# Photosynthesis and production of West Indian cherry irrigated with saline waters under nitrogen/potassium fertilization

Evandro Manoel da Silva[1]*[iD], Hans Raj Gheyi[2][iD], Reginaldo Gomes Nobre[3][iD],
Joicy Lima Barbosa[4][iD], Valeska Karolini Nunes Oliveira[1][iD], Luderlândio de Andrade Silva[1][iD]

[1]Federal University of Campina Grande, Campina Grande, Brazil
[2]Federal University of Recôncavo da Bahia, Cruz das Almas, Brazil
[3]Federal University of the Semi-Arid, Caraúbas, Brazil
[4]Federal University of Alagoas, Rio Largo, Brazil
*Corresponding author, e-mail: evandroagroman@hotmail.com

**Abstract**

Fertilization management is a technique that has been studied for the mitigation of salt stress in plants. In this perspective, this work aimed to evaluate the effects of combinations of potassium and nitrogen fertilization on the photosynthetic pigments, photosynthetic efficiency, and production of the West Indian cherry irrigated with waters of different salinities, between 420 and 550 days after transplanting. The experiment was conducted in an open field at the Center of Sciences and Agrifood Technology of the Federal University of Campina Grande, using 60 L lysimeters, in a randomized block design with a 5 x 4 factorial scheme, corresponding to five salinity levels of the irrigation water – ECw (0.3; 1.3; 2.3; 3.3, and 4.3 dS m$^{-1}$) and four combinations of nitrogen and potassium fertilization (C1 = 70% N + 50% K$_2$O; C2 = 100% N + 75% K$_2$O; C3 = 130% N + 100% K$_2$O, and C4 = 160% N + 125% K$_2$O) of the dose recommended for the West Indian cherry, with three replications and one plant per plot. The cv. Flor Branca was studied through its grafting on a rootstock of the cv. Junco. The irrigation with ECw above 0.3 dS m$^{-1}$ reduced the contents of chlorophyll $a$ and $b$ in the leaves, the photochemical efficiency, CO$_2$ assimilation rate, instantaneous carboxylation efficiency, instantaneous water-use efficiency, and the number of fruits per plant. The combined fertilization with 70% of N + 50% of K$_2$O increased the photosynthetic pigments, photosynthesis, and the number of fruits per plant, as well as mitigated the deleterious effects of water salinity on the production per plant up to the ECw of 2.3 dS m$^{-1}$, which revealed to be the most adequate combination for West Indian cherry fertilization.

**Keywords:** *Malpighia emarginata* D. C., salinity, nitrogen, potassium

**Introduction**

Fruit farming is an activity of great importance for the semiarid region of Northeastern Brazil, and the West Indian cherry (*Malpighia emarginata* D. C.) ranks among the fruit species of highest economic interest in the region due to the high content of vitamin C and the presence of antioxidant compounds in its fruits (Calgaro & Braga, 2012).

In this region, where the production system depends on irrigation, water quality may become a limiting factor since nearly 53% of the water sources (ponds, dams, and wells) present salinity levels above 3.0 dS m$^{-1}$, constituting a severe degree of restriction for irrigation (Morais et al., 1998).

Excess of salt in the irrigation water causes the degradation of photosynthetic pigments and chloroplasts, closure of stomata, reduction in the use of luminous energy and the carboxylation of the Rubisco enzyme, and also the activity of the enzymes of the Calvin-Benson cycle, reducing the photosynthetic activity and crop production (Parihar et al., 2015).

Nitrogen and potassium are involved in physiological and biochemical processes, with direct effect on plant growth and production, considering that the first constitutes the structure of proteins, enzymes, amino acids, nucleic acids, etc. (Leghari et al., 2016); and the second acts in the control of cell turgidity, activation of enzymes involved in respiration and photosynthesis, regulating the opening and closing of stomata, and in the transportation of carbohydrates (Wang et al., 2013). In this perspective, it is necessary to search for fertilization management strategies with the combination of these nutrients, aiming at decreasing the nutritional deficiencies induced by salt stress and promoting gains in production.

It is highlighted that the adequate combinations of doses of nitrogen and potassium in plants under saline

conditions can induce the $Cl^-/NO_3^-$ and $Na^+/K^+$ ratios in the leaves, with mitigation of the toxic effects of the $Na^+$ and $Cl^-$ ions and reestablishment of the nutritional balance (Andrade Júnior et al., 2011), generating favorable conditions for the physiological and biochemical activities, with a positive impact on crop production.

Studies demonstrated that increases of 40% in the contents of N and P (Sá et al., 2019a) and of 125% in $K_2O$ (Lima et al., 2019a), in relation to the recommended dose, mitigated the negative effects of the ECw up to 3.8 dS $m^{-1}$ on growth, photosynthesis, and production of the West Indian cherry cv. BRS Jaburu, in the first year of cultivation.

Studies are still incipient regarding the effect of the NK combination on photosynthesis and the production of this crop under salt stress, in the second year of cultivation. In this perspective, this study aimed to evaluate the effects of combinations of nitrogen and potassium fertilization on the photosynthetic pigments, photosynthetic efficiency, and production of the West Indian cherry irrigated with waters of different salinities, from 420 to 550 days after transplanting.

**Material and Methods**

The experiment was conducted in the field, with plants cultivated in 60 L lysimeters installed in an experimental area belonging to the Center of Sciences and Agrifood Technology (CCTA) of the Federal University of Campina Grande (UFCG) in the municipality of Pombal, PB. The geographic coordinates of the place are 6° 48'16" South latitude, 37° 49'15" West longitude, and mean elevation of 144 m. According to the classification by Köppen-Geiger, adapted to Brazil, the climate of the region is classified as BSh, hot semiarid (Peel et al., 2007), whose rainfall, mean temperature, and air relative humidity during the experimental period were 8.0 mm; 27.7 °C, and 44.8%, respectively (INMET, 2018).

