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Levels of nitrate, pigments and thermographic analysis of lettuce under different temperatures of nutrient solution

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The temperature of the nutrient solution in hydroponic crops of lettuce is a determinant of biomass yield and affects several physiological mechanisms of the plant. The aim of this study is to evaluate the influence of nutrient solution temperature on the levels of nitrate and pigments in leaves, as well as green mass yield and thermal flow in lettuce crop hydroponically. The experiment was conducted in a greenhouse at the Federal Technological University of Paraná. The nutrient solution was maintained at three temperatures (15 and 25°C and environmental temperature). The accumulated nitrate in leaf and photosynthetic pigment level were evaluated five times (28, 35, 42, 49 and 56 days after sowing). The total green mass yield and thermal flow behavior in the plants were assessed through thermographic analysis. The temperature of the solution influenced the levels of nitrate, chlorophyll and carotenoids in lettuce leaves, and green mass, which were larger at 25°C treatment. The root and stem had thermal equilibrium with the nutrient solution, while the temperature at the middle and upper part of the plant is similar to that of the environment.

Key words: *Lactuca sativa*, pigments, nitrate, heat conduction, green mass.

INTRODUCTION

Hydroponic is a production technique that allows the control of many variables. With the current available technologies, it is of great benefit to conduct research that enables the maximization of productivity in this cropping system.

Among the leafy vegetables that adapted more to this system, lettuce is mostly grown by farmers and consumed in Brazil because of its nutritional value, taste, and availability throughout the year (Geisenhoff et al., 2009). However, it is a very sensitive to adverse

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environmental conditions, as temperature of the nutrient solution is one of the factors that mostly influence the growth and development of plants.

Among the variables that can be affected by the temperature of the solution, there is the absorption of nutrients (Feltrim et al., 2009). Once the plant does not absorb excess water, the stomatal opening is not affected (Costa and Marenco, 2007). Thus, high transpiration flow carries more nutrients up to the leaves, mainly nitrogen (N) in nitrate form. With the satisfactory amounts of N accumulated in the leaves, the plant tends to produce more biomass and larger molecules formed by this element. Chlorophyll is one of the most present in the plant and most known. The amount of nitrate in the leaves must be accompanied often, because its residuality is an indicator of it being toxic to human health as a potential carcinogen (Luz et al., 2008).

Plants with high amount of heat in the leaves need transpiration to reduce their temperature (Taiz and Zeiger, 2009), and one of the tools that can be used in monitoring the changes associated with the pattern of leaf cooling model is thermal analysis, which is done remotely and instantly. Jones (1999b) suggests the potential use of thermographic images as estimation tools even for stomatal conductance. Thus, this work aims to evaluate the influence of the nutrient solution temperature on lettuce crop grown hydroponically.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the Federal Technological University of Paraná - UTFPR (25 ° 17'58.06 " S and 54 ° 06'52.28 " O, 417 m altitude), in the City of Medianeira –PR. The climate is Cfa, humid with hot summers, based on Koppen classification. The average annual temperature is 21°C and rainfall is 1,880 mm per year.

The lettuce crops were placed in nutrient solution using NFT (Nutrient Film Technique) method. A wooden bench of 1 m and inclination of 3% were used. Two types of cultivation channels were used: Nursery channels with polyethylene of 6 m long and 40 mm in diameter and definitive cultivation channels with polyethylene of 6 m long and 75 mm in diameter. Definitive channels were placed 15 cm apart from each other.

Three boxes of polyethylene water with a capacity of 250 L were used as the nutrient solution reservoirs. They were installed below the level of the bench, and semi buried in the ground. This allows the return of the nutrient solution per gravity, forming a closed system. The nutrient solution pumping from the reservoirs to the cultivation channels was conducted using three electric pumps with 41 W powers. It was drowned and individually actuated by means of a timer. The flow at the channel inlet was 1.25 L min^{-1}, which was controlled per 1/4 valve and monitored twice a day (7h and 9h) by means of graduated cylinder and chronometer. The separation of the irrigation system was needed to ensure control of the nutrient solution temperature.

