

Nitrate concentration in lettuce leaves depending on photosynthetic photon flux and nitrate concentration in the nutrient solution

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ABSTRACT

The nitrate accumulation in plant tissues can occur due to low light availability. However, published studies have not linked nitrate accumulation to photosynthetic photon flux (PPF) measured during the growing period. This study aimed to evaluate the effect of the reduction of photosynthetic photon flux and the concentration of nitrate in the nutrient solution on agronomic characteristics and accumulation of nitrate in lettuce grown in hydroponics. The trial design was entirely randomized in a factorial scheme (4 x 2) with three repetitions, and four shading levels: 0, 30, 50, and 80% and two nitrate levels in nutrient solution: 5 and 10 mmol L⁻¹. The dry matter production decreased directly and linearly with the reduction of light. The lowest level in nitrate solution also led to reduction in dry mass yield. The maximum accumulation of nitrate reached 966.3 mg kg⁻¹ fresh mass, with PPF of 118 μmol/m²/s and 140 mg L⁻¹ of N-NO₃⁻¹ (below the maximum levels recommended by the European Union), and the minimum of 200 mg kg⁻¹ with PPF of 455 μmol/m²/s and 70 mg L⁻¹ of N-NO₃⁻¹ in the nutrient solution. The reduction of nitrate in the nutrient solution from 140 to 70 mg L⁻¹ led to the reduction of nitrate accumulation in shoots, but also caused a decrease in the production of phytomass of shoots, as well as reduced leaf area significantly.

Keywords: *Lactuca sativa* L., hydroponic crop, shading, nitrate accumulation.

RESUMO

Teores de nitrato em alface sob fluxo de fótons fotossintéticos e concentração de nitrato na solução nutritiva

O acúmulo de nitrato nos tecidos vegetais pode ocorrer em função da baixa disponibilidade luminosa. Entretanto, trabalhos publicados não têm relacionado acúmulo de nitrato com o fluxo de fótons fotossintéticos (PPF) medidos durante o período de cultivo. Neste trabalho objetivou-se avaliar características agrônomicas e acúmulo de nitrato em plantas de alface cultivadas em hidroponia em função da redução no fluxo de fótons fotossintéticos e da concentração de nitrato na solução nutritiva. O delineamento experimental foi inteiramente ao acaso, em esquema fatorial 4 x 2, com três repetições, sendo quatro níveis de sombreamento: 0, 30, 50 e 80% e dois níveis de nitrato na solução: 5 e 10 mmol L⁻¹. A produção de massa seca caiu direta e linearmente com a redução da luminosidade. O menor nível de nitrato na solução também acarretou redução no rendimento de massa seca. A concentração máxima de NO₃⁻ observada alcançou 966,3 mg kg⁻¹ de massa fresca, com o PPF de 118 μmol/m²/s e 140 mg L⁻¹ de N-NO₃⁻¹ (abaixo dos níveis máximos preconizados pela União Européia) e a mínima, 200 mg kg⁻¹ com PPF de 455 μmol/m²/s e 70 mg L⁻¹ de N-NO₃⁻¹ na solução nutritiva. A redução do nitrato na solução nutritiva de 140 para 70 mg L⁻¹ levou à redução do acúmulo de nitrato na parte aérea, porém também promoveu a redução na produção da fitomassa da parte aérea, bem como a redução na área foliar de forma significativa.

Palavras-chave: *Lactuca sativa* L., cultivo hidropônico, sombreamento, acúmulo de nitrato.

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Lettuce (*Lactuca sativa*) is the most cultivated vegetable in Brazil. In hydroponics, lettuce is also the highest expression crop, both by the quantity and the quality that reaches compared to conventional tillage. This demonstrates the preference of the population in the consumption of lettuce in relation to other broadleaves (Cometti & Furlani, 2009a). Lettuce leaves are usually eaten raw and without any restriction to daily intake. However, lettuce has a great ability to accumulate nitrate in their leaves (Eysinga & Van Roorda, 1984), which can be harmful to human health

(Wright & Davison, 1964). Nitrate is regarded as a possible precursor of carcinogenic nitrosamines (Addiscott, 2006; Cometti & Furlani, 2009a). However, it is still not consensus, nor is it known precisely, if the intake of nitrate is beneficial or harmful to human health (Light *et al.*, 2008).

