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Chlorophyll Fluorescence and Fruit Quality Response of Blueberry to Different Mulches

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Abstract: Mulch is widely used in blueberry cultivation for weed control; however, there is still uncertainty as to how the use of different types of mulch alters leaf photosynthetic behavior and the quality and productivity of blueberry fruit. The objective of our research was to evaluate the effect of different types of mulch on the physiological, quality and yield characteristics of blueberries. Three treatments were established: T1 (control), T2 (pine bark) and T3 (geotextile) in two cultivars: Ochlockonee and Legacy. The parameters measured were: the photochemical quantum yield of photosystem II (Y_{II}), the maximum photochemical efficiency of photosystem II (F_v/F_m), electron transport rate (ETR), fruit quality and yield parameters. The results show lower soil temperature in T1 during the morning ($p < 0.05$) compared to the two mulch treatments, which was the opposite during the afternoon, the temperatures were more stable and closer to the optimum (21 °C) in T2 and T3, with mulch favoring root and foliar development. On the other hand, the treatments with mulch favored a higher photosynthetic efficiency of photosystem II (Y_{II}) at the end of afternoon and were associated with an increased firmness of the fruit; the firmness of all fruits was higher than that in the control treatment ($p < 0.05$) in the Legacy cultivar, but without differences between them, with values of 73 and 75 gf mm^{-1} for T2 and T3, respectively, and 67 gf mm^{-1} for the Control. In addition, it was observed that the use of mulch only increased the fruit yield in the Legacy cultivar, both in T2 and T3, with both being superior to T1 ($p < 0.05$). It can be concluded that the use of mulch decreases soil temperature in the midday and late afternoon, improving the edaphoclimatic conditions during the development of the blueberry. In addition, plants with mulch have lower stomatal conductance, which promotes greater photosynthetic efficiency during the day, increasing both firmness and fruit yield.

Keywords: size; stomatal conductance; firmness; photosystem II; leaf area index

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1. Introduction

One of the main constraints in the production of blueberry is weed control [1], which brings with it a decrease in fruit quality and yield due to nutrient and light competition [2]. This problem has been addressed in fruit orchards through the application of herbicides [3]. However, in organic or ecological management, there is no effective product for weed control. The use of covers is one of the most efficient organic weed control techniques, based upon the use of the crop residue of wheat, corn, rice, as well as polyethylene, biodegradable plastic, among others [4–8]. This technique has mainly been studied in vegetables [9–15], cereals [13,15–19], some fruit species [1,4,8,20,21] and in forestry [6]. Several studies have demonstrated effects on weed control [18], fruit yield [6,13–15,18], fruit weight, plant development [1], the absorption and accumulation of nutrients in plants [4,10,11], water use efficiency [4,11,15–17,22], changes in soil temperatures [16,17] and cover effects on root growth [23]. However, it is also necessary to evaluate fruit quality (firmness and size) and physiological characteristics to minimize stress in the plant, influenced by the use of mulches.

There are studies that indicate the use of mulch generated from plant residues, such as wheat straw or pine sawdust, diminishes the need for labor in weed control [7,24], provides organic matter, increases soil moisture conservation [25,26] and changes the rate of water infiltration [27], as well as soil fertility [28]. However, other authors point out that pine mulch decreases the percentage of soluble solids in fruit. This is attributed to lower photosynthetic efficiency under certain environmental and edaphic conditions, such as a decrease in soil temperature, pH, and electrical conductivity [11,19,22,29].

The use of black polyethylene mulch in vegetables increases yield by 20% and its marketability by 14% [25]. In studies carried out on cherry tomatoes, in which different types of mulch were evaluated, stomatal conductance and chlorophyll concentration were not affected by their use [30], despite the fact that in other crops such as *Glycine max*, the use of pine bark mulch increased the chlorophyll content by 25% [31]. In other fruits, such as *Citrus reticulata* B., it was found that at a soil depth of 0.05 m, the temperature was significantly reduced by 6 °C with the use of organic mulch, resulting in an increased yield of 25 % [32]. Based upon this previous research, we hypothesized that temperature changes during the day in sandy soils when using different types of mulch in blueberry cultivation would decrease the degree of photoinhibition in blueberry plants, thus altering the physiological and quality characteristics of blueberry fruit. The objective in this study was to evaluate the effect of different types of mulch on physiological characteristics, such as quality and yield.

2. Materials and Methods

2.1. Location and Characteristics of the Study

The study was carried out in a commercial blueberry orchard, with two cultivars, rabbiteye cv. Ochlockonee (*Vaccinium virgatum* Aiton) and highbush cv. Legacy (*Vaccinium corymbosum* L.), in a field located in the Biobío Region of Chile (39°55′ S 41°16′ W). The study was carried out during two consecutive seasons. The soil is classified as Isotic Typic Xeropsamment [33], the soil chemical characteristics of which, according to analysis carried out in the soil chemistry laboratory of the Universidad de Concepción, Chillan, Chile, were: pH: 6.0; electrical conductivity: 0.08 dS m^{-1}; organic matter: 1.8%; available nitrogen: 7 mg kg^{-1}; available phosphorus: 16 mg kg^{-1}; available Potassium: 151 mg kg^{-1}; exchangeable calcium: 4.1 cmol kg^{-1}; and exchangeable magnesium: 0.76 cmol kg^{-1}. The climate is temperate Mediterranean, with hot, dry summers and cold, wet winters. The average rainfall in the area is 672 mm, concentrated in winter and early spring [34]. The planting density was 3.2 m × 1.0 m (3125 plants ha^{-1}) for both cultivars, and the average height of the plants was 1.75 and 1.5 m, for cv. Ochlockonee and Legacy, respectively. The experiment was conducted in a randomized complete block (RCB) design for each cultivar, with a total of three treatments and three replicates, corresponding to T1: control treatment (without mulch); T2: pine bark mulch (from *Pinus radiata*, of the 2015/2016 season); and T3: geotextile mulch (3220WO, Agritela Green, Arrigoni, Italy). For all cases in T1, T2 and T3, a manual control of the total number of weeds was carried out before the implementation of the treatments, leaving all the experimental plots free of weeds and considering a total width of 1.2 m over the row. Subsequently, six manual weed controls were carried out for each year of evaluation, always keeping the plots weed-free, so that they would not influence the experiment.