The control of the nutrient solution temperature was carried out by means of digital thermostat coupled to a sounder, which measured the temperature at the entrance of the discharge pump. This thermostat controls the cooling system as heat varies from 1°C more or less per minute.

The heating system is composed of a resistance heater encapsulated in glass. The cooling system was formed by a compressor (outer part) and an evaporator coil with copper (internal part). The copper coil was in contact with the nutrient solution and to avoid contamination of the solution with copper, it was coated with a polytetrafluoroethylene layer.

A completely randomized design with three treatments and 10 replications were used in this work. All the treatments used the following temperatures: 15, 25 and ambient temperature (21.8°C). Each plot consisted of a cultivation channel containing ten plants, wherein the first orifice channel was used to observe the flow, and the two as its surrounding. Also, a cultivation channel was used on each side of the borders.

Pelleted seeds of the cultivar Vera were used. The sowing was done in phenolic foam cells of 1.9 × 1.9 × 2.0 cm; they were washed and accommodated at the bottom of a corrugated pan, which was maintained in dark storage and moistened with water only for 48 h. After germination, the seedlings were exposed to light in the greenhouse and received diluted nutrient solution three times a day with electrical conductivity of 700 µS cm^{-1}. At 14 days after sowing (DAS), the seedlings were transferred to the nurseries channels; initially, they received nutrient solution of 700 µS cm^{-1} and then it increased from 100 µS cm^{-1} each day to 1,400 µS cm^{-1}. During this period, the plants received nutrient solution in ambient temperature with circulation time or resting time of 15 min from 7 am to 19 h. At night, the nutrient solution was supplied for 15 min with intervals of 105 min rest. At 21 DAS, the more uniform plants were transferred to the definitive cultivation channels, where the temperature of the nutrient solution was controlled and the circulation time was similar to that of the nurseries channels for 56 DAS.

The nutrient solution used in the experiment was recommended by Furlani et al. (1999). Its chemical composition of macro and micronutrients is presented in Table 1. The level of the reservoir, electrical conductivity and pH were monitored twice a day (7 and 19 h). The reservoir level was maintained above 50% of its capacity, the pH was maintained between 5 and 7 and the electrical conductivity between 1,000 and 1,400 µS cm^{-1}.

Nitrate concentration, chlorophyll content and carotenoid content were analyzed at 28, 35, 42, 49 and 56 days after sowing (DAS). For the determination of nitrate levels, we used the colorimetric method described by Cataldo et al. (1975), from the nitration of salicylic acid and read in spectrophotometer at 410 nm. For the extraction and quantification of chlorophyll and carotenoids, the method described by Lichtenthaler and Welburn (1983) was used. 10 leaf discs per treatment (one disk per plant) were used. They were collected around the middle of plant, avoiding the ribs. At the end of the cultivation (56 DAS), the total green mass with precision scale was analyzed. Analyses were done in Food and Water Analysis Laboratory, Campus Medianeira of the Federal Technological University of Paraná, accredited by the Secretary of State for Agriculture and Supply of Paraná.

The thermal images were obtained at 55 DAS by means of a brand imager of Testo® model 881, whose reading occurred from 19 to 20 h with the irrigation maintained in continuous flow. The reading started 10 min at the start of the irrigation. After removing the plants from the cultivation channel, they were accommodated in a support to reduce the thermal interference of the medium and the reading was realized by positioning the imager at a distance of 1 m from the plant in less than 30 s. When this time was extrapolated the plant was returned to the channel and a new measurement was realized. Four points in the plant were used for thermal comparison: Root temperature (Tr), stem temperature (Ts) temperature of the middle leaf (Tm) and temperature at the end of the leaf (Tex). The temperature and relative humidity during the measurement were 25.5°C and 84%, respectively. Image processing was carried out by Testo IRSoft® 3.2 software.

The averages of the variables: Nitrate concentration, chlorophyll content, carotenoid content and green mass yield were compared using confidence intervals overlap ($p<0.05$), while the temperature

Table 1. Chemical composition of the nutrient solution recommended for leafy vegetables for Furlani et al. (1999).

