Journal of Plant Nutrition

ISSN: 0190-4167 (Print) 1532-4087 (Online) Journal homepage: www.tandfonline.com/journals/lpla20

# Elevated carbon dioxide and substrate composition affect growth, nutrient uptake, and water use efficiency in blueberry cultivation under greenhouse

Nidia Ortiz-Delvasto & Micaela Carvajal

**To cite this article:** Nidia Ortiz-Delvasto & Micaela Carvajal (2025) Elevated carbon dioxide and substrate composition affect growth, nutrient uptake, and water use efficiency in blueberry cultivation under greenhouse, Journal of Plant Nutrition, 48:12, 2137-2154, DOI: 10.1080/01904167.2025.2470388

**To link to this article:** https://doi.org/10.1080/01904167.2025.2470388

JOURNAL OF PLANT NUTRITION
2025, VOL. 48, NO. 12, 2137–2154
https://doi.org/10.1080/01904167.2025.2470388

Taylor & Francis
Taylor & Francis Group

# Elevated carbon dioxide and substrate composition affect growth, nutrient uptake, and water use efficiency in blueberry cultivation under greenhouse

Nidia Ortiz-Delvasto and Micaela Carvajal

Aquaporins Group. Plant Nutrition Department, Centro de Edafología y Biología Aplicada del Segura (CEBAS, CSIC), Murcia, Spain

**ABSTRACT**

Blueberries (*Vaccinium corymbosum* L.) are a valuable crop with growing demand due to their nutritional and health benefits. Optimizing their cultivation under controlled environmental conditions as the use of supplemental carbon dioxide ($CO_2$), offers a promising avenue to enhance growth. However, the interaction between elevated $CO_2$ levels and substrate composition on physiological parameters and nutrient dynamics in blueberry plants remains underexplored. Therefore, the objective of the present study was to investigate $CO_2$ supplementation in the growth and production under controlled conditions in a greenhouse with different substrate composition. Physiological parameters, mineral composition, production, water use efficiency (WUE), nutrient use efficiency (NUE), and nitrite and nitrate concentration were determined in plants grown in 3 substrates with different physical-chemical characteristics. Results showed that $CO_2$ (1000 ppm) positively influenced the growth and production of blueberry. The plants grown in the S2 (100% coconut fiber) or S3 (90% coconut fiber and 10% perlite) under elevated $CO_2$ presented higher the photosynthetic rates. This result in relation to S1 (70% coconut fiber and 30% peat) attributed to the composition of the substrate. However, S1 or S3 presented higher WUE and NUE under elevated $CO_2$, increase water use and carbon assimilation. Elevated $CO_2$ did not increase macronutrients and micronutrients concentration in leaf and fruit. However, plants grown in S2 have a low nitrogen absorption and adaptation to assimilate. Therefore, the results indicate that the addition of perlite in S3 followed by peat in S1 were more suitable than S2 under elevated $CO_2$, since the plants were able to absorb nitrogen and water more effectively providing higher yield in the S3.

**ARTICLE HISTORY**
Received 22 March 2024
Accepted 12 February 2025

**KEYWORDS**
Greenhouses; mineral nutrition; nitrogen assimilation; substrate physical properties; water uptake

## Introduction

The impact of increased $CO_2$ levels on enhancing crop growth has been widely reported (Shaheen, Mustafa, and Ulfat. 2022; Van der Kooi et al. 2016; Bishop, Leakey, and Ainsworth 2014; Yang et al. 2022). This phenomenon has been applied in commercial greenhouse farming after scientific research using a variety of $CO_2$ enrichment methods, including greenhouses, growth chambers, and gradient tunnels (Medina et al. 2016; Shanmugam et al. 2013; Wei et al. 2018; Kimball et al. 2007). Initially studies reported by Kimball (1983) revealed an average 33%

**CONTACT** Micaela Carvajal mcarvaja@cebas.csic.es

boost in agricultural yield through $CO_2$ enrichment. The knowledge gathered from later experiments revealed that stimulation of photosynthetic rate, increasing crop yield and reduction of transpiration rate occurred at high $CO_2$ (Liu et al. 2019; Shaheen, Mustafa, and Ulfat. 2022; Han et al. 2023). Considering that $CO_2$ is key to photosynthesis, $CO_2$ enrichment is a widely accepted technique to improve photosynthesis, thereby increasing yield and commercial income (Pan et al. 2019; Curtis and Wang 1998; Jacotot et al. 2018; Han et al. 2023).

Also, it has been observed that high concentrations of $CO_2$ can increase tomato biomass by developing higher number of branches and the average leaf area (Mamatha et al. 2014; Pan et al. 2019; Fathurrahman 2023). Increased photosynthesis and reduced stomatal aperture are mainly induced by an increase in Rubisco activity and depolarization of the guard cell membrane potential, possibly leading to reduced transpiration and significantly increasing WUE (Wei et al. 2018; Da Silva Fortirer et al. 2017). At the same time, findings have been reported that there are different changes in the C, N and P stoichiometry of woody and herbaceous plants when exposed to high $CO_2$ concentrations, (Du et al. 2019). Furthermore, it has been reported recently that although there are known advantages of providing $CO_2$ in greenhouses, it could also have negative effects on nutrient absorption, particularly causing a decrease in the concentration of nitrogen in tissues (Gojon et al. 2023).

Carbon and nitrogen are the most abundant elements in cells, which intensifies the need for coordination mechanisms to avoid metabolic inefficiencies (Li et al. 2022). On the other hand, nitrogen assimilation depends on the availability of a carbon and, consequently, limitation or oversupply of one element strongly affects the metabolism of the other (Zhang et al. 2018; Baslam et al. 2020). Architectural features such as plant height, branches and panicles were reported to be affected not only by N, but also by the distribution of N in the different organs (Luo, Zhang, and Xu 2020). Moreover, it has been observed that blueberry fruit quality was related to a high carbon/nitrogen ratio (Yang et al. 2023). Therefore, dynamic variations in carbon and nitrogen metabolism in plants directly or indirectly affect the formation and transformation of photosynthates (Wu et al. 2022).

Blueberry (*Vaccinium sp. L.*), an economically important member of the *Ericaceae* family and widely distributed geographically, has become an important fruit crop worldwide. Spain represent first place in the ranking of blueberry producers in the European Union (Junta de Andalucia 2021). For optimal development, this crop must have a low pH (4.5 to 5.5) (Smrke et al. 2021). Compared to other non-ericaceous crops, blueberry plants have low mineral nutrient concentrations and, therefore, tend to have lower nutrient requirements than other fruit crops (Doyle, Nambeesan, and Malladi 2021). Due to these nutritional requirements, producers are searching for novel methods to grow plants in regions with suboptimal soil conditions, an alternative being cultivation in containers with soilless substrate and highly controlled fertilization and irrigation systems (Kingston et al. 2017).

Given the specific pH and organic matter requirements of blueberry, and the fact that agricultural soils rarely meet these criteria, growers frequently amend fields with sulfur, organic matter, or other inputs. Despite these efforts, the narrow soil preferences of blueberry limit its suitable cultivation areas. Recently, container-based production has gained popularity, as the use of soilless substrates allows cultivation in a broader range of environments. These substrates enable precise control of nutrient levels in the rhizosphere and allow cultivation on land with issues such as soil-borne pests, salinity, infertility, or chemical residues. Container production also provided flexibility, allowing growers to move plants and adjust spacing as they grow (Fang et al. 2020). Currently, blueberry plants are grown in containers using substrates supplemented with peat and synthetic minerals (such as vermiculite and expanded clay) to achieve the desired physical and chemical properties. However, preparing optimal substrate mixtures remains challenging, as these materials can exhibit characteristics that could affect plan physiology (Zárate et al. 2017).

In soilless culture, blueberries have high productivity from the first year of cultivation, and one of the main reasons for achieving this growth pattern is that soilless substrates allow the roots area to be improved by controlling the water and nutrient content (Heller and Nunez 2022). Thus, innovative horticultural practices with controlled environmental conditions such as light, humidity, temperature and $CO_2$ concentrations have allowed profitable crops in Spain, Morocco, Mexico, Chile, China, Peru and Argentina, where crops from temperate zones were not common a few decades ago (Bañados 2009; Fang et al. 2022; Lobos and Hancock 2015).

Although nurseries have been using soilless substrates for blueberry production for many years, the concept of commercial fruit production in containers is relatively new. Substrate mixes used in nurseries often include peat, coir, bark, and/or perlite, but it has not yet been established whether these components are equally suitable for long-term blueberry fruit production. (Fulcher et al. 2015).

Therefore, the aim of this work was to investigate $CO_2$ supplementation on blueberry growth and production under greenhouse-controlled conditions with different substrates. As the choice of peat/coconut fiber in substrates for growing blueberry has pointed to influence various physiological responses affecting yield and quality (Ortiz-Delvasto et al. 2024). In this study, experiments were conducted using tree different soilless substrates, one of which varied in its peat, coconut fiber and perlite composition. For that, the growth, fruit yield, gas exchange, mineral nutrition, water use efficiency (WUE) and nutrient use efficiency (NUE) was determined and related to mineral nutrients composition and to N assimilation.

## Material and methods

### Plant material and growth conditions

The experiments were carried out with plants of Blueberry (*V. corymbosum*, L. cv. Legacy). Blueberry plants were supplied by a commercial nursery (Viveros el Cierrón, Asturias, Spain) as plants of approximately 60 cm in height after growing in commercial greenhouse conditions.

