column Followed by the same letter are not statistically significant differences.

^z First application on December 14, 2011.

As the temperature in the berry pulp (Table 9), During the four evaluation dates, the Lowest observed temperature in pulp berries was located on the east side (shaded) of the back, with temperatures ranging Between 24 and 29.9 ° C. During the season , Followed by fruit in clusters covered by shading net, with temperatures Between 31 and 34.4 ° C. The berries with Nufresh applications presented, in general, slightly lower temperatures without application berries and bunches exposed to sunlight directly. As the behavior of the reflectance in the UV region, in the second and fourth evaluation date, the application on the bunches of Nufresh Showed a positive effect on the temperature control of the setting Control Regarding the pulp.

Table 9. Berry pulp Temperature Accuracy Measured by a thermocouple thermometer (TR di Turoni & C., Forli, Italy). As at noon for the various treatments.

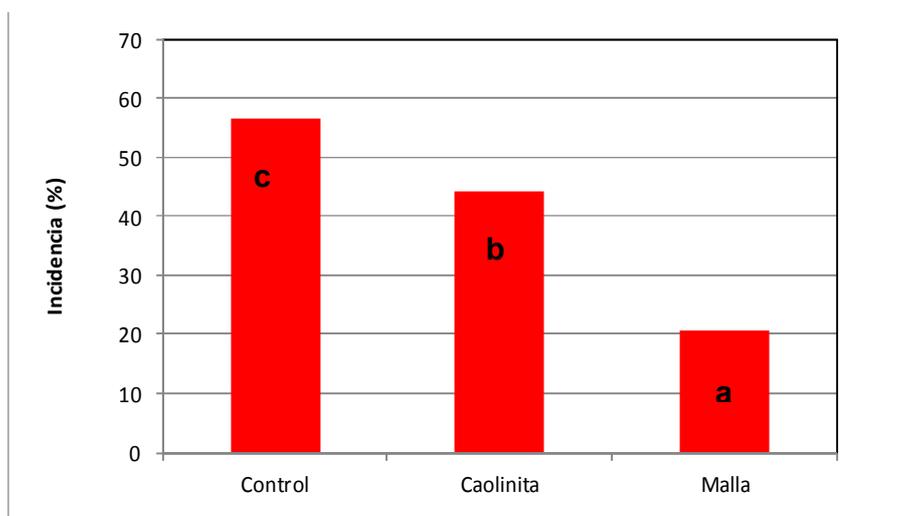


Figure 9 Effect of sunburn damage in clusters of cv. Cabernet Sauvignon, 2011-2012 season, Viña San Pedro, Molina. Different letters among treatments indicate significant differences (*), with $p < 0.01$ as determined by Tukey HSD test.

The results obtained for the severity of sunburn, were analyzed by dividing them into different categories. In the case of severity levels 0, 2, 3 and 4 were significant differences ($p < 0.05$) between treatments, but not in the level 1 where there was no difference ($p < 0.05$) (Figure 10). For level 0 (no damage, severe 0% of affected berries) mesh treatment recorded the highest value with 79.3% of clusters in this range, while Nufresh and control treatments showed values of 55.7 and 43.3% respectively. At level 1 (range 1-25% severity among affected berries) control treatments, and mesh Nufresh not statistically different ($p < 0.05$) between them, with values of 19.7, 20.3 and 13, 7% respectively. Level 2 (range 26-50% severity among affected berries) mesh treatment (4.5% severity) statistically different ($p < 0.05$) than the control treatments (15.3% severity) and Nufresh (12.5% severity). At level 3 (range 51-75% severity among

affected berries), the three treatments differed from each other ($p < 0.05$), where the mesh treatment has the lowest percentage of severity with 2.6%, and then followed by Nufresh control, which showed values of 8.1 and 15.2% respectively. Within the Layer 4 (severity range between 76-100%) with mesh treatment was statistically different ($p < 0.05$) than the other two treatments showing a severity value of less than 1%.

