



Growing highbush blueberries (*Vaccinium corymbosum* L.) in a protected environment—How much does a microclimate matter?

Tina Smrke^{a,*}, Dominik Vodnik^b, Robert Veberic^a, Helena Sircelj^b, David Lenarcic^b, Jerneja Jakopic^a

^a University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair for Fruit Growing, Viticulture and Vegetable Growing, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

^b University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair for Applied Botany, Ecology, Plant Physiology and Informatics, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

ARTICLE INFO

Article History:

Received 12 April 2023

Revised 26 June 2023

Accepted 11 July 2023

Available online xxx

Edited by L. Sebastiani

Keywords:

Vaccinium corymbosum L.

Duke

High tunnel

Hail net

Photosynthesis

Fluorescence

Phenolics

Photosynthetic pigments

ABSTRACT

In a two-year experiment, the aim of our study was to evaluate the physiological and chemical response of highbush blueberry (*Vaccinium corymbosum* L.) 'Duke' plants to changed microclimatic conditions that may occur during production in a protected environment—in this case, high tunnels and a hail net. Measurements were performed from 7.00 a.m. to 7.00 p.m. on days with different weather conditions in 2020 and 2021. In the second year of the experiment, both photosynthetic pigments and phenolics were measured in the leaves. The air temperature, relative humidity, photosynthetic photon flux density (PPFD), substrate temperature, and water content were dramatically altered by the protected environment. Along with an increased air temperature and VPD (leaf vapor pressure deficit), significant reductions in net photosynthesis (Pn), stomatal conductance (gs), and Fv'/Fm' (actual PS II efficiency) were detected under both protected environments, with higher values under the high tunnel. Elevated zeaxanthin and AZ/VAZ concentrations were detected in the leaves from 7.00 a.m. to 12.00 p.m., after which these concentrations reduced and continued to do so until the evening. Significance was also observed between high-tunnel and hail-net conditions. Individual and total phenolic concentrations in the leaves did not significantly change during the daytime, but significantly higher values were detected under the hail net. Our results indicate that, regarding photosynthetic pigments and phenolics, modified microclimatic conditions, i.e. elevated air temperature and reduced light quantity under different types of protected environments strongly affect the physiological status of highbush blueberry 'Duke' plants and the chemical composition of leaves.

© 2023 The Authors. Published by Elsevier B.V. on behalf of SAAB. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

1. Introduction

The northern highbush blueberry (*Vaccinium corymbosum* L.) originates from the eastern and mid-western parts of the USA, where winters are severe and summers are moderate (Lobos and Hancock, 2015). However, in the last two decades, the world production volume of blueberries dramatically increased due to their potential health benefits and consequently higher consumption by the consumer (Retamales and Hancock, 2018). Production areas, along with others spread into environments with warmer climates (Lobos and Hancock, 2015). In addition, global warming caused the average air temperatures to increase in recent decades, which is associated with changes in weather patterns and extreme weather events, such as

severe droughts, heavy rain, frequent hailstorms, and spring frosts (Houston et al., 2018). In order to protect the plants and fruit from unexpected weather conditions, different types of protected environments, such as high tunnels and hail nets, are gaining more interest among growers in intensive blueberry production. They protect the fruit from bird attacks, and high tunnels protect developing leaves and flowers from spring frosts, therefore allowing production to expand towards cooler areas (Lobos and Hancock, 2015), despite passive ventilation and heating (Lamont, 2009). Protection under a high tunnel is enabled by a layer of polyethylene covering the high tunnels and generates changes in environmental conditions, such as air temperature, relative humidity, and light (Retamal-Salgado et al., 2015). According to recent studies, under a high tunnel, air temperatures under the high tunnel are from 3.2 to 10.4 °C higher (Li and Bi, 2019), the relative humidity is from 10% to 20% lower (Retamal-Salgado et al., 2015), and light quantity is up to 40% lower compared to ambient conditions (Lobos et al., 2012). All these changes can cause stress in

* Corresponding authors at: University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, Ljubljana, Slovenia.
E-mail address: tina.smrke@bf.uni-lj.si (T. Smrke).

plants by affecting their physiological and chemical processes, such as leaf transpiration, stomatal conductivity, and therefore net photosynthesis (Hao et al., 2019; Ogden and van Iersel, 2009; Retamal-Salgado et al., 2015; Righi et al., 2012). However, blueberry plants usually benefit from production under the high tunnels, which is reflected in earlier (Smrke et al., 2021) and higher yields (Retamal-Salgado et al., 2015).

Among environmental factors that are altered by high tunnels, air temperature has the greatest influence on blueberries, as yield is further increased, and specimens can reach up to 40 °C or higher (Retamal-Salgado et al., 2015). Each blueberry cultivar possesses a specific temperature range for optimal growth and development (Moon et al., 1987) and, in northern highbush blueberries, usually varies between 20 and 25 °C (Hancock, 2009), which is quite low and could result from properties of the climate from which the northern highbush blueberry originates (Lobos and Hancock, 2015).

Temperatures above the individual cultivar threshold cause injuries that primary include a reduction of net photosynthesis by damaging the chloroplast structure and suppressing Photosystem II (PS II), which is highly thermolabile (Chen et al., 2012; Hao et al., 2019; Zheng et al., 2017). At the same time, inhibition of the photosystem usually leads to increased chlorophyll fluorescence, which suggests that fluorescence measurements are a valid method for the detection and evaluation of changes in photosynthetic apparatuses as a result of altered microclimatic conditions (Chen et al., 2012; Hao et al., 2019; Maxwell and Johnson, 2000). Additionally, cellular membrane injuries, senescence, or cellular death can occur (Xu et al., 2006).

Reduced yield (Yang et al., 2019) and plant growth (Hao et al., 2019) are reported to be frequent outcomes of high temperature conditions, while Retamal-Salgado et al. (2015) provided opposite results, where blueberry plants reached higher yields when grown in conditions with elevated air temperature. At the same time, the chemical composition of leaves, including phenolics and photosynthetic pigments (Asanica et al., 2017; Estrada et al., 2015), changes under constant exposure to high temperature, since their synthesis represents the defense mechanism of plants against stress, and their degradation indicates severe stress (Hasanuzzaman et al., 2020; Sircelj, 2008).

Only a few studies on the physiological response of the northern highbush blueberry to modified microclimatic conditions have been conducted with an emphasis on high temperatures (Chen et al., 2012; Hancock et al., 1992; Hao et al., 2019; Zheng et al., 2017), but not on plants grown in vivo under a protected environment. Additional research is required to determine the effect of modified air temperature, light quantity, and relative humidity on the performance of an individual cultivar in real production conditions and consequently for appropriate cultivar selection in individual production areas. More information about plant response to high temperatures is still needed for a better understanding of the physiological and chemical processes in blueberry plants when exposed to high temperatures. The aim of our study was to evaluate the physiological status of plants and the chemical composition of leaves, located either under a high tunnel or a hail net, on two different days in weather that modifies microclimatic conditions under a high tunnel to different extents. We believe that our research will provide a better understanding of the blueberry plant's response to modified microclimatic conditions under a protected environment in terms of physiological status and leaf chemical composition.

2. Materials and methods

2.1. Plant material

A 2-year experiment was performed in 2020 and 2021 on 3 and 4-year-old 'Duke' plants located in the test field of the Biotechnical Faculty, in Ljubljana, Slovenia (latitude: 46°05' N, longitude: 14°47' E,

altitude: 295 m). Thirty plants were planted in 60 L black pots located either under a high tunnel or a hail net (15 plants each), both of which were 6 m wide and 24 m long. Planting distance was 0.6 m within the row. The substrate contained soil, peat, and pine sawdust, one third each by volume. The floor was covered with white polypropylene foil, in order to prevent weed growth. A drip irrigation system was established, with four drippers per plant (1.8 L per dripper h⁻¹). Plants were fertigated nine times in season with mineral fertilizers, from the beginning of April to mid-June. Hail net was placed above the plants at the end of flowering until leaf senescence. The high tunnel (Schwarzmann) was covered with a transparent polypropylene foil, with natural ventilation. Microclimatic conditions were not artificially modified and were constantly monitored with different sensors (air temperature, relative humidity, light quantity).

2.2. Monitoring of environmental conditions

Environmental condition monitoring with different sensors was established to constantly control air temperature, relative humidity (SHT11 integrated sensor, Sensirion Inc., Staefa, Switzerland), substrate temperature (DS18B20, Dallas Semiconductor, Dallas, Texas), water content (SWC; EC5, Decagon Devices, Pullman, USA), and light quantity (Li-Cor quantum sensor, Lincoln, USA) under the high tunnel and the hail net. Data were stored in a measuring instrument and were automatically sent to the IoT cloud. Substrate water content was calculated from EC-5 output (mV) to volumetric water content (VWC;%). Light quantity was additionally expressed as a day light integral (DLI; [mmol m⁻² day⁻¹]).

2.3. Water potential measurements

For measurements, 10 uniform and healthy plants were chosen in individual treatments. The first and last plants in the row were automatically eliminated. Measurements were conducted on days with different weather conditions (July 29, 2020—hot/sunny; September 2, 2020—moderate warm/sunny; August 6, 2021—hot/sunny with transient early afternoon cloudiness; August 12, 2021—hot/sunny) after the ripening period was complete. Midday shoot water potential (ψ ; [MPa]) was measured in current-year, fully developed shoots, which were cut from the plants gradually and immediately before measurements. Measurements were performed using a Scholander pressure chamber and were conducted to determine the water status of the plants (Hladnik et al., 2009).