For both evaluation periods, all treatments were standardized with phytosanitary management and conventional fertilization, and the levels of the extraction and distribution of nutrients for both cultivars was based on those indicated by Hirzel et al. [35]. Both the frequency and amount of water applied in each treatment was the same, and it was replaced based on the estimated potential evapotranspiration of the crop, using the data obtained from the meteorological station of the Institute of Agricultural Research (INIA), Human, Los Angeles, Chile, adjacent to the place of study [34]. Each repetition or experimental unit had seven plants, from which the three central plants were evaluated, leaving the two plants at the ends as border plants. To improve pollination, bumblebee hives with 7 hives ha^{-1} were installed (Natupol ^{TM}, class C hives, Koppert Biological Systems, Holland, The Netherlands).

2.2. Characterization of the Edaphoclimatic Conditions of the Treatments

For each treatment and cultivar, and in each evaluation period, the air temperature (Ta; °C) and relative humidity (Rh, %) were recorded every 15 min throughout the development of the crop, for which automatic sensor Key Tag models were used (Model HAXO-8, Key Tag Recorders, Auckland, New Zealand). The sensors were located at a height of 1.2 m above the ground in the plantation row on a support with protection for the sensor, so that direct sunlight was avoided. The variation of light conditions was quantified in terms of photosynthetically active radiation (PAR), according to the method proposed by Al-Helal and Abdel-Ghany [36], with an AccuPAR LP-80 (Decagon Devices Inc., Pullman, Washington, DC, USA.) ceptometer, which delivered an average of 80 quantum sensors. The photosynthetically active photon density (PPFD, μmol m^{-2} s^{-1}) was quantified at three times of the day: 09:00, 12:00 and 16:00, on a sunny day, in both cultivars. In parallel, the soil temperature was measured (Ts; °C) with a digital thermometer (Multi Thermometer, Shanghai, China) three times a day (9:00, 12:00 and 16:00). The thermometer was located at a depth of 0.1 m in the row and between the plants in each treatment. With an OS-5p portable fluorimeter (Opti-Sciences, Hudson, NH, USA), leaf temperature was estimated (Tf; °C) at 09:00, 12:00 and 16:00 h. In each experimental unit, the average of 3 plants was considered, and for each plant, 4 sub-samples were made from each of the cardinal points of the plant at a height average of 1.2 m, always considering leaves exposed to the sun, and the second third of the season offshoot were considered for all treatments.

2.3. Chlorophyll Fluorescence and Stomatal Conductance

For each of the cultivars and treatments evaluated, the efficiency of the photosystem II (PS_{II}) and the rate of electron transfer (ETR) of PS_{II} was measured in leaves fully exposed to the sun, both mature and seasonal growths, located in the second third of the season offshoot. A total of 10 measurements per plant were made in different leaves located in the cardinal directions, at an average height of 1.2 m [37]. In parallel, the maximum intensity of fluorescence was measured (F_m) as well as the minimum intensity of chlorophyll fluorescence (F_0), with the help of a portable model OS-5p fluorimeter (Opti-Sciences, Hudson, NH, USA) during a sunny day, at three times of the day (9:00, 12:00 and 15:00 h), according to Kooten and Snell [38]. F_0 as well as F_m were determined after the leaves had adapted to the dark for 30 minutes [39,40], using leaf clips which included mobile obturation plates. With these parameters, the maximum photochemical efficiency of photosystem II was quantified (F_v/F_m), using the following relationship proposed by Maxwell and Johnson [41]: $F_v/F_m = (F_m - F_0)/F_m$. The degree of photoinhibition was quantified by the ratio F_v/F_m at different times of the day, related to the value recorded in the morning, also using the same frequency and instrument used for photochemical quantum yield measurements of photosystem II (Y_{II}) and electron transport rate (ETR) on light-adapted leaves [41]. At the same time, stomatal conductance was measured (gs, mmol m^{-2} s^{-1}) with a portable porometer, model SC-1 (Decagon Devices INC., Washington, DC, USA). The gs measurements were made on fully illuminated leaves of the same plant, located in the second third of the season's twig, during a sunny day, at three times of the day (9:00, 12:00, and 15:00 h) [40,42].

2.4. Yield, Fruit Quality and Foliar Indices

For each experimental unit, when fruit was 100% blue, the harvest of each experimental unit began, and the total weight (g) of fruits per plant was quantified, for which a Precisa model precision balance was used (Precisa instruments AG, Dietikon, Switzerland). From each experimental unit, 20 fruits were randomly selected to determine the equatorial diameter (Ed, mm), using a digital caliper, with a precision of $+/-$ 0.03 mm (Electronic Digital Caliper, Altraco, Los Angeles, CA, USA). The same fruits used for the Ed were used for the measurement of soluble solids (°Brix), for which a refractometer was used (Carl Zeiss, Jena, Germany). For the foliar indices, the chlorophyll content of the leaf was determined by measuring SPAD units using the Minolta SPAD-502DL Plus equipment (Konica Minolta, INC, Osaka, Japan) at midday. Fifty leaves per treatment and repetition were randomly selected in the different plants of the experimental unit, always located in the second third of the twig of the current season, exposed to light, located between 1.0 to 1.2 m from the ground [43]. Leaf area index (LAI) readings were estimated with an AccuPAR LP-80 ceptometer (Decagon Devices Inc., Pullman, Washington, DC, USA). These measurements were carried out at noon, simultaneously to the measurements of chlorophyll fluorescence, according to the methodology proposed by Sonnentag et al. [44], obtaining the average of the two plants of each treatment and repetition. Fruit firmness (FF; gf mm^{-1}) was determined from 20 fruits for each treatment and repetition, randomly selected in each harvest, and measured with CherryTex Cv-2 equipment (CherryTex Cv-2 model, Universidad de Concepción, Chillán, Chile), which corresponded to the grams of force necessary to deform the fruit by one millimeter [45].

