

# Ecophysiology and vegetative and productive behavior of 'Chardonnay' vines under protected cultivation systems in Serra Catarinense

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## ABSTRACT

**Aims:** This study aimed to evaluate the effect of protected cultivation, with anti-hail screen or plastic cover, on phenological behavior, water potential, gas exchange, vegetative growth, and vine production under protected cultivation systems in an altitude region of Santa Catarina.

**Place and Duration of Study:** The experiment was carried out in São Joaquim, SC, in the 2018/19, 2019/20, and 2020/21 harvests, with the cultivar Chardonnay on Paulsen 1103 rootstock.

**Methodology:** The treatments consist of an uncovered environment (control) and crops protected with white screen anti-hail (4 mm x 7 mm) and clear plastic raffia cover (160 µm).

**Results:** The anti-hail net did not interfere in the phenological stages, photosynthesis, and gas exchange of the plants and reduced the RFA, vegetative growth and production, about the plastic cover, and similar development conditions to the control treatment (without cover). The plastic cover accelerated the maturation process, reduced photosynthetically active radiation (RFA), promoted vegetative growth, delayed leaf senescence, and increased productivity in relation to plants without cover, but did not change the photosynthesis of plants and other attributes related to the plants. gas exchange. The highest red to far-red ratio was observed in an uncovered system. The water potential of the plants was not affected. Net photosynthesis, gas exchange, CO<sub>2</sub> ratio did not show significant differences. The light saturation point was higher in plants in the cultivation system protected with plastic cover. Chlorophyll a, b, a+b, and the chlorophyll a/b ratio were higher in plants under a plastic cover in the 2018/19 harvest. For the years 2019/20 and 2020/21, these variables of net photosynthesis, gas exchange, and CO<sub>2</sub> ratio did not show differences between treatments, with the exception of the 2020/21 harvest, in which the plastic cover showed a higher value for chlorophylls a + b. Plants under plastic cover showed an anticipation of fruit maturation in relation to the screen and without cover. Plants under plastic cover showed higher values than plants without cover in terms of bunch weight (116%), number of bunches per plant (49%), and weight of bunches per plant (65%). The cultivation protected with anti-hail net maintained these attributes similar to the cultivation without cover.

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**Keywords:** *Altitude wines; microclimate; wine tourism; hail net; plastic cover.*

## 1. INTRODUCTION

Although Brazil does not have a tradition in wine production, as with many European countries, there is a considerable area dedicated to the cultivation of grapes and wine production, such as the temperate production zones of Campanha in the Rio Grande do Sul, the Serra Gaúcha and the Serra Catarinense [1].

The wines produced in the Serra Catarinense region (above 800 m altitude) are highlighted in the national scenario [2]. The factors that make the cultivation of high-altitude wine grapes interesting, culturally and economically, are associated with the differentiated flavor and aroma, which, combined with wine tourism, make the culture increasingly attractive to the consumer and profitable to the producer [3].

However, places with an altitude of more than 700 m are more prone to hail and late frosts [4], in addition to high rainfall at the time of sprouting and harvest [5], high relative humidity, and excessive leaf wetness, conditions that require the application of chemical products to control diseases, a factor that affects the quality of wines [6]. Another limiting factor is the anticipation of harvest in many vintages, not allowing the grapes to reach the appropriate ripening period, which affects the productivity and oenological attributes of the wines produced [6, 7].

Considering the aspects of climate and grape production in regions of the Serra Catarinense, many producers are adopting cultivation in a protected environment with the use of anti-hail screen [8] or plastic cover [9], aimed at protecting plants from hail and late frosts [10]. However, the impacts of protected cultivation on plant physiology and fruit quality in altitude regions for grape cultivation are still scarce in the literature. The implementation of the plastic cover and anti-hail mesh in the Serra Catarinense region requires studies, mainly on factors related to physiological and microclimatic responses, vegetative and productive behavior [11].

The use of plastic covers increases the temperature inside the canopy and reduces humidity, evapotranspiration, and incident solar radiation [12, 13, 14]. In regions of Rio Grande do Sul, which have a tradition of protected cultivation and the production of quality wines, the works are more advanced regarding the interference of the use of plastic covering. However, in the Serra Catarinense region, little is known about the effects of using this technology.

The objective of this work was to evaluate the effects of microclimatic conditions, phenology, gas exchange, light interception, water potential in plants, vegetative and productive behavior of vines managed in an uncovered management system, **undercover** with an anti-hail screen and transparent plastic tarpaulin in altitude region in the Serra Catarinense.