2.4. Aboveground biomass

The growth of each plant was calculated as the difference between autumn (October 27, 2021) and spring (March 30, 2021) plant volume measurements. With measuring tape, we measured plant height and two widths at half the height (one in the row direction and one at a 90° angle). Plant volume was calculated as

$$V(\text{dm}^3) = \frac{\text{height (cm)} \times 1/2 \text{ width 1 (cm)} \times 1/2 \text{ width 2 (cm)} \times \pi}{1000}$$

2.5. Net photosynthesis, chlorophyll fluorescence, and gas exchange measurements

One fully developed, healthy, sun-exposed leaf, located at half the height on the edge of the bush, was marked for measurements on each of the chosen plants. Net photosynthesis (P_n ; [$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$]) measurements were conducted at 7.00 a.m. (morning), 12.00 p.m. (noon), and 5.00 p.m. (evening) in 2020 and at 12:00 a.m. (noon) in 2021, on the same days on which the ψ measurements were taken, with an LI-6400 (Li-Cor, Lincoln, USA) portable photosynthesis system with an IRGA detector. In 2021, measurements were conducted

only at 12.00 p.m. due to the additional all-day chlorophyll fluorescence and gas exchange measurements. Measurements of Pn were recorded when steady state conditions were reached. During the measurements, the reference CO₂ concentration was maintained at 400 $\mu\text{mol mol}^{-1}$, and the photosynthetic photon flux density (PPFD) was maintained at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (saturating light intensity), while temperature and relative humidity were only monitored at the ambient level. Intercellular CO₂ leaf concentration (Ci; [$\mu\text{mol CO}_2 \text{mol}^{-1}$]) was also monitored (Douthé et al., 2018).

Chlorophyll fluorescence measurements were conducted in 2021 on the same days and on the same leaves as those of the Pn measurements from 7.00 a.m. until 7.00 p.m. every 20 min using an LI-600 (Li-Cor, Lincoln, USA) portable porometer/fluorometer. Actual PS II efficiency (F_v'/F_m') was measured at ambient conditions on light-adapted leaves after saturating an irradiance pulse of 10,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. F_m' is the maximal fluorescence yield of a light-adapted leaf after applying a saturating light pulse on the prevailing ambient light. F_v' is the variable fluorescence yield on a light-adapted leaf. Together with the actual PS II efficiency, stomatal conductance (gs; [$\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$]) and transpiration (E; [$\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$]) were measured. The leaf temperature ($^{\circ}\text{C}$), leaf vapor pressure deficit (VPD; [kPa]), and PPFD were also recorded (Mishra, 2018).

2.6. Leaf sampling for chemical analyses

Leaf samples were collected for phenolics and photosynthetic pigment extraction on both measuring days in 2021, at 7.00 a.m., 12.00 a.m., and 7.00 p.m., to determine the concentrations at different temperatures and lighting conditions. For individual treatment, one randomly chosen, fully developed leaf per plant with no injuries was selected, immediately frozen in liquid nitrogen, lyophilized, ground to a fine powder in a cooled grinder, and stored in a firmly sealed plastic containers at -20°C until extraction.

2.7. Extraction and determination of photosynthetic pigments and phenolics

Photosynthetic pigment extraction from previously ground leaves was performed under dim lighting in five replicates based on a method described by Tausz et al. (2003). For the extractions, 0.2 g of dry leaf powder was mixed with 4 mL of ice-cold 100% acetone in glass test tubes. Samples were homogenized by an Ultra-Turrax T25 homogenizer (IKA, Staufen, Germany) for 30 s. After that, the samples were filtered through polyamide filters (Minisart, SRP 15, PTFE, Sartorius Stedim Biotech, Göttingen, Germany) into vials and immediately subjected to HPLC analysis.

Pigment separation took place on HPLC-DAD (Thermo Finnigan, San Jose, California, USA) on a Spherisorb S5 ODS-2 column ($250 \times 4.6 \text{ mm}$) with an S5 ODS-2 ($50 \times 4.6 \text{ mm}$) precolumn (Alltech Associates, Inc., Deerfield, Illinois, USA), with detection at 440 nm. Mobile Phase A consisted of acetonitrile, water, and methanol (100/10/5; v/v/v), and Mobile Phase B consisted of acetone and ethyl acetate (2/1; v/v). The gradient used was, sequentially, from 10% B to 75% B from 0 to 18 min, from 75% B to 70% B from 18 to 25 min, and from 70% B to 100% B from 25 to 30 min, returning then to initial conditions from 30 to 32 min. The flow rate was maintained at 1 mL min^{-1} (Tausz et al., 2003). Pigment concentrations were calculated from corresponding external standards, based on peak area and expressed in mg g^{-1} of DW.

Leaf phenolics were extracted from previously ground samples in five replicates, according to the previously described method by Mikulic-Petkovsek et al. (2010), where 0.7 g of the sample was mixed with 5 mL of 100% methanol. Samples were thoroughly mixed by vortexing and put into a cooled ultrasonic bath (0°C) for 1 h, followed by centrifugation at 9000 g at 4°C for 10 min (5810 R, Eppendorf centrifuge, Hamburg, Germany). The purchased supernatant was filtered

through polyamide filters (0.2 μm pores, Chromafil AO-20/25, Macherey-Nagel, Düren, Germany) into the vials and stored at -20°C until high-performance liquid chromatography (HPLC) was conducted.

The separation and detection of individual phenolics were performed using a Dionex UltiMate 3000 HPLC (Thermo Scientific, Waltham, Massachusetts, USA) system (Mikulic-Petkovsek et al., 2010). An injected sample that amounted to 20 μL was analyzed for 50 min at a 0.6 mL min^{-1} flow. Detection was set at an absorbance of 280 nm, 350 nm, and 530 nm. Column temperature (Gemini C18, $150 \times 4.6 \text{ mm}$, 3 μm , Phenomenex, California, USA) was maintained at 25°C , and the autosampler temperature was maintained at 10°C . Mobile Phase A consisted of 3% acetonitrile and 0.1% formic acid in bi-distilled water (v/v/v), and Mobile Phase B consisted of 3% bi-distilled water and 0.1% formic acid in acetonitrile (v/v/v). The gradient used was, sequentially, 5% Solvent B from 0 to 15 min, 5%–20% B from 15 to 20 min, 20%–30% B from 20 to 30 min, 30%–90% B from 30 to 35 min, 90%–100% B from 35 to 45 min, and 100%–5% B from 45 to 50 min.

An LTQ XL linear ion trap mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) was used to identify individual phenolics based on mass fragmentation, and their retention times were compared with external standards on HPLC (Mikulic-Petkovsek et al., 2010). A mass spectrometer was operating in negative ion mode with electrospray ionization and m/z scanning from 115 to 1600. Capillary temperature was maintained at 250°C , source voltage at 4 kV, sheath gas at 20 units, and auxiliary gas at 8 units. The flow rate of the samples was 0.6 mL min^{-1} , and the injection volume was 10 μL . The individual phenolic content was calculated from a corresponding external standard or a similar compound curve equation and expressed in mg g^{-1} of dry weight (DW).

2.8. Statistics

Statistical analysis was performed in R Commander i386 4.0.4 (Vienna, Austria) (“R: A language and environment for statistical computing,” 2021). Statistically significant differences between treatments were determined by one-way analysis of variance (ANOVA). Different letters represent significant differences between treatments. In the first year of the experiment, two variables regarding net photosynthesis measurements were observed: the protected environment with two levels (a high tunnel and a hail net, significance marked as *) and the three measuring times (7.00 a.m., 12.00 p.m., and 5.00 p.m., significance marked as #). Each variable was analyzed separately by ANOVA. In the second year of the experiment, net photosynthesis measurements were conducted only at 12.00 p.m. under the high tunnel and the hail net (two levels), suggesting that only one variable was studied. The leaf chemical composition (photosynthetic pigments and phenolics) was evaluated three times a day under both protected environments, indicating the same statistical analysis as that for the net photosynthesis in the first year of the experiment. Linear regression was used to estimate the correlations between VPD as an independent variable and both net photosynthesis (Pn) and stomatal conductance (gs) as dependent variables.

3. Results

3.1. Environmental conditions

Figs. 1 and 2 represent environmental conditions in both protected environments and in Ljubljana, from May 1st to September 6th, in 2020 and 2021. With the red lines, the measuring days are marked. From both figures, it is evident that 2021 was warmer than 2020, with more days reaching values close to 45°C under the high tunnel. Greater fluctuations in relative humidity were detected in 2021.

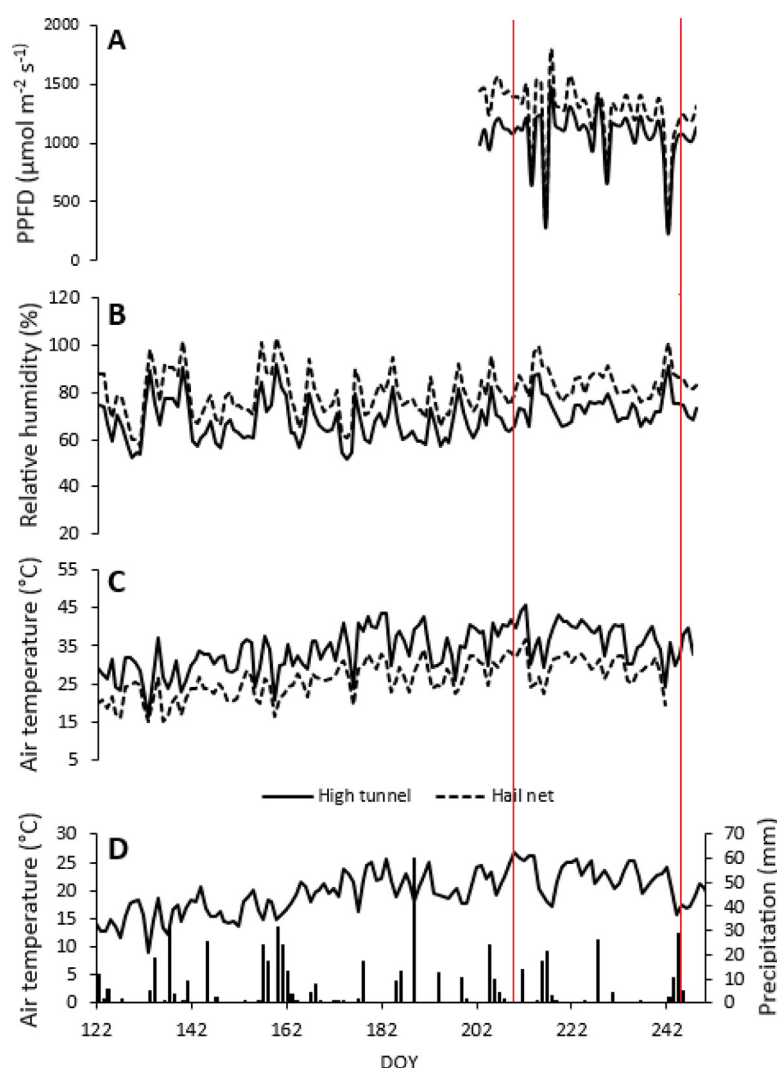


Fig. 1. Microclimatic conditions (PPFD, which started on July 22 (A), relative humidity (B), and air temperatures (C)) under the high tunnel and the hail net, together with air temperature and precipitation (D) for outdoor conditions at the meteorological station Ljubljana, Bežigrad, from May 1 to September 6, 2020 (DOY, day of the year). Measuring days are marked with red lines.