The experimental growth stage was carried out during the months of June and July, in an automated greenhouse located in the CEBAS-CSIC experimental field in Santomera (Murcia, Spain), under regulated conditions of temperature by a cooling system that maintained temperatures of 25 °C and 20 °C (day/night). The humidity was stable ranging from 60-70%. The controlled conditions experimental phase and the elevated $CO_2$ experimental phase was maintained under these same conditions.

Plants were grown into 30-L pots with 3 different experimental substrates (provided by Projar, Valencia, Spain) composed of: (S1) 70% coconut fiber and 30% peat, (S2) 100% coconut fiber, (S3) 90% coconut fiber and 10% perlite. The experimental design was completely randomized with 3 cultivation lines (one line per substrate), with 30 plants, randomly distributed in 10 plants per substrate. The substrates were irrigated through continuous dripping at regular 7-min intervals, 4 times a day, with one dripper per container emitting water at of $3\,L\ h^{-1}$. The irrigation solution containing $KNO_3$ (6 mM), $Ca(NO_3)_2$ (4 mM), $KH_2PO_4$ (1 mM), and $MgSO_4$ (1 mM), $H_3BO_3$ (25 µM), $MnSO_4$ (2 µM), $ZnSO_4$ (2 µM), $CuSO_4$ (0.5 µM), $(NH_4)_6Mo7O24$ (0.5 µM) and Fe-EDDHA (20 µM) (Hoagland and Arnon 1938).

The plants grew under these conditions during 16 wk, until the plants reached the stage of production. After that period, the experimental design consisted in two different periods of one month each. The first period called Control conditions (ambient $CO_2$), where the plants grown in the different substrates (S1, S2 and S3) were with ambient $CO_2$ (evaluated during the first 8 wk), and the second period called Elevated $CO_2$ (evaluated during the last 4 wk) where the concentration of $CO_2$ was maintained to 1000 ppm. $CO_2$ was emitted through ventilation filters and gradually reaching a concentration of 1000 ppm of $CO_2$. The gas emission period was for 12 h each day, throughout the daylight hours (from 8:00 to 20:00). Two days before the end of both the control conditions period and the period under elevated $CO_2$ conditions, 5 plants from each substrate were harvested and samples were collected for subsequent analysis.

### Relative growth rate (RGR) and fruit production

Relative growth rate was determined in leaves of control conditions and high $CO_2$ content treatments, during 24 days with an interval of 8 days between each measurement. Leaves were selected (3 per plant in different branches) in 5 plants per substrate. This procedure was carried out *in situ* by drawing the outline of the leaf on a sheet of paper and later using the ImageJ program to calculate the area, to afterwards obtain the relative growth rate with the following equation (Primo-Millo and Agustí 2020):

$$RGR = \frac{(\mathrm{LnA2} - \mathrm{LnA1})}{(\mathrm{t1} - \mathrm{t2})}$$

Where A1 is the initial leaf area and A2 is the leaf area determined at time 2. t1 and t2 are the dates of data collection (intervals of 8 days).

Fruit production was determined during each month of control conditions (ambient $CO_2$) and elevated $CO_2$ conditions. All fruits from each plant was harvested at commercial maturation point and biomass were determined per substrate. The results are presented as the average g or number per plant per month.

### Leaf gas exchange

Gas exchange parameters such as net photosynthesis rate ($A_n$), stomatal conductance ($g_s$) and internal $CO_2$ concentration ($C_i$) were measured in fully developed leaves using a TPS-2 Portable Photosynthesis System gas exchange meter (PP Systems, Inc., Amesbury, MA, USA) on a total of 4 leaves per plant from 3 plants for each substrate in each growth period (control conditions and elevated $CO_2$). Measurements were taken one hour after the start of the photoperiod. All observations were recorder at a PPFD (photosynthetic photon flux density) of 1500 $\mu$mol $m^{-2}$ $s^{-1}$ and cuvette temperature of 25 °C with 2.0 $cm^2$ of leaf area exposed for gas exchange. All gas exchange measurements were taken between 09:00 and 12:00 (local time).

### Intrinsic water use efficiency (iWUE) and nutrient use efficiency (NUE)

Intrinsic water use efficiency measures differences related to the leaves ability to regulate photosynthesis and stomatal conductance, and which are independent of atmospheric conditions at the time of measurement. The intrinsic efficiency of water use was calculated using the formula $A_n/g_s$ (with the units µmol $CO_2$ $mmol^{-1} H_2O$) (Fischer and Turner 1978), where ($A_n$) is the net photosynthesis of the leaf and ($g_s$) the stomatal conductance.

Nutrient use efficiency was determined from crop yield per unit of nutrient applied and was calculated using the following formula (McDonald et al. 2013).

$$Nutrient\ \ use\ \ efficiency = \frac{Yield(Kg^{-1}Kg^{-1})}{Quantity\ \ of\ \ fertilizer\ \ applied(Kg^{-1}Kg^{-1})}$$

### Analysis of mineral elements

Minerals contents (macronutrients and micronutrients) were determined according the ISO 11.885 (1996) by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) using a

Thermo ICAP 6500 Duo equipment (Thermo Fisher Scientific, Waltham, MA, USA). Lyophilized samples of leaf and fruit from 3 selected plants of each cultivation were ground into a fine powder using a grinder (Taurus Aromatic, Lleida, Spain). For each ground sample, 200 mg were added in a microwave furnace equipment to a 25 mL tube with a mixture of 4 mL of $HNO_3$ (68% purity) and 1 mL $H_2O_2$ (33% purity) for their subsequent digestion. 300 mL high-purity de-ionized water, 30 mL $H_2O_2$ (33% purity) and 2 mL $H_2SO_4$ (98% purity) were also added in the Teflon reactor. The microwave heating digestion program consisted of 3 steps: starting at 20 °C and 40 bar; increasing 10 bar/minute for 30 min up to 220 °C; and keeping 220 °C for 20 min. After cooling, the mineralized sample were transferred to double gauge tubes of 10 mL (micro minerals) and 25 mL (macro minerals), and the volume made up with high-purity de-ionized water. A multimineral standard solution containing 31 minerals supplied by SCP Science (Quebec, Canada) was used to prepare calibration standards in high-purity de-ionized water. For ICP-OES analyses, two control samples containing high-purity de-ionized water and a multimineral standard were used. Each mineral determination was performed at specific wavelengths ranging from 167.1 to 670.8 nm. The concentration of macro and micro minerals were calculated according the formula ‘$\text{mg kg}^{-1}$ or $\text{g kg}^{-1}$ = (C x D)/W’; where C was mineral concentration, D was the dilution factor and W was sample weight.

### Determination of total carbon and nitrogen

The total carbon and nitrogen content were determined by the DUMAS method, using the elemental analyzer model TRUSPEC CN628 (LECO Corporation, MI, USA) (Matejovic 1993). Lyophilized samples of leaf and fruit from 3 selected plants of each cultivation were ground into a fine powder using a grinder (Taurus Aromatic, Lleida, Spain). For each ground sample, 0.1 g of the solid sample was placed in a tin capsule, which was then closed and introduced into the autosampler of the equipment. The results expressed as percentage of weight ($\text{g } 100\,\text{g}^{-1}$).

### Determination of nitrite and nitrate

The determination of nitrite and nitrate was carried out in accordance with ISO 10304-1 (2007) by ion chromatography (Michalski and Kurzyca 2014), using a Metrohm 850 Ion Chromatograph (Metrohm AG, Switzerland). Samples of leaf, and fruit from 3 selected plants of each cultivation substrate and $CO_2$ conditions were frozen in liquid nitrogen and lyophilized. After that they were ground into a fine powder using a grinder (Taurus Aromatic, Lleida, Spain). Of each ground sample, 0.2 g was added to 40 mL of milliQ water. The sample was shaken on a mechanical shaker for 30 min and then centrifuged at 3000 rpm for 10 min.

The sample was filtered through a 0.45 micron nylon filter. Measurement was then performed in the chromatograph. A multimineral standard solution (commercial solution of different anions) supplied by SCP Science (Quebec, Canada) was used to prepare calibration standards in highly purified deionized water. The anion concentration is expressed in mg/L.

### Statistical analysis

Data analyses were performed using the SPSS 25.0.0.1 software package (IBM Corp., Armonk, NY, USA). All the parameters were analyzed using one-way ANOVA, followed by post hoc Duncan’s New Multiple Range Test.

## Results

### Relative growth rate (RGR) and fruit production

The RGR of blueberry plants in elevated $CO_2$ conditions were significantly higher than those of Control conditions plants (Figure 1(a)). However, no differences were observed between the plants grown in the different substrates.

Blueberry plants under control conditions (ambient $CO_2$) or under elevated $CO_2$, presented significant changes in fruit production when grown in the different substrates. In control conditions (ambient $CO_2$), the plants grown in S1 have higher production, compared to the plants grown in S2 and S3, which did not present significant differences. However, under elevated $CO_2$ conditions, all plants experienced a significant increase in production compared to control conditions plants (Figure 1(b)). In the case of elevated $CO_2$ plants grown in S3 had higher production in relation to the plants grown in S1 or S2.