## 2. MATERIAL AND METHODS

The experiment was carried out in the 2018/19, 2019/20, and 2020/21 cycles with the cultivar 'Chardonnay', at the Monte Agudo Winery, located in São Joaquim, SC, at 1,264 m altitude, with geographic coordinates 28°14'54' 'S and 49°47'52"W. The vineyard has been implanted for 11 years (2007 to 2018), on Paulsen 1103 rootstock. The conduction system is "Y", with a spacing of 2.90 m between rows and 1.30 m between plants, obtaining a density of 2,870 plants per hectare. Pruning is mixed with four sticks per plant and 4 to 6 buds per stick.

The climate classification of the region of São Joaquim, SC, is Cfb, according to Köppen [15], with moderate temperature and well-distributed rain, mild summer, the occurrence of frosts in winter and autumn, with average temperatures below 20°C, except in summer, when temperatures exceed 28°C. In winter, the average temperature is below 14°C, with minimum temperatures below 8°C.

The air under the plastic cover had lower values of relative humidity in both seasons. This condition can be attributed to the barrier that the plastic imposes to leaf wetness.

The treatments evaluated were the control, without the use of plastic cover, with anti-hail screen and plastic cover.

The material used in the cover was plastic braided transparent polyethylene canvas (160 µm thick), waterproofed in the first year (2018) of use in the vineyard. After the grapes were harvested, the material (plastic tarpaulin) was removed, to increase durability, and replaced immediately after pruning in the next agricultural cycle (allowing durability of up to 20 years). The plastic cover was arranged along the planting line, following a north-south orientation, seated on metallic wires and these over steel arches (tunnels with a height of 1.30 m from the plant to the plastic and 3 m wide), fixed on the structure of the "Y" system.

The white anti-hail screen, with a mesh opening of 4 mm x 7 mm, fixed in the same arrangement, seated on the same metallic structure used for the plastic, on metallic wires, and these on steel arches (tunnels with a height of 1,30 from the plant to the plastic and 3 m wide), fixed on the structure of the "Y" system (only placed for experimental purposes).

Phenology evaluations were carried out from the beginning of sprouting to the end of the cycle, plant water potential, gas exchange, the intensity of different wavelengths, photosynthetically active radiation, photosynthetic pigments, vegetative and productive attributes.

After performing the winter pruning in the productive cycles, after 14 days, evaluations were carried out to estimate the sprouts of the plants for the phenology calculations. Data were collected from 1 plant of each repetition, at 14-day intervals, throughout the vegetative/productive cycle of the 2018/19 and 2019/20 harvests. Phenology was expressed as a

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percentage of branches per point of the phenological scale developed by Lorenz et al. [16], on the evaluation dates. In the same plant, the number of branches and total leaves per plant were evaluated.

For the microclimatic characterization, the maximum, minimum, average temperatures and relative humidity were obtained using a data logger (NOVUS, LOGBOX-RHT-LCD, country?) at the height of the bunches. The equipment was in the field from August to September in the 2019/20 and 2020/21 harvests.

In the evaluations of microclimatic variables in the two seasons (2019/20 and 2020/21), changes caused by the use of plastic covering on the crop rows were observed. Plants under plastic cover had higher maximum, average and minimum air temperatures compared to those uncovered and under the screen (Table 1). The average temperature difference between the plants under the uncovered system and the anti-hail screen was 0.2 °C, and 0.5 °C between the uncovered system and the plastic cover in the year 2019/20. In the year 2020/21, these differences were 0.1 °C in the uncovered system and anti-hail screen, and 0.6 °C between the uncovered and the plastic cover, respectively. Study carried out by Pedro Júnior et al. [13] cite higher temperature in the bunch of 'Syrah' vines produced under the plastic cover, compared to the uncovered environment. It can be seen, in general, that the plastic cover delays the loss of heat in comparison to the uncovered system and the anti-hail screen. Therefore, temperature directly affects plant development and the hormonal balance of fruit trees [17], as well as the vegetative development and quality of the grapes produced [18].

Table 1. Microclimatic variables of maximum, minimum, average temperatures and relative humidity (%) in an uncovered cultivation environment, covered with anti-hail screen and with plastic cover, in the 'Chardonnay' cultivar, from September to October, in the 2019 harvests /20 and 2020/21, São Joaquim, SC.