PPFD, maximum air temperature, and relative humidity under protected environment conditions on all measuring days in 2020 and 2021 are shown in Fig. 3. In 2020, PPFD was constantly higher under the hail net on July 29, while some variation between treatments occurred on September 2, from 7.00 a.m. to 13.00 p.m. Mean differences in PPFD and DLI between treatments were 23% and 22.7% on the first day and 14.1% and 16% on the second day, respectively. The maximum air temperature was higher in July 29, compared to September 2. Differences between the high tunnel and the hail net of approximately 17.7% and 18.2% were also found on the July and September measuring date, respectively, with higher values under the high tunnel. The opposite trend was observed under the hail net regarding relative humidity measurements, where values were about 25.8% higher on July 29 and 18.2% higher on September 2.

In 2021, a similar trend was observed in environmental condition measurements as in 2020 (Fig. 3). PPFD and DLI were 21.3% and 21.8% higher, respectively, under the hail net compared to the high tunnel on August 6. A noticeable drop in light quantity occurred in the afternoon, around 15.00 p.m. A similar difference in PPFD and DLI between treatments was found on August 12, where the values were 19.1% and 14.7%, respectively. Maximum air temperature was approximately 21.5% and 22.2% higher under the high tunnel on the first and second measuring date, respectively. Relative humidity was

about 22% and 25.2% higher under the hail net on both measuring days in 2021.

The substrate water content and temperature are presented in Table 1. A significant difference in both observed parameters was determined between plants under the high tunnel and the hail net on both measuring days in both years. In 2020, higher values of water content and temperature were measured under the high tunnel. On the contrary, in 2021, the substrate under the hail net contained more water than pots under the high tunnel on both days, but substrate temperature was still higher under the high tunnel.

3.2. Water potential

Midday shoot water potential (ψ) measurements are shown in Table 2. Significant differences between protected environments were detected on the hot and sunny day in both years. Significantly less negative values were measured in plants under the hail net at both times.

3.3. Aboveground biomass

The mean values of the aboveground biomass measurements of plants included in the experiment are shown in Table 2. In 2020, the

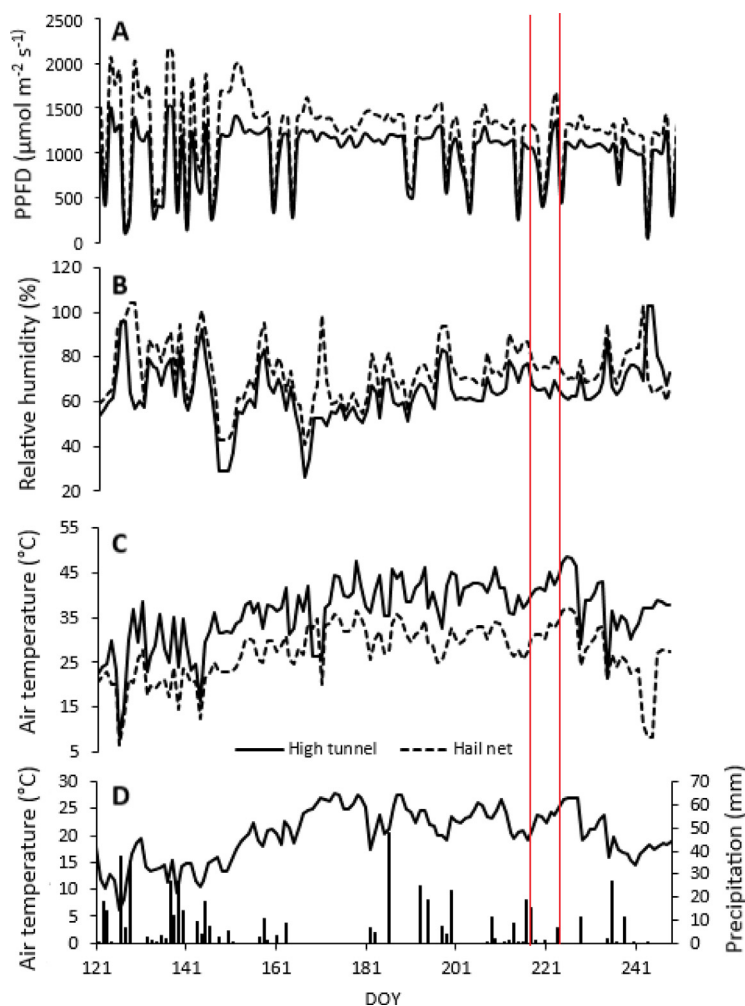


Fig. 2. Microclimatic conditions (PPFD (A), relative humidity (B), and air temperatures (C) under the high tunnel and the hail net, together with air temperature and precipitation (D) for outdoor conditions at the meteorological station Ljubljana, Bežigrad, from May 1 to September 6, 2021 (DOY, day of the year). Measuring days are marked with red lines.

plants grew statistically the same in both protected environments. However, a significant difference was detected in 2021, where the plants grew significantly more intensely under the high tunnel, compared to the hail net.

3.4. Net photosynthesis, chlorophyll fluorescence, and gas exchange

Fig. 4 represents the Pn measurements and the corresponding parameters in 2020 that were conducted at three different measuring times during the day. On July 29, VPDl and leaf temperature values significantly increased from 7.00 a.m. onwards. In the morning and at noon, significant differences were found between treatments in transpiration, with higher values under the high tunnel. Ci significantly changed in leaves under the hail net from the morning onwards. Nevertheless, treatments did not differ in Ci at the same measuring time. Significant drops in Pn and gs were detected in leaves from both protected environments between the morning and evening measurements, together with a significant difference between treatments in the morning and at noon. An opposite trend as was found in the case of VPDl and leaf temperature was observed in WUE, where the values significantly decreased during the day, with no significance between treatments.

Lower values with similar daily variations and statistical results in VPDl and leaf temperature were found on September 2, compared to the measuring day in July (Fig. 4). Daily variations in

gs, E, Pn, and Ci coincide with each other, where significance was detected in both protected environments, with the exception of the E of leaves under the hail net. When comparing both treatments, at the same measuring time, significant differences were found only at noon. In the WUE measurements, significance was found, on a daily level, in the variation of individual protected environments, together with significant differences between treatments at noon.

According to the data shown in Fig. 5, Pn and gs significantly decreased as VPDl increased in 2020. At the same time, higher values were detected under the high tunnel. In Fig. 6, the dependence of Pn and gs on VPDl in 2021 is shown. Pn and gs are shown to decrease as VPDl increased, while no significance was detected in the mean values of those two parameters between the high tunnel and the hail net (Table S1). Pn ranged from 5.09 to 7.71 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under the high tunnel and from 4.75 to 7.42 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under the hail net on both measuring days. High tunnel measurements are similar to those on a hot day in July 2020, while Pn under the hail net exhibited noticeably lower values on that day (2.49 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). However, in plants under the hail net, the change occurred faster.

In 2021, a significant difference between protected environments was found only in Ci on the first measuring day, while other measured parameters did not significantly differ between the high tunnel and the hail net (Table S1). In 2020, at the same measuring time

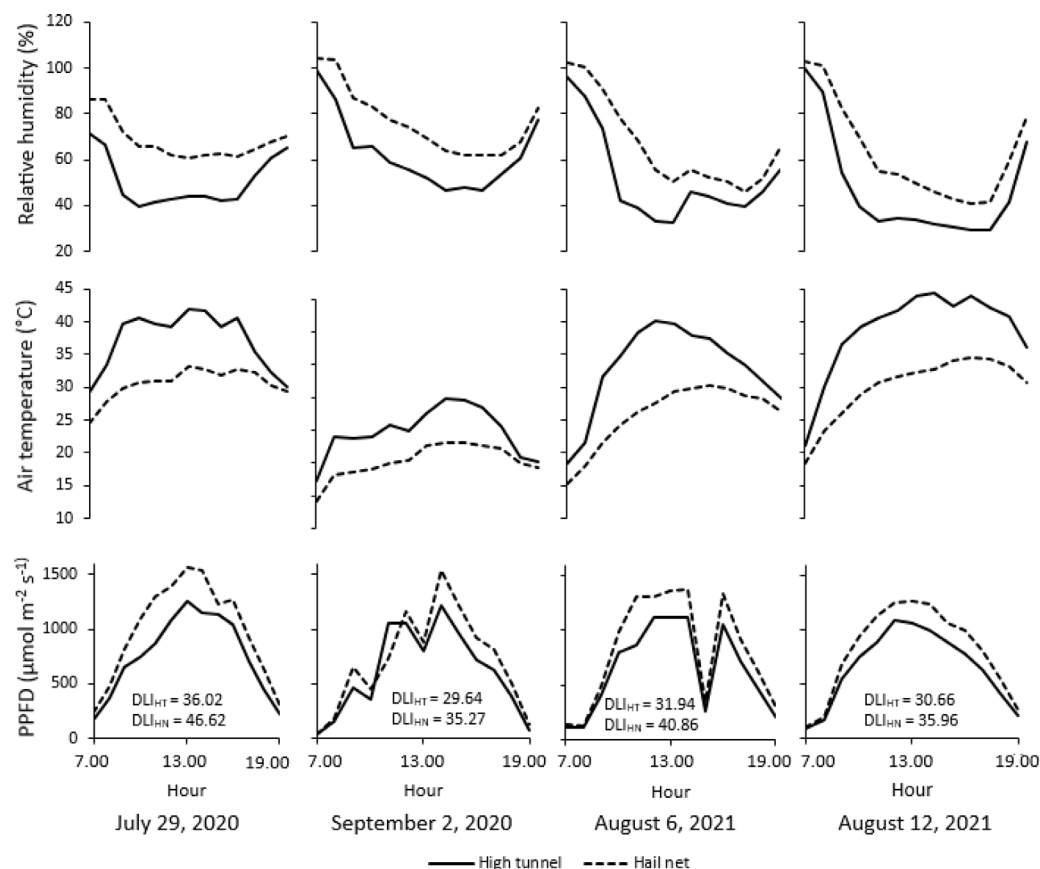


Fig. 3. Microclimatic conditions (PPFD, air temperature and relative humidity) under the high tunnel and the hail net on two different days, each with different weather conditions, in 2020 (July 29–hot/sunny; September 2–moderate warm/sunny) and 2021 (August 6–hot/sunny with transient early afternoon cloudiness; August 12–hot/sunny). DLI_{HT} = day light integral under the high tunnel; DLI_{HN} = day light integral under the hail net; $mmol\ m^{-2}\ day^{-1}$.