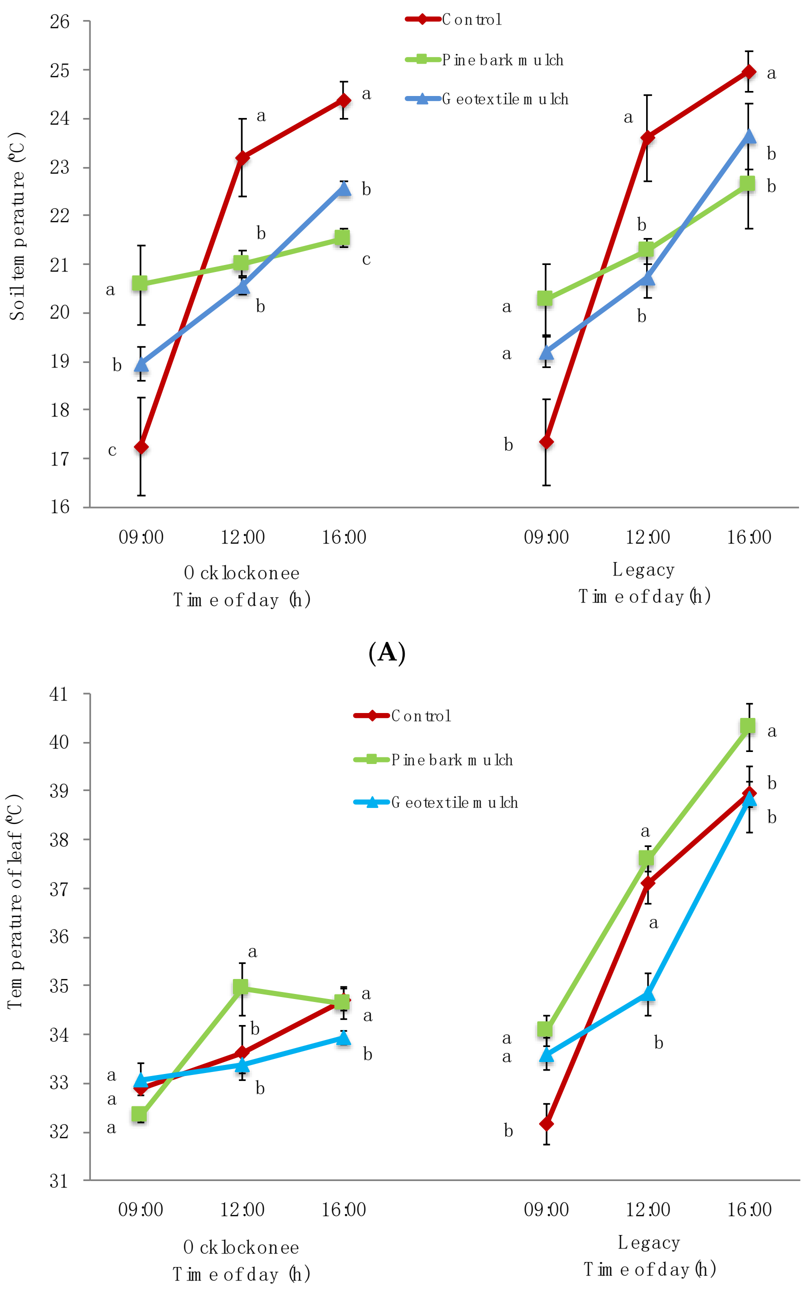
2.5. Statistic Analysis

To quantify the effect of the treatments on the measured variables at different hours of the day, an analysis of variance (ANOVA) with a post hoc comparison Tukey test was used (after the data normality check), with a significance level of 0.05. The statistical analysis of the data was performed using the general SAS model (Version 9.1; SAS Institute, Cary, NC, USA) [46].

3. Results and Discussion

3.1. Edaphoclimatic Variations of the Different Treatments

Figure 1A shows soil temperatures at different times of the day for the two cultivars studied, demonstrating that in the early hours there were significant differences between the control treatment (T1) < pine bark mulch (T2) < geotextile mulch (T3), ($p < 0.05$) for cv. Ochlockonee, with the average estimated Ts being 17, 19 and 21 °C, respectively. However, at midday, this response was reversed, with T1 being greater ($p < 0.05$) than T2 and T3 and these last treatments being equal to each other. The same trend was observed for 16:00 (h), but with Ts close to 22, 23 and 25 °C, for T2, T3 and T1, respectively (Figure 1A). Regarding the cv. Legacy, it showed a trend and values to those of cv. Ochlockonee, at 9:00, 12:00 and 16:00 h, with T2 and T3 being equal at all hours ($p < 0.05$), but both were greater at 9:00 h compared to the control and lower at 12:00 and 16:00 h (Figure 1A). The temperature trend towards the end of the day between the different types of mulch coincided with what was stated by other authors [2,23,47,48], who pointed out that sawdust mulch lowers soil temperature, favoring root growth in a temperature range of 14 to 18 °C. In addition, Spiers [23] and Cox [48] pointed out that there was a negative correlation in the vegetative development of the plant as the soil temperature increased from 16 °C to 38 °C. It is for this reason that the soil temperatures observed in this study in the control treatment (Figure 1A; Ts > 24 °C) could have restricted root development and affected the vegetative and productive development of the plant. This is corroborated by Bryla et al. [2], who determined that root growth is regulated by temperature.