Macronutrients	**N-NO_3^-**	**N-NH_4^+**	**P**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**S**
(g 1,000 L^{-1})	174	24	32.7	193	183	39.4	52
Micronutrients	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**Zn**	
(g 1,000 L^{-1})	0.3	0.004	3.6	0.3	0.08	0.11	

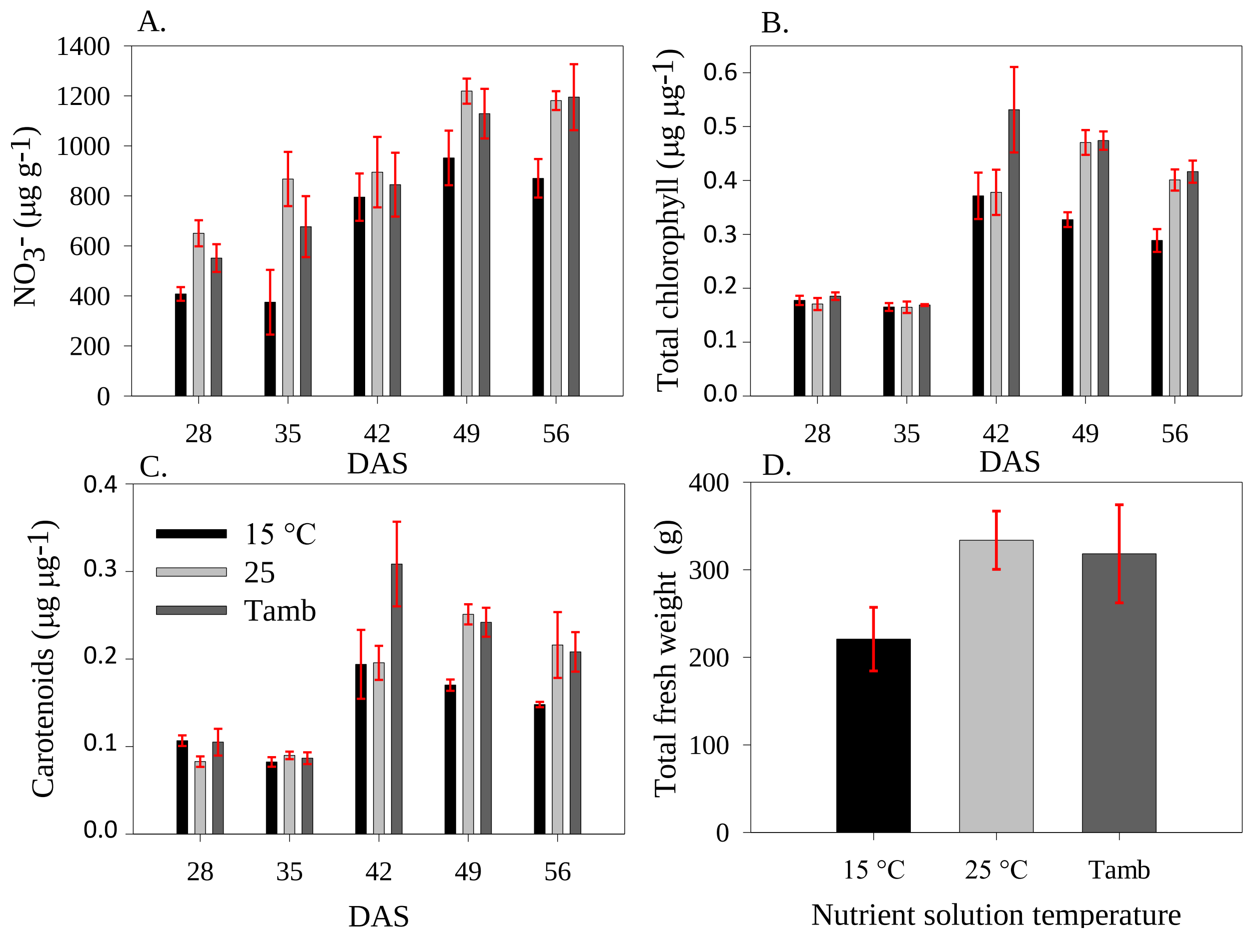

Figure 1. Nitrate levels (A), total chlorophyll (B), and carotenoids (C) leaves at 28, 35, 42, 49 and 56 DAS and total fresh weight (D) lettuce grown in nutrient solution temperature of 15 and 25°C and ambient temperature. The averages differ by no overlap of the bars of their respective confidence intervals ($p < 0.05$).

values measured in different parts of the plant in each treatment were submitted to Tukey test (5%).