(12.00 p.m.), significance was detected in gs, E, Pn, and Ci on September 2 (Fig. 4).

On August 6, 2021, comparable daily variation in VPD, leaf temperature, and PPFD was found under both protected environments (Fig. 7). However, a more noticeable difference was observed in PPFD between the high tunnel and the hail net, while the other two observed parameters mostly coincide, with minor fluctuations. In E, great variation was observed between the high tunnel and the hail

net. According to the data presented in Fig. 7, gs and F_v/F_m' reached higher values under the high tunnel for most of the day.

A trend similar to that found on August 6 was found on August 12 in all measured parameters, shown in Fig. 7.

3.5. Photosynthetic pigments and phenolics in leaves

The results for photosynthetic pigments are presented in Table S2 and S3 and Fig. 8. The foliar photosynthetic pigment concentrations significantly differed between protected environments and measuring times. On both measuring days, the β -carotene/total chlorophylls ratio remained the same during the day under the high tunnel, while it significantly increased from 12.00 p.m. to 7.00 p.m. under the hail net on August 6. Consequently, a significant difference was found between both protected environments at 7.00 p.m. on the first day of measurements. On August 12, significance in β -carotene/total chlorophylls ratio between high tunnel and hail net was determined throughout the day. A similar daily variation was detected between the VAZ/total chlorophylls ratio and VAZ on August 6, where significant differences were measured between both treatments and at all measuring times, with significantly the highest values under the high tunnel and at 12.00 p.m. On the contrary, on the second measuring day at 12.00 p.m., VAZ/total chlorophylls ratio reached significantly higher values under the hail net, while no significance was detected regarding VAZ. Total chlorophylls did not significantly change during the daytime under individual treatments; however, significance was detected at 7.00 a.m. and 12.00 p.m. between the high tunnel and the hail net on August 6 and no significance was determined on August 12. The AZ/VAZ ratio and the zeaxanthin content significantly

Table 1

Substrate water content (VWC) and temperature under the high tunnel and the hail net on two different days, each with different weather conditions, in 2020 (29 July–hot/sunny; September 2–moderate warm/sunny) and 2021 (August 6–hot/sunny with transient early afternoon cloudiness; August 12–hot/sunny).

2020	Location	VWC (%)	Substrate temperature (°C)
July 29	High tunnel	27.31 ± 0.42	32.3 ± 1.5
	Hail net	25.93 ± 0.75	28.2 ± 1.4
September 2	High tunnel	28.23 ± 0.42	21.2 ± 1.3
	Hail net	25.77 ± 0.15	17.9 ± 0.7
2021	High tunnel	25.52 ± 0.68	26.7 ± 1.8
	Hail net	29.54 ± 1.43	21.6 ± 1.2
August 12	High tunnel	25.16 ± 0.64	33.4 ± 1.0
	Hail net	30.16 ± 1.93	29.1 ± 0.3

Data are mean values $\pm$ standard error from 7.00 a.m. to 7.00 p.m. Significant differences were estimated using the *t*-test ($p < 0.05$).

** $p < 0.01$; *** $p < 0.001$.

Table 2

Midday stem water potential (Ψ) under the high tunnel and the hail net on two different days, each with different weather conditions, in 2020 (July 29—hot/sunny; September 2—moderate warm/sunny) and 2021 (August 6—hot/sunny with transient early afternoon cloudiness; August 12—hot/sunny) and aboveground biomass growth in 2020 and 2021.

2020	Location	Ψ (MPa)	Aboveground biomass (dm ³)
July 29	High tunnel	-0.97 ± 0.12	71.3 ± 21.6
	Hail net	-0.81 ± 0.05	59.3 ± 14.2
	<i>p</i>	**	NS
September 2	High tunnel	-0.77 ± 0.13	71.3 ± 21.6
	Hail net	-0.71 ± 0.05	59.3 ± 14.2
	<i>p</i>	NS	NS
2021			
August 6	High tunnel	-0.63 ± 0.04	75.1 ± 17.3
	Hail net	-0.64 ± 0.06	39.3 ± 8.4
	<i>p</i>	NS	***
August 12	High tunnel	-0.75 ± 0.06	75.1 ± 17.3
	Hail net	-0.67 ± 0.06	39.3 ± 8.4
	<i>p</i>	**	***

Data are mean values $\pm$ standard error of 10 replicates. Significant differences were estimated using the *t*-test ($p < 0.05$).

** $p < 0.01$; *** $p < 0.001$.

increased under the high tunnel and the hail net, reaching the highest values at 12.00 p.m. on both days of measurements. However, in AZ/VAZ, significance between protected environments was detected only at 7.00 a.m. on the first, and at 12.00 p.m. on the second measuring day, while significance in zeaxanthin content was found at 12.00 p.m.

Fig. 9 represents the groups of phenolics found in blueberry leaves, and Table S4 and S5 present the statistical analysis of individual phenolics. No significant increase or decrease in individual groups of phenolics was detected during the daytime on both measuring days. Some exceptions were found among individual phenolics, but these differences did not significantly contribute to the overall group content. On the other hand, significance was observed between the high tunnel and the hail net at all three measuring times. In August 6, significantly higher levels of phenolic acids, flavonols, and total phenolics were measured in leaves from plants under the hail net. On the contrary, a higher flavan-3-ol content was measured under the high tunnel. Most individual phenolics correspond to the overall group content. Some exceptions were found, such as the phenolic acids 5-galloylquinic acid, *p*-coumaroylquinic acid, and di-caffeoylquinic acid 2, the flavan-3-ol procyanidin dimer 1, and, among the flavonols, myricetin derivatives, quercetin-3-*O*-glucoside, quercetin-3-*O*-malonyl hexoside, isorhamnetin derivatives, and syringetin-3-*O*-glucoside.

A trend similar to that found on August 6 was found on August 12 in the differences in the content of groups of phenolics (Fig. 9) and individual phenolics (Tables S4 and S5) between the high tunnel and the hail net. However, a pronounced difference between both of these days was found in flavan-3-ols content, where significantly higher concentrations were measured on the hot and sunny day (August 12) under the hail net.

4. Discussion

To date, only a few studies on the physiological status of blueberry plants under modified microclimates in natural conditions have been conducted (Retamal-Salgado et al., 2015). In the present study, higher temperatures led to a significantly warmer substrate and a lower relative humidity under the high tunnel, when compared with the hail net, which is in accordance with previously reported results (Li and Bi, 2019; Ogden and van Iersel, 2009; Smrke et al., 2021). Higher temperatures and consequently lower relative humidity values under the high tunnels result from passive solar radiation and restricted natural

ventilation (Lamont, 2009; Ogden et al., 2011). Lower PPFD was also detected under the high tunnel, with the most pronounced differences found on clear, sunny days. Substrate water content differed between the high tunnel and the hail net in both years, despite irrigation and constant water fluctuation monitoring.

It is already known that growing blueberries under a high tunnel does not negatively affect aboveground biomass development. Growing under a high tunnel prolongs the growing period before leaf senescence and brings forth bud swelling, which could explain the higher growth rates in 2021 (Ciordia et al., 2002; Retamal-Salgado et al., 2015). Blueberries originate from shady locations, so the exposure of plants to full sun could cause radiation stress. Transparent polypropylene foil, by which the high tunnel was covered, reduced the PPFD that would have otherwise reached the plants and cause plant stress, thus allowing for undisturbed growth (Smrke et al., 2021). Spring frost occurred in 2021, which affected plant aboveground biomass development under the hail net.

In recent years, more sunny days with temperatures over 35 °C have been occurring during the growing period (Houston et al., 2018), and this temperature can be additionally increased by high tunnels (Retamal-Salgado et al., 2015; Smrke et al., 2021). During the hot and sunny weather on July 29, 2020, leaf temperatures did not differ between protected environments, probably due to the significantly higher transpiration (*E*) measured under the high tunnel, which cooled the plants and brought the temperature of the leaves closer to those under the hail net (Hao et al., 2019). At the same time, the high temperatures and low relative humidity substantially increased the VPD (1.5 kPa to 3.5 kPa) during the day at both sites and therefore decreased the *g_s* (Hao et al., 2019; Retamal-Salgado et al., 2015; Zheng et al., 2017). The latter two parameters are strongly correlated, since a higher stomatal conductance indicates a higher gas exchange between the leaf and the atmosphere, which includes CO₂ assimilation and transpiration (Hao et al., 2019). This is also evident from our results, where both parameters under the high tunnel were higher in the morning (Fig. 4; Retamal-Salgado et al., 2015). We can relate this response to soil water content, which was, despite the fact that pots were irrigated in both treatments, slightly lower under the hail net than under the high tunnel (Table 1). On the other hand, we could not confirm this relation with water potential (Ψ) measurements (Table 2).

A significant reduction in stomatal conductivity was detected from morning until noon under the high tunnel, while no significant change was detected in transpiration. This could be a strategy employed by plants to survive changed and possibly stressful growing conditions (Ben-Asher et al., 2008; Zheng et al., 2017).

A higher stomatal conductance in plants grown under the high tunnel contributed to higher transpiration and enabled higher photosynthetic rates (*P_n*). Under saturating light, *P_n* was higher in high tunnel plants in the morning and at midday, while both treatments, at the end of the day, when evaporative demand was still very high, had a similar *P_n* level, the lowest in a 12 h period.