(A)

(B)

Figure 1. Effects of the different types of mulch: (**A**) soil temperature (°C); (**B**) leaf temperature (°C), at three times of the day. For each different the time of day, lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean ($n = 9$).

It can be observed, in Figure 1A, that in the Ochlockonee and Legacy cultivars, the time variations of the soil were lower in the treatments with some type of mulch (pine bark and geotextile) compared to the control. This coincides with the results obtained in other studies carried out on different species that used the same types of mulch [4,29,49]. This could favor the adaptability of the plant to extreme time variations at the ground level, such as those observed in the control treatment of the present study [23]. It is important to point out that in most of the previously published studies on crop plant growth and performance, bare soils were not evaluated [1,2,20,21], Therefore, the present study provided relevant information on the behavior of the edaphoclimatic parameters of the different types of mulch in a soil belonging to the Arenales series [33], when comparing mulch treatments to bare soil. Additionally, the Ts reached in this study at T2 and T3 did not coincide with the findings of Strik et al. [1] and Strik et al. [21], who point out that soil in a treatment with geotextile mulch reached temperatures close to 27 °C, in contrast to our study, in which the Ts reached values close to 25 °C (Figure 1A), which favors the use of mulch in sandy soil to reduce soil temperature to values closer to the optimum for root development (16 < Ts < 18 °C) [2].

On the other hand, leaf temperature variation for both cultivars was observed. For cv. Ochlockonee, all treatments were the same at 9:00 h ($p > 0.05$), with mean values of 32 °C. However, at noon, a 2 °C higher temperature was observed between T2 (35 °C) and T1 and T3, in both of which the temperature was 33 °C. It should be noted that all treatments showed a tendency to increase Tf towards the end of the day, reaching mean values of 34 °C. A similar trend was observed in cv. Legacy, in which all treatments showed an increase in Tf from values close to 32 °C at the beginning of the day, reaching a maximum temperature of mean values of 40 °C, with, on average, Tf in the Legacy cultivar being 6 °C higher than in the Ochlockonee cultivar (Figure 1B). These temperature values recorded in both cultivars were above the optimum temperature for plant development (T < 28 °C), which could have generated stress, altered other physiological parameters [50] and increased susceptibility to photoinhibition [51–53]. This may negatively affect the fruit expansion rate during its last phase of development (40 to 70 DDPF) [40]. However, according to Zhen et al. [54], the optimum temperature for development and photosynthetic processes for some blueberry cultivars can be as high as 35 °C, and above this temperature, there is a decrease in net photosynthesis as well as in the rate of transpiration. This has even been observed to begin to decrease over 38 °C [54]. Furthermore, the Legacy cultivar could experience irreversible fruit damage, as pointed out by Yang et al. [55], which could translate into a loss of commercial quality, given the extreme temperatures and radiation as a result of the effects of climate change [23,36,40,52]. With climate warming predicted to increase, this should be a focus of future research.

3.2. PSII Chlorophyll Fluorescence Parameters and Stomatal Conductance

The observed values of Y_{II} for both cultivars (Table 1A) showed that there were no significant differences ($p > 0.05$) between treatments T2 and T3 with respect to the control for the first two measurements of the day. Our results are in line with the findings of other authors [39,56], in which early in the morning, the mean values of 0.35 were observed, and at noon, they decreased towards the mean values of 0.21. However, towards the end of the day, in the control treatment (T1), Y_{II} decreased strongly, being inferior to treatment T2 and T3 ($p < 0.05$), which showed a lower recovery of the photosynthetic apparatus in both cultivars in the control treatment. It was shown that at the end of the afternoon, plants in the pine bark mulch treatment experienced a greater recovery of Y_{II} (both cultivars), surpassing the treatment without mulch (T1) by 44 to 109%. This was a consequence of the decrease in temperature (Figure 1A) and photoinhibition observed in this study (Table 1B), coinciding with other studies [37,50].

On the other hand, there was no significant difference ($p > 0.05$) among treatments with respect to F_v/F_m, with the mean values being close to 0.8 in the course of the morning for both cultivars (Table 1B); these values coincided with the findings of other authors in the early hours of the morning for similar development conditions [40,42,50]. It is apparent that there was some degree of stress in both cultivars. The values of F_v/F_m with respect to the value in the morning [42,57] ranged from the mean values of 0.8 to 0.76 for the different mulch treatments ($p > 0.05$). However, T3 exhibited a recovery of stress (Table 1B) for both cultivars. Therefore, the pine bark treatment might have resulted in a greater photochemical efficiency of photosystem II (Table 1A) associated with the stress recovery of the plant in the Legacy cultivar, which presented a negative and significant correlation with Tf (Figure 2A; $p < 0.001$) and with an $R^2 = 0.43$, which corroborated previously indications. It should be noted that this Y_{II} response could also have been affected by other factors such as gs. In Table 1C, it can be seen that the ETR showed the same trend as F_v/F_m, which decreased for all treatments and cultivars during the course of the day; this trend is consistent with those found by other authors [57,58].

Table 1. Mean parameters of chlorophyll fluorescence: (A) photochemical quantum yield of photosystem II (Y_{II}), (B) maximum photochemical efficiency of photosystem II (F_v/F_m) and (C) electron transport rate (ETR).