The treatments were arranged in a 5 x 4 factorial scheme, referring to five salinity levels of the irrigation water (ECw): 0.3; 1.3; 2.3; 3.3, and 4.3 dS $m^{-1}$, and four combinations of doses of nitrogen and potassium: C1 = 70% N + 50% $K_2O$; C2 = 100% N + 75% $K_2O$; C3= 130% N + 100% $K_2O$, and C4= 160% N + 125% $K_2O$ of the recommended dose for the West Indian cherry (Cavalcanti, 2008), in a randomized block design, with three replications, and the plot composed of a lysimeter containing one plant, totaling 60 lysimeters in the experiment.

The saline levels were based on the study of Sá et al. (2019a), in which they verified that the irrigation with water of salinity up to 2.2 dS $m^{-1}$ did not compromise the growth, photosynthetic activity, and production of the West Indian cherry cv. BRS 336 Jaburu, in the first year of cultivation.

The saline waters of the treatments from 1.3 to 4.3 dS $m^{-1}$ were prepared by observing the relationship between the ECw and the concentration of salts ($mmol_c$ $L^{-1}$ = CE x 10) (Rhoades et al., 1992) through the dissolution of NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$, maintaining the equivalent proportion of 7:2:1, respectively, in the water of the treatment with the lowest salinity level (0.3 dS $m^{-1}$).

Initially, the plants were irrigated with water of electrical conductivity of 0.3 dS $m^{-1}$, aiming at promoting good acclimatization to field conditions. The application of saline waters began at 41 days after transplanting (DAT), a period in which the irrigation was manually performed daily with the water of the respective treatment and based on the drainage lysimetry principle, in which the volume to be applied was determined by the difference between the volume applied and the volume drained in the previous irrigation, and the value of this difference is equivalent to the water volume needed for the soil to reach its maximum water retention capacity (field capacity). A 15% leaching fraction was applied every 15 days, based on the water volume applied in this period with the purpose to decrease the excessive accumulation of salts in the root zone of the plants.

The application of fertilization combinations began at 20 DAT, being simultaneously performed by topdressing in a 20 cm radius in relation to the base of the plant. A fertilization recommendation for the West Indian cherry cultivar "Flor Branca" cultivated under irrigation conditions corresponding to 100 g of N and 80 g of $K_2O$ per plant per year was adopted (Cavalcanti, 2008), equivalent to the doses of the treatments with 100% of nitrogen and potassium. The amounts of N and $K_2O$ applied during the conduction of the experiment are presented in Table 1.

The lysimeters were spaced in the field at 1.8 x 2.0 m, between plants and planting rows, respectively, occupying an area of 216 $m^2$. At the base of the lysimeters, a drainage system composed of a 3.0 cm layer of fine gravel, 2.0 cm of washed sand followed by 56 $dm^3$ of soil. At the bottom, a drain with a diameter of 12.7 mm was installed, connected to a plastic bottle with a volumetric capacity of 2 L. The physical and chemical attributes (Table 2) were determined in the Laboratory of Irrigation and Salinity of the CTRN/UFCG following methodologies recommended by Donagema et al. (2011).

The seedlings were accquired from a commercial plant nursery registered in the National Registry of Seeds and Seedlings, located in the District of São Gonçalo,

**Table 1.** Amounts of N and $K_2O$ applied through the treatments of combinations of nitrogen and potassium fertilization and phosphate fertilization, carried out in the first and second year of cultivation, up to 550 days after transplanting (DAT).

| Fertilizer combinations | Quantities applied per plant | | |
|---|---|---|---|
| | 1st year (up to 365 DAT) | 2nd year (365 - 550 DAT) | Total (up to 550 DAT) |
| C1 = 70% N + 50% $K_2O$ | 70 g N + 40 g $K_2O$ | 35 g N + 20 g $K_2O$ | 105 g N + 60 g $K_2O$ |
| C2 = 100% N + 75% $K_2O$ | 100 g N + 60 g $K_2O$ | 50 g N + 30 g $K_2O$ | 150 g N + 90 g $K_2O$ |
| C3 = 130% N +100% $K_2O$ | 130 g N + 80 g $K_2O$ | 65 g N + 40 g $K_2O$ | 195 g N + 120 g $K_2O$ |
| C4 = 160% N + 125% $K_2O$ | 160 g N + 100 g $K_2O$ | 80 g N + 50 g $K_2O$ | 240 g N + 150 g $K_2O$ |
| Phosphate fertilization ($P_2O_5$) | | | |
| - | 20 g | 10 g | 30 g |

Note: The application of combinations of doses of N and $K_2O$ was done in an interval of 15 days, using urea (45% N) and KCl (60% $K_2O$) as sources. The phosphate fertilization in the first year was made with simple superphosphate (18% $P_2O_5$), mixing the fertilizer with the soil of the lysimeter and in the second year divided into 12 applications in equal parts, in an interval of 15 days, using monoammonium phosphate - MAP (61% $P_2O_5$ and 12% N), amount of N was subtracted from the doses of treatments with nitrogen fertilization.

**Table 2.** Physical and chemical attributes of the soil used in the experiment, before applying the treatments, collected from the 0-0.20-m layer in Lot 14, Sector I, of the Irrigated Perimeter of Várzeas de Sousa-PB, Brazil.

| Textural Class | | | | | | Exchange complex | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ad | Tp | O.M | N | P assimilable | $Ca^{2+}$ | $Mg^{2+}$ | $Na^+$ | $K^+$ | $H^+ + Al^{3+}$ |
| SL | (kg $dm^{-3}$) | --------- (%) -------- | | | (mg $dm^{-3}$) | ---------------- (cmol$_c$ $dm^{-3}$) ---------------- | | | | |
| | 1.44 | 47.63 | 0.41 | 0.02 | 41.00 | 3.50 | 1.70 | 0.14 | 0.30 | 0.00 |
| Saturation extract | | | | | | | | | | |
| pHse | ECse | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ | $Na^+$ | $Cl^-$ | $SO_4^{2-}$ | $CO_3^{2-}$ | $HCO_3^-$ | SM |
| | (dS $m^{-1}$) | ------------------------------------ (mmol$_c$ L) ------------------------------------ | | | | | | | | (%) |
| 7.11 | 1.28 | 1.39 | 3.23 | 0.38 | 5.78 | 9.00 | Absent | 0.00 | 1.40 | 20.80 |
| SAR (mmol $L^{-1})^{0.5}$ | | ESP | | Salinity | | | Soil Class | | | |
| 3.80 | | 2.48 | | Non saline/ non sodic | | | Normal | | | |