Measurements on September 2, 2020, showed that stomatal conductance, transpiration, and net photosynthesis increased from morning to noon, and significant differences emerged in the same way as in July. As opposed to July, however, a significantly higher intercellular CO₂ concentration was measured in leaves under the high tunnel (Fig. 4). This suggests that a higher leaf gas exchange provided the plants with sufficient CO₂ for optimal carbon assimilation. This was most likely strongly influenced by the air temperature, since the optimal temperature for CO₂ intake in 'Duke' is approximately 32 °C (Zheng et al., 2017), while the air temperature during measurements did not exceed 30 °C.

In 2021, when *P_n* measurements were conducted only at noon, no significant differences in stomatal conductance, transpiration, or *P_n* were observed between the plants from different environments, probably due to a higher data variability (Table S1). However, linear

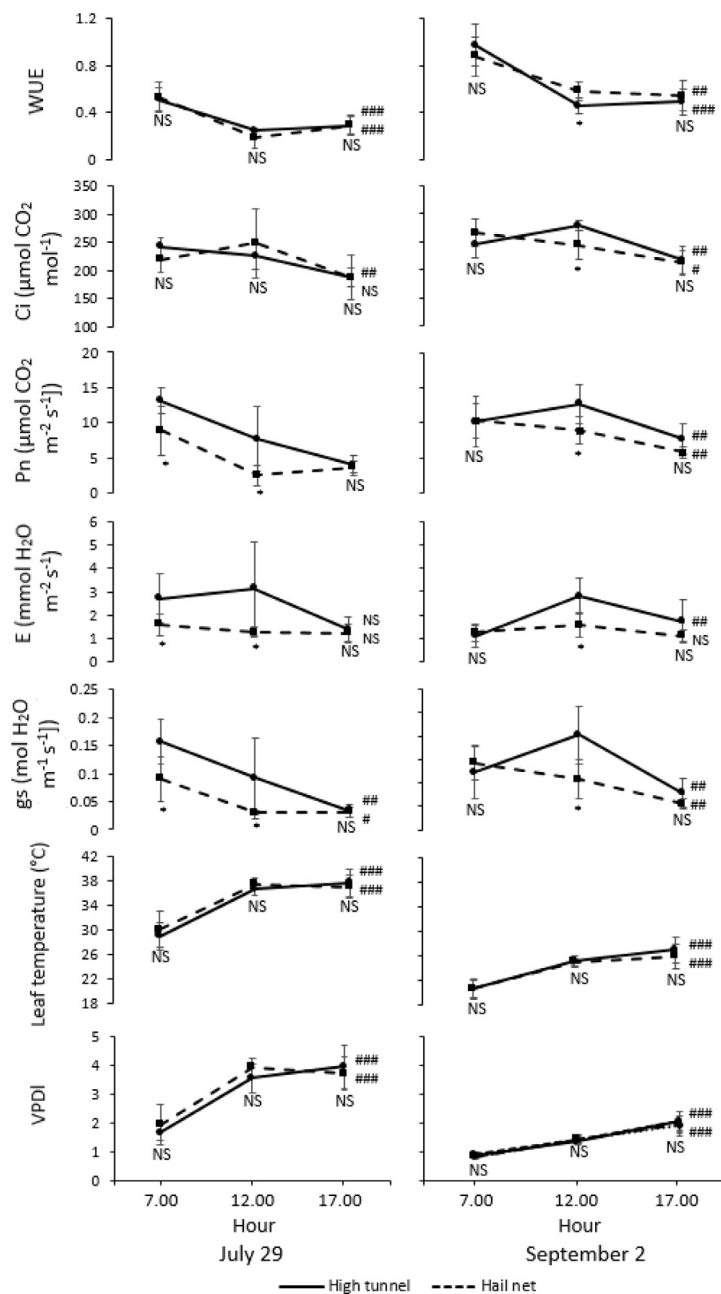


Fig. 4. VPD, leaf temperature, gs, E, Pn, Ci, and WUE measurements under the high tunnel and the hail net at 7.00 a.m., 12.00 p.m., and 5.00 p.m., on two different days, each with different weather conditions, in 2020 (July 29—hot/sunny, September 2—moderate warm/sunny).

Data are mean values $\pm$ standard error of 10 replicates. Significant differences between measuring times within individual protected environments were estimated using the LSD test (#, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$; NS, not significant) and between protected environments within individual measuring times using the t -test (*, $p < 0.05$; NS, not significant).

analysis showed a negative correlation between stomatal conductance and VPD and between plant photosynthesis and VPD (Ferlan et al., 2016; Retamal-Salgado et al., 2015). The drop in those parameters occurred faster under the hail net (Fig. 6), indicating a greater responsiveness in those plants to changed growing conditions, due to the greater variation in PPFD and relative humidity (Li and Bi, 2019; Retamal-Salgado et al., 2015). A noticeable, similar drop in F_v/F_m' measurements on both sites was observed during the 12 h measurements (Fig. 7), suggesting that monitoring chlorophyll fluorescence, together with xanthophyll cycle pigment concentrations and their ratios (violaxanthin, antheraxanthin, and zeaxanthin), is a valid method of indicating changed microclimatic or even stressful conditions (Chen et al., 2012). On August 6, a transient light decrease

occurred in the afternoon, and this reduced PPFD, leaf temperature, and VPD and increased F_v/F_m' , indicating the quick responsiveness of PS II to environmental changes (Chen et al., 2012; Maxwell and Johnson, 2000). In addition, higher zeaxanthin and VAZ concentrations measured under the high tunnel (Table S2) indicate an elevated elimination of excess energy (i.e., heat) from the photosynthesis apparatus (Sircelj, 2008; Vosnjak et al., 2021), thus enabling plants to survive under stressful conditions caused by higher air temperatures. Among xanthophyll cycle pigments, an increase in zeaxanthin, together with violaxanthin de-epoxidation, plays a crucial role in protecting the photosynthesis apparatus from heat damage. Transformation from violaxanthin to zeaxanthin occurs in a matter of seconds, while a reverse reaction can require a range from several

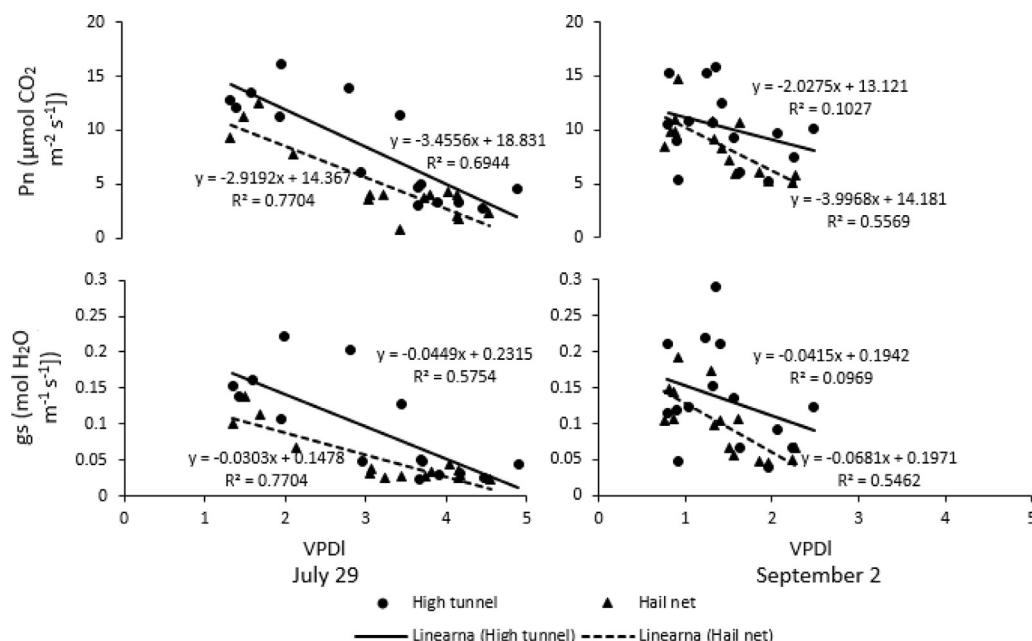


Fig. 5. Relationship between Pn and gs measurements and VPDl under the high tunnel and hail net at 7.00 a.m., 12.00 p.m. and 5.00 p.m., on two weather different days in 2020 (July 29 – hot/sunny, September 2 – moderate warm/sunny).

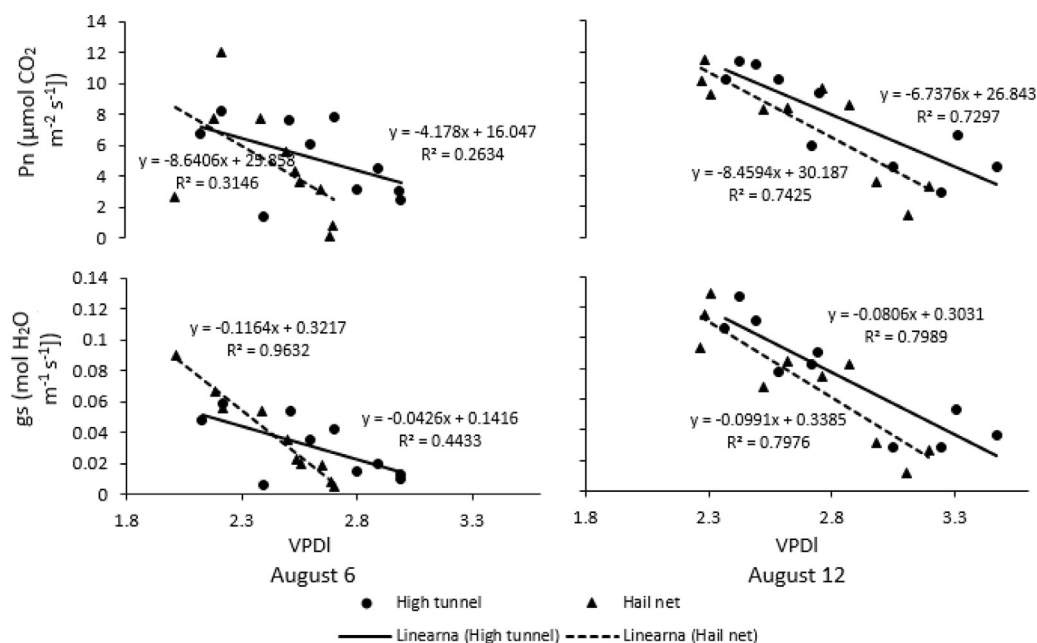


Fig. 6. Relationship between Pn and gs measurements and VPDl under the high tunnel and the hail net at 12.00 p.m., on two different days, each with different weather conditions, in 2021 (August 6—hot/sunny with transient early afternoon cloudiness; August 12—hot/sunny).

minutes to several hours (Sircelj, 2008). For the same reason xanthophyll cycle pigments, from 12.00 p.m. to 7.00 p.m., did not fully return to the initial conditions they were in the morning. Fast reactions in the xanthophyll cycle are associated with the resistance of plants to stress, which was additionally confirmed by a positive correlation between increasing air temperature, PPFD, zeaxanthin content, and consequently VAZ and the AZ/VAZ ratio in leaves (Depka et al., 1998). Increased VAZ is the first defense of plants against stressful conditions (Fernández-Marín et al., 2018; Yin et al., 2010), while AZ/VAZ expresses the ability to dissipate excess solar energy as heat, in order to protect PS II from photoinhibition (Yin et al., 2010).