Parameter		Treatments	Ochlockonee Time of Day (h)			Legacy Time of Day (h)		
			9:00	**12:00**	**16:00**	**9:00**	**12:00**	**16:00**
(A)	Y_{II}	Control	0.35 a	0.22 a	0.18 c	0.36 a	0.25 a	0.11 c
		Pine bark mulch	0.39 a	0.21 a	0.33 a	0.38 a	0.26 a	0.28 a
		Geotextile mulch	0.39 a	0.20 a	0.26 b	0.36 a	0.24 a	0.23 b
(B)	F_v/F_m	Control	0.79 a	0.77 a	0.74 b	0.79 a	0.75 a	0.73 c
		Pine bark mulch	0.79 a	0.77 a	0.78 a	0.80 a	0.76 a	0.77 a
		Geotextile mulch	0.78 a	0.76 a	0.75 b	0.80 a	0.76 a	0.76 a
(C)	ETR	Control	98.82 b	91.30 a	8.87 b	89.42 b	78.65 a	6.47 b
		Pine bark mulch	82.20 b	84.62 a	53.17 a	83.32 b	88.23 a	43.74 a
		Geotextile mulch	127.27 a	87.77 a	12.82 b	110.46 a	85.12 a	11.27 b

Different lowercase letters in each column indicate significant differences ($p < 0.05$) compared with (A) photochemical quantum yield of photosystem II, (B) maximum photochemical efficiency of photosystem II and (C) electron transport rate, according to Tukey's test ($p < 0.05$). Data are the mean of n = 10. T1: control treatment; T2: pine bark mulch; and T3: geotextile mulch.

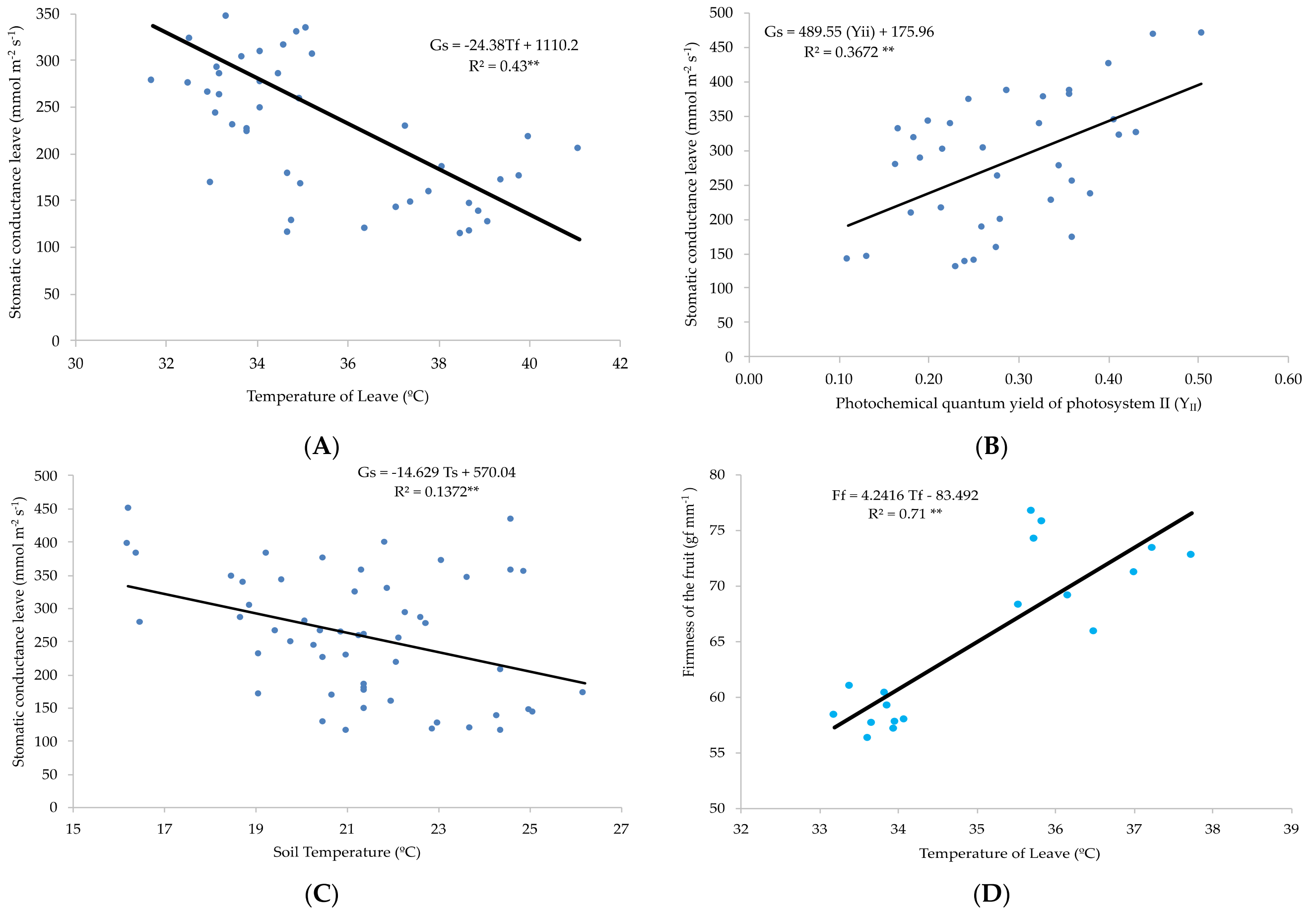


Figure 2. Correlation of stomatal conductance (gs) in different blueberries cultivars grown with different mulches, (**A**) temperature of leaves (°C), (**B**) soil temperature, (**C**) photochemical quantum yield of photosystem II (Y_{II}) and (**D**) firmness of fruit and temperature of leaves. ** Significant at $p < 0.001$.