### Gas exchange

Figure 2 shows the results of the gas exchange parameters. The photosynthetic rate ($A_n$, Figure 2(a)) showed no significant changes in the plants grown in the different substrates in control conditions (ambient $CO_2$). However, under elevated $CO_2$, all values were significantly higher than in control conditions plants independently of the substrates. Furthermore, on elevated $CO_2$ substrates S2 and S3 showed a significantly higher photosynthetic rate relative to plants grown on S1.

The internal $CO_2$ concentration ($C_i$, Figure 2(b)) did not show significant differences among substrates in control conditions. However, this parameter increased significantly in all plants grown in different substrates subjected to elevated $CO_2$, compared to control conditions plants. The plants grown in S2 substrate presented the highest Ci in elevated $CO_2$ conditions in relation to the plants grown in S1 and S3, with the plants grown in S3 providing the lowest $C_i$ values.

Stomatal conductance ($g_s$, Figure 2(c)) increased significantly in plants grown on all substrates under elevated $CO_2$ conditions relative to control conditions plants. The plants grown in control conditions did not show significant differences within the different substrates. Under elevated $CO_2$ conditions, plants grown in S2 substrate presented a significant increase in relation to plants

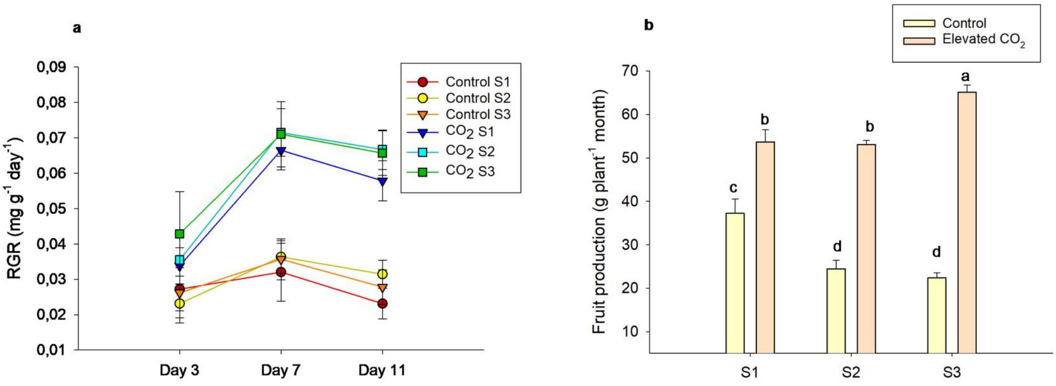


Figure 1. (a). Relative growth rate of leaves of the blueberry plant with substrates S1, S2 and S3 under control conditions *(ambient $CO_2$)* and under conditions of elevated $CO_2$. measured for 11 days with an interval of 4 days between each measurement. Points indicate the mean ± SE of 3 samples with 3 measurements each. (b). Fruit production per month of the blueberry plant with substrates S1, S2 and S3 during 30 days under control conditions and under conditions of elevated $CO_2$. Each value represents the average production of 5 plants ± SE. Means followed by different letters were significantly different according to duncan's test.

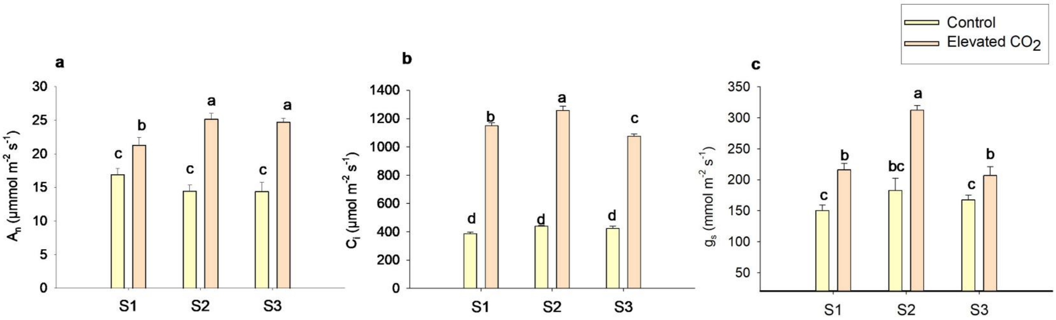


**Figure 2.** Gas exchange parameters of blueberry plant with substrates S1, S2 and S3 under control conditions and under conditions of elevated $CO_2$. (a) Photosynthesis rate ($A_n$, μmol m$^{-2}$ s$^{-1}$), (b) internal $CO_2$ concentration ($C_i$, μmol m$^{-2}$ s$^{-1}$), (c) stomatal conductance ($g_s$, mmol m$^{-2}$s$^{-1}$). Values are means ± SE ($n = 3$). For each treatment, different letters show significant differences according to duncan's test.

grown in S1 and S3 substrate. No significant differences were found between plants grown in S1 and S3.

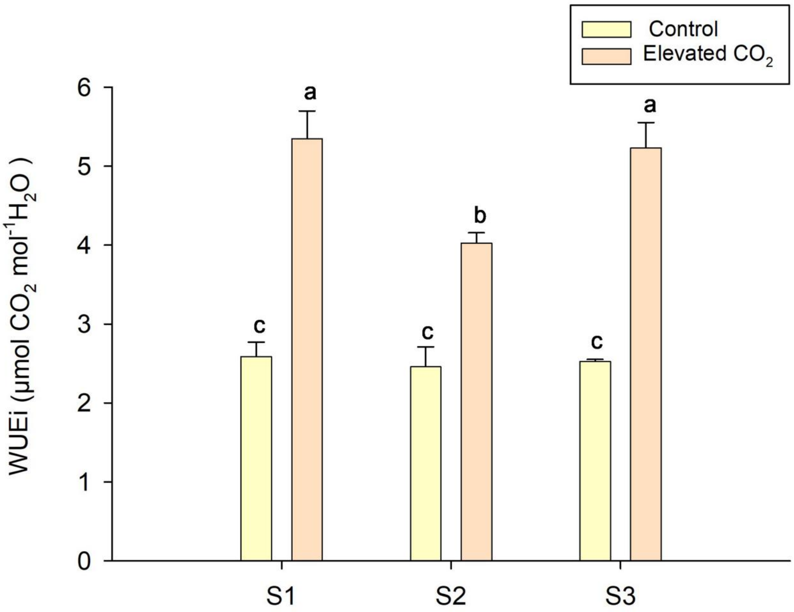
### Intrinsic water use efficiency (WUEi) and nutrient use efficiency (NUE)

The water use efficiency did not present significant differences in the plants grown in different substrates under control conditions (ambient $CO_2$). However, under elevated levels of $CO_2$, they showed significant increases in relation to the control conditions plants. Plants grown on substrates S1 or S3 under elevated $CO_2$ conditions showed a significant increase in WUEi compared to S2, and these plants had the lowest WUEi.

In relation to the nutrient use efficiency, plants showed a significant increase when they were subjected to elevated $CO_2$ conditions compared to control conditions independently of the type of substrate. Under control conditions, plants grown in S3 substrate presented a greater NUE values, in all individual nutrients, in relation to plants grown in S1 or S2. Plants grown in S2 presented the lowest values. Also, significant differences observed between plants grown in S1, S2 and S3, under elevated $CO_2$ conditions. The S3 substrate on elevated $CO_2$ conditions presented a greater NUE, in relation to the plants grown in S1 or S2, with the plants grown in S2 substrate being the ones that presented the lowest NUE (Figure 3).

### Concentration of mineral elements

The blueberry plants subjected to high concentration of $CO_2$ levels showed a generalized decrease of the main macronutrients and micronutrients, both in leaves and fruits of plants grown in S1, S2 or S3, in relation to plants grown under control conditions (plants grown in S1, S2 or S3 under ambient $CO_2$ conditions) (Table 1). Regarding the macronutrients of the plants subjected to elevated $CO_2$, Ca is one of the few elements that showed an increase in concentration in leaves of plants grown in S1 and S2 substrate. In relation to the fruit, the concentration of Ca was lower in plants grown under elevated $CO_2$ conditions (independently of the substrates) compared with those grown in control conditions (with ambient $CO_2$). The concentration of K presented a higher concentration in the leaves of the plants grown in all substrate under control conditions (ambient $CO_2$) compared to high $CO_2$. The concentration of this nutrient showed no changes in fruits. The concentration of Mg in the leaves presents a significant increase in plants grown with S1 substrate when subjected to elevated $CO_2$ conditions. In relation to the fruit, this nutrient did not present significant differences either in control and elevated $CO_2$ in the different substrates. The concentration of P presented in leaf and fruit, in all S1, S2 and S3 substrates, did not show



| | Nutrients | S1 | | S2 | | S3 | |
|---|---|---|---|---|---|---|---|
| | | Control | Elevated $CO_2$ | Control | Elevated $CO_2$ | Control | Elevated $CO_2$ |
| $kg\ kg^{-1}$ | $KNO_3$ | 1.190 ± 0.019e | 1.794 ± 0.010b | 0.888 ± 0.005f | 1.382 ± 0.006d | 1.725 ± 0.015c | 2.520 ± 0.029a |
| | $Ca(NO_3)_2$ | 0.772 ± 0.009e | 1.148 ± 0.014b | 0.574 ± 0.013f | 0.891 ± 0.011d | 1.102 ± 0.004c | 1.632 ± 0.010a |
| | $KH_2PO_4$ | 4.591 ± 0.013e | 6.922 ± 0.011b | 3.426 ± 0.014f | 5.326 ± 0.015d | 6.656 ± 0.014c | 9.792 ± 0.017a |
| | $MgSO_4$ | 2.465 ± 0.012d | 3.712 ± 0.008b | 1.841 ± 0.022d | 2.866 ± 0.014c | 4.2267 ± 0.621b | 5.276 ± 0.034a |
| $g\ kg^{-1}$ | Fe-EDTA | 32.135 ± 0.004e | 48.543 ± 0.374b | 23.967 ± 0.007f | 37.023 ± 0.243d | 46.810 ± 0.261c | 68.366 ± 0.135a |
| | Micronutrients | 32.054 ± 0.072d | 47.443 ± 0.960b | 23.967 ± 0.058e | 36.991 ± 0.066c | 46.810 ± 0.310b | 68.113 ± 0.031a |