The concentrations of nitrate in commercial lettuce vary considerably. Nitrate levels ranging from 26 to 2568 mg kg⁻¹ fresh weight have been found. Plants grown in hydroponic systems showed higher levels compared to those grown in conventional systems

(Beninni *et al.*, 2002). Among the foods consumed by humans, plants represent between 72 and 94% of daily intake of nitrate (Santamaria, 1997). The acceptable nitrate content in lettuce for human consumption vary with the season and have not been stipulated by the Brazilian law, therefore European indexes have been adopted in Brazil. Thus, the European Union establishes the maximum permissible levels from 3,500 to 4,500 mg N-NO₃⁻ kg⁻¹ fresh weight for the winter season and 2,500 mg of N-NO₃⁻ kg⁻¹ for the summer crops (Europe, 2009). The accumulation of

nitrate in plant tissues occurs when there is an imbalance between the absorption and assimilation of this ion or ammonium, and surplus quantities are stored in the vacuoles to be assimilated later (Andriolo, 1999). The main factors that affect nitrate accumulation in plants are genetic, environmental, amount and proportion of N-carrier and the amount of molybdenum provided. Light intensity seems to be among the environmental factors that most influences nitrate accumulation in plants, because plants usually accumulate more nitrate when they are subjected to low light intensity (Ohse, 2000). This explains why nitrate levels in vegetables are higher in countries with low availability of radiation, such as Northern Europe. Despite the fact that Brazil's conditions of solar radiation apparently do not promote accumulation of nitrate in lettuce, samples of hydroponic lettuce with levels close to the limits by European laws have been reported by Benini *et al.* (2002) who found out samples reaching 2,568 mg kg⁻¹ fresh weight. Hydroponic crops are associated with the use of sun protection, usually done with agricultural plastic and shade screen. The study of Novo *et al.* (2008) correlates accumulation of nitrate with shading (percentage), using the unit % of shading. This unit, however, does not allow extrapolation to areas with different intensities of solar radiation. Thus, it is necessary to study the accumulation of nitrate as a function of photosynthetic photon flux. This is a variable that can be measured with simple equipment, enabling the extrapolation of the shade effect on the accumulation of nitrate to other locations.

The objective of this study was to evaluate agronomic characteristics and nitrate accumulation in lettuce grown in hydroponics as a function of photosynthetic photon flux and nitrate content in the nutrient solution.

MATERIAL AND METHODS

The experiment was carried out from September 23th to October 16th 2009 (early spring), in a greenhouse covered with transparent plastic film

and a thermal reflective screen inside (Aluminet[®]), at the Centro de Ciências Agrárias of Universidade Federal do Espírito Santo, located in Alegre, Espírito Santo state, Brazil (20°45'S, 41°32'W, altitude 271 m). The climate is Cwa (Köppen), characterized by a dry winter and rainy summer. Lettuce cultivar Vera was seeded in phenolic foam (cubes of 2x2x2 cm), irrigated with water until germination. From then, plants received nutrient solution (Furlani *et al.*, 1999) with EC= 1.59 mS m⁻¹ and 1.44 mS m⁻¹ for the nutrient solutions 1 and 2, respectively. Thinning was done at 21 days after sowing (DAS), leaving one plant per cell. Then the seedlings were transplanted to plastic pots containing three liters of nutrient solution.

The experimental design was entirely randomized in a factorial 2 x 4, with two nutrient nitrate concentration solutions (140 and 70 mg L⁻¹) and four shading levels (0, 30, 50, 80%), which average of photosynthetic photon flux (PPF)

were, respectively 455, 243, 146 and 118 mmol/m²/s (Table 1). There were three replicates and a pot per plot. Shading was carried out in small bamboo houses (0.8 m high, 0.7 m wide and 1.0 m in length), covered with polyethylene UV screens (Sombrite[®]) to reduce brightness in different shading treatments (30, 50 and 80%).