For the stomatal conductance (gs), significant variation was observed during the day for both cultivars (Figure 3). Early in the morning (9:00), in the Ochlockonee cultivar, the gs were all significantly different with T1 > T2 > T3, with 410, 343 and 266 mmol m^{-2} s^{-1} ($p < 0.05$), respectively. For the cv. Legacy, the control treatment was the one that recorded the lowest gs value ($p < 0.05$), with T3 = T2 > T1, with values of 255, 236 and 173 mmol m^{-2} s^{-1}, respectively (Figure 3). Therefore, treatments T3 and T2 initiated the photosynthetic process in the early hours of the day in more optimal conditions than T1, since T2 and T3 ended the day with higher Y_{II} (Table 1A); this was shown by the significant positive correlation ($R^2 = 0.37$; $p < 0.001$) between gs and Yii (Figure 2B). The higher stomatal conductance indicated a higher gas exchange capacity of the leaf, and therefore a higher CO_2 assimilation rate [58]. From noon (12:00), T3 decreased gs in cv. Ochlockonee, being significantly different from T2 = T1 ($p < 0.05$), with values of 257, 351 and 400, respectively (Figure 3). This was due to the fact that T3 increased the Tf (Figure 1B) with a value of 35 °C, resulting in a decrease in the gas exchange capacity, as observed in the negative correlation between gs and Tf (Figure 2A), with a value of $p < 0.001$ and $R^2 = 0.43$; similar results were reported by Lobos et al. and Rho et al. [57,58]. Towards the end of the day (16:00 h) (Figure 3), T1 continued to decrease stomatal conductance, being significantly different from T2 = T3 ($p < 0.05$), agreeing with the findings of Kim et al. [59]. Therefore, for T1 in cv. Ochlockonee, with no mulch, an increase in Ts was allowed, stimulating an increase in gs, which could have generated an increase in water demand, as various authors pointed out in their results [58,60,61]. Despite the high variability of gs in response to soil temperature ($R^2 = 0.13$; Figure 2C, the significance ($p < 0.001$) showed that the response of gs had a negative tendency to increase the predictor variable (Ts), which validates the statements in the previous paragraph. On the other hand, Figure 4 shows that plants of both cultivars, Ochlockonee and Legacy, significantly increased the chlorophyll content, reaching levels 25% higher than SPAD levels in the pine bark mulch treatment. This change in chlorophyll content in the leaves could have impacted the net photosynthetic capacity of the leaves, as indicated by Cunha et al. [43]. This could have had effects on the production of the blueberry crop.

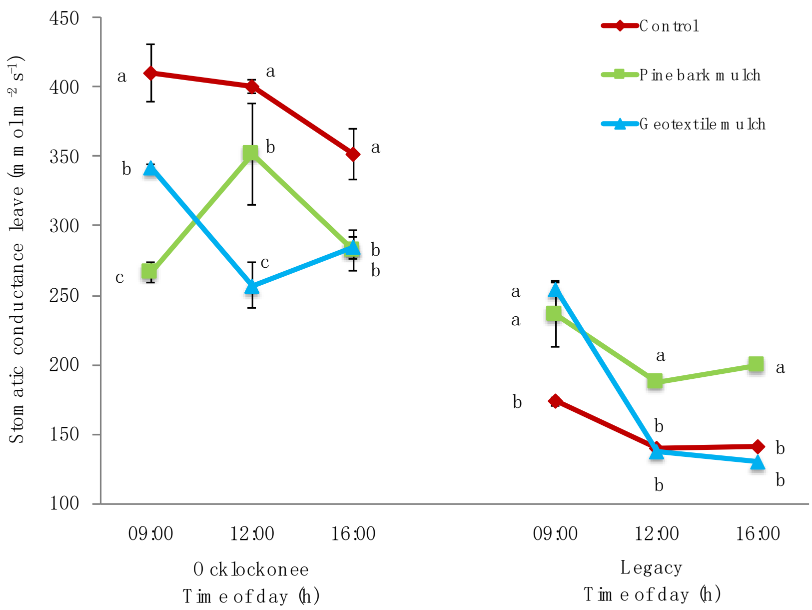


Figure 3. Effects of the different types of mulch on the stomatal conductance in leaves (mmol m^{-2} s^{-1}) at three times of the day. For each different the time of day, lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean (n = 9).

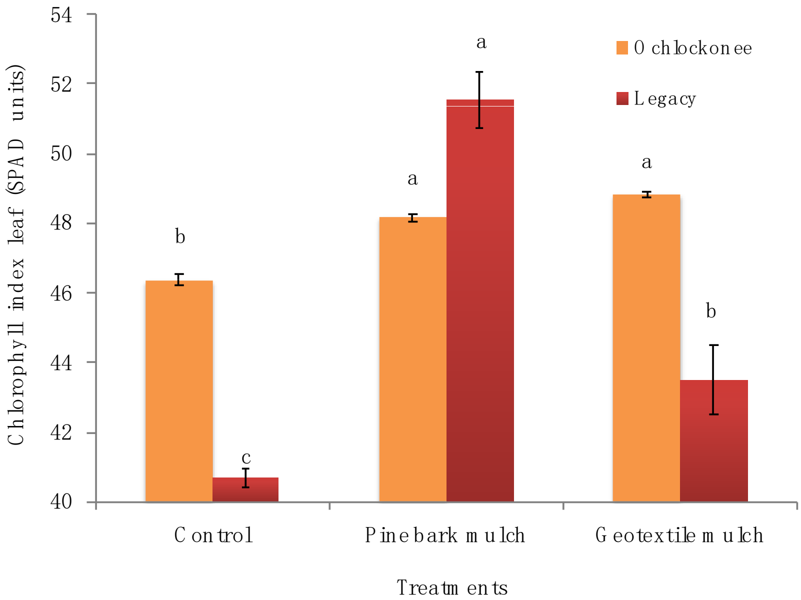


Figure 4. Effects of the different types of mulch on the chlorophyll index in leaves (SPAD units) at three times of the day. For each cultivar, different lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean ($n = 9$).