Microclimatic Variables	Uncovered	Screen	Plastic cover
	Crop 2019/20		
Max. canopy (°C)	34,5	34,2	34,7
T. min. canopy (°C)	7,1	7,1	7,5
T. average canopy (°C)	20,6	20,4	21,1
UR. canopy (%)	79,1	81,4	77,9
	Crop 2020/21		
Max. canopy (°C)	32,2	32,1	32,4
T. min. canopy (°C)	8,1	8,1	8,7
T. average canopy (°C)	20,0	20,1	20,6
UR. canopy (%)	81,2	80,6	79,6

The radiation spectrum was obtained with a spectrophotometer (APOGEE INSTRUMENTS – model SS – 110), with detection of wavelengths from 340 to 810 nm, at midday. From the data obtained, the total radiation available in the control treatment and the radiation available for plants under the anti-hail screen and plastic cover were quantified. The total radiation (without interference from the physical barrier) was quantified as 100% of the available light, and the other values were calculated as a function of the total radiation, and then, the percentage of light retained by the physical barrier was determined. The spectroradiometer measured the amount of light in the ultraviolet (300-390 nm), blue (450-490 nm), green (490-580 nm), red (620-700 nm), far-red (700-750 nm) ranges. The red/far-red (V/Vd) ratio was calculated considering these last two wavelength ranges. Photosynthetically Active Radiation (RFA) was determined with a cepometer, Licor model leaf area index (LAI-2200C – Licor, USA).

The determination of leaf water potential ( $\Psi_{\text{foliar}}$ ) was performed with a pressure chamber (PMS Instrument Co, model 1000, USA). The evaluations were carried out on leaves opposite the grape bunches, from 7 am to 3 pm, in December of the 2019/20 and 2020/21 harvests, with intervals of two hours between the evaluations.

The number of branches and leaves was estimated per plant. The variables were analyzed according to the vegetative stage of the plants. Four representative branches were marked on the plants, with the aid of graduated rulers, and measurements were performed on one plant by repetition. The branches and leaves were not removed from the plants to avoid causing damage and injuries. The leaf attributes were quantified through the evaluation of the area, dry mass, and specific area, using ten leaves in each repetition. Leaf area ( $\text{cm}^2$ ) was quantified using a leaf area integrator (Li-Cor, model LI-3100, USA). The dry mass (g) was quantified on an analytical balance, after drying in an oven at 65°C, with forced air circulation, for 72 hours. The specific leaf area ( $\text{cm}^2 \text{g}^{-1}$ ) was calculated by dividing the area ( $\text{cm}^2$ ) by the dry mass (g) of the leaves. The fresh weight of leaves on a plant ( $\text{kg plant}^{-1}$ ) was estimated by the number of leaves on the plant and multiplied by the fresh mass (g).

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The levels of chlorophyll a, b and total (a+b), a/b and carotenoids ratio were obtained by collecting three disks of 26.6 mm in circumference and placed in amber glasses (10 mL), covered with aluminum foil and tape adhesive, with 7 mL of DMSO (extractor reagent - dimethyl sulfoxide), incubated at 65°C for two hours. After total chlorophyll extraction, the liquid was pipetted into Elisa plates, and reading was performed in a microplate reader (brand, country model), at chlorophyll wavelengths at 649 nm, 665 nm and 480 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. The levels of chlorophyll a, b and total and carotenoids were obtained by the formulas: Chl a ( $\mu\text{L}^{-1}$ ) =  $12.47 \times (665 \text{ nm}) - 3.63 \times (649 \text{ nm})$ . Chl a ( $\text{g L}^{-1}$ ) =  $((\text{Chl a} \times (\text{DMSO volume}) / 1000) / \text{leaf disc leaf area (mm}^2)) \times 10000$ ; Chl b ( $\text{g L}^{-1}$ ) =  $25.06 \times (640 \text{ nm}) - 6.5 \times (665 \text{ nm})$ . Chl b =  $((\text{Chl b} \times (\text{DMSO volume}) / 1000) / \text{leaf area of the sheet (mm}^2)) \times 10000$ ; Chl a + b =  $21.44 \times (649 \text{ nm}) + 5.97 \times (665 \text{ nm})$ . Chl a+b ( $\text{g L}^{-1}$ ) =  $((\text{Chl a} \times (\text{DMSO volume}) / 1000) / \text{leaf disc leaf area (mm}^2)) \times 10000$ ; the a/b ratio by dividing the values of a and b, carotenoids =  $(1000 \times (470 \text{ nm}) - 2.14 \times (\text{Chl a}) - 70.16 \times (\text{Chl b})) / 220$ , Carotenoid ( $\text{g L}^{-1}$ ) =  $((\text{carotenoids} \times (\text{DMSO volume}) / 1000) / \text{leaf area of leaf disc (mm}^2)) \times 10000$  adapted [19].

Net photosynthesis ( $A$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance (gs);  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), ratio between  $\text{CO}_2$  (Ci/Ca), internal concentration of  $\text{CO}_2$  (Ci), quantum yield of photosystem I (FS1) and maximum quantum yield of photosystem II (FSII), relative rate of electron transport (ETR) were generated with an infrared gas analyzer (LI-6400, LI-COR, USA), equipped with an open-top chamber. These evaluations were performed on the color change of the berries.