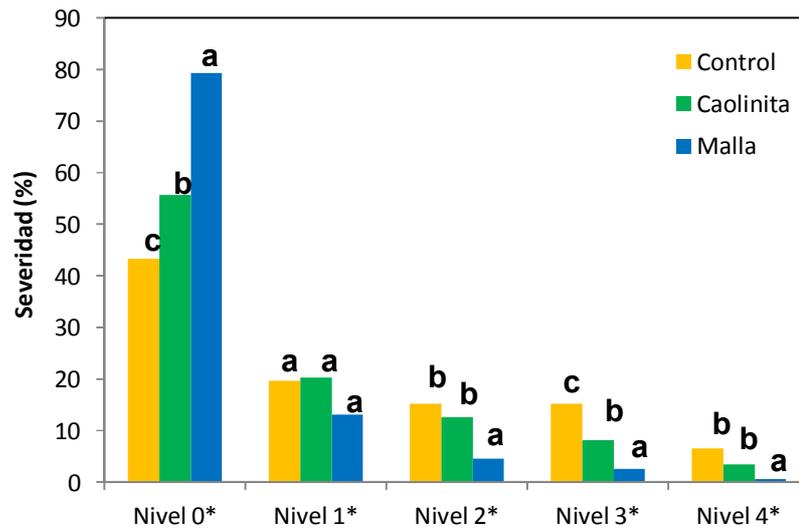


Figure 10. Analysis of sunburn damage in clusters of cv. Cabernet Sauvignon by level of severity, 2011-2012 season, Viña San Pedro, Molina.* Levels =% of damaged berries per cluster: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%. At each level, different letters indicate significant differences (*), with $p < 0.01$ as determined by Tukey HSD test.

In general, the severity of damage by sunburn treatment was lower in the mesh, followed by treatment with Nufresh, and finally the control treatment was the one that presented the greatest damage (Figure 10). Given these results, it is noted in the field do not see saw an optimal coverage of the first applications Nufresh, which together with the rapid growth of the berry, probably caused some damage that occur at soft shoulder cluster, which were observed towards the end of the ripening period of the grapes.

CHEMICAL COMPOSITION OF BERRIES

In the case of the juice composition variables (density, pH, titratable acidity and assimilable nitrogen), no statistically significant differences ($p < 0.05$) between treatments at harvest (Table 10).

Table 10. Variables composition of grape juice treated Nufresh and shading nets compared to a control treatment

Tratamiento	Densidad g/L	pH	AT g/l ac. Sulf. Eq.	FAN mg/L
Control	1103,00	3,55	4,16	300,53
Nufresh	1105,00	3,62	3,93	307,74
Malla	1103,66	3,55	4,07	301,63
Significancia	n.s.	n.s.	n.s.	n.s.

In the case of phenolic skins concentration and absorbance at 520 nm, corresponding to red pigments, measured after extraction in acidic solution, measurements showed no statistically significant differences ($p < 0.05$) for the fruit at the time of harvest (Table 11).

The above results indicate that treatment with mesh or Nufresh not affect much of the chemical composition variables commonly used to decide when to harvest the fruit.

Table 11. Phenols and absorbance at 520 nm corresponding to an extract of grape skins treated with various treatments

Treatment	phenol totals	Abs. 520 nm
	g/L EAG	UA
Control	0,2003	0,1107
Nufresh	0,1993	0,1323
Mesh	0,1780	0,1117
Importance	n.s.	n.s.

CONCLUSIONS

Both; use of Nufresh and use of black colored shading nets, show no significant changes in physiological variables of gas exchange (stomatal conductance, photosynthesis and transpiration) in different stages of fruit ripening. However, it is possible to see some gradient in the absolute values of assimilation and transpiration (Control> Nufresh> Mail).

As for the physiological variables of gas exchange, both treatments applied to control sunburn (mesh and Nufresh) no significant changes on xylem water potential (Ψ_x) in plants at different growth stages during the period of fruit ripening. In general, plants of the three treatments were maintained at a level of light to moderate water stress (-0.5 MPa to -0.8 MPa) in all measurement dates.

As for the effect on the reflection, over Nufresh light energy and temperature of skin and flesh, its effect depends on the valuation date, but seems to be more clear the closer to the harvest date. This is probably due to the successive applications Nufresh, will improve the level of coverage with the product during the season. It is also likely that the association between reduction in temperature and increased UV reflector can together, minimizing damage to soft level. However, further studies are required to conclude this with certainty.