Phenolics are known for their protective role in plants; however, the synthesis of individual phenolics is elevated under UV radiation

(Jakopic et al., 2009; Smrke et al., 2021). The content of flavonols was on both measuring days higher under the hail net, while the highest flavan-3-ols contents differed between August 6 and August 12. This suggests a specific anti UV-radiation activity of flavonols (Fig. 9, Table S4) (Jakopic et al., 2009). Interestingly, no significant variation in individual groups of phenolics was found on a daily level within individual protected environments. Plants have to adapt to changed and possibly stressful microclimatic conditions where they grow, and the accumulation of phenolic compounds in leaves is considered an adaptation of plants on these conditions (Naikoo et al., 2019; Pereira, 2016). According to these results, we can conclude that light quantity was probably a crucial factor, altering phenolic accumulation in 'Duke' leaves (Naikoo et al., 2019).

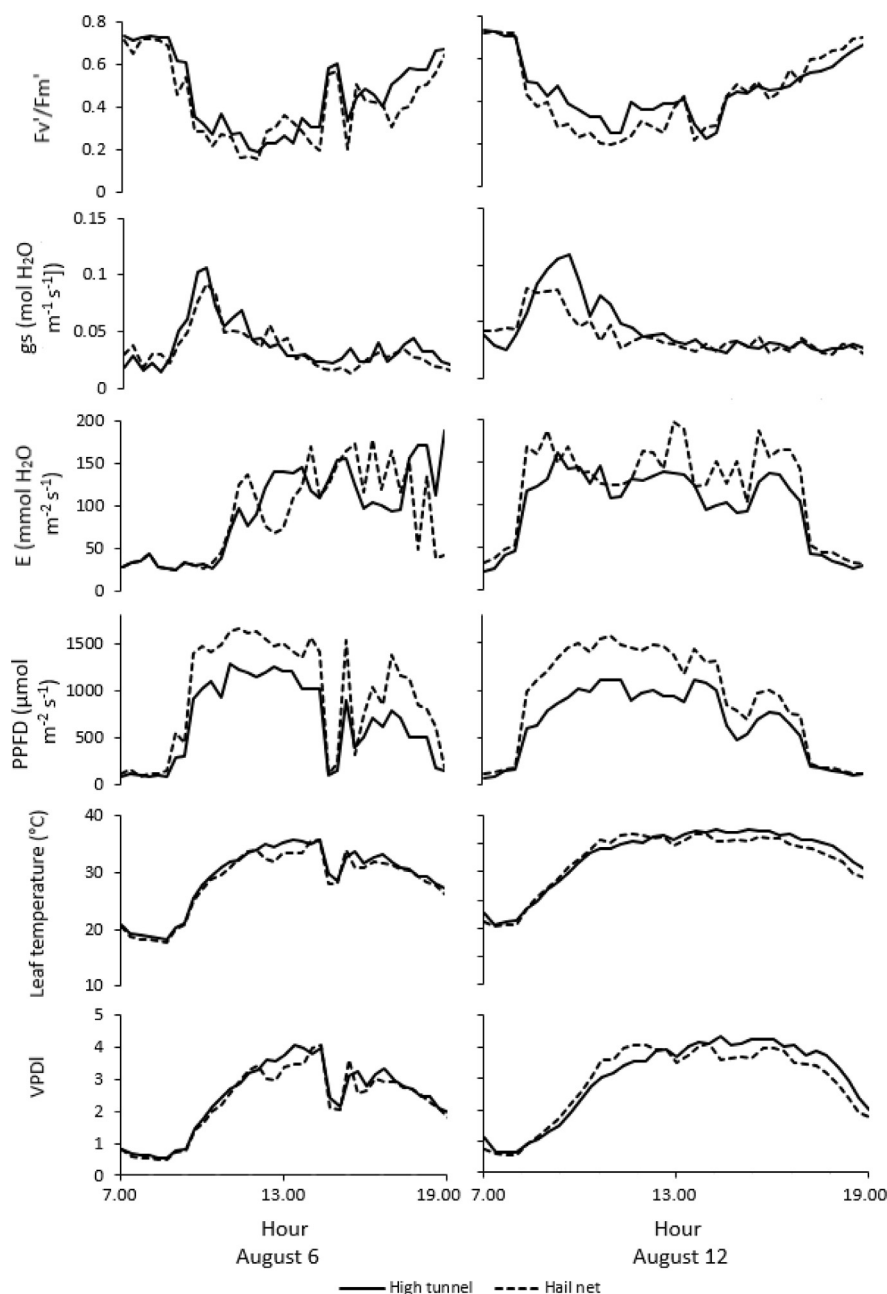


Fig. 7. VPD, leaf temperature, PPFD, E , g_s , and F_v/F_m' measurements under the high tunnel and the hail net from 7.00 a.m. to 7.00 p.m. on two different days, each with different weather conditions, in 2021 (August 6—hot/sunny with transient early afternoon cloudiness; August 12—hot/sunny).

A greater divergence in F_v/F_m' between the high tunnel and the hail net was observed on the second measuring day (August 12), when higher values were detected in plants under the high tunnel (Fig. 7). That day was sunny and warm from the morning onwards, while August 6 was still foggy until 9.20 a.m. For that reason, the plants under the hail net had to export excess solar energy through chlorophyll fluorescence already in the morning, when the values were close to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, while these values were approximately half as low under the high tunnel (Chen et al., 2012; Maxwell and Johnson, 2000). Moreover, the difference in PPFD between both protected environments decreased, and so did the difference in F_v/F_m' . These results cannot be correlated with substrate water content and Ψ , since significant higher values were measured under the hail net, despite irrigation. Interestingly, zeaxanthin content was higher under the hail net at 12.00 p.m., and this is probably a result of the progressive increase in PPFD from the early morning onwards (Table

S3). The plants can adapt to slowly progressing stressful conditions by enhanced carotenoids synthesis, including β -carotene and xanthophyll cycle pigments (Sircelj et al., 2007).

A protected environment alters microclimatic conditions by surrounding the plants but can sometimes cause plant stress (Li and Bi, 2019; Retamal-Salgado et al., 2015). This is reflected in concentrations of chloroplast pigments and their ratios (Asanica et al., 2017; Vosnjak et al., 2021). In our leaf samples, neoxanthin and lutein, which are structural pigments, significantly differed between the high tunnel and the hail net on August 6, 2021 (Table S2). Their content is usually unchanged; however, if a decrease occurs, it is at the expense of elevated β -carotene due to chemical transformations (Saini et al., 2015), which was confirmed by our results. Total chlorophyll content (chlorophyll $a + b$) was significantly higher in leaves under the high tunnel, which is probably associated with the lower radiation energy reaching the plants. Plants often adapt to low light