As presented in Figure 5, there was an increase in LAI in T2 and T3 in the cv. Ochlockonee, with respect to the control treatment, in which both were significantly higher ($p < 0.05$) than T1, being 2.0 on average compared with 0.9 for T1. For the Legacy cultivar, the treatment with pine bark mulch (T2) was also shown to be superior to T1 and T3 ($p < 0.05$). These LAI values coincided with the findings obtained in studies by Muñoz-Vega et al. [62], who reported that blueberries grown under mulch had an increased leaf area. This increase in LAI also coincided with the findings of Sonnentag et al. [44] and Iqbal et al. [63], who showed that the use of mulch maintained soil moisture and soil temperature close to the optimum (T < 28 °C), favoring net photosynthesis, and therefore, vegetative and productive development. No significant differences were observed between the size of the fruits in all the treatments and both cultivars (Figure 6), with values close to 13 mm for both the Ochlockonee and Legacy cultivars. These results are opposed to results from other authors. This difference may be related to the different growing conditions such as soil type [17], the environmental conditions [29], cultivars used [3], types of mulch [29], and different conditions in the control treatments [20,48].

Figure 7 shows that berries with the highest firmness were observed in the Legacy cultivar, in the treatments T3 > T2 > T1 ($p < 0.05$), with the mean values of 75, 72 and 67 gf mm^{-1}, respectively. Regarding the cv. Ochlockonee, no significant differences were observed between the different treatments, with firmness values being 59.9, 58.4 and 57.8 gf mm^{-1}, for T3, T2 and T1, respectively. For the cv. Legacy, there was greater firmness in treatments T3 and T2 (Figure 7). According to studies carried out in other investigations, the use of mulch favors plant hydration, which was improved by maintaining higher evapotranspiration levels compared to the control treatment in the early hours of the day (Figure 3), which could alter the firmness of the fruit [63,64], as observed in the study (Figure 7). However, in the cv. Ochlockonee, the decrease in soil and leaf temperature was not enough to improve fruit firmness, probably due to genetically predefined firmness [63,64], which overshadowed the contributions of the use of mulch. On the contrary, in the cv. Legacy, there were other factors, such as leaf temperature, that correlated (Figure 2D) negatively and significantly with fruit firmness ($R^2 = 0.71$; $p < 0.001$), which showed that the use of mulch improved the firmness of the fruit by reducing gas exchange (Figure 2A) as a result of the decrease in leaf temperature.

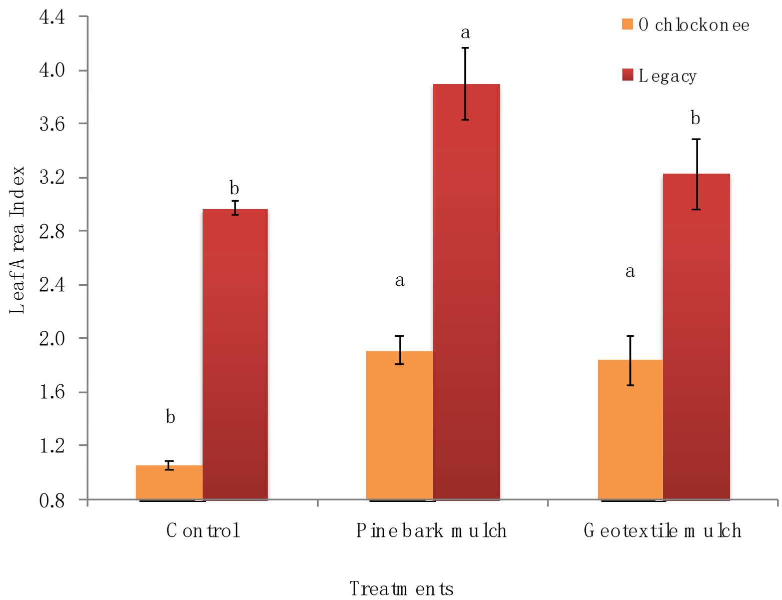


Figure 5. Effects of the different types of mulch on the leaf area index. For each cultivar, different lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean ($n = 9$).

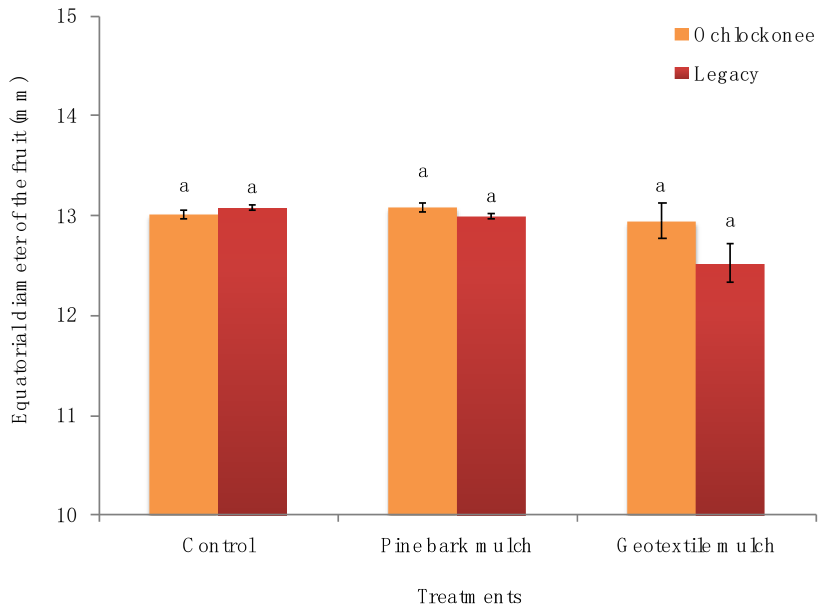


Figure 6. Effects of the different types of mulch on the equatorial diameter of the fruit. For each cultivar, different lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean ($n = 9$).

Figure 8 shows the yield per plant, in which only the cv. Legacy was characterized by a higher yield under the geotextile mulch treatment, with a 100% increase and an average yield of the mean value of 950 g per plant.