SL – Sandy loam; Ad – Apparent density; Tp – Total porosity; pHse - pH of the saturation extract; ECse - Electrical conductivity of the saturation extract of soil at 25 °C; SM - Saturated soil moisture content (mass basis); SAR - Sodium adsorption ratio; ESP - Exchangeable sodium percentage; P, $K^+$ and $Na^+$ extracted by Mehlich 1; $Ca^{2+}$ and $Mg^{2+}$ extracted by 1.0 M KCl at pH 7.0; $H^+ + Al^{3+}$ extracted by 0.5 M CaOAc at pH 7.0; O.M - Organic matter estimated by Walkley-Black wet digestion.

municipality of Sousa – PB, cultivated in polyethylene bags with dimensions of 10 x 20 cm and volumetric capacity of 0.5 L, subjected to wedge grafting using rootstock and scion of the cultivars Junco and Flor Branca, respectively.

Transplanting was performed when the seedlings reached 30 cm of height, 120 days after grafting, in the soil with moisture content at field capacity. During the conduction of the experiment, a 4.0 cm layer of grass mulch was added.

The photosynthetic pigments were evaluated at 540 DAT, determined through the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *total*), and carotenoids (CAR), using the following methodology: 6 plant tissue discs with 9-mm diameter were removed from the leaf blade, in the fourth pair of leaves of a branch located at the middle region of the canopy. The discs were immersed in 6 mL of 80% acetone, contained in glass recipients with capacity for 10 mL, in which the samples remained totally in the dark for 48 hours, in a refrigerator, at a temperature of 8 °C for pigment extraction. Afterward, the contents of photosynthetic pigments were quantified with spectrophotometry, with absorbance (A) readings in the wavelengths of 663, 646, and 470 nm, using 80% acetone as a blank. The contents of chlorophyll *a*, *b, total*, and carotenoids were obtained by Eq.1; Eq.2; Eq.3, and Eq.4, according to the methodology by Lichtenthäler (1987):

The photosynthetic parameters were also

$$Chl\ a = 12.21\ A663 - 2.81\ A64 \tag{1}$$

$$Chl\ b = 20.13\ A646 - 5.03\ A663 \tag{2}$$

$$Chl\ total = 17.3\ A646 + 7.18\ A663 \tag{3}$$

$$CAR = \frac{(1000\ A470 - 1.82\ Chl\ a - 85.02\ Chl\ b)}{198} \tag{4}$$

measured at 540 DAT through the photochemical efficiency and gas exchanges, measured between 6:00 a.m. and 9:00 a.m. in a leaf located in the region of the plant canopy from which the leaf discs were removed for pigment analysis.

The photochemical efficiency was analyzed through the fluorescence parameters of chlorophyll a, including: initial fluorescence (Fo), maximum fluorescence (Fm), and variable fluorescence (Fv), using the PEA equipment – Hansatech, with the placement of metallic tweezers in the fourth fully-expanded leaf, from the apex to the base of the branch, where it remained for 30 minutes of adaptation to the dark, followed by the reading.

The gas exchanges were determined through the transpiration (*E*), stomatal conductance (*gs*), $CO_2$ assimilation rate (*A*), intercellular $CO_2$ concentration (*Ci*), instantaneous carboxylation efficiency (*EiCi*), and instantaneous water use efficiency (*WiUE*). The first four variables were measured using a handheld infrared carbonic gas analyzer (IRGA), model LCPro + Portable

Photosynthesis System®, operating with temperature adjusted to 25 °C, irradiance of 1200 µmol photons $m^{-2}$ $s^{-1}$, airflow of 200 mL $min^{-1}$, and concentration of atmospheric $CO_2$. With these data, the instantaneous carboxylation efficiency (*EiCi*) - (*A/Ci*) and the instantaneous water use efficiency were calculated (*WiUE*) - (*A/E*) (Silva et al., 2014).

The number of fruits (NF) and the production per plant (PROD) were obtained from harvests performed when the fruits presented a red-colored pellicle, at five-days interval, from 420 to 550 DAT. The NF was determined through the counting of all fruits. The PROD was obtained by summing the weight of all fruits produced per plant during the harvest period.

The means of the variables were subjected to analysis of variance by the F test at the 0.05 and 0.01 levels of probability, with the data of the salinity levels analyzed by regression studies, and the means of the combination of doses of nitrogen and potassium compared by Tukey's test (p<0,05), using the Sisvar statistical software, version 5.6, for the processing of the data (Ferreira, 2014).

**Results and Discussion**

There was no significant effect (p>0.05) for the interaction salinity of the irrigation water x combination of NK fertilization on the contents of photosynthetic pigments and the fluorescence of chlorophyll *a* (Table 3). However, there was an isolated significant effect (p<0.01 or p<0.05) of the water salinity on the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, maximum fluorescence, and variable fluorescence, whereas the combinations of NK fertilization significantly affected (p<0.01) the contents of chlorophylls and carotenoids. The initial fluorescence was not influenced (p>0.05) by any of the studied factors.

The increase in the salinity of the irrigation water linearly increased the contents of Chl *a*, Chl *b*, and total Chl in the West Indian cherry leaves (Figures 1A, 1B, and 1C), decreasing these values by 9.47; 7.80, and 8.73%, respectively, per unitary increment of the ECw. For the

contents of CAR, the data adjusted to the quadratic model, whose maximum value (0.0061 g $m^{-2}$) was obtained with the ECw of 2.5 dS $m^{-1}$, being later reduced by the increment in water salinity (Figure 1D).

The decrease in the chlorophyll contents may be related to the inhibition of the synthesis of the 5-aminolevulinic acid, a precursor molecule of chlorophyll, and to the increase in the activity of the chlorophyllase enzyme, which degrades the molecules of these photosynthetic pigments under salt stress conditions (Freire et al., 2013; Silva et al., 2017; Sá et al., 2019b).