It is possible to hypothesize that the damage started to be generated from fruit set onwards (not measured in this study), so it is highly recommended to advance the first application and increase the number of them while the fruit is in its early stages of development, perhaps even before the turn of berry color.

The use of two strategies for controlling sunburn, a black color shading net (35% shade) and applying Nufresh on clusters and foliage significantly decreased the incidence of sunburn on berries compared to treatment control without any control technique. The incidence rates of sunburn were 21%, 44% and 84% for treatments with mesh Nufresh and control, respectively. In addition to the severity of damage to significant differences severity ranges: healthy, moderate, advanced and severe, being generally less affected berries mesh treatment, and finally followed by Nufresh control.

As to the chemical composition variables, no significant differences, which implies that the treatment, the conditions used in this test, no significant changes occur to the chemical composition variables common evaluated to decide when to harvest grapes.

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ANNEXES

CLIMATE CHARACTERISTICS 2011/2012 SEASON

Figure A1 shows average temperatures, minimum and maximum were recorded among phenological stages of bud cottony to harvest during the season 2011/2012. The maximum temperature recorded was 35.8 ° C (27 December 2011), while the lowest was 1.8 ° C (2 September 2011). During this season, 97% of average daily air temperature was above 10 ° C, temperature value is considered minimal thermal threshold of growth and development for the cultivation of the vine by several authors (Lozano, 2000 , Mullins et al. 1992; Ortega et al., 2002, Williams et al., 1985; Elizondo, 2001). Also the average temperatures were highest during the first fortnight of January, which skirted the 21.8 ° C.

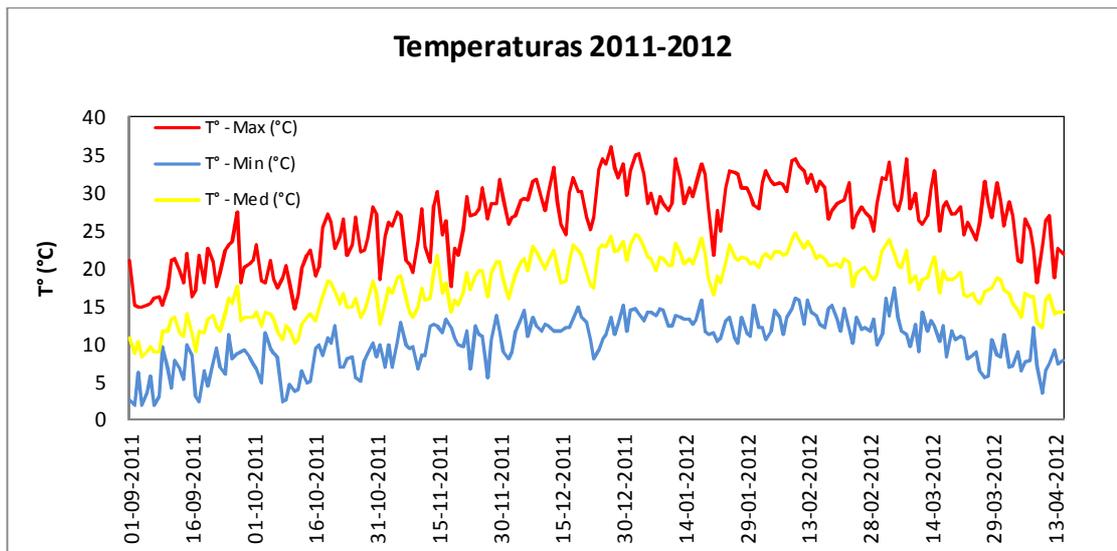


Figure A1 Temperature (° C) minimum, average and maximum during the 2011-2012 season weather station recorded in Viña San Pedro, Molina.

On the behavior of the maximum and minimum relative humidity during the season 2011/2012, we can say that these ranged from 12.3% to 100%, with the highest values during the months of September and October, while the lowest values occurred in the months of December, January and February, while coinciding with the period of higher temperatures (Figure A2).