Nutrient use efficiency (NUE)

**Figure 3.** Intrinsic water use efficiency (WUEi) (µmol $CO_2$ $mol^{-1}$ $H_2O$) and nutrient use efficiency (NUE) macronutrients (kg $kg^{-1}$) and micronutrients (g $kg^{-1}$) of blueberry plant with substrates S1, S2 and S3 under control conditions and under conditions of elevated $CO_2$. Values are means ± SE (*n* = 3). For each treatment, different letters show significant differences according to Duncan's test.

significant differences under both $CO_2$ conditions. The concentration of S present in the leaves of the plants showed lower values under high $CO_2$ than under ambient $CO_2$. In relation to the concentration in the fruit, there were no significant differences in any of the substrates and in any of the $CO_2$ conditions (ambient $CO_2$) or elevated $CO_2$ conditions.

Regarding the micronutrients in plants subjected to elevated $CO_2$ contents, a decrease in the concentration of B observed in both the leaves and fruits of plants grown in all substrates. Fe presented higher values in the foliar and fruit concentration of plants grown control conditions (ambient $CO_2$) than in high $CO_2$. Manganese increased the concentration in leaves of plants grown in S2 and S3 substrates under elevated $CO_2$ conditions. However, in fruits, the concentration decreased in plants grown in all substrates, when subjected to elevated $CO_2$ levels. Finally, Zn presented higher values in the leaves of plants grown in S1 substrate under control conditions (ambient $CO_2$). In relation to the fruit, this nutrient did not present significant differences under both $CO_2$ conditions.

**Table 1.** Concentration of mineral elements. Macronutrients (g kg$^{-1}$DW) and micronutrients (mg kg$^{-1}$DW) measured in leaf and fruit of blueberry plant with substrates S1, S2 and S3 in control conditions and elevated $CO_2$ conditions.

| | | S1 | | | | S2 | | | | S3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaf | | Fruit | | Leaf | | Fruit | | Leaf | | Fruit | |
| | Element | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ |
| Macronutrients | Ca | 1.54 ± 0.65b | 1.94 ± 0.27a | 0.42 ± 0.02c | 0.26 ± 0.02c | 1.57 ± 0.28b | 2.31 ± 0.28a | 0.41 ± 0.03c | 0.28 ± 0.04c | 2.02 ± 0.86a | 2.06 ± 0.04a | 0.38 ± 0.03c | 0.20 ± 0.01c |
| (g kg$^{-1}$ DW) | K | 12.96 ± 1.16a | 7.02 ± 0.02 cd | 7.22 ± 0.13 cd | 6.29 ± 0.22 cd | 12.28 ± 0.16ab | 6.46 ± 0.46 cd | 7.43 ± 0.16 cd | 6.33 ± 0.11 cd | 11.27 ± 0.95b | 6.51 ± 0.12 cd | 7.15 ± 0.17 cd | 5.90 ± 0.01d |
| | Mg | 0.87 ± 0.07b | 1.05 ± 0.03a | 0.29 ± 0.02c | 0.27 ± 0.02c | 0.91 ± 0.04b | 0.89 ± 0.07b | 0.28 ± 0.02c | 0.24 ± 0.01c | 0.94 ± 0.06ab | 0.90 ± 0.03b | 0.29 ± 0.01c | 0.24 ± 0.01c |
| | P | 2.10 ± 0.57a | 2.13 ± 0.25a | 0.88 ± 0.02a | 0.75 ± 0.04a | 1.25 ± 0.03a | 1.66 ± 0.09a | 0.94 ± 0.04a | 0.83 ± 0.05a | 1.76 ± 0.21a | 1.77 ± 0.06a | 1.04 ± 0.04a | 0.79 ± 0.04a |
| | S | 1.64 ± 0.06a | 1.30 ± 0.17c | 0.61 ± 0.01d | 0.54 ± 0.04d | 1.62 ± 0.10a | 1.24 ± 0.06c | 0.63 ± 0.01d | 0.51 ± 0.02d | 1.55 ± 0.09b | 1.36 ± 0.08bc | 0.64 ± 0.02d | 0.47 ± 0.02d |
| Mlicronutrients | B | 40.39 ± 1.86a | 19.51 ± 0.78b | 6.87 ± 0.06d | 6.46 ± 0.04e | 38.05 ± 1.06a | 18.51 ± 0.25bc | 8.04 ± 0.20de | 6.12 ± 0.07e | 44.62 ± 12.46a | 17.67 ± 0.43bc | 8.11 ± 0.39d | 5.85 ± 0.09e |
| (mg kg$^{-1}$ DW) | Fe | 100.57 ± 56.10a | 34.24 ± 2.14b | 27.50 ± 0.89b | 8.33 ± 0.19c | 40.49 ± 5.90b | 42.98 ± 1.50b | 30.33 ± 0.36b | 12.04 ± 0.24c | 47.08 ± 11.68c | 50.17 ± 0.50b | 22.85 ± 1.45b | 7.85 ± 0.10c |
| | Mn | 39.30 ± 5.75e | 43.88 ± 2.81de | 7.46 ± 0.33f | 5.95 ± 0.04 g | 59.02 ± 13.61c | 90.68 ± 1.26a | 13.00 ± 1.53f | 9.25 ± 0.82f | 56.69 ± 1.22 cd | 75.71 ± 1.57b | 13.35 ± 1.49f | 9.19 ± 0.11f |
| | Zn | 30.09 ± 6.66a | 21.91 ± 1.45b | 5.03 ± 0.19c | 5.50 ± 0.60c | 21.05 ± 1.85b | 16.05 ± 2.29b | 5.38 ± 0.34c | 5.56 ± 0.31c | 19.77 ± 0.80b | 22.94 ± 3.16b | 5.78 ± 0.33c | 6.54 ± 0.27c |

Results are expressed as the mean of three biological replicates ± standard error. Different letters show significant differences according to Duncan's test.

## Determination of total carbon and nitrogen

The N concentration (Figure 4) in the leaves of plants grown in all S1, S2 and S3 substrates did not show significant variations under elevated $CO_2$ conditions compared to the control conditions (ambient $CO_2$). In fruits, the N concentration showed a significant increase in the fruits of plants grown in S2, when they were exposed to elevated $CO_2$. The fruits of the plants grown in S1 or S3 did not show significant differences when subjected to elevated $CO_2$ when compared with ambient $CO_2$ conditions.

The leaf C concentration showed a significant increase in the leaves of the plants in all S1, S2 and S3 under elevated $CO_2$ in relation to the plants grown in ambient $CO_2$ conditions. In relation to C concentration in the fruits, a significant increase was observed in the plants grown in S1 or S2, when they were subjected to elevated $CO_2$ concentrations, compared to the control conditions. Plants grown in S3 showed no significant differences between the ambient $CO_2$ conditions and those subjected to elevated $CO_2$.

## Determination of nitrite and nitrate

The nitrite ($NO_2^-$) concentration (Table 2) in leaves and fruits increased significantly to a similar value in the plants grown in S1, S2 and S3 when subjected to elevated $CO_2$ conditions as compared to control conditions. In control conditions leaves and fruits, the values were very low