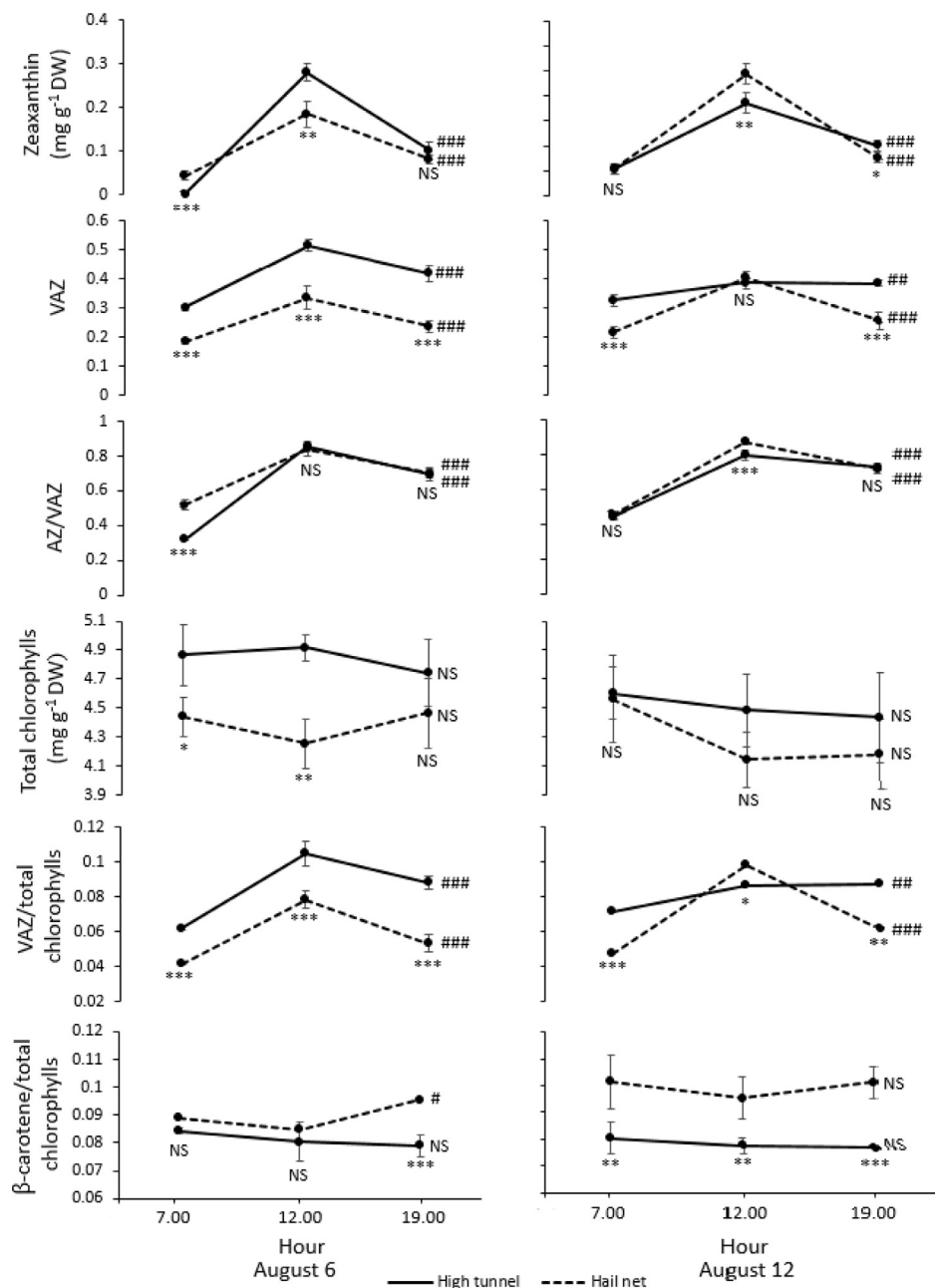


Fig. 8. β -carotene/total chlorophylls, VAZ/total chlorophylls, and AZ/VAZ ratios, together with total chlorophylls, VAZ, and zeaxanthin concentrations in blueberry leaves collected from plants under the high tunnel and the hail net at 7.00 a.m., 12.00 p.m. and 7.00 p.m. on August 6 and August 12, 2021.

Data are mean values $\pm$ standard error of 4 replicates. Significant differences between measuring times within individual protected environments were estimated using the LSD test (#, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$; NS, not significant) and between protected environments within individual measuring times using the t -test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, not significant).

intensities in order to, by increasing their chlorophyll content in their leaves, capture more solar energy (Johnson et al., 2005). The chlorophyll a/b ratio usually varies between 2.2 (shade) and 4.2 (sun) (Fernández-Marín et al., 2018). These values can be reached under high amounts of light or elevated stressful conditions, which, in our experiment, occurred during the 12 h interval only under the high tunnel, indicating that those environmental conditions caused plant stress (Camejo et al., 2005).

The total chlorophylls and the chlorophyll a/b ratio did not differ between plants from different protected environments on August 12 (Table S3), probably due to the higher variability of the results and the equally irradiated leaf samples, respectively. However, like August 6, an increase in the chlorophyll a/b ratio

was again found during the daytime measurements—this time on both sites. When stress is severe, VAZ needs help in dissipating excess energy, which is provided by β -carotene. This is evident from an elevated β -carotene/total chlorophylls ratio (Fernández-Marín et al., 2018), which, in this study, was significantly increased in plant leaves on August 12 under the hail net, where a higher PPFD was measured.

5. Conclusions

Growing blueberry plants under protected environments, such as high tunnels, alters the microclimatic conditions surrounding the plants. Differences in those conditions between the high

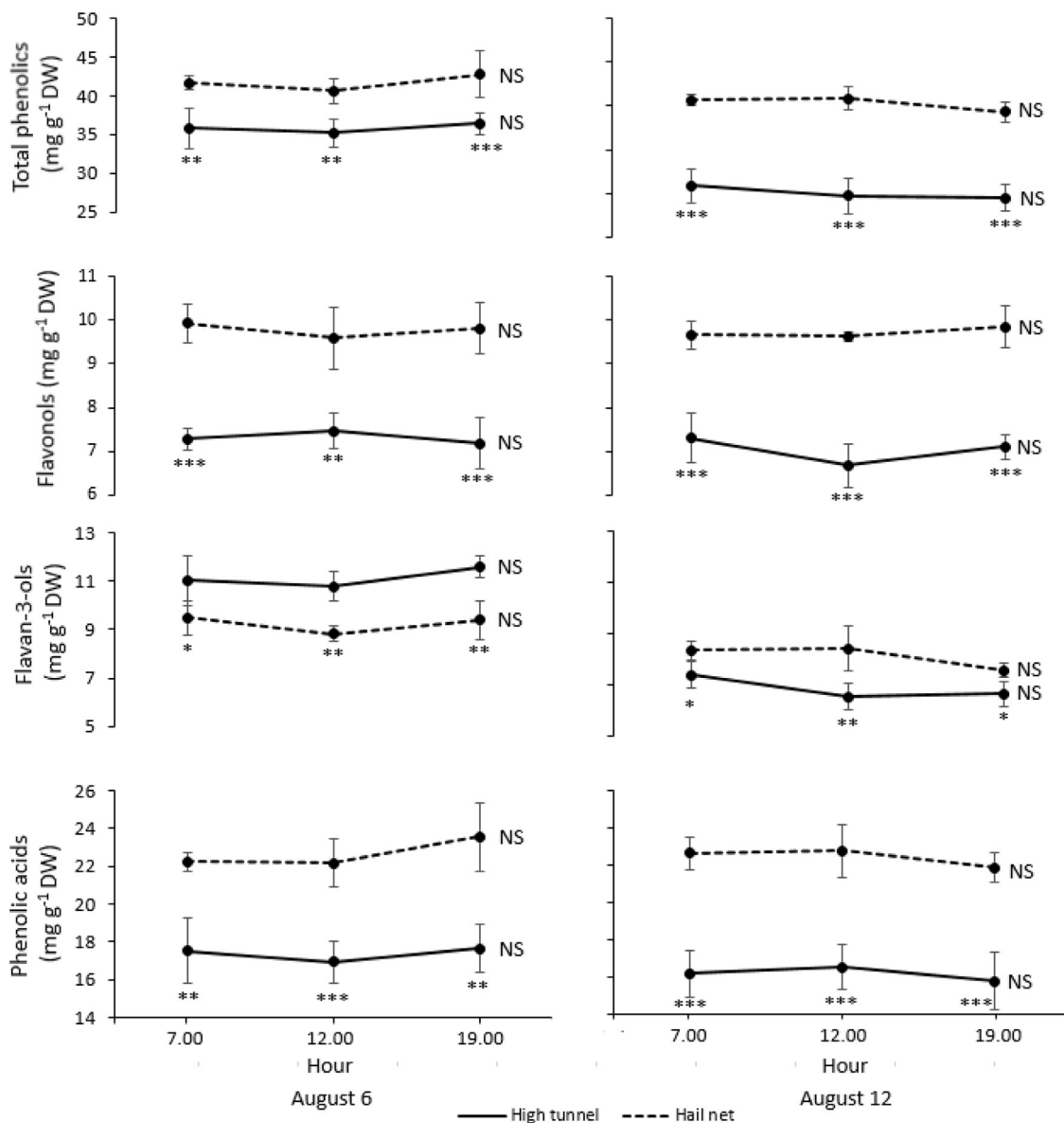


Fig. 9. Phenolic acids, flavan-3-ols, flavonols, and total phenolics in blueberry leaves collected from plants under the high tunnel and the hail net at 7.00 a.m., 12.00 p.m. and 7.00 p.m. on August 6 and August 12, 2021.

Data are mean values $\pm$ standard error of 4 replicates. Significant differences between measuring times within individual protected environments were estimated using the LSD test ($p < 0.05$) and between protected environments within individual measuring times using the t -test ($p < 0.05$).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant.

tunnel and the hail net were most prominent on hot and sunny days, when plants faced transient stress and responded with stomatal closure and photoprotective mechanisms. No pronounced difference was detected in the leaf physiological processes of high-tunnel- or hail-net-grown plants. However, the amplitude and dynamics of the measured physiological traits differed slightly between the two sites depending on the specific microclimatic conditions. Despite the fact that leaf-level measurements did not reveal chronic stress in blueberries in the high tunnel, we cannot recommend the use of this protected environment for production under climatic conditions similar to those typical of the geographic region studied. More detailed growth analyses should be conducted to examine the possible effects of lower DLI in the high tunnel on the carbon balance of the plant. In this context, the issue of increased soil temperature and possible negative effects on roots should also be considered.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Tina Smrke: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Dominik Vodnik:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Robert Veberic:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Helena Sircelj:** Methodology, Funding acquisition, Supervision, Writing – review & editing. **David Lenarcic:** Data curation, Formal

analysis, Writing – review & editing. **Jerneja Jakopic:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Acknowledgements

The authors acknowledge the financial support of the Slovenian Research and Innovation Agency within the research programs Horticulture (P4-0013) and Agroecosystems (P4-0085) and the infrastructural center IC RRC AG (10-0022-0481-001).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2023.07.023.