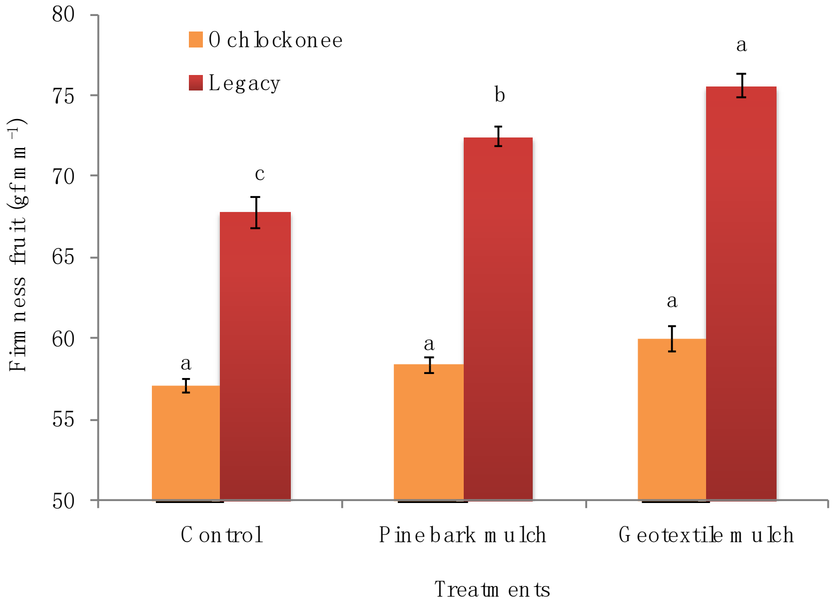


Figure 7. Effects of the different types of mulch on the firmness of the fruit. For each cultivar, different lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean ($n = 9$).

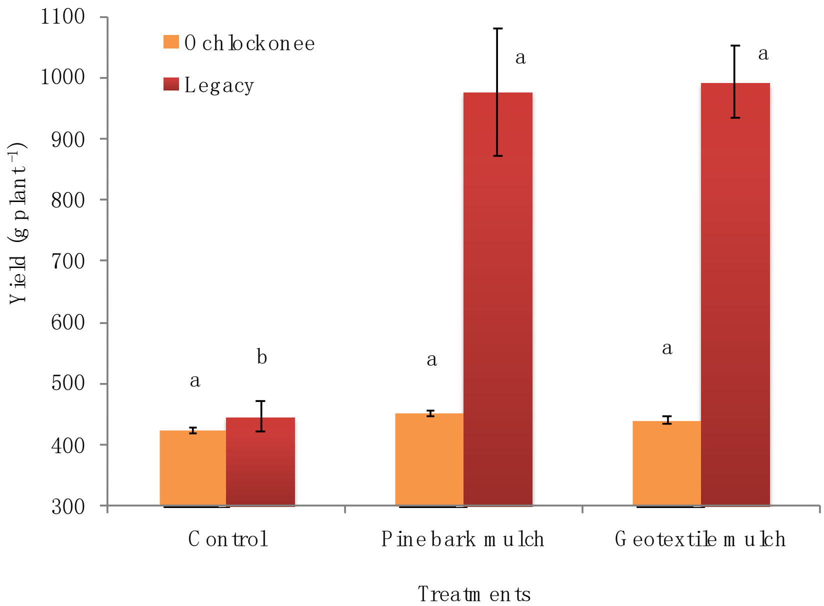


Figure 8. Effects of the different types of mulch on the yield per plant (kg plant^{-1}). For each cultivar, different lowercase letters indicate significant differences for different types of mulch, according to the Tukey test ($p < 0.05$). The vertical bars correspond to ±the standard error of the mean ($n = 9$).

In cv. Ochlockonee, there was no treatment effect, probably because this cultivar is less demanding in terms of nutrient requirement, soil quality and environmental conditions than cv. Legacy, as reported in other studies [2,23,47,48]. Regarding the cv.Legacy, the magnitude of the effect of the mulch treatments (T2 and T3) was 90% greater than in the control treatment. The greater increase in yield in T2 and T3 could be associated with an increase in photosynthetic efficiency, as reflected in Y_{II} and F_v/F_m (Table 1A,B), as proposed by Lobos et al. [57] in their study of photosynthetic efficiency in blueberries. An explanation for this might be that mulch increases the availability of water in the soil due to less direct evaporation from the soil; this has been found to be the case in other research [1,2]. However, several authors pointed out that a decrease in chlorophyll content decreases the net production of photoassimilates [48], which could also affect performance.

4. Conclusions

The rabbiteye blueberry (*Vaccinium virgatum* Aiton cv. Ochlockonee) and Higbush (*Vaccinium corymbosum* L. cv. Legacy) responded favorably to the cultural practices of using pine bark mulch, reducing the Ts by approximately 5 °C during the period of highest daytime ambient temperature, and increasing Ts during the morning by 3 °C, stabilizing the temperature during the day at around 21 °C.

In the cv. Legacy, the use of pine bark mulch increased fruit production by over 90%, both due to a lower degree of stress on the plant during the day and the down-regulation of stomatal conductance levels. This favored greater water use efficiency during the day. This stimulated the cv. Legacy to produce a higher level of chlorophyll content and leaf area in blueberry plants grown in pine bark and geotextile mulches. In addition, the geotextile mulch resulted in a greater firmness of the fruit. Because of this, the geotextile mulch is recommended for the cultivation of the blueberry cv. Legacy.

For cv. Ochlockonee, although the use of mulch increased leaf area and leaf chlorophyll content, it did not have a significant effect on yield and fruit firmness. Therefore, from an agronomic point of view, the technique of using geotextile and pine bark as mulch to stimulate greater production and fruit quality is not recommended if adopted only from this point of view. However, the mulches did reduce weeds and decrease evaporation losses as well, and pine bark promote soil health in the long term.

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