Solutions had the following nutrient concentrations in mg L⁻¹: the first with 140 N-NO₃⁻; 26.18 P-H₂PO₄⁻; 171.47 K⁺; 128.67 Ca⁺⁺; 17.46 Mg⁺⁺; 0.5 B; 0.05 Cu; 0.5 Mn; 0.05 Mo; 0.1 Zn; 24.37 S and 2 Fe; the second nutrient solution was the same except that contained 70 N-NO₃⁻. The sources of major nutrients were commercial fertilizers: potassium nitrate, magnesium sulfate and monopotassium phosphate (MKP). For the other nutrients, p.a. salts were used: 4H₂O calcium nitrate, calcium chloride, boric acid, 5.H₂O copper sulfate, manganese sulfate, anhydrous sodium molybdate, 7.H₂O zinc sulfate and Fe-EDTA (13%). The pH was

Table 1. Temperature of leaves and nutrient solution, and photosynthetic photon flux under shading treatments (temperatura de folha e da solução nutritiva, e fluxo de fótons fotossintéticos sob os tratamentos de sombreamento). Alegre, UFES, 2009.

[NO ₃ ⁻]	Hour	0	Shading (%)		
		(unshaded)	30	50	80
Leaf temperature (°C)					
140	09:00	26.8	27.0	27.9	27.7
140	12:00	27.5	27.7	27.5	29.5
140	15:00	26.4	26.9	27.3	28.5
70	09:00	26.2	26.7	27.0	28.0
70	12:00	27.2	27.8	29.2	29.7
70	15:00	26.5	27.0	28.0	28.7
Temperature of nutrient solution (°C)					
140	09:00	29.8	24.6	25.0	24.3
140	12:00	29.0	27.3	27.6	26.9
140	15:00	30.0	27.7	28.5	27.2
70	09:00	24.9	24.3	24.3	24.2
70	12:00	28.4	27.2	27.1	27.0
70	15:00	30.3	28.5	28.3	27.4
Photosynthetic photon flux (μmol/m²/s)					
	09:00	621	322	190	153
	12:00	529	285	175	146
	15:00	173	102	59	80
	Average*	455	243	146	118

*Average used as independent variable for regressions (valores médios utilizados como variável independente nas regressões).

adjusted to 5.5 with NaOH solution. The amount of solution was refilled daily and completely replaced every week. The solutions were calculated using a spreadsheet from Furlani & Cometti (2009b).

Leaf temperature was determined on the fifth leaf from the apex to the base, and nutrient solution temperature was measured using an infrared digital thermometer, model Ad Therm Kids brand HuBDIC. The photosynthetic photon flux was measured by a radiometer Quantum Meter, model SS-QMSW from Apogee Instruments Inc, USA, positioning the bar with 12 sensors toward the zenith over the plant canopy. Air temperature was determined using a mercury thermometer fixed inside the greenhouse. Leaf temperature, temperature of nutrient solution and air temperature (Table 1), and the PPF were measured every 2 days after transplanting seedlings to pots. The mean air temperature inside the greenhouse during trial was 30, 34, and 32°C at 9:00 am, 12:00, and 3:00 pm, respectively.

Plants were harvested 47 days after sowing, and divided into roots and shoots to determined: shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM) and root dry mass (RDM), plant height (PH), plant diameter, stem length (SL), root length (RL) and leaf area (LA) and leaf number (LN) of plants. Three leaves of lettuce from each plot were collected to determine the leaf area, being one of the apical third, one of the middle and another of the basal third. These leaves were weighed to obtain mass and scanned (with Multifunction Samsung SCX-4100®) to obtain the area of three sheets expressed in m². The total area of the plant was calculated proportional to the mass of the plant. Nitrate content in dry matter was determined after extraction with water (Cataldo *et al.*, 1975). The data were submitted to analysis of variance (SigmaStat, SPSS Science, Chicago, Ill.) and analyzed by regression (SigmaPlot, SPSS, Chicago, Ill), using the average measured photosynthetic photon flux (Table 1) as the independent variable for analysis of the shading treatments. T-test was used to compare the nitrate levels in

the nutrient solution by adopting a 5% probability level.

RESULTS AND DISCUSSION

There were no significant interactions between levels of shading and nitrate content in the nutrient solution for all variables.