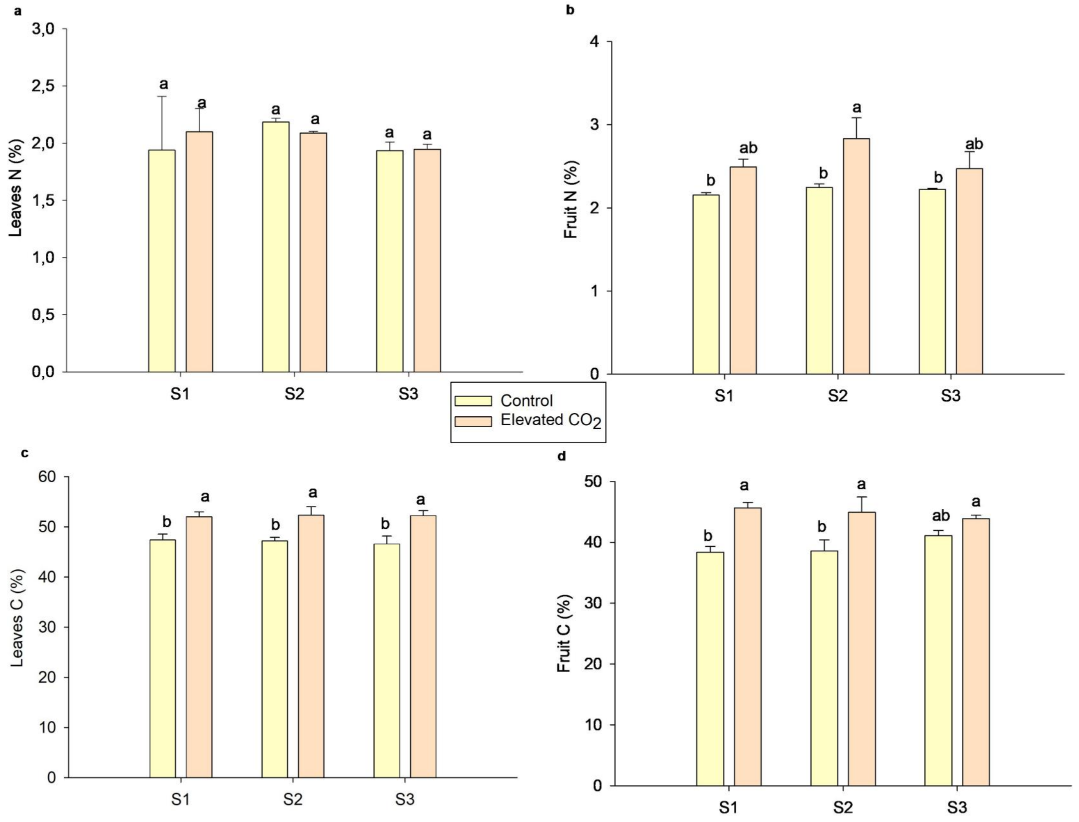


**Figure 4.** Concentration of total carbon and nitrogen. (a) percentage of N in leaves, (b) percentage of N in fruit, (c) percentage of C in leaves, (d) percentage of C in fruit, measured in plants grown with substrates S1, S2 and S3 in control conditions and elevated $CO_2$ conditions. Results are expressed as the mean ± SE ($n = 3$). Columns with different letters differ significantly according to duncan's test.

showing <0.01 mg 100 $g^{-1}$D.W. in all leaves of the different substrates, and in fruits of S1 and S3 substrates. However, the concentration was detectable in fruits of S2 in control conditions (0.22 mg 100 $g^{-1}$D.W).

The results of nitrate concentration ($NO_3^-$) revealed a different pattern between leaf and fruit. In the case of leaves, a significant decrease was observed in plants grown in both S1, S2 and S3, when subjected to elevated $CO_2$ concentrations compared to the control conditions. On the contrary, in the fruits of plants grown in all S1, S2 and S3 substrate, a significant increase was observed when they were subjected to elevated $CO_2$ conditions in relation to the control conditions.

## Discussion

It is known that high $CO_2$ increase the growth and development of herbaceous plants (Roy, Kapoor, and Mathur 2024). However, the studies of the response of woody plants is very limited. In this way, the studies reports that, in general, elevated $CO_2$ produced higher photosynthetic rate, generating increases in growth and productivity, but the responses will depend on water uptake and mineral nutrition (Mndela et al. 2022). In this sense, our blueberry plants presented a positive effect on growth by elevated $CO_2$ in all substrates, increasing the speed of growth and production. These results coincide with studies carried out on mulberry plants (*Morus* sp) where they found persistent increases in the photosynthetic rate when subjecting them to high $CO_2$ conditions (Sekhar et al. 2014). Photosynthesis has been reported to be significantly improved due to the carboxylation role of Rubisco being favored to the detriment of its oxygenating activity, increasing photosynthetic efficiency (Lobo et al. 2022). This enhancing results have been previously reported in various studies on woody plants as *Camellia sinensis* L. grown in chambers (Ahammed et al. 2020), in *Cornus drummondii* C.A. Mey., *Rhus glabra* L., *Gleditsia triacanthos* L., *Juniperus osteosperma* Torr. grown in a greenhouse (O'Connor et al. 2022) and bitter orange trees planted in open chambers with plastic for 17 years, with elevated $CO_2$ (Kimball et al. 2007). The level of $CO_2$ is also a question of debate since projections of atmospheric $CO_2$ concentration will range between about 450 and 600 ppm by the year 2050 since we are strongly dependent on future scenarios of anthropogenic emissions (Woodward 2002). However, the fact that this high levels were only applied for one month should be taken into account.

Our blueberry plants showed an increase in net photosynthesis rate ($A_n$), stomatal conductance ($g_s$) and internal $CO_2$ concentration ($C_i$) under elevated $CO_2$ conditions, in all S1, S2 and S3 substrates, relative to control conditions plants. However, the plants grown in S2 or S3 subjected to elevated $CO_2$ conditions presented the highest growth and highest values of the photosynthetic rate, in relation to S1. This result can be attributed to the composition of the substrate and its physical characteristics reported previously (Ortiz-Delvasto et al. 2024), since S2 and S3 were composed mainly of coconut fiber which has the capacity to retain more water and provide greater aeration compared to other substrates (Gómez-Bellot et al. 2020). On the other hand, as Ci did not show changes in control conditions (ambient $CO_2$), but an increase in this parameter was observed similarly in plants grown in either S1 or S3 under elevated $CO_2$.

Various studies have shown that the behavior of Gs under conditions of elevated $CO_2$ is contradictory and could be specific to each specie (Purcell et al. 2018). Accordingly, measurements on woody plants, demonstrated that Gs could increase in response to elevated $CO_2$ levels under specific climatic conditions (high temperature and low humidity) but depending on water availability (Purcell et al. 2018). In blueberry, previous works reported the effect of source-sink balance on foliar traits and found a positive correlation between stomatal conductance and photosynthesis (Jorquera-Fontena et al. 2016), demonstrating that blueberry plants could have the capacity to respond to changes in the demand by photoassimilates by altering stomatal, photochemical, and biochemical parameters (Petridis et al. 2020). In this sense, we observed that

**Table 2.** Concentration of $NO_2^-$ and $NO_3^-$ measured in leaf and fruit of blueberry plant with substrates S1, S2 and S3 in control conditions and elevated $CO_2$ conditions.

| | | S1 | | | | S2 | | | | S3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaf | | Fruit | | Leaf | | Fruit | | Leaf | | Fruit | |
| | Element | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ | Control | $CO_2$ |
| (mg 100 g$^{-1}$ DW) | $NO_2^-$ | <0.01 | 0.50 ± 0.01d | <0.01 | 0.61 ±0.02 bc | <0.01 | 0.64 ± 0.01ab | 0.22 ± 0.02 f | 0.59 ± 0.02c | <0.01 | 0.45 ± 0.01e | <0.01 | 0.65 ± 0.00 a |
| | $NO_3^-$ | 1.92 ± 0.00 a | 0.64 ± 0.02 f | 0.34 ± 0.02 g | 1.01 ± 0.01c | 1.43 ± 0.01b | 0.78 ± 0.01d | 0.76 ± 0.01de | 1.03 ± 0.02c | 1.45 ± 0.02 b | 1.04 ± 0.02c | 0.73 ± 0.01e | 1.01 ± 0.01c |

Results are expressed as the mean of three biological replicates ± SE. Values within a column followed by different letters are significantly different according to Duncan’s tests.

blueberry plants in S2 grown under elevated $CO_2$ conditions somehow had the ability to increase Gs by the 100% of coconut fiber in the substrate. It has been reported that the increase of AW in soils could improve stomatal conductance and net assimilation rate, resulting in increased photosynthetic capacity in species such as black locust (*Robinia pseudoacacia* L.) (Liu et al. 2013), but the fact that S1 and S3 showed similar $g_s$ values indicate that, again, other parameter is influencing.

Substrates S1 and S3 presented higher WUE and NUE in elevated $CO_2$ conditions, indicating that these plants have the ability to balance water use and carbon assimilation to a higher extent than S2. The higher values of water use efficiency (WUE) and nutrient use efficiency (NUE), in each individual nutrient, in S1 and S3 were possibly occurring due to the composition of peat (S1) and perlite (S3) that was promoting higher water and nutrient availability (Ortiz-Delvasto et al. 2024), being the nitrogen compounds those that provide higher NUE increases (Domec, Duncan, and McCulloh 2017; McCarthy et al. 2010).

Elevated $CO_2$ caused a significant failed to increase in the concentration of macronutrients, K and S, and micronutrients, B and Fe, in blueberry leaves and fruits grown in all S1, S2 and S3 substrates, in relation to plants grown under control conditions (ambient $CO_2$). As it has been reported that the increase in yield caused by elevated $CO_2$ can reduce the mineral quality in the edible parts of crops (Dong et al. 2018) in our case, it occurred at a very low extent in fruits for K, S, and B. However, Fe and Zn was observed to be limited in leaves but Fe greatly decrease fruits with all substrates. The suppression of Fe and Zn has been reported in rice (*Oryza sativa* L.) under elevated $CO_2$ (Chumley and Hewlings 2020) and also in cotton (*Gossypium herbaceum* L.) (Prior et al. 1998), but they could not asses the mechanism leading to such reduction. Although, several hypotheses have been proposed, any of them could fit with our results. Some reports indicated that the low nutrient concentrations is a result of nutrient dilution, which occurs because the increase in carbohydrates synthesis is greater than the increase in nutrient absorption under elevated $CO_2$ conditions (Guo et al. 2015). Accordingly, also Ca was suppressed in the fruits of blueberry plants subjected to high $CO_2$ conditions could be think in opposite to the study carried out by Fangmeier et al. (1999), where they found an increase in Ca and Fe in the grain of wheat under elevated $CO_2$ conditions. However, the fact that they blueberry are fruits and wheat are seed could reduce the confronting results.