RESULTS AND DISCUSSION

The nitrate content in lettuce leaves differed significantly between treatments at different temperatures of the nutrient solution during the cultivation. The maximum values ranged from 951.6 to 1,218.7 µg of NO_3^- per gram (g) of green mass leaf at 15 and 25°C, respectively (Figure 1A). Thus, plants irrigated with nutrient solution at 25°C accumulated, on average, 2 and 28% higher nitrate than plants with 15°C solution and ambient temperature (Tamb), respectively.

This behavior is due to the increase of nutrient solution temperature, which results in increased electrical conductivity thereof. A higher electrical conductivity favors increased nitrate content in lettuce leaves, because the conductivity of the nutritive solution does not only influence the absorption of water, but also the absorption of nutrients, both of which are closely linked (Steidle et al., 2005; Genuncio et al., 2012; Cometti et al., 2013).

Table 2. Test medium for the temperatures obtained in points of the plant and for the temperature of the nutrient solution [(1)].

Temperature of nutrient solution	Temperature at the measuring point in the plant (ºC)				
	Tr	Ts	Tm	Tex	CV (%)
15 °C	15.01[b]	14.85[b]	17.97[a]	17.96[a]	1.19
25 °C	24.96[a]	25.44[a]	23.26[b]	23.21[b]	3.89
T_{amb}	21.65[b]	22.09[b]	22.57[ab]	23.20[a]	6.12

Tr - Root temperature, Ts - Stem temperature, Tm - Temperature of the middle of the plant, Tex - Temperature of the extremity of the plant. [(1)] Means followed by different letters in the lines differ by Tukey test at 5% probability.

This association is due to the fact that as temperature increases in the leaf, the stomata of the lettuce tends to open more for the cooling process, transferring heat to the water and releasing it in vapor form, a process called transpiration. Thus, the higher the transpiration, the greater the cooling of the leaf; in other words, the higher the energy of the water in the leaf, the greater is the stomatal opening degree and time. As a result, the absorption and transport of nutrients to the shoot through transpirational flow occurs at higher rates, so that there is enhanced absorption in the root, transmission via the xylem and accumulation of nitrate in the leaves (Taiz and Zeiger, 2009).

The nitrate levels in leaf increase in the crop until it reaches its maximum in the last two collections. There is a tendency for these values to decrease when the plant is in its senescence period. Therefore, the values used for comparison with the limits tolerated for human consumption were the highest, which were observed at 49 DAS at 15 and 25°C and 56 DAS at Tamb. However, these values were lower than those recommended by the World Organization for Food and Agriculture (WHO, 2012) and the European Community legislation (CE, 2006), remaining within the standards allowed by standard-setting bodies and close to the values found in lettuce for several researchers (Escoín - Peña et al., 1998; Fernandes et al., 2002; Takahashi et al., 2007; Luz et al., 2008; Oshe et al., 2009; Aprigio et al., 2012).

Oshe et al. (2009) observed high nitrate content of 80.2 mg kg^{-1} in the shoot of the cultivar Vera, hydroponically without shading. However, Aprigio (2008) found 1,330 mg kg^{-1} and Takahashi et al. (2007) quantified 2,314 mg kg^{-1}.

Due to the variation in nitrate accumulation in the leaves at different temperatures of the nutrient solution, there were also significant changes in total chlorophyll content from the 49 DAS, when the plant was already well formed (Figure 1B). Considering the maximum levels of chlorophyll at 15 and 25°C treatments, there was 27% increase in the higher temperature treatment, a value which confirms the increase in nitrate concentration (28%).

As nitrogen is part of the constitution of chlorophyll molecules, there is direct relationship between the availability of N and the formation of chlorophyll in leaves (Taiz and Zeiger, 2009). Soratto et al. (2004) observed that the chlorophyll content is viable to indicate N deficiency in the plant. In some crops, the measurement of chlorophyll content through portable meters is a good alternative to indicate the amount of N to be applied (Argenta et al., 2011).