The number of bunches and weight of bunches (kg) per plant were obtained by collecting all bunches from two plants, which were counted and weighed, and the average of these values was obtained. The individual bunch weight (g) was estimated by the total weight of bunches and divided by the number of bunches. Yield ( $\text{kg ha}^{-1}$ ) was estimated by harvesting two plants and then weighting yield to the total number of plants in one hectare.

The design was in a completely randomized design, with three treatments and five replications per treatment, with two plants per replication. The program for statistical analysis was SISVAR 2.0, with LSD test ( $p < 0.05$ ).

### 3. RESULTS AND DISCUSSION

A reduction of RFA was observed for plants under a plastic cover and anti-hail screen, in relation to uncovered plants, respectively, of 39% and 36% in the 2019/20 crop, between uncovered plants and under anti-hail screen; the difference was 58% and 35% in the 2020/21 crop, between uncovered plants and plants under plastic cover (Table 2). The greater reduction of RFA under a plastic cover in the second crop may be related to the accumulation of dirt and loss of transparency of the plastic placed in the cultivation line. Although the material is removed at the end of the harvest to increase its durability, the condition of accumulation of residues from the previous year is a relevant factor. Other studies carried out in Brazil also demonstrated the reduction of RFA under the plastic cover. Azevedo et al. [20] observed a decrease in the number of grape plants with the cover, at 50 cm above the plant canopy. Mota et al. [21], Cardoso et al. [22, 23], Chavarria et al. [24] also found a reduction in RFA under plastic cover. According to Chavarria et al. [25], the reduction of RFA can be 30% in covered vineyards, compared to the uncovered system. This condition is due to the imposition of the physical barrier imposed by the plastic, which resulted in a decrease in the availability of light [26]. According to Leitão et al. [12], the RFA was reduced by 40%, at 50 cm from the canopy, on vines under a plastic cover in the São Francisco river valley.

The quantity and quality of light incident on the canopy of plants greatly affects the development of vines, as they are directly linked to all physiological processes of plants [27]. The amount of available light is essential for the synthesis of photosynthetic pigments in plants, since the chlorophyll content of the leaves is directly linked to the reaction centers of the photosystems, a condition that leads to the growth and development processes of plants [28].

Table 2. Photosynthetically active radiation (RFA;  $\text{W m}^{-2}$ ) incident on the height of vine bunches of the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December 2019 harvests /20 and 2020/21. Sao Joaquin, SC.

Treatment	Crop 2019/20	Crop 2020/21
Uncovered	2317 a	1613 a
anti-hail screen	1492 b	1142 b
Cob. Plastic	1416 b	872 b
CV %	17,7	21,0

Means followed by the same letter, in the columns, did not differ by the LSD test ( $p < 0.05$ ).

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It was observed that the plastic cover was able to intercept the ultraviolet (UV) light incident on the canopy (Table 3). Lights in the blue, red, and far-red bands were also intercepted by the plastic cover, but at a lower intensity than the UV. Red light is another factor that slows down the leaf senescence process. The reduction of available red light in the canopy allows leaves to remain active for longer periods of time [28], as it presents late leaf senescence. The ratio of red and far-red (V/Vd) was higher for plants without the plastic cover. Plants under hail net had a lower V/Vd ratio, a condition that causes the plant to have an increase in vegetative growth, changing its characteristics to adapt to the light restriction imposed by the cover [24, 27, 29]. Plants subjected to shading have low respiratory rates and absorb available light, which makes them more efficient in terms of the use of radiation, a condition that reduces the waste of photons in the leaf, due to the smaller number of cells. According to Batista et al. [30], the quality of light available in plant canopy is directly linked to the production of structural genes that contribute to the adaptation of plants to shaded environments.

Table 3. Light intensity at different wavelengths, and V/Vd ratio, at the height of vine bunches in the 'Chardonnay' cultivar, in an uncovered, covered cultivation system, with anti-hail screen and with plastic cover, in December 2019/20 harvests, Sao Joaquin, SC

Radiation attributes	Uncovered	Screen	Plastic cover	CV %
Ultraviolet	76,7 a	76,6 a	12,1 b	11,3
Blue	27,8 a	21,2 b	16,2 c	13,0
Green	67,9 a	37,4 b	40,4 b	21,7
Red (V)	60,8 a	37,4 b	39,1 b	20,3
Far-red (Vd)	37,3 a	27,5 b	26,7 b	15,1
V/Vd Ratio	1,63 a	1,29 c	1,47 b	5,92

Means followed by the same letter, in the lines, do not differ by the LSD test ( $p < 0.05$ ).

It was observed in Figure 1 that the water potential of the plants was adequate for the development of the vine, in the three conduction systems, which must be between -0.2 and -0.6 MPa. This condition of no water deficit for plants under plastic cover is explained by the high rainfall that occurs in the region where the work was carried out.