References

- Asanica, A., Delian, E., Tudor, V., Teodorescu, R.I., 2017. Physiological activity of some blueberry varieties in protected and outside conditions. *Agric. Sci. J.* 6, 31–39.
- Ben-Asher, J., Garcia, Y., Garcia, A., Hoogenboom, G., 2008. Effect of high temperature on photosynthesis and transpiration of sweet corn (*Zea mays* L. var. rugosa). *Photosynthetica* 46, 595–603. <https://doi.org/10.1007/s11099-008-0100-2>.
- Camejo, D., Rodríguez, P., Morales, M.A., Dell'Amico, J.M., Torrecillas, A., Alarcón, J.J., 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* 162, 281–289. <https://doi.org/10.1016/j.jplph.2004.07.014>.
- Chen, W., Cen, W., Chen, L., Di, L., Li, Y., Guo, W., 2012. Differential sensitivity of four highbush blueberry (*Vaccinium corymbosum* L.) cultivars to heat stress. *Pakistan J. Bot.* 44, 853–860.
- Ciordia, M., Díaz, M.B., García, J.C., 2002. Blueberry culture both in pots and under italian-type tunnels. *Acta Hort.* 123–127. <https://doi.org/10.17660/actahortic.2002.574.17>.
- Depka, B., Jahns, P., Trebst, A., 1998. β -Carotene to zeaxanthin conversion in the rapid turnover of the D1 protein of photosystem II. *FEBS Lett* 424, 267–270. [https://doi.org/10.1016/S0014-5793\(98\)00188-4](https://doi.org/10.1016/S0014-5793(98)00188-4).
- Douthé, C., Gago, J., Ribas-Carbo, M., Nunez, R., Pedrol, N., Flexas, J., 2018. Measuring photosynthesis and respiration with infrared gas analysers. In: Sanchez-Moreiras, A.M., Regiosa, M.J. (Eds.), *Advances in Plant Ecophysiology Techniques*. Springer, Cham, pp. 51–75.
- Estrada, F., Escobar, A., Romero-Bravo, S., González-Talce, J., Poblete-Echeverría, C., Caligari, P.D.S., Lobos, G.A., 2015. Fluorescence phenotyping in blueberry breeding for genotype selection under drought conditions, with or without heat stress. *Sci. Hortic.* 181, 147–161. <https://doi.org/10.1016/j.scienta.2014.11.004>.
- Ferlan, M., Eler, K., Simončič, P., Batič, F., Vodnik, D., 2016. Carbon and water flux patterns of a drought-prone mid-succession ecosystem developed on abandoned karst grassland. *Agric. Ecosyst. Environ.* 220, 152–163. <https://doi.org/10.1016/j.agee.2016.01.020>.
- Fernández-Marín, B., García-Plazaola, J.I., Hernández, A., Esteban, R., 2018. Advances in plant ecophysiology techniques. In: Sánchez-Moreiras, Adela M., Regiosa, M.J. (Eds.), *Advances in Plant Ecophysiology Techniques*. Springer International Publishing AG, USA, pp. 29–50.
- Hancock, J., 2009. Highbush blueberry breeding. *Latv. J. Agron.* 12, 35–38.
- Hancock, J.F., Haghghi, K., Krebs, S.L., Flore, J.A., Draper, A.D., 1992. Photosynthetic heat stability in highbush blueberries and the possibility of genetic improvement. *HortScience* 27, 1111–1112. <https://doi.org/10.21273/hortsci.27.10.1111>.
- Hao, L., Guo, L., Li, R., Cheng, Y., Huang, L., Zhou, H., Xu, M., Li, F., Zhang, X., Zheng, Y., 2019. Responses of photosynthesis to high temperature stress associated with changes in leaf structure and biochemistry of blueberry (*Vaccinium corymbosum* L.). *Sci. Hortic.* 246, 251–264. <https://doi.org/10.1016/j.scienta.2018.11.007>.
- Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Al Mahmud, J., Fujita, M., Fotopoulos, V., 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. *Antioxidants* 9, 1–52. <https://doi.org/10.3390/antiox9080681>.
- Hladnik, J., Eler, K., Kržan, K., Pintar, M., Vodnik, D., 2009. Short-term dynamics of stomatal response to sudden increase in CO₂ concentration in maize supplied with different amounts of water. *Photosynthetica* 47, 422–428. <https://doi.org/10.1007/s11099-009-0065-9>.
- Houston, L., Capalbo, S., Seavert, C., Dalton, M., Bryla, D., Sagili, R., 2018. Specialty fruit production in the Pacific Northwest: adaptation strategies for a changing climate. *Clim. Change* 146, 159–171. <https://doi.org/10.1007/s10584-017-1951-y>.
- Jakopic, J., Stampar, F., Veberic, R., 2009. The influence of exposure to light on the phenolic content of “Fuji” apple. *Sci. Hortic.* 123, 234–239. <https://doi.org/10.1016/j.scienta.2009.09.004>.
- Johnson, D.M., Smith, W.K., Vogelmann, T.C., Brodersen, C.R., 2005. Leaf architecture and direction of incident light influence mesophyll fluorescence profiles. *Am. J. Bot.* 92, 1425–1431. <https://doi.org/10.3732/ajb.92.9.1425>.
- Lamont, W.J.J., 2009. Overview of the use of high tunnels worldwide. *HortTechnology* 19, 25–29.
- Li, T., Bi, G., 2019. Container production of southern highbush blueberries using high tunnels. *HortScience* 54, 267–274. <https://doi.org/10.21273/HORTSCI13639-18>.
- Lobos, G.A., Hancock, J.F., 2015. Breeding blueberries for a changing global environment: a review. *Front. Plant Sci.* 6, 1–14. <https://doi.org/10.3389/fpls.2015.00782>.
- Lobos, G.A., Retamales, J.B., Hancock, J.F., Flore, J.A., Cobo, N., del Pozo, A., 2012. Spectral irradiance, gas exchange characteristics and leaf traits of *Vaccinium corymbosum* L. “Elliott” grown under photo-selective nets. *Environ. Exp. Bot.* 75, 142–149. <https://doi.org/10.1016/j.envexpbot.2011.09.006>.
- Maxwell, K., Johnson, N.G., 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51, 659–668. <https://doi.org/10.1093/jexbot/51.5.659>.
- Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., Veberic, R., 2010. The influence of organic/integrated production on the content of phenolic compounds in apple leaves and fruits in four different varieties over a 2-year period. *J. Sci. Food Agric.* 90, 2366–2378. <https://doi.org/10.1002/jsfa.4093>.
- Mishra, A.N., 2018. Chlorophyll fluorescence: a practical approach to study ecophysiology of green plants. In: Sanchez-Moreiras, A.M., Regiosa, M.J. (Eds.), *Advances in Plant Ecophysiology Techniques*. Springer, Cham, pp. 77–97.
- Moon, et al., 1987. No Title Genotypic differences in the effect of temperature on CO₂ assimilation and water use efficiency in blueberry. *J. Am. Soc. Hortic. Sci.*, 112, pp. 170–173.
- Naikoo, M.I., Dar, M.I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F.A., Naushin, F., 2019. Role and regulation of plants phenolics in abiotic stress tolerance: an overview. *Plant Signal. Mol. Role Regul. Under Stress. Environ.* 157–168. <https://doi.org/10.1016/B978-0-12-816451-8.00009-5>.
- Ogden, A.B., Kim, J., Radcliffe, C.A., Van Iersel, M.W., Donovan, L.A., Sugiyama, A., 2011. Leaf and bud temperatures of southern highbush blueberries (*Vaccinium corymbosum*) inside high tunnels. *Acta Hort.* 893, 1319–1326. <https://doi.org/10.17660/actahortic.2011.893.155>.
- Ogden, A.B., van Iersel, M.W., 2009. Southern highbush blueberry production in High Tunnels: temperatures, development, yield, and fruit quality during the establishment years. *HortScience* 44, 1850–1856. <https://doi.org/10.21273/hortsci.44.7.1850>.
- Pereira, A., 2016. Plant abiotic stress challenges from the changing environment. *Front. Plant Sci.* 7, 2013–2015. <https://doi.org/10.3389/fpls.2016.01123>.
- R: A Language and Environment for Statistical Computing. .
- Retamal-Salgado, J., Bastías, R.M., Wilckens, R., Paulino, L., 2015. Influence of microclimatic conditions under high tunnels on the physiological and productive responses in blueberry ‘O’Neal’. *Chil. J. Agric. Res.* 75, 291–297. <https://doi.org/10.4067/S0718-58392015000400004>.
- Retamales, J.B., Hancock, J.F., 2018. *Blueberries*, 2nd ed. Cabi, Boston, MA, USA.
- Righi, E.Z., Buriol, G.A., Angelocci, L.R., Heldwein, A.B., Tazzo, I.F., 2012. Relationships of photosynthetic photon flux density, air temperature and humidity with tomato leaf diffusive conductance and temperature. *Braz. Arch. Biol. Technol.* 55, 359–370. <https://doi.org/10.1590/S1516-89132012000300005>.
- Saini, R.K., Nile, S.H., Park, S.W., 2015. Carotenoids from fruits and vegetables: chemistry, analysis, occurrence, bioavailability and biological activities. *Food Res. Int.* 76, 735–750. <https://doi.org/10.1016/j.foodres.2015.07.047>.
- Sircelj, H., 2008. Karotenoidi v fotosinteznem aparatu in odziv na stres. *Acta Agric. Slov.* 91, 271–282.
- Smrke, T., Veberic, R., Hudina, M., Zitko, V., Ferlan, M., Jakopic, J., 2021. Fruit Quality and Yield of Three Highbush Blueberry (*Vaccinium corymbosum* L.) Cultivars Grown in Two Planting Systems under Different Protected Environments.
- Sircelj, H., Tausz, M., Grill, D., Batič, F., 2007. Detecting different levels of drought stress in apple trees (*Malus domestica* Borkh.) with selected biochemical and physiological parameters. *Sci. Hortic.* 113, 362–369. <https://doi.org/10.1016/j.scienta.2007.04.012>.
- Tausz, M., Wonisch, A., Grill, D., Morales, D., Soledad Jiménez, M., 2003. Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. *J. Exp. Bot.* 54, 1505–1510. <https://doi.org/10.1093/jxb/erg175>.
- Vosnjak, M., Sircelj, H., Hudina, M., Usenik, V., 2021. Response of chloroplast pigments, sugars and phenolics of sweet cherry leaves to chilling. *Sci. Rep.* 11, 1–13. <https://doi.org/10.1038/s41598-021-86732-y>.
- Xu, S., Li, J., Zhang, X., Wei, H., Cui, L., 2006. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. *Environ. Exp. Bot.* 56, 274–285. <https://doi.org/10.1016/j.envexpbot.2005.03.002>.
- Yang, Q., Liu, E., Fu, Y., Yuan, F., Zhang, T., Peng, S., 2019. High temperatures during flowering reduce fruit set in rabbiteye blueberry. *J. Am. Soc. Hortic. Sci.* 144, 339–351. <https://doi.org/10.21273/JASHS04650-19>.
- Yin, Y., Li, S., Liao, W., Lu, Q., Wen, X., Lu, C., 2010. Photosystem II photochemistry, photoinhibition, and the xanthophyll cycle in heat-stressed rice leaves. *J. Plant Physiol.* 167, 959–966. <https://doi.org/10.1016/j.jplph.2009.12.021>.
- Zheng, Y., Li, R., Sun, Y., Xu, M., Zhang, H., Huang, L., Zhu, Y., Wang, H., Li, G., Liu, L., Li, F., Guo, L., Zhang, X., 2017. The optimal temperature for the growth of blueberry (*Vaccinium corymbosum* L.). *Pakistan J. Bot.* 49, 965–979.