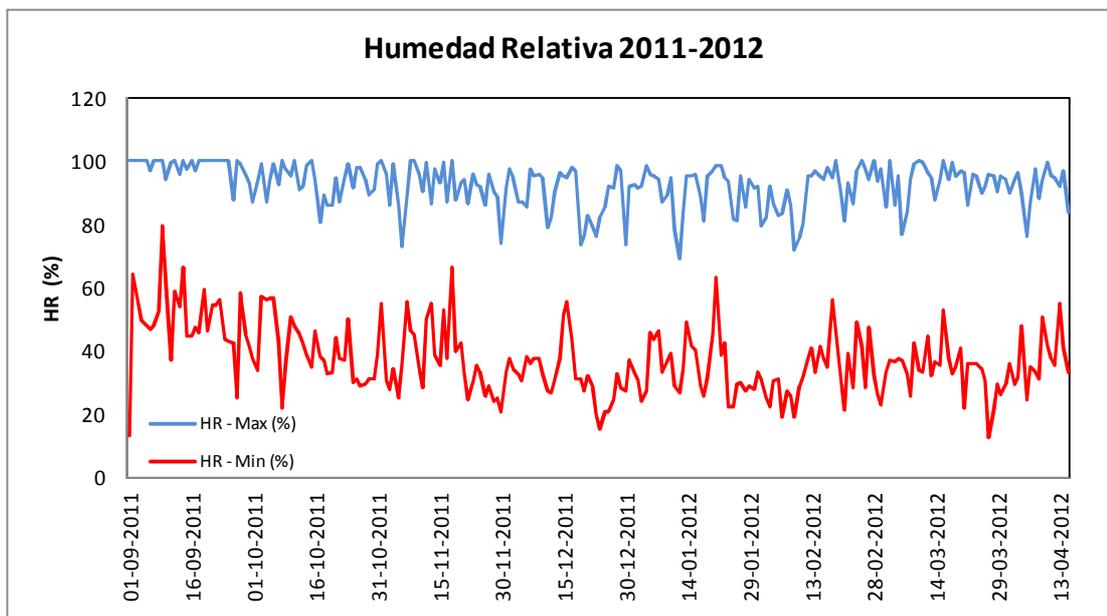


Figure A2 humidity (%) minimum and maximum for the 2011-2012 season weather station recorded in Viña San Pedro, Molina.

During the 2011-2012 season were a total of 1902.1 degree days accumulated, measured from the month of May (2011) to the first half of April (2012) (Figure A3), which corresponded to the harvest of Cabernet Sauvignon . These results agree with those obtained by Ortega et al. (2002), Verdugo (2011) and Lozano (2000), who defined a thermal storage for Cabernet Sauvignon in Talca commune of 1,558, 1,383 and 1,497 respectively accumulated degree days.

Recorded in this season is above the above, differing from 22 to 27%. This could be explained by several reasons:

- i) The seasons they were made phenology studies Verdugo (2011) and Lozano (2000) were probably the coldest seasons 2011/12.
- ii) The geographical conditions of this memory test is the town of Molina is probably a little different to the Talca and
- iii) Automatic weather station is installed Molina beside a dead vegetation at least 10 meters around. Instead Panguilemo EMA is installed under reference conditions with permanent vegetative cover.

We recorded a total of 1,460 hours of cold (base 7.2 ° C) accumulated throughout the season, from May to April, which is more than the minimum (1400 hours accumulated cold) grapevine cultivars most demanding chilling requirement during winter break (and Pszczółkowski Gil, 2007).