Figure 1. Water potential of leaves in the cultivar Chardonnay, measured at three periods of the day (7:00, 9:00, 11:00, 13:00, 15:00 hours), in the month of December, in the 2019/2019 harvests 20 and 2020/21, in uncovered systems, under screen and plastic cover, in the city of São Joaquim, SC.

For the variables net photosynthesis (A), stomatal conductance (gs),  $CO_2$  (Ci/Ca),  $CO_2$ (Ci), Photosystem I (FS I), the maximum quantum yield of photosystem II (PhiPS2), and relative rate of electron transport (ETR) there was no difference between treatments (Table 4). These results show that there is no damage to plants grown under plastic cover in terms of photosynthetic capacity. The quality of light is directly linked to the photosynthetic processes of plants, an example of this condition according to WANG et al [28] is that reduction in blue light delays the process of leaf senescence, as there is no degradation of chlorophyll present in chloroplasts. Plants are able to effectively compensate for the reduced availability of light for their development. This condition improves the  $CO_2$  assimilation characteristics, supporting the transport of electrons and maintaining the efficiency of photosystem II. Plants can change their leaf morphology in terms of the arrangement of chloroplasts [31] and by changing the thickness of leaf tissues, responding according to the amount of light in the environment [26]. According to Da Silva et al. [17], the increase in temperature and reduction in relative humidity can provide a lower rate of net photosynthesis, a condition that directly reflects on the stages of plant flowering and berry maturation.

Table 4. Gas exchange (gs) in vine bunches of the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December 2018/19 and 2019/20 harvests São Joaquim, SC.

Gas exchange	Uncovered	Screen	Plastic cover	CV %
	Crop 2018/219			
A ( $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	13,5 ns	13,5	14,6	8,87
gs ( $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	0,19 ns	0,19	0,20	9,21
Ci/Ca	0,67 ns	0,68	0,66	7,50

Ci	254,9 ns	258,6	248,1	3,32
Fs	574,0 ns	545,5	585,4	8,64
Fv/Fm'	0,52 ns	0,53	0,56	6,21
PhiPS2	0,32 ns	0,35	0,35	12,9
ETR ( $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ )	135,4 ns	144,4	152,7	10,5

**Crop 2020/21**

A ( $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	14,5 ns	14,4	15,2	9,24
gs ( $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	0,23 ns	0,23	0,24	13,8
Ci/Ca	0,67 ns	0,67	0,67	5,32
Ci	267,1 ns	267,2	266,4	4,82
Fs	706,1 ns	703,3	700,0	28,3
Fv/Fm'	0,52 ns	0,53	0,57	8,62
PhiPS2	0,29 ns	0,30	0,28	16,4
ETR ( $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ )	136,3 ns	149,3	154,4	16,2

Means followed by the same letter, in the lines, do not differ by the LSD test ( $p < 0.05$ ). A – Net carbon assimilation rate. g – Stomatal conductance. Ci/Ca – ratio between indoor and ambient CO<sub>2</sub> concentrations. Internal ci-concentration of CO<sub>2</sub>. Fs- photosystem I. Fv/Fm' - maximum quantum yield of photosynthesis II. PhiPS2 - photosynthesis II. ETR – the relative rate of electron transport. \*means followed by the same letter do not differ statistically by the LSD test (5% probability). \*CV % - coefficient of variation.

Chlorophyll a contents and chlorophyll a/b ratio in leaves were higher for plants under a plastic cover in the 2018/19 crop year. For chlorophyll b, the highest content was obtained in plants under hail net and plastic cover (Table 5). Plants with higher chlorophyll a/b ratios, according to Wang et al. [28] and Hairmansis et al [32], present greater disorders in chloroplasts. This condition explains the need for adaptation of cell arrangements in leaves to maintain their photosynthetic capacity. The highest levels of chlorophyll b in shaded environments is related to the evaluation of plants and their adaptation to environmental changes [33]. In the 2019/2020 crop year there was no difference between treatments for all variables related to chloroplast pigments. In the 2020/21 crop year, there was a difference only for the chlorophyll a+b variable, which was higher in plants under the plastic cover, compared to plants under hail and uncovered screens. Kong et al. [34], in a study, carried out with the quality of light, temperature, and photosynthesis in cherry tomatoes, observed that plants subjected to higher temperatures had higher values of chlorophyll a, b and a+b. The data found in this work for the protected cultivation of vines corroborate those presented by Kong et al. [34]. Work carried out by Chavarria et al. [26] also showed higher levels of photosynthetic pigments in vines produced under the plastic cover. According to Streit et al. [31], plants subjected to high light intensities can undergo the process of photoinhibition, as a way of protecting the plant from excess light and, consequently, reducing chlorophyll levels. According to Wang et al. [26], plants subjected to a higher incidence of blue light tend to reduce the production of chlorophyll in the leaves, a condition observed in Table 5, which shows plants without cover present greater availability of blue light and, consequently, lower production of chlorophyll. The levels of carotenoids in the leaves did not differ between treatments (Table 5).