The increase in the contents of carotenoids up to the ECw of 2.5 dS $m^{-1}$ may be involved with the protection of photosynthetic machinery to the effects of salt stress since, besides the capture of light and dissipation of the excess of luminous energy (Leghari et al., 2016) in plants exposed to salinity, this pigment acts as an antioxidant agent, protecting the membrane lipids of the oxidative stress in the chloroplasts (Falk & Munné-Bosch, 2010). However, it is highlighted that, above this level, the water salinity may have increased the degradation of ß-carotene and the reduction of the synthesis of zeaxanthin, resulting in the decrease of the contents of carotenoids (Freire et al., 2013; Sá et al., 2019b).

Regarding the combinations of NK fertilization, it was observed that the Chl *a*, Chl *b*, Chl *total*, and CAR (Figures 2A, 2B, 2C, and 2D) obtained higher values under fertilization with the C1 (70% N + 50% $K_2O$) and C2 combinations (100% N + 125% $K_2O$), followed by a reduction in the C3 combination (130% N + 100% $K_2O$), although with no statistical difference except Car. Furthermore, it is verified that the fertilization with the C4 combination (160% N + 125% $K_2O$), decreased the contents of photosynthetic pigments, especially when compared statistically with the plants that received the C1 and C2 combinations.

The supply of nitrogen and potassium, especially in the combinations of 70% N + 50% $K_2O$ and 100% N + 75% $K_2O$, promoted a greater synthesis of photosynthetic pigments, especially chlorophylls, considering that the

**Table 3.** Summary of F test for the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *total*), carotenoids (CAR), initial fluorescence (Fo), maximum fluorescence (Fm) and variable fluorescence of chlorophyll a (Fv) in West Indian cherry plants irrigated with saline water, under combinations of nitrogen and potassium fertilization, at 540 DAT.

| Source of variation | F test significance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DF | Chl *a* | Chl *b* | Chl *total* | CAR[1] | Fo | Fm | Fv |
| Salinity (S) | 4 | ** | ** | ** | * | ns | ** | ** |
| NK combination (C) | 3 | ** | ** | ** | ** | ns | ns | ns |
| Interaction S x C | 12 | ns | ns | ns | ns | ns | ns | ns |
| Blocks | 2 | ns | ns | ns | ns | ns | ns | ns |
| Erro | 38 | 0.0005 | 0.0003 | 0.0016 | 0.0004 | 9424.8 | 106492.9 | 98051.1 |
| CV (%) | - | 23.72 | 23.62 | 23.28 | 35.56 | 11.72 | 12.35 | 17.27 |

ns, * and **, respectively not significant, significant at p < 0.05 and p < 0.01; DF - Degrees of freedom; CV - Coefficient of variation. [1] statistical analysis performed after data transformation into √X.
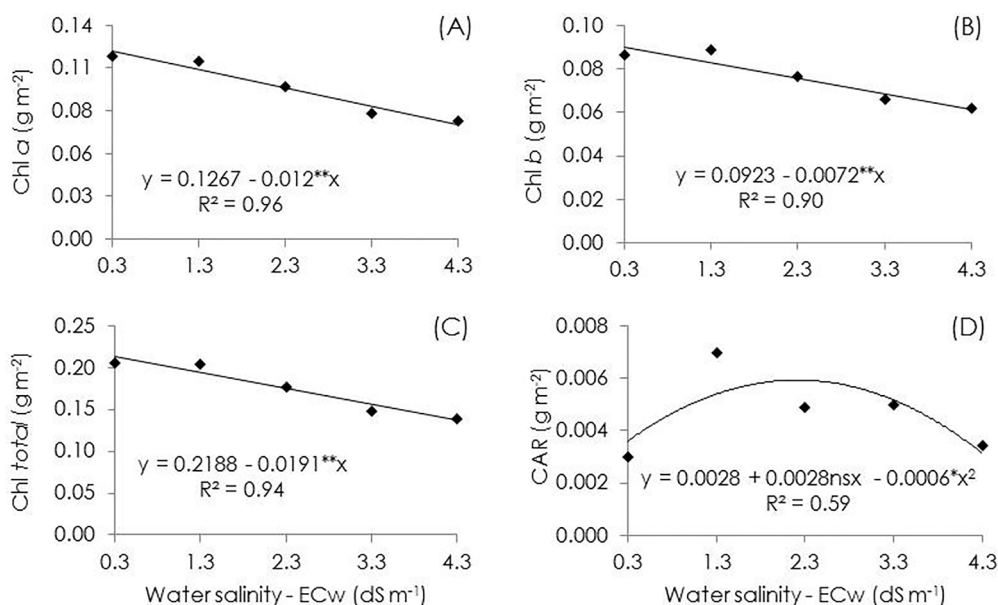
**Figure 1.** Contents of Chlorophyll *a* - Chl *a* (A), chlorophyll *b* - Chl *b* (B), total chlorophyll - Chl *total* (C) and carotenoids - Car (D) in West Indian cherry leaves irrigated, as a function of the electrical conductivity of irrigation water (ECw) at 540 days after transplanting.
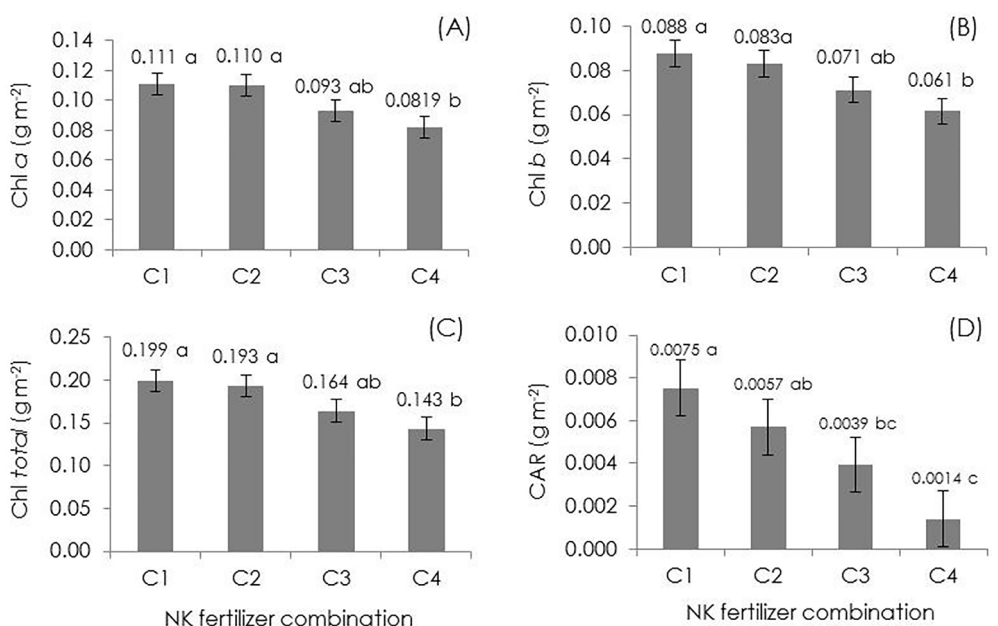


C1= 70% N + 50% $K_2O$; C2= 100% N + 75% $K_2O$; C3= 130% N + 100% $K_2O$ e C4 = 160% N + 125% $K_2O$ of recommendation.