Our study demonstrated that elevated $CO_2$ did not affect the concentration of total N in the leaves or fruit, but the concentration of total N in fruit increased in plants grown in S2 under elevated $CO_2$ conditions. This action was probably because S2 is composed only of coconut fiber, which has a high water retention capacity, good aeration capacity, which allows greater water availability and therefore greater nutrient retention (Ortiz-Delvasto et al. 2024). Accordingly, He et al. (2014) found that elevated $CO_2$ in soybeans increased the entry of labile C into the soil, so there are more microbial carbon sources, which increases the abundance of genes in the N cycle. Poorter et al. (1997) observed that the decrease in total nitrogen in leaves under elevated $CO_2$ conditions was lower when plants were grown in hydroponics than in pots, which suggests it is related to the improvement of nitrogen nutrition in hydroponic cultivation.

Under control conditions (ambient $CO_2$), plants grown in S1 presented a higher concentration of $NO_3^-$ in the plant leaf, in relation to plants grown in S2 or S3. This result can be attributed to the high cation exchange capacity of peat-based substrates, which primarily affects the retention of cations but has little impact on nitrate, making it more available and influencing overall plant dynamics. This likely explains the increased nitrate concentration in S1 plants (Bar-Tal et al. 2019). However, the lower nitrate concentrations observed under high $CO_2$ conditions indicate more efficient nitrogen metabolism.

Research suggests that the N uptake preference of blueberry plants is for ammonium rather than nitrate, probably due to an evolutionary mechanism resulting from the adaptation of blueberries to habitats characterized by acid soils, where ammonium is the most abundant inorganic N ion available (Claussen and Lenz 1999; Poonnachit and Darnell 2004; Nunez, Olmstead, and Darnell 2015; Bryla and Strik 2015). However, it is not clear whether the improved performance of blueberry plants at high $NH_4^+$ concentrations was due to increased N uptake or to increased uptake of microelements such as iron, manganese or zinc, which are more accessible at low rhizosphere pH (Tamir et al. 2021). In our trial, we used $NO_3^-$ as the main source of N with high rates of absorption possibly due to the pH adjustment of the nutrient solution.

Differences were found in the concentration of $NO_2^-$, $NO_3^-$ ions when the plants were subjected to elevated $CO_2$ conditions in all the substrates. A generalized limitation in the concentration of leaf $NO_3^-$ was observed in plants grown in S1, S2 and S3. Various studies report a decrease in the concentration of $NO_3^-$ in plants grown under elevated $CO_2$ conditions (Purvis, Peters, and Hageman 1974; Yelle, Gosselin, and Trudel 1987; Geiger et al. 1998; Stitt and Krapp 1999). On the contrary, regardless of the substrate, an increase in $NO_2^-$ concentration was observed in both leaves and fruit under elevated $CO_2$ conditions. Taken together, these changes in the concentration of total N, nitrate and nitrite under conditions of elevated $CO_2$ can indicate that high $CO_2$ can cause greater nitrogen absorption, provided that the nitrogen supply is adequate, but, the assimilation of inorganic nitrogen could be modified (Stitt and Krapp 1999). To understand the decrease in nitrogen concentration, Taub and Wang (2008) found that the reduced uptake of nitrogen is likely caused by a decreased demand for nitrogen by the plant tissues and a reduced ability of the soil/root system to supply nitrogen to the rest of the plant. In our substrates, it can be observed that plants grown in S2 has low ability to absorb nitrogen and low adaptation to assimilate nitrogen since $NO_2^-$ was found at higher concentrations than with the rest of substrates either in leaves or fruits.

## Conclusion

In our study with blueberry plants, it was observed that elevated $CO_2$ levels (1000 ppm) produced faster growth and increased fruit production in relation to enhanced photosynthesis, internal $CO_2$ concentration, and stomatal conductance. Also, elevated $CO_2$ positively influenced overall plant nutrition by improving nutrient use efficiency (NUE) and water use efficiency (WUE). However, the nutritional status of the plants was strongly influenced by the substrate composition, which affected how efficiently nutrients were absorbed. Accordingly, the composition of the substrates played a key role in plant development. Substrates S3 (90% coconut fiber and 10% perlite) and S1 (70% coconut fiber and 30% peat) were found to be more suitable for blueberry cultivation under elevated $CO_2$ conditions, as they facilitate nutrient and water uptake, likely due to the inclusion of perlite and peat. Also, the nitrate concentration in leaves of blueberry of plants grown in S1 revealed a relation to peat composition and the cationic exchange capacity. Considering the results related to the suppression of K, S, B and Fe by high $CO_2$, further investigation is needed to understand the mechanisms which is independent of the substrate composition.

## Acknowledgements

The authors thank PROJAR, S.L. for supplying the substrates.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This research was funded by Spanish Ministerio de Ciencia e Innovacion, CDTI with the 0 E-Missions Project (MIP-20201045).

## ORCID

Nidia Ortiz-Delvasto http://orcid.org/0000-0002-2224-8707
Micaela Carvajal http://orcid.org/0000-0001-7321-4956

## References

Ahammed, G. J., L. Xin, L. Airong, and C. Shuangchen. 2020. Physiological and defense responses of tea plants to elevated CO2: A review. *Frontiers in Plant Science* 11 (March):305. doi: 10.3389/fpls.2020.00305.

Bañados, M. P. 2009. Expanding blueberry production into non-traditional production areas: Northern Chile and Argentina, Mexico and Spain. *Acta Horticulturae* 810 (810):439–45. doi: 10.17660/ActaHortic.2009.810.57.

Bar-Tal, A., U. Saha, M. Raviv, and M. Tuller. 2019. Chapter 7 - Inorganic and synthetic organic components of soilless culture and potting mixtures. In *Soilless culture*, ed. Michael Raviv, J. Heinrich Lieth, and Asher Bar-Tal. 2nd ed., 259–301. Boston: Elsevier. doi: 10.1016/B978-0-444-63696-6.00007-4.

Baslam, M., T. Mitsui, K. Sueyoshi, and T. Ohyama. 2020. Recent advances in carbon and nitrogen metabolism in C3 plants. *International Journal of Molecular Sciences* 22 (1):1–39. doi: 10.3390/ijms22010318.

Bishop, K. A., A. Leakey, and E. A. Ainsworth. 2014. How seasonal temperature or water inputs affect the relative response of C3 crops to elevated [CO2]: A global analysis of open top chamber and free air CO2 enrichment studies. *Food and Energy Security* 3 (1):33–45. doi: 10.1002/fes3.44.

Bryla, D. R., and B. C. Strik. 2015. Nutrient requirements, leaf tissue standards, and new options for fertigation of northern highbush blueberry. *HortTechnology* 25 (4):464–70. doi: 10.21273/HORTTECH.25.4.464.

Claussen, W., and F. Lenz. 1999. Effect of ammonium or nitrate nutrition on net photosynthesis. *Plant and Soil* 208 (1):95–102. https://link.springer.com/content/pdf/10.1023/A%3A1004543128899.pdf. doi: 10.1023/A:1004543128899.

Curtis, P. S., and X. Wang. 1998. A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. *Oecologia* 113 (3):299–313. doi: 10.1007/s004420050381.

Chumley, H., and S. Hewlings. 2020. The effects of elevated atmospheric carbon dioxide [CO2] on micronutrient concentration, specifically iron (Fe) and zinc (Zn) in rice; a systematic review. *Journal of Plant Nutrition* 43 (10):1571–8. doi: 10.1080/01904167.2020.1739303.

Da Silva Fortirer, J., A. Grandis, D. Pagliuso, C. De Toledo Castanho, and M. Silveira Buckeridge. 2023. Meta-analysis of the responses of tree and herb to elevated $CO_2$ in Brazil. *Scientific Reports* 13 (1):15832. doi: 10.1038/s41598-023-40783-5.

Da Silva, J. R., A. E. Patterson, W. P. Rodrigues, E. Campostrini, and K. L. Griffin. 2017. Photosynthetic acclimation to elevated CO2 combined with partial rootzone drying results in improved water use efficiency, drought tolerance and leaf carbon balance of grapevines (Vitis Labrusca). *Environmental and Experimental Botany* 134:82–95. doi: 10.1016/j.envexpbot.2016.11.007.

Domec, J. C., D. S. Duncan, and K. A. McCulloh. 2017. A synthesis of the effects of atmospheric carbon dioxide enrichment on plant hydraulics: Implications for whole-plant water use efficiency and resistance to drought. *Plant, Cell & Environment* 40 (6):921–37. doi: 10.1111/pce.12843.

Dong, J., X. Qiao, G. Nazim, C. Wenying, L. Xun, and D. Zengqiang. 2018. Elevated and super-elevated CO2 differ in their interactive effects with nitrogen availability on fruit yield and quality of cucumber. *Journal of the Science of Food and Agriculture* 98 (12):4509–16. doi: 10.1002/jsfa.8976.

Doyle, J. W., S. U. Nambeesan, and A. Malladi. 2021. Physiology of nitrogen and calcium nutrition in blueberry (Vaccinium Sp.). *Agronomy* 11 (4):765. doi: 10.3390/agronomy11040765.