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Table 5. Levels of photosynthetic pigments in vines in the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December 2018/19, 2019/20, and 2020/21 harvests, Sao Joaquin, SC

Photosynthesizers pigments	Uncovered	Screen	Plastic cover	CV%
<b>Crop 2018/19</b>				
Chlorophyll a ( $\text{g L}^{-1}$ )	2,50 c	3,14 b	3,23 a	2,02
Chlorophyll b ( $\text{g L}^{-1}$ )	1,33 b	1,60 a	1,57 a	2,45
Chlorophyll a+b	3,83 b	4,74 a	4,80 a	1,41
a/b ratio	1,88 b	1,97 b	2,06 a	3,20
Carotenoids ( $\text{g L}^{-1}$ )	0,55 ns	0,59	0,56	7,36
<b>Crop 2019/20</b>				
Chlorophyll a ( $\text{g L}^{-1}$ )	1,93 ns	1,75	2,08	28,8
Chlorophyll b ( $\text{g L}^{-1}$ )	1,17 ns	1,13	1,25	29,4

Chlorophyll a+b	3,09 ns	2,88	3,33	35,7
a/b ratio	1,66 ns	1,54	1,66	31,7
Carotenoids (g L <sup>-1</sup> )	0,38 ns	0,31	0,38	37,8
<b>Crop 2020/21</b>				
Chlorophyll a (g L <sup>-1</sup> )	1,35 ns	1,22	1,43	34,4
Chlorophyll b (g L <sup>-1</sup> )	0,81 ns	0,74	0,82	30,0
Chlorophyll a+b	2,16 ab	1,97 b	2,25 a	20,6
a/b ratio	1,66 ns	1,64	1,75	30,5
Carotenoids (g L <sup>-1</sup> )	0,29 ns	0,28	0,30	34,3

Means followed by the same letter, in the lines, do not differ statistically by the LSD test (5% probability). CV % - coefficient of variation.

In the 2019/20 **crop**, the cultivar Chardonnay presented leaf development (1), inflorescence appearance (5), flowering (6), and fruit development (7) **was** two days earlier in the plastic cover in relation to the screen, and seven days earlier than the overdraft. In the 2018/19 harvest, fruit maturation (8) occurred early for plants under **the** plastic cover.

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This difference can be attributed to the increase in temperature under **the** plastic cover. A similar condition occurred for Alonso et al. [35], with seedless grapes produced in a greenhouse. Chavarria et al. [25] described the same behavior for 'Moscatto Giallo' grapes under protected cultivation in **the** Serra Gaúcha, and it accelerated the sprouting process and allowed reaching the early maturation stage, considering that the period of regulation in the flowering and fruit ripening period, favors the ripening of grapes under **a** plastic cover in 10 to 50 days [36, 37]. For Salem et al. [38], seedless grapes produced under plastic cover showed **an** anticipation of harvest from 17 to 22 compared to the uncovered system.

The increase in the average temperature under plastic cover resulted in the anticipation of some phenological stages (Table 6). The anticipation in the development stages of plants conducted under **a** plastic cover in relation to uncovered systems and under **the** screen in the 2019/2020 harvest is related to the occurrence of frost in November 2019. This caused the death of shoots, freezing of plant tissues, and plant defoliation by up to 90% in uncovered and under-screen systems (data not shown). This climatic phenomenon causes loss of tissue turgor, reduction in cell volume, cell dehydration, and plant death [39]. Although the grapes reach the ripening point in advance in **the** plastic cover, when compared to other systems, this condition allows for greater permanence of the bunches in the field, providing **a** better quality of the berries, due to the accumulation of sugars and phenolic compounds, conditions that are influenced by the canopy temperature [24].

Plants under plastic cover remained for longer periods with healthy and photosynthetically active leaves, which culminated in the delay of stage 9 (beginning of dormancy) in both agricultural seasons (Table 6). When there is no reduction in the chlorophyll content in the leaves, the photosynthetic rate does not show a drastic reduction [28]. According to Cardoso et al. [23] and Comarin et al. [40], the permanence of leaves in vines grown under plastic cover is longer than in uncovered plants. This condition can be attributed to the non-occurrence of leaf wetness and, consequently, lower incidence and severity of diseases in vine leaves (data not shown). According to Pedro Júnior and Hernandez [41], grape cultivars managed with plastic cover showed a reduction in the severity of fungal diseases and a decrease in plant defoliation.