The content of carotenoids in the leaves, as well as chlorophyll, had significant change between treatments at 15 and 25°C from 49 DAS (Figure 1C). However, this difference is related to the need for heat loss by the plant, once carotenoids known as xanthophylls are responsible for heat dissipation. Since at 25°C treatment the water was already absorbed with a large amount of heat, it possibly might have reduced the cooling capacity for latent heat through transpiration. Thus, the plant could produce more carotenoids of this kind to convert the excess light energy absorbed into heat, preventing damage to the chloroplast photosynthetic apparatus, a process known as non-photochemical quenching (Taiz and Zeiger, 2009).

The green mass yield also increased significantly by increasing the temperature of the solution from 15 to 25°C, where the difference was equal to 51% in relation to treatment at 15°C. This could be due to the fact explained above, in which there was necessity for the plant stomata to have been opened longer for leaf cooling. Through the stomatal opening there is a control of CO_2 in the leaf, which directly affects photosynthesis (Liberato et al., 2006). With sufficient water and heat on the leaves, the stomata opening may have promoted greater absorption of CO_2, causing higher photosynthetic rates and consequently higher green mass yield.

Malorgio et al. (1990) observed an increase in the fresh weight of lettuce in NFT with temperature of 25°C in the area of the root system compared to lower temperatures.

The temperature readings realized in the several measuring points in the plant differed significantly according to Tukey test ($p<0.05$) for each treatment (Table 2). It is observed that in the treatment with 15°C the temperature tended to increase from the middle of the plant, while in the treatment with 25°C this effect occurred inversely.

In all treatments it was observed that the temperature of the root and stem is at equilibrium with the temperature of the nutrient solution. However, the temperature of the greenhouse and the plant tip tend to approach the