**Figure 2.** Contents of chlorophyll *a* - Chl *a* (A), chlorophyll *b* - Chl *b* (B), total chlorophyll - Chl *total* (C) and carotenoids - Car (D) in West Indian cherry leaves, as a function of different combinations of nitrogen and potassium fertilization at 540 days after transplanting. Means followed by different letters indicate that the treatments differ by Tukey test (p < 0.05). Vertical bars represent the standard error of the mean (n = 3).

doses of nitrogen applied were more adequate for the synthesis of chlorophyll, considering that this mineral nutrient presents a high correlation with the content of this pigment in the leaves and is one of the main constituents of the chlorophyll molecule (Leghari et al., 2016). The use of the C4 combination (160% N + 125% $K_2O$) may have caused a nutritional imbalance and the intensification of the osmotic effect, with the decrease of water absorption. These effects may have occurred due to the application

of high doses of N and K through urea and KCl, which possess high saline index corresponding to 75 and 115%, respectively (Alvarenga et al., 2019). A similar result was found for the guava crop (Silva et al., 2017).

It is verified, in Figures 3A and 3B, that the increment in water salinity reduced the maximum fluorescence (Fm) and the variable fluorescence (Fv) of the chlorophyll *a*, which decreased by 3.57 and 4.62%, respectively, with per unit increase in the ECw of irrigation.

The decrease in the maximum fluorescence indicates a deficiency in the photoreduction of quinone A (QA), which may promote the deactivation of the photosystem II and directly affect the flow of electrons between photosystems I and II in the membrane of the thylakoids, compromising the photochemical efficiency (Silva et al., 2017; Sá et al., 2019a).
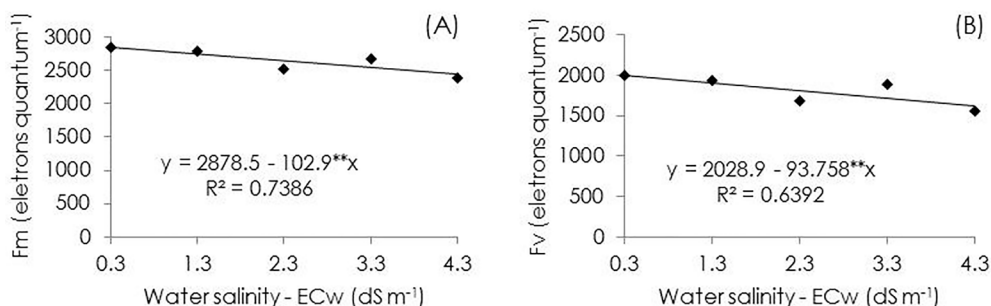


**Figure 3.** Maximum fluorescence of chlorophyll a (Fm) and variable fluorescence (Fv) in West Indian cherry leaves irrigated, as a function of the electrical conductivity of irrigation water (ECw) at 540 days after transplanting.

The decrease of the variable fluorescence (Figure 3B) reflects a reduction of the energy transfer capacity of the electrons ejected from the molecules of the pigments for the formation of the NADPH and ATP reducers, which are used during $CO_2$ assimilation in the biochemical phase of photosynthesis (Tatagiba et al., 2014; Sá et al., 2019a). Thus, it is generally affirmed that the increment in the salinity of the irrigation water decreases the transportation of electrons used in photosynthesis, with a probable reduction of the photochemical efficiency in the West Indian cherry plants.

It was observed (Table 4) that the effect of interaction between irrigation water salinity x NK combination was significant (p<0.01) on the production per plant. Furthermore, there was a significant isolated effect (p<0.01) of the water salinity and combinations of NK fertilization (p<0.05) on the $CO_2$ assimilation rate, intercellular $CO_2$ concentration, instantaneous carboxylation efficiency, instantaneous water-use efficiency, and the number of fruits per plant, whereas transpiration and stomatal conductance were not significantly affected by any of the studied factors (p>0.05).

**Table 4.** Summary of F test for transpiration rate (*E*), stomatal conductance (*gs*), $CO_2$ assimilation rate (*A*), $CO_2$ intercellular concentration (*Ci*), instantaneous efficiency carboxylation (*EiCi*), instantaneous water use efficiency (*WiUE*) at 540 DAT and number of fruits (NFR) and production per plant (PROD) of harvests between 420 and 550 DAT, in West Indian cherry irrigated with saline water, under combinations of nitrogen and potassium fertilization.

| Source of variation | F test significance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DF | *E* | *gs* | *A* | *Ci* | *EiCi* | *WiUE* | *NFR* | *PROD* |
| Salinity (S) | 4 | ns | ns | * | ** | ** | * | ** | ** |
| NK combination (C) | 3 | ns | ns | ** | * | ** | ** | ** | ** |
| Interaction S x C | 12 | ns | ns | ns | ns | ns | ns | ns | ** |
| Blocks | 2 | ** | ** | ** | ** | ** | * | ns | ns |
| Erro | 38 | 0.114 | 0.0003 | 3.047 | 912.03 | 0.0001 | 2.974 | 34379.0 | 0.145 |
| CV (%) | - | 35.66 | 35.66 | 36.85 | 15.76 | 44.59 | 33.90 | 42.10 | 37.74 |

ns, * and **, respectively not significant, significant at p < 0.05 and p < 0.01; DF - Degrees of freedom; CV - Coefficient of variation.