Table 6. Phenological stages (days after pruning to reach each stage), and vines in the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December 2018/19 and 2019/20. Sao Joaquin, SC.

Phenological stages	Days after pruning			
	<b>Crop 2018/ 2019</b>			CV%
	Uncovered	Screen	Plastic cover	
0	25 ns	28	23	24,6
1	44 ns	45	38	20,7
5	57 ns	57	55	11,2
6	79 ns	76	76	10,0
7	135 ns	130	128	6,8
8	210 a	205 a	197 b	2,2
9	225 b	221 b	240 a	2,1
<b>Crop 2019/2020</b>				

0	52 ns	56	52	8,7
1	81 a	81 a	72 b	6,1
5	109 a	109 a	83 b	10,6
6	140 b	138 b	125 a	4,1
7	148 b	148 b	133 a	3,6
8	199 b	207 a	196 b	5,5
9	221 b	221 b	255 a	2,0

\*(0)-budding, (1)-leaf development, (5)-inflorescence appearance, (6)-flowering, (7)-fruit development, (8)-fruit maturation, (9)-beginning of dormancy. \*Evaluations carried out only in the 2018/19 and 2019/20 harvests. \*Means followed by the same letter do not differ statistically by the LSD test (5% probability). \*CV (%) - coefficient of variation.

There was no difference between treatments for the number of **branches** per plant in **the** 2018/19, 2019/20, and 2020/21 **crop** years, and in the 2019/20 year, there was no difference for specific leaf area (AFE). The number of leaves per plant and the PF of leaves per plant were higher for plants treated with plastic cover in the three seasons. The AFE in the 2018/19 and 2020/21 harvests was higher in plants conducted under **the** plastic cover, differing from plants conducted in an uncovered system and under **a** hail net (Table 7). According to Salem et al. [38] and Chavarria et al. [25], vines under plastic cover have higher AFE. This condition is directly linked to the increase in temperature and the availability of water [42]. Another factor is the quality of light incident on the canopy since the greater vegetative growth under the plastic cover is related to the lower V/Vd ratio, as well as the greater availability of blue and red radiation, which falls on the canopy and favors the synthesis of pigments. photosynthetic agents [28].

Table 7. Vegetative attributes measured in December, in **the** 2018/19, 2019/20, and 2020/21 harvests, in uncovered systems, under screen and plastic cover in the 'Chardonnay' cultivar, in the city of São Joaquim, SC.

Vegetative attributes	Crop 2018/19			CV %
	Uncovered	Screen	Plastic cover	
Number of <b>branches</b> plant <sup>-1</sup>	26 ns	25	27	8,8
No. of leaves plant <sup>-1</sup>	623 b	531 c	731 a	7,9
PF of leaves plant <sup>-1</sup> (kg)	3,277 a	2,915 b	4,102 a	9,7
AFE (cm g <sup>-1</sup> )	14 b	15 b	18 a	11,0
<b>Crop 2019/20</b>				
Number of <b>branches</b> plant <sup>-1</sup>	14 ns	14	15	7,8
No. of leaves plant <sup>-1</sup>	399 b	381 b	472 a	7,3
PF of leaves plant <sup>-1</sup> (kg)	1,223 b	1,332 b	1,793 a	8,1
AFE (cm g <sup>-1</sup> )	15 ns	18	16	21,0
<b>Crop 2020/21</b>				
Number of <b>branches</b> plant <sup>-1</sup>	26 ns	25	27	8,8
No. of leaves plant <sup>-1</sup>	614 b	514 b	726 a	6,9
PF of leaves plant <sup>-1</sup> (kg)	1,948 b	1,638 b	3,139 a	15,5
AFE (cm g <sup>-1</sup> )	15 b	15 b	22 a	3,5

\*Number-number. \*PF – fresh weight. AFE – specific leaf area. Means followed by the same letter do not differ statistically by the LSD test (5% probability). CV % - coefficient of variation.

Plants grown under plastic cover had a higher number of clusters per plant compared to plants in an uncovered system and under **the** screen, in the 2018/19 harvest. In the 2019/20 and 2020/21 **crops**, plants under **a** plastic cover and anti-hail screen did not differ from each other, however, both covered environments provided a greater number of clusters per plant compared to the uncovered system. In the three seasons of study, the weight of bunches per plant and productivity were higher in plants under the plastic cover, in relation to plants under the screen, and in an uncovered system, which there was no difference between the last two systems of conduction. In the 2019/20 and 2020/21 **crop** years, the weight of individual bunches was higher for plants in a plastic cover system compared to the system under **the** screen and uncovered, which showed no differences between them. However, in the 2018/19 **crop** year, plants under **a** plastic cover and under hail net did not show differences between them, and there was no difference between the screen cover and **the** uncovered system (Table 8). In the 2018/19 **crop**, no differences were observed for plant bunch weight between plants grown under plastic cover and screen.