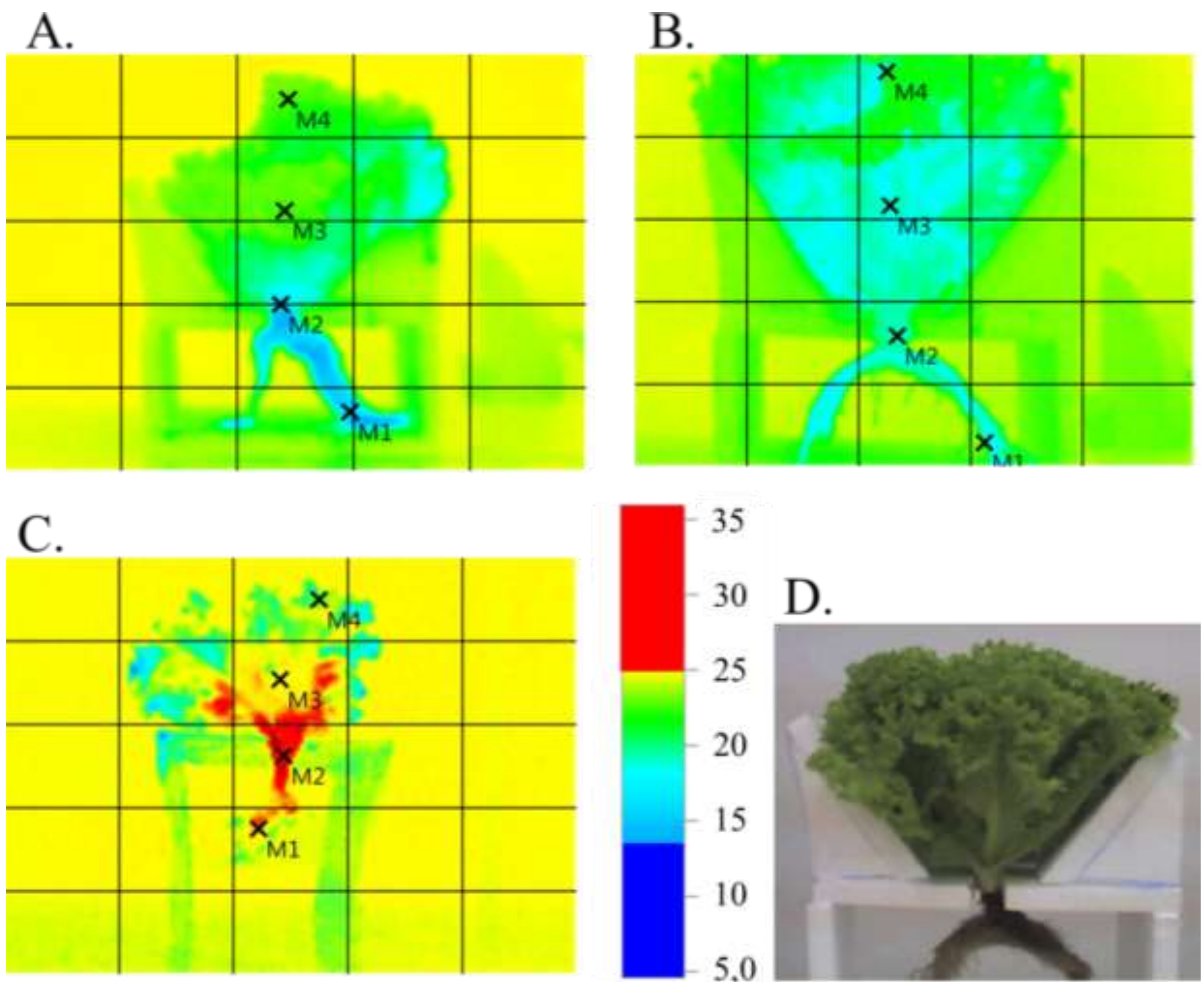

Figure 2. Thermographic images of lettuce plants submitted to nutrient solution at 15°C (A), ambient temperature (B) and 25°C (C), in detail for the accommodation of the plant for measuring the temperature in the several points (D); M1 - plant root , M2 - the plant stem, M3 - middle of the plant and M4 - extremity of the leaves

ambient temperature (Figure 2).

At 15°C treatment, the water of the solution was absorbed by the roots of low thermal level, but to be transported to the leaves, the temperature was increased. This is because it has received a great deal of latent heat derived from photosystems and cellular respiration (Figure 2A). Beyond that, as the leaf mass was higher than the root, the temperature at the upper part of the plant was increased by the need for thermal equilibrium solution-plant-atmosphere.

With the average thermal image of treatment with nutrient solution at ambient temperature, it is observed that the temperatures of the root, stem, middle of the plant and extremity of the leaves were maintained very close (Figure 2B). However, the plant temperature was below ambient temperature. This is due to the fact that for the plant to absorb water solution at ambient temperature, heat needs to be transferred to the water. It remained at equilibrium in its several parts. After losing latent and sensible heat to the environment, its temperature was lowered to absorb more water than this.

In thermography, average of the plant was irrigated with nutrient solution at 25°C. It is observed that temperatures of roots and stem were very close to the temperature of the nutritive solution (Figure 2C). The temperature of the middle of the plant, while achieving a smaller coverage area, was also close to the temperature of the nutrient solution, and the temperature at the end of the leaf had a tendency to become milder. In this case, the water was absorbed with a large amount of heat and reached the leaves. This heat may have been dissipated by the greater transpiration flow, making the leaf temperature lower than that of air. Furthermore, in this treatment there was a major yield of carotenoids, which may have contributed effectively to transfer the excess heat from the plant for the water and thereby reduce the leaf temperature (Taiz and Zeiger, 2009).

Thermography is an effective technique for monitoring the heat content in plants. So it can be used as an important tool for the temperature of the nutrient solution to be adjusted carefully to avoid heat stress on the plant and accumulation of nitrate above the maximum level recommended for human health.

Conclusion

1. Increasing the nutrient solution temperature promotes greater accumulation of nitrate and higher levels of chlorophyll and carotenoids on lettuce leaves, as well as increased green mass yield.
2. Independently, the thermal condition, the temperature of the root and stem remain at equilibrium with the temperature of the nutrient solution, while the temperature of the middle and end of the plant tends to approach ambient temperature.

Conflict of Interests

The authors have not declared any conflict of interests.

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