The non-significant effect of water salinity on stomatal conductance and transpiration may be related with the maintenance of turgor pressure in the guard cells that regulate the opening and closing of stomata, by effect of the osmotic adjustment, as already observed in the West Indian cherry crop irrigated with saline waters (Alvarenga et al., 2019).

For the intercellular $CO_2$ concentration, a linear positive effect (Figure 4A) was verified by the increment of water salinity, resulting in an increase of 11.63% for each unitary increment of the ECw. Such a fact is associated with the accumulation of $CO_2$ in the intercellular space of the foliar mesophyll, with a probable effect on the decrease of carbon fixation in the synthesis of carbohydrates, during photosynthesis (Silva et al., 2018; Lima et al., 2019a). This generally occurs due to the elevation of the C1 concentration on the leaves, which reduces the carboxylation efficiency of the Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) enzyme, responsible for the fixation of carbon in the biochemical phase of photosynthesis (Silva et al., 2018).

The increase in the salinity of the irrigation water

decreased the $CO_2$ assimilation rate, the instantaneous carboxylation efficiency, and the instantaneous water-use efficiency (Figures 4B, 4C, and 4D), with linear

decrease rates equivalent to 7.98, 12.89, and 8.23%, respectively, for each unitary increment in the ECw.
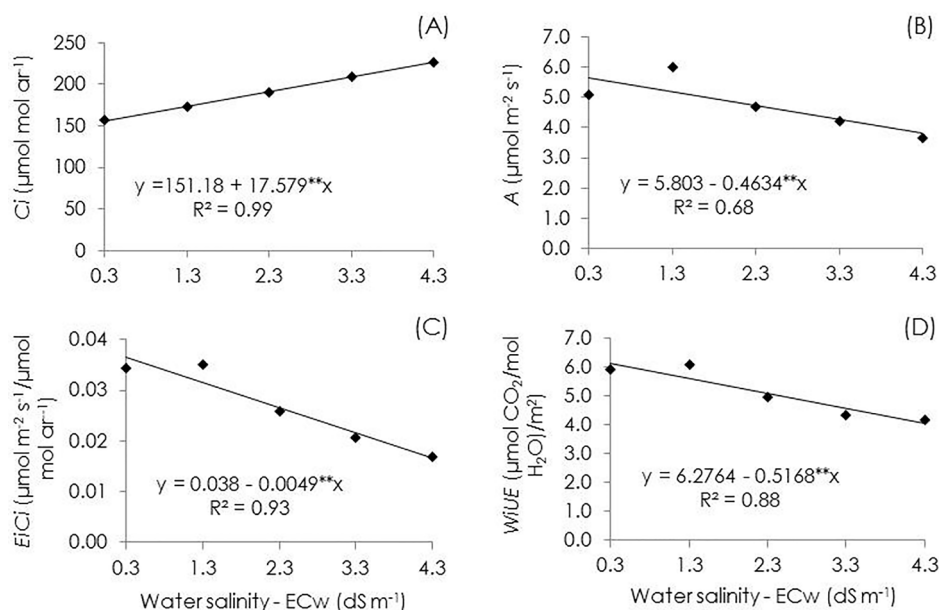


**Figure 4.** $CO_2$ intercellular concentration – $Ci$ (A), $CO_2$ assimilation rate – $A$ (B), instantaneous efficiency carboxylation - $EiCi$ (C) and instantaneous water use efficiency – $WiUE$ (D) in West Indian cherry, as a function on the electrical conductivity of irrigation water (ECw) at 540 days after transplanting.

Some studies demonstrate that the decrease of the $CO_2$ assimilation rate by the increase of salt stress (Figure 4B) occurs due to the reduction in the stomatal opening, which restricts the inflow of $CO_2$ to the leaves (Sá et al., 2019a; Lima et al., 2019b). However, this was not the case of the present study since the stomatal conductance ($gs$) was not affected by the ECw (Table 4), indicating that the decrease of the $CO_2$ assimilation rate is related to non-stomatal factors, such as the reduction of the activity of the rubisco enzyme, which performs $CO_2$ fixation (Parihar et al., 2015).

The decrease of the $EiCi$ (Figure 4C) reinforces the previous justification since this variable is used to investigate if non-stomatal factors, notably of biochemical origin, are affecting the photosynthetic activity (Silva et al., 2014). According to these authors, it represents the amount of carbon that the plant fixes to perform photosynthesis per unit of unfixed carbon, which is accumulated in the intercellular spaces of the mesophyll, as verified in Figure 4A.

The decrease of the instantaneous water use efficiency - $WiUE$ (Figure 4D) was associated with the decrease of the $CO_2$ assimilation rate since the transpiration ($E$) was not altered by the increase in salinity, indicating that there was no alteration in the water loss.

With regard to the combinations of NK fertilization (Figure 5A), it is noted that the plants fertilized with the

C3 (130% N + 100% $K_2O$) and C4 combinations (160% N + 125% $K_2O$) resulted in a higher intercellular $CO_2$ concentration, whereas the lowest values were observed in the plants that received the C1 combination (70% N + 50% $K_2O$). Since this variable represents the accumulation of $CO_2$ in the intercellular spaces of the mesophyll that is not being used by photosynthesis (Silva et al., 2014), it is highlighted that this is associated with the higher doses of N and K applied in the C3 and C4 combinations, which became harmful to carbon fixation for the photosynthetic activity, probably due to the reduction of the enzymatic activity of the $CO_2$-fixing enzyme (rubisco), resulting in a higher intercellular $CO_2$ concentration (Figure 5A) and, consequently, in the reduction of photosynthesis (Alvarenga et al., 2019), whose effects can be observed through the $CO_2$ assimilation rate (Figure 5B).

The highest values of the $CO_2$ assimilation rate, instantaneous carboxylation efficiency, and instantaneous water-use efficiency (Figures 5B, 5C, and 5D) of the plants fertilized with the C1 combination (70% N + 50% $K_2O$), and significantly lower values when using the remaining combinations, indicate that high doses can damage the carboxylation efficiency and, consequently, the instantaneous water-use efficiency. The doses of N and K considered sufficient, such as those applied through the C1 combination, promoted the nutritional balance in the plants, with a positive impact on these variables.