**Comment [A21]:** please indicate – on the row or column

**Comment [A22]:** this phrase is too hard to follow – please reformulate

The gain in the number of bunches per plant under the plastic cover, compared to the uncovered system, was 28%, 17%, and 50% in the 2018/19, 2019/20, and 2020/21 crops, respectively. For plants conducted under anti-hail nets, the gains, in relation to the uncovered system, were 3%, 17%, and 29%, in the 2018/19, 2019/20, and 2020/21 harvests, respectively. For the weight of bunches (g) plants under the plastic cover, in relation to uncovered, they showed an increase of 10%, 49%, and 30%, in the 2018/19, 2019/20, and 2020/21 harvests, respectively. For the anti-hail net, gains were lower, 6%, 11%, and 0.9% compared to uncovered, in the 2018/19, 2019/20, and 2020/21 harvests, respectively. The results of the present work corroborate those of Pedro Júnior and Hernades [41], who reported higher values for the number of bunches, the weight of bunches per plant, and individual weight of bunches in plants grown under the plastic cover.

**Comment [A23]:** this is the correct name of the author?

These differences between the numbers of bunches can be attributed to the way of management in the pruning, conduction, and thinning of bunches. Another important factor was the occurrence of late frost in November 2019, a condition that caused the death of shoots and defoliation in up to 90% in plants conducted in an uncovered system and under an anti-hail screen. This condition directly affected the productivity of the 2019/20 crop, and provided a reduction in production in the 2020/21 crop, as the physiological condition and the amount of reserve were compromised for the following year. The plastic cover provided productivity increases of 49%, 116%, and 65%, compared to uncovered, in the 2018/19, 2019/20, and 2020/21 harvests, respectively. The anti-hail net increased productivity by 17%, 18%, 60%, compared to uncovered, in the 2018/19, 2019/20, and 2020/21 harvests, respectively.

**Comment [A24]:** Please use another term – it is not proper

The productivity gains corroborate the results found by Azevedo et al. [20] with grapes produced under a plastic cover in the São Francisco River Valley, and for grapes produced in Bento Gonçalves [40]. Pedro Júnior and Hernades [41] also observed higher yields in grapes produced under the plastic cover, in summer and winter crops. These authors attributed the productivity gains to the lower incidence of diseases and lower defoliation in the plants.

**Comment [A25]:** Please try to reformulate – is hard to follow!

Table 8. Productive attributes of vines in the cultivar 'Chardonnay', in uncovered cultivation systems, covered with anti-hail net and with plastic cover, in December 2018/19, 2019/20 and 2020/21 harvests. Sao Joaquin, SC.

Productive attributes	Uncovered	Screen	Plastic cover	CV %
Crop 2018/19				
No. of plant bunches <sup>-1</sup>	31 b	32 b	43 a	11,1
Weight bunches kg plant <sup>-1</sup>	3,9 b	4,6 b	6,4 a	15,0
bunch weight (g)	130,8 b	138,2 ab	145,3 a	6,77
Productivity (Mg.ha <sup>-1</sup> )	11,221 b	13,460 b	18,482 a	15,0
Crop 2019/20				
No. of plant bunches <sup>-1</sup>	4,7 b	6,2 a	5,9 a	11,0
Weight bunches kg plant <sup>-1</sup>	0,08b	0,08b	67,3a	52,0
bunch weight (g)	83,9 b	67,2 b	128 a	16,7
Productivity (Mg.ha <sup>-1</sup> )	0,228 b	0,251 b	2,158a	56,0
Crop 2020/21				
No. of plant bunches <sup>-1</sup>	19 b	37 a	38 a	21,0
Weight bunches kg plant <sup>-1</sup>	2,1 b	0,9 b	6,1 a	34,0
bunch weight (g)	111 b	112 b	159 a	8,9
Productivity (Mg.ha <sup>-1</sup> )	6,104 b	2,441 b	17,449 a	34,2

**Comment [A26]:** Please explain this unit measure

Means followed by the same letter, in the lines, do not differ statistically by the LSD test (5% probability). CV % - coefficient of variation.

#### 4. CONCLUSION

The objective of the work was "to evaluate the effect of protected cultivation on phenology, water relations, gas exchange, vegetative growth and production in 'Chardonnay' vine." Thus, it must conclude on how the plastic cover and the screen influenced phenology, water relations, gas exchange, vegetative growth, and production in 'Chardonnay' vines.

**Comment [A27]:** The conclusion section is way too short and, in this form, it does not highlight the interesting and useful results presented in the previous section – Results and Discussion

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**Comment [A29]:** The reference from 22 to 41 are missing!

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