Du, C., X. Wang, M. Zhang, J. Jing, and Y. Gao. 2019. Effects of elevated CO2 on plant C-N-P stoichiometry in terrestrial ecosystems: A meta-analysis. *The Science of the Total Environment* 650 (Pt 1):697–708. doi: 10.1016/j.scitotenv.2018.09.051.

Fang, Y., G. Nunez, M. Da Silva, D. Phillips, and P. Munoz. 2020. A review for southern highbush blueberry alternative production systems. *Agronomy* 10 (10):1531. doi: 10.3390/agronomy10101531.

Fang, Y., G. Nunez, P. Fisher, and P. Munoz. 2022. Effect of container size, substrate composition, and genotype on growth and fruit quality of young southern highbush blueberry in a container-based intensive production system. *Scientia Horticulturae* 302 (March):111149. doi: 10.1016/j.scienta.2022.111149.

Fangmeier, A., L. De Temmerman, L. Mortensen, K. Kemp, J. Burke, R. Mitchell, M. Van Oijen, and H. Weigel. 1999. Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO2 concentrations and stress conditions in the european, multiple-site experiment 'ESPACE-Wheat'. *European Journal of Agronomy* 10 (3-4):215–29. doi: 10.1016/S1161-0301(99)00012-X.

Fathurrahman, F. 2023. Effects of carbon dioxide concentration on the growth and physiology of *Albizia saman* (Jacq.) merr. *Journal of Ecological Engineering* 24 (9):302–11. doi: 10.12911/22998993/169145.

Fischer, R. A., and N. C. Turner. 1978. Plant productivity in the arid and semiarid zones. *Annual Review of Plant Physiology* 29 (1):277–317. doi: 10.1146/annurev.pp.29.060178.001425.

Fulcher, A., N. Gauthier, W. Klingeman, F. Hale, and S. White. 2015. Blueberry culture and pest, disease, and abiotic disorder management during nursery production in the southeastern U.S.A Review. *Journal of Environmental Horticulture* 33 (1):33–47. doi: 10.24266/0738-2898-33.1.33.

Geiger, M. P., P. Walch-Liu, C. Engels, J. Harnecker, E. Schulze, F. Ludewig, U. Sonnewald, W. Scheible, and M. Stitt. 1998. Enhanced carbon dioxide leads to a modified diurnal rhythm of nitrate reductase activity in older plants, and a large stimulation of nitrate reductase activity and higher levels of amino acids in young tobacco plants. *Plant, Cell & Environment* 21 (3):253–68. doi: 10.1046/j.1365-3040.1998.00277.x.

Gojon, A., O. Cassan, L. Bach, L. Lejay, and A. Martin. 2023. The decline of plant mineral nutrition under rising CO2: Physiological and molecular aspects of a bad deal. *Trends in Plant Science* 28 (2):185–98. doi: 10.1016/j.tplants.2022.09.002.

Gómez-Bellot, M., B. Lorente, M. Sánchez-Blanco, M. Ortuño, P. Nortes, and J. Alarcón. 2020. Influence of mixed substrate and arbuscular mycorrhizal fungi on photosynthetic efficiency, nutrient and water status and yield in tomato plants irrigated with saline reclaimed waters. *Water (Switzerland)* 12 (2):438. doi: 10.3390/w12020438.

Guo, J., M. Zhang, X. Wang, and W. Zhang. 2015. A possible mechanism of mineral responses to elevated atmospheric CO2 in rice grains. *Journal of Integrative Agriculture* 14 (1):50–7. doi: 10.1016/S2095-3119(14)60846-7.

Han, X., Y. Sun, J. Chen, Z. Wang, H. Qi, Y. Liu, and Y. Liu. 2023. Effects of CO2 enrichment on carbon assimilation, yield and quality of oriental melon cultivated in a solar greenhouse. *Horticulturae* 9 (5):561. doi: 10.3390/horticulturae9050561.

He, Z., J. Xiong, A. Kent, Y. Deng, K. Xue, G. Wang, L. Wu, J. Van Nostrand, and J. Zhou. 2014. Distinct responses of soil microbial communities to elevated CO2 and O3 in a soybean agro-ecosystem. *The ISME Journal* 8 (3):714–26. doi: 10.1038/ismej.2013.177.

Heller, C., and G. Nunez. 2022. Preplant fertilization increases substrate microbial respiration but does not affect southern highbush blueberry establishment in a coconut coir-based substrate. *HortScience* 57 (1):17–21. doi: 10.21273/HORTSCI16220-21.

Hoagland, D. R., and D. I. Arnon. 1938. The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station* 347:39.

Jacotot, A., C. Marchand, S. Gensous, and M. Allenbach. 2018. Effects of elevated atmospheric CO2 and increased tidal flooding on leaf gas-exchange parameters of two common mangrove species: Avicennia marina and Rhizophora stylosa. *Photosynthesis Research* 138 (2):249–60. doi: 10.1007/s11120-018-0570-4.

Jorquera-Fontena, E., M. Alberdi, M. Reyes-Díaz, and N. Franck. 2016. Rearrangement of leaf traits with changing source-sink relationship in blueberry (*Vaccinium corymbosum* L.) leaves. *Photosynthetica* 54 (4):508–16. doi: 10.1007/s11099-016-0207-9.

Junta de Andalucia. 2021. Red Fruit, Sector Basic Data on Blueberry. Campaign Red Fruit Sector 2020–2021.

Kimball, B. 1983. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations 1. *Agronomy Journal* 75 (5):779–88. doi: 10.2134/agronj1983.00021962007500050014x.

Kimball, B., S. Idso, S. Johnson, and M. Rillig. 2007. Seventeen years of carbon dioxide enrichment of sour orange trees: Final results. *Global Change Biology* 13 (10):2171–83. doi: 10.1111/j.1365-2486.2007.01430.x.

Kingston, P., C. Scagel, D. Bryla, and B. Strik. 2017. Suitability of sphagnum moss, coir, and douglas fir bark as soilless substrates for container production of highbush blueberry. *HortScience* 52 (12):1692–9. doi: 10.21273/HORTSCI12374-17.

Li, Q., Y. Ren, H. Fu, Z. Li, F. Kong, and J. Yuan. 2022. Cultivar differences in carbon and nitrogen accumulation, balance, and grain yield in maize. *Frontiers in Plant Science* 13 (September):992041. doi: 10.3389/fpls.2022.992041.

Liu, X., H. Zhang, J. Wang, X. Wu, S. Ma, Z. Xu, T. Zhou, N. Xu, X. Tang, and B. An. 2019. Increased CO2 concentrations increasing water use efficiency and improvement psii function of mulberry seedling leaves under drought stress. *Journal of Plant Interactions* 14 (1):213–23. doi: 10.1080/17429145.2019.1603405.

Liu, X., Y. Fan, J. Long, R. Wei, R. Kjelgren, C. Gong, and J. Zhao. 2013. Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of Robinia pseudoacacia seedlings. *Journal of Environmental Sciences (China)* 25 (3):585–95. doi: 10.1016/S1001-0742(12)60081-3.

Lobo, A., I. Catarino, E. Silva, D. Centeno, and D. Domingues. 2022. Physiological and molecular responses of woody plants exposed to future atmospheric co2 levels under abiotic stresses. *Plants (Basel, Switzerland)* 11 (14):1880. doi: 10.3390/plants11141880.

Lobos, G., and J. Hancock. 2015. Breeding blueberries for a changing global environment: A review. *Frontiers in Plant Science* 6 (SEPTEMBER):782. doi: 10.3389/fpls.2015.00782.

Luo, L., Y. Zhang, and G. Xu. 2020. How does nitrogen shape plant architecture? *Journal of Experimental Botany* 71 (15):4415–27. doi: 10.1093/jxb/eraa187.

Mamatha, H., N. Srinivasa Rao, R. Laxman, K. Shivashankara, R. Bhatt, and K. Pavithra. 2014. Impact of elevated CO2 on growth, physiology, yield, and quality of tomato (Lycopersicon esculentum Mill) Cv. Arka Ashish. *Photosynthetica* 52 (4):519–28. doi: 10.1007/s11099-014-0059-0.

Matejovic, I. 1993. Determination of carbon, hydrogen, and nitrogen in soils by automated elemental analysis (dry combustion method). *Communications in Soil Science and Plant Analysis* 24 (17-18):2213–22. doi: 10.1080/00103629309368950.

McCarthy, H., R. Oren, H. Johnsen, A. Gallet-Budynek, S. Pritchard, C. Cook, S. Ladeau, R. Jackson, and A. Finzi. 2010. Re-assessment of plant carbon dynamics at the duke free-air CO2 enrichment site: Interactions of atmospheric [CO2] with nitrogen and water availability over stand development. *The New Phytologist* 185 (2):514–28. doi: 10.1111/j.1469-8137.2009.03078.x.

McDonald, G., W. Bovill, C. Huang, and D. Lightfoot. 2013. Nutrient use efficiency. In *Genomics and breeding for climate-resilient crops*. Vol. 2:333–93. Berlin, Heidelberg: Springer Berlin Heidelberg. doi: 10.1007/978-3-642-37048-9_10.

Medina, S., R. Vicente, A. Amador, and J. L. Araus. 2016. Interactive effects of elevated [CO2] and water stress on physiological traits and gene expression during vegetative growth in four durum wheat genotypes. *Frontiers in Plant Science* 7 (NOVEMBER2016):1738. doi: 10.3389/fpls.2016.01738.