This probably occurred due to the positive influence of adequate N and K doses on the activity of the rubisco enzyme, which performed a higher $CO_2$ fixation.
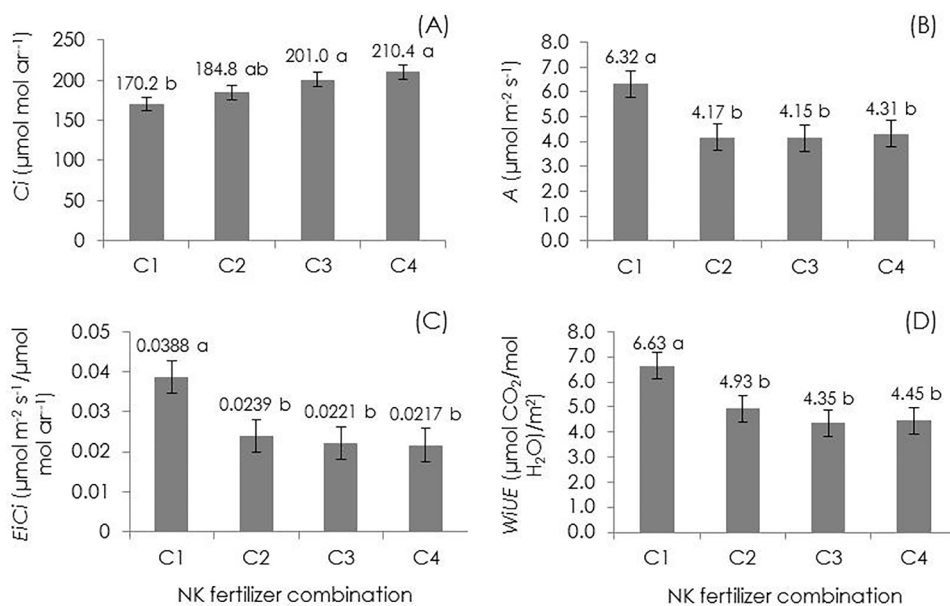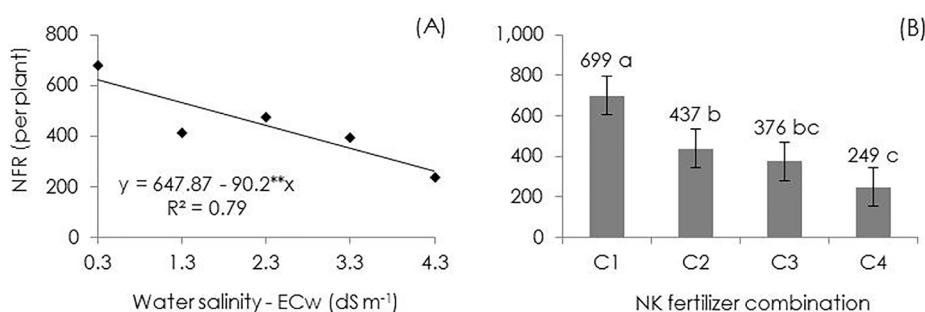


C1= 70% N + 50% $K_2O$; C2= 100% N + 75% $K_2O$; C3= 130% N + 100% $K_2O$ e C4 = 160% N + 125% $K_2O$ of recommendation.

**Figure 5.** $CO_2$ intercellular concentration – *Ci* (A), $CO_2$ assimilation rate – *A* (B), instantaneous efficiency carboxylation - *EiCi* (C) and instantaneous water use efficiency – *WiUE* (D) in West Indian cherry, as a function of different combinations of nitrogen and potassium fertilization at 540 days after transplanting. Means followed by different letters indicate that the treatments differ by Tukey test (p < 0.05). Vertical bars represent the standard error of the mean (n = 3).

The increase in the salinity of the irrigation water linearly reduced the number of fruits per plant by 13.92% for each unitary increment of water salinity (Figure 6A). This is related to the osmotic and toxic effect of salts, which minimized the water absorption, nutrient availability, and photosynthetic activity of plants, impacting negatively the emission of flowers and fruit survival rate (Lima et al., 2019b; Sá et al., 2019a).



C1= 70% N + 50% $K_2O$; C2= 100% N + 75% $K_2O$; C3= 130% N + 100% $K_2O$ e C4 = 160% N + 125% $K_2O$ of recommendation;

**Figure 6.** Number of fruits per plant - NFR of West Indian cherry of harvests carried out between 420 and 550 days after transplanting, as a function of the electrical conductivity of irrigation water (ECw) (A) and different combinations of nitrogen and potassium fertilization (B). Means followed by different letters indicate that the treatments differ by Tukey test (p < 0.05). Vertical bars represent the standard error of the mean (n = 3).

Highest number of fruits (699) were obtained in the plants fertilized with the C1 combination (70% N + 50% $K_2O$), occurring a gradual reduction with the fertilization with the C2 (100%N + 75%$K_2O$), C3 (130% N + 100% $K_2O$), and C4 combinations (160%N + 125%$K_2O$) (Figure 6B). The availability of N and K in the C1 combination probably promoted better conditions for the physiological and biochemical activities, reflecting on greater flower emission and fruit survival. With the increase in the doses of N and K applied through the other combinations, it is likely that soil pH alterations and the intensification of salt stress may have occurred due to the fertilizers applied in excess, resulting in an osmotic effect and nutritional imbalance, with a negative effect on fruit survival

(Marques et al., 2012; Oliveira et al., 2014).