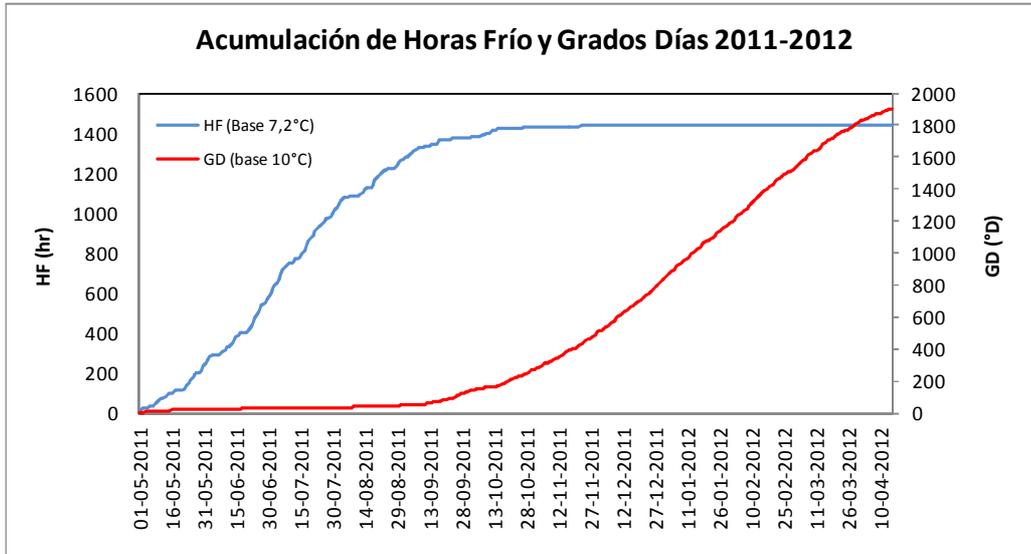


Figure A3 cold hours (base 7.2 ° C) and degree days (base 10 ° C) during the 2011-2012 season weather station recorded in Viña San Pedro, Molina.

Rainfall from September (budding) to April (harvest) occurred during the season 2011/2012 was, 25 mm, of which the majority (86.4%) were concentrated in spring and daily maximum value in that period was 8 mm, occurred in November. Moreover, the reference of evapotranspiration (ETo) estimated using the Penman-Monteith (Ortega-Farias et al., 1997), reached their peak for the season from November until the first half of January, recording a maximum of 6.1 mm in the second half of November, coinciding with the months that recorded the highest temperatures (figure A4).

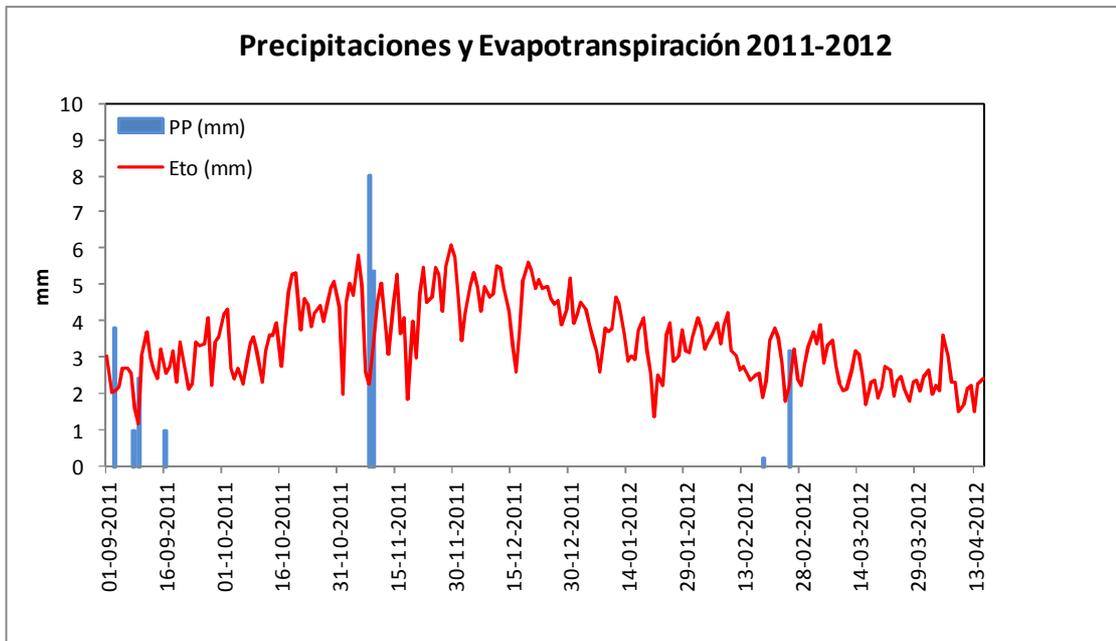


Figure A4 Rainfall (mm) and potential evapotranspiration (mm) during the 2011-2012 season and calculated recorded weather station data Viña San Pedro, Molina.