Michalski, R., and L. Kurzyca. 2014. Determination of nitrogen species (nitrate, nitrite and ammonia ions) in environmental samples by ion chromatography determination of nitrogen species (nitrate, nitrite and ammonia ions) in environmental samples by ion chromatography. *Polish Journal of Environmental Studies* 15 (July):5–18. http://www.pjoes.com/pdf/15.1/5-18.pdf.

Mndela, M., J. Tjelele, I. Madakadze, M. Mangwane, I. Samuels, F. Muller, and H. Pule. 2022. A global meta-analysis of woody plant responses to elevated CO2: Implications on biomass, growth, leaf n content, photosynthesis and water relations. *Ecological Processes* 11 (52):1–21. doi: 10.1186/s13717-022-00397-7.

Nunez, G., J. Olmstead, and R. Darnell. 2015. Rhizosphere acidification is not part of the strategy i iron deficiency response of vaccinium arboreum and the southern highbush blueberry. *HortScience* 50 (7):1064–9. doi: 10.21273/HORTSCI.50.7.1064.

O'Connor, R. C., D. M. Blumenthal, T. W. Ocheltree, and J. B. Nippert. 2022. Elevated CO2 counteracts effects of water stress on woody rangeland-encroaching species. *Tree Physiology* tpac150. doi: 10.1093/treephys/tpac150

Ortiz-Delvasto, N., P. Garcia-Gomez, M. Carvajal, and G. Bárzana. 2024. Aquaporins-mediated water availability in substrates for cannabis cultivation in relation to CBD yield. *Plant and Soil* 495 (1-2):469–85. doi: 10.1007/s11104-023-06341-8.

Pan, T., J. Ding, G. Qin, Y. Wang, L. Xi, J. Yang, J. Li, J. Zhang, and Z. Zou. 2019. Interaction of supplementary light and CO2 enrichment improves growth, photosynthesis, yield, and quality of tomato in autumn through spring greenhouse production. *HortScience* 54 (2):246–52. doi: 10.21273/HORTSCI13709-18.

Petridis, A., J. Van der Kaay, J. Sungurtas, S. Verrall, S. McCallum, J. Graham, and R. Hancock. 2020. Photosynthetic plasticity allows blueberry (Vaccinium corymbosum L.) plants to compensate for yield loss under conditions of high sink demand. *Environmental and Experimental Botany* 174 (March):104031. doi: 10.1016/j.envexpbot.2020.104031.

Poonnachit, U., and R. Darnell. 2004. Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in Vaccinium species. *Annals of Botany* 93 (4):399–405. doi: 10.1093/aob/mch053.

Poorter, H., Y. Van Berkel, R. Baxter, J. Den Hertog, P. Dijkstra, R. Gifford, K. Griffin, C. Roumet, J. Roy, and S. Wong. 1997. The effect of elevated CO2 on the chemical composition and construction costs of leaves of 27 C3 species. *Plant, Cell & Environment* 20 (4):472–82. doi: 10.1046/j.1365-3040.1997.d01-84.x.

Primo-Millo, E., and M. Agustí. 2020. Charper 10: Vegetative growth. In *The Genus Citrus*, ed. M. Talon, M. Caruso, and F. Gmitter, 193–217. Woodhead Publishing. doi: 10.1016/B978-0-12-812163-4.00010-3.

Prior, S., H. Torbert, G. Runion, G. Mullins, H. Rogers, and J. Mauney. 1998. Effects of carbon dioxide enrichment on cotton nutrient dynamics. *Journal of Plant Nutrition* 21 (7):1407–26. doi: 10.1080/01904169809365492.

Purcell, C., S. Batke, C. Yiotis, R. Caballero, W. Soh, M. Murray, and J. McElwain. 2018. Increasing stomatal conductance in response to rising atmospheric CO2. *Annals of Botany* 121 (6):1137–49. doi: 10.1093/aob/mcx208.

Purvis, A., D. Peters, and R. Hageman. 1974. Effect of carbon dioxide on nitrate accumulation and nitrate reductase induction in corn seedlings. *Plant Physiology* 53 (6):934–41. doi: 10.1104/pp.53.6.934.

Roy, S., R. Kapoor, and P. Mathur. 2024. Revisiting changes in growth, physiology and stress responses of plants under the effect of enhanced CO2 and temperature. *Plant & Cell Physiology* 65 (1):4–19. doi: 10.1093/pcp/pcad121.

Sekhar K.M., Rachapudi V.S., Mudalkar S. and Reddy A.R. 2014. Persistent stimulation of photosynthesis in short rotation coppice mulberry under elevated CO2 atmosphere. *Journal of Photochemistry and Photobiology B: Biology* 137: 21–30. doi: 10.1016/j.jphotobiol.2014.05.001

Shaheen, H., A. Mustafa, and A. Ulfat. 2022. Chapter 6: Crop production in response to elevated $CO_2$: Grain yield and quality. In *Sustainable Crop Productivity and Quality Under Climate Change*, ed. F. Liu, L. Xiangnan, P. Hogy, D. Jiang, M. Brestic, and B. Liu, 91–101. doi: 10.1016/B978-0-323-85449-8.00009-9.

Shanmugam, S., K. Kjaer, C. Ottosen, E. Rosenqvist, D. Kumari, and B. Wollenweber. 2013. The alleviating effect of elevated co2 on heat stress susceptibility of two wheat (Triticum aestivum L.) cultivars. *Journal of Agronomy and Crop Science* 199 (5):340–50. doi: 10.1111/jac.12023.

Smrke, T., R. Veberic, M. Hudina, D. Stamic, and J. Jakopic. 2021. Comparison of highbush blueberry (Vaccinium corymbosum l.) under ridge and pot production. *Agriculture (Switzerland)* 11 (10):929. doi: 10.3390/agriculture11100929.

Stitt, M., and A. Krapp. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: The physiological and molecular background. *Plant, Cell & Environment* 22 (6):583–621. doi: 10.1046/j.1365-3040.1999.00386.x.

Tamir, G., G. Afik, S. Zilkah, N. Dai, and A. Bar-Tal. 2021. The use of increasing proportions of N-NH4+ among the total applied inorganic N to improve acidification and the nutritional status and performance of blueberry plants in soilless culture. *Scientia Horticulturae* 276 (September 2020):109754. doi: 10.1016/j.scienta.2020.109754.

Taub, D., and X. Wang. 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO2? a critical examination of the hypotheses. *Journal of Integrative Plant Biology* 50 (11):1365–74. doi: 10.1111/j.1744-7909.2008.00754.x.

Van der Kooi, C., M. Reich, M. Löw, L. De Kok, and M. Tausz. 2016. Growth and yield stimulation under elevated CO2 and drought: A meta-analysis on crops. *Environmental and Experimental Botany* 122Elsevier B.V.: :150–7. doi: 10.1016/j.envexpbot.2015.10.004.

Wei, Z., T. Du, X. Li, L. Fang, and F. Liu. 2018. Interactive effects of CO2 concentration elevation and nitrogen fertilization on water and nitrogen use efficiency of tomato grown under reduced irrigation regimes. *Agricultural Water Management* 202 (February):174–82. doi: 10.1016/j.agwat.2018.02.027.

Woodward, F. 2002. Potential impacts of global elevated CO2 concentrations on plants. *Current Opinion in Plant Biology* 5 (3):207–11. doi: 10.1016/S1369-5266(02)00253-4.

Wu, Y., L. Ma, L. Zhang, Y. Zhang, H. Zhou, Y. Wang, and Y. Liu. 2022. Photosynthetic carbon and nitrogen metabolism of Camellia oleifera abel during acclimation to low light conditions. *Journal of Plant Physiology* 278 (April):153814. doi: 10.1016/j.jplph.2022.153814.

Yang, H., Y. Wu, Y. Duan, C. Zhang, Z. Huang, W. Wu, L. Lyu, and W. Li. 2022. Metabolomics combined with physiological and transcriptomic analyses reveal regulatory features associated with blueberry growth in different soilless substrates. *Scientia Horticulturae* 302 (December 2021):111145. doi: 10.1016/j.scienta.2022.111145.

Yang, H., Z. Wei, Y. Duan, Y. Wu, C. Zhang, W. Wu, L. Lyu, and W. Li. 2023. Transcriptomic and metabolomic investigation of the adaptation mechanisms of blueberries to nitrogen deficiency stress. *Scientia Horticulturae* 321 (July):112376. doi: 10.1016/j.scienta.2023.112376.

Yelle, S., A. Gosselin, and M. Trudel. 1987. Effect of atmospheric CO2 concentration and root-zone temperature on growth, mineral nutrition, and nitrate reductase activity of greenhouse tomato. *Journal of the American Society for Horticultural Science* 112 (6):1036–40. doi: 10.21273/JASHS.112.6.1036.

Zárate, B., M. Alonso, V. Morales, A. Vásquez, P. Benito, and N. Arellanes. 2017. Physical and chemical characterization of pine bark for the production of blueberry (Vaccinium corymbosum L.) in containers. *Acta Horticulturae* 1180 (1180):503–10. doi: 10.17660/ActaHortic.2017.1180.71.

Zhang, C., C. Zhou, R. Burnap, and L. Peng. 2018. Carbon/Nitrogen metabolic balance: Lessons from cyanobacteria. *Trends in Plant Science* 23 (12):1116–30. doi: 10.1016/j.tplants.2018.09.008.