In the follow-up effect of the salinity of the irrigation water within each combination of NK fertilization, it is verified (Figure 7A) that the increment in the ECw promoted a quadratic effect on production per plant (PROD) obtaining highest production (3.345 kg)
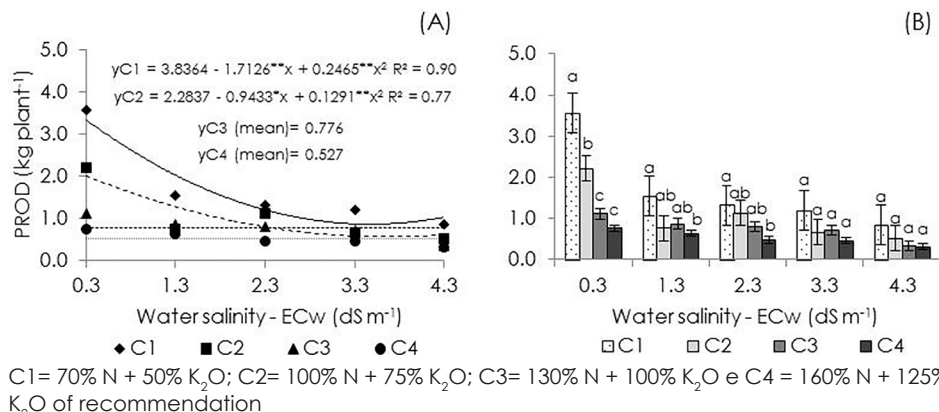
with the use of the C1 (70% N + 50% $K_2O$) and occurring a decrease until obtaining the lowest values (0.862 and 0.563 kg plant$^{-1}$) at the ECw levels of 3.5 and 3.7 dS m$^{-1}$, respectively, from which there was a small variation, with a stabilization trend of production per plant.



(A)

$yC1 = 3.8364 - 1.7126^{**}x + 0.2465^{**}x^2$  $R^2 = 0.90$
$yC2 = 2.2837 - 0.9433^{**}x + 0.1291^{**}x^2$  $R^2 = 0.77$
yC3 (mean)= 0.776
yC4 (mean)= 0.527

(B)

◆ C1   ■ C2   ▲ C3   ● C4

C1= 70% N + 50% $K_2O$; C2= 100% N + 75% $K_2O$; C3= 130% N + 100% $K_2O$ e C4 = 160% N + 125% $K_2O$ of recommendation

□ C1   □ C2   ■ C3   ■ C4

**Figure 7.** Follow-up effect of irrigation water salinity in each NK fertilization combination (A) and NK fertilization combinations within each salinity level (B) for production per plant - PROD of West Indian cherry, of harvests between 420 and 550 days after transplanting. Means followed by different letters indicate that the treatments differ by Tukey test (p < 0.05). Vertical bars represent the standard error of the mean (n = 3).

The reduction in the production of plants fertilized with the C1 and C2 combinations is related to the decrease of photochemical efficiency, $CO_2$ assimilation, reduction of the number of fruits per plant, and mass accumulation in these fruits, which were negatively affected by the osmotic and toxic effects of the salts in the irrigation water. On the other hand, the stabilization of production from the ECw level of 3.5 dS m$^{-1}$ may have resulted from the acclimatization mechanisms of the crop to salinity, as also verified by Alvarenga et al. (2019) on the growth of the West Indian cherry cv. Flor Branca irrigated until the water salinity level of 4.3 dS m$^{-1}$.

In the plants fertilized with the C3 (130% N + 100% $K_2O$) and C4 combinations (160% N + 125% $K_2O$), it was observed (Figure 7A) that the production per plant (PROD) was not affected by the increase of water salinity, obtaining means of 0.776 and 0.527 kg plant$^{-1}$, respectively. Possibly, the combinations became harmful at the lowest ECw levels of the irrigation water (0.3; 1.3, and 2.3 dS m$^{-1}$), causing production to be reduced and thus equated those of the plants irrigated with the highest saline levels (3.3 and 4.3 dS m$^{-1}$), considering that the application of high doses of N and K, using urea and KCl, can acidify the soil and cause an osmotic effect (Alvarenga et al., 2019).

In the unfolding of the combinations of NK fertilization, it was observed, within each level of water salinity (Figure 7B), that the fertilization with the C1

combination (70% N + 50% $K_2O$) promoted greater production (3.567 kg per plant) when irrigated with a ECw level of 0.3 dS m$^{-1}$, in relation to the remaining combinations. It is also verified that the plants irrigated with the ECw levels of 1.3 and 2.3 dS m$^{-1}$ and fertilized with the C1 combination also obtained higher values of PROD (1.542 and 1.323 kg plant$^{-1}$, respectively), although not differing statistically from the C2 and C3 combinations, whose values at the ECw of 1.3 dS m$^{-1}$ were 0.758 and 0.872 kg plant$^{-1}$, and 1.127 and 0.799 kg plant$^{-1}$ in the irrigation with ECw of 2.3 dS m$^{-1}$ (Figure 7B).

The higher production values for the plants fertilized with these combinations (especially C1) through the irrigation with ECw up to 2.3 dS m$^{-1}$ probably occurred due to the better nutritional balance in the plants, established by the doses of N and K applied in these combinations. These doses may have maintained better proportions of the $NO_3^-/Cl^-$ and $K^+/Na^+$ ratios in the leaf tissues (Andrade Júnior et al., 2012), promoting a greater synthesis of organic solutes capable of regulating the osmotic potential and mitigating the effect of salt stress (Sá et al., 2019a and 2019b; Lima et al., 2019b), with a probable effect on production.

In the plants irrigated with waters of 3.3 and 4.3 dS m$^{-1}$, it was verified that the use of different combinations of NK fertilization did not cause a significant effect on production per plant (PROD) (Figure 7B), although with a trend for higher values in the plants that received the C1

combination (70% N + 50% $K_2O$); however, it is highlighted that none of the combinations of N and $K_2O$ was able to mitigate the harmful effects of these salinity levels of the irrigation water on the production of West Indian cherry plants.

## Conclusions

The irrigation with water of electrical conductivity above 0.3 dS $m^{-1}$ reduced the contents of chlorophyll *a, b,* and total in the leaves, as well as the photochemical efficiency, $CO_2$ assimilation rate, instantaneous carboxylation efficiency, instantaneous water-use efficiency, and the number of fruits per plant, regardless of the fertilization combination employed.

The combination of 70% of N + 50% of $K_2O$ increased the photosynthetic pigments in the leaves, the photosynthesis parameters, and the number of fruits per plants; furthermore, it mitigated the deleterious effects of water salinity on the production per plant up to the electrical conductivity of 2.3 dS $m^{-1}$, becoming the most adequate combination for West Indian cherry fertilization between 420 and 550 days after transplanting.

## Acknowledgments

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