The production of fresh and dry shoot mass and root (SFM, RFM, SDM, and RDM) increased with the photosynthetic photon flux (PPF). The elevation of the PPF 118 (with 80% shading) to 455 mol/m²/s (without shading) increased SFM from 39.6 to 152.7, SDM from 1.2 to 8.7, RFM from 6.0 to 21.8 and RDM from 0.4 to 1.6, g/plant, with 140 mg L⁻¹ of NO₃⁻. With 70 mg L⁻¹ of NO₃⁻,

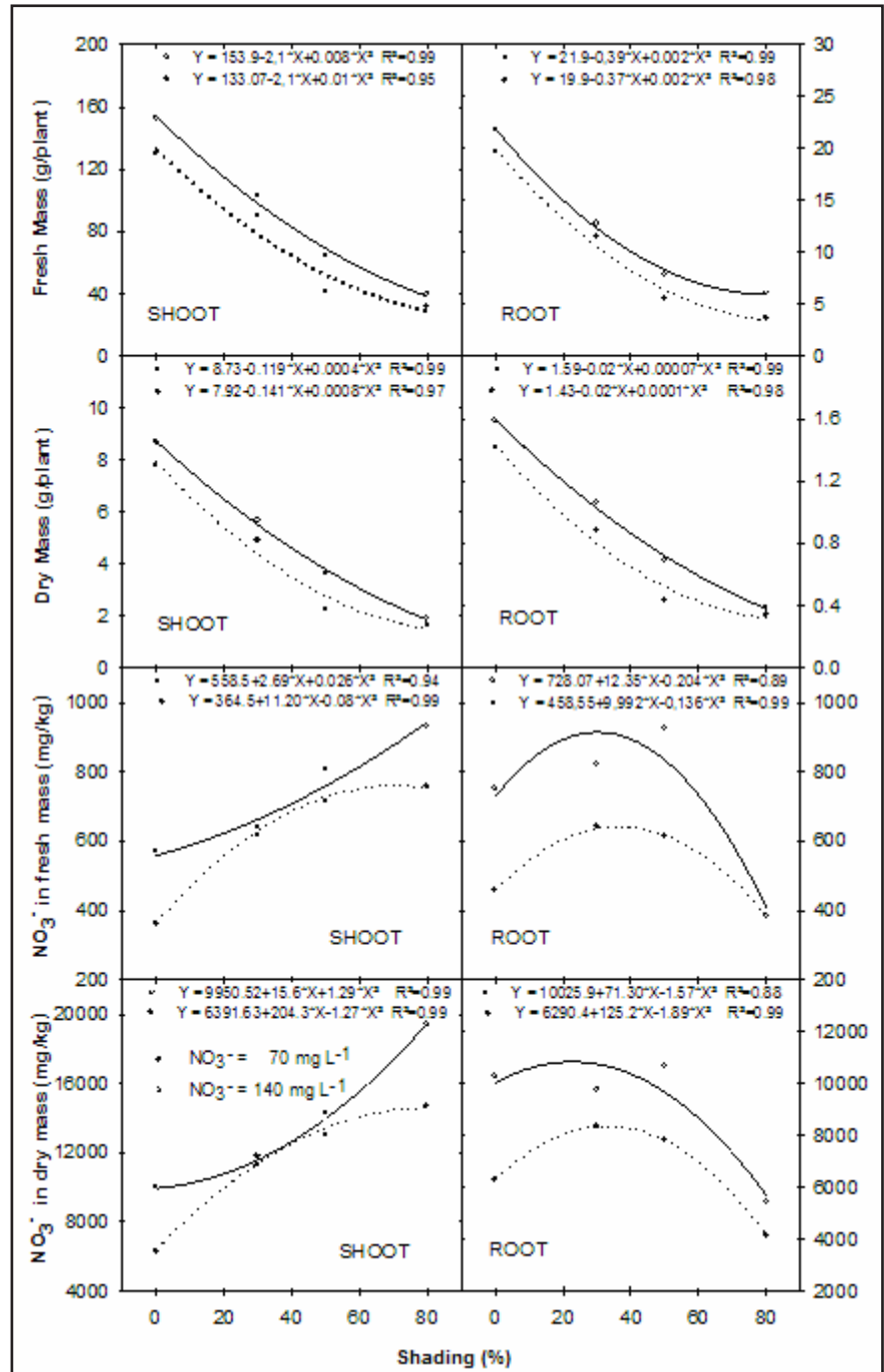


Figure 1. Production of phytomass and nitrate accumulation by lettuce in hydroponics at different levels of photosynthetic photon flux and N-NO₃⁻¹ in the nutrient solution (produção de fitomassa e acúmulo de nitrato na alface cultivada em hidroponia em diferentes níveis de fluxo de fótons fotossintéticos e N-NO₃⁻¹ na solução nutritiva). Alegre, UFES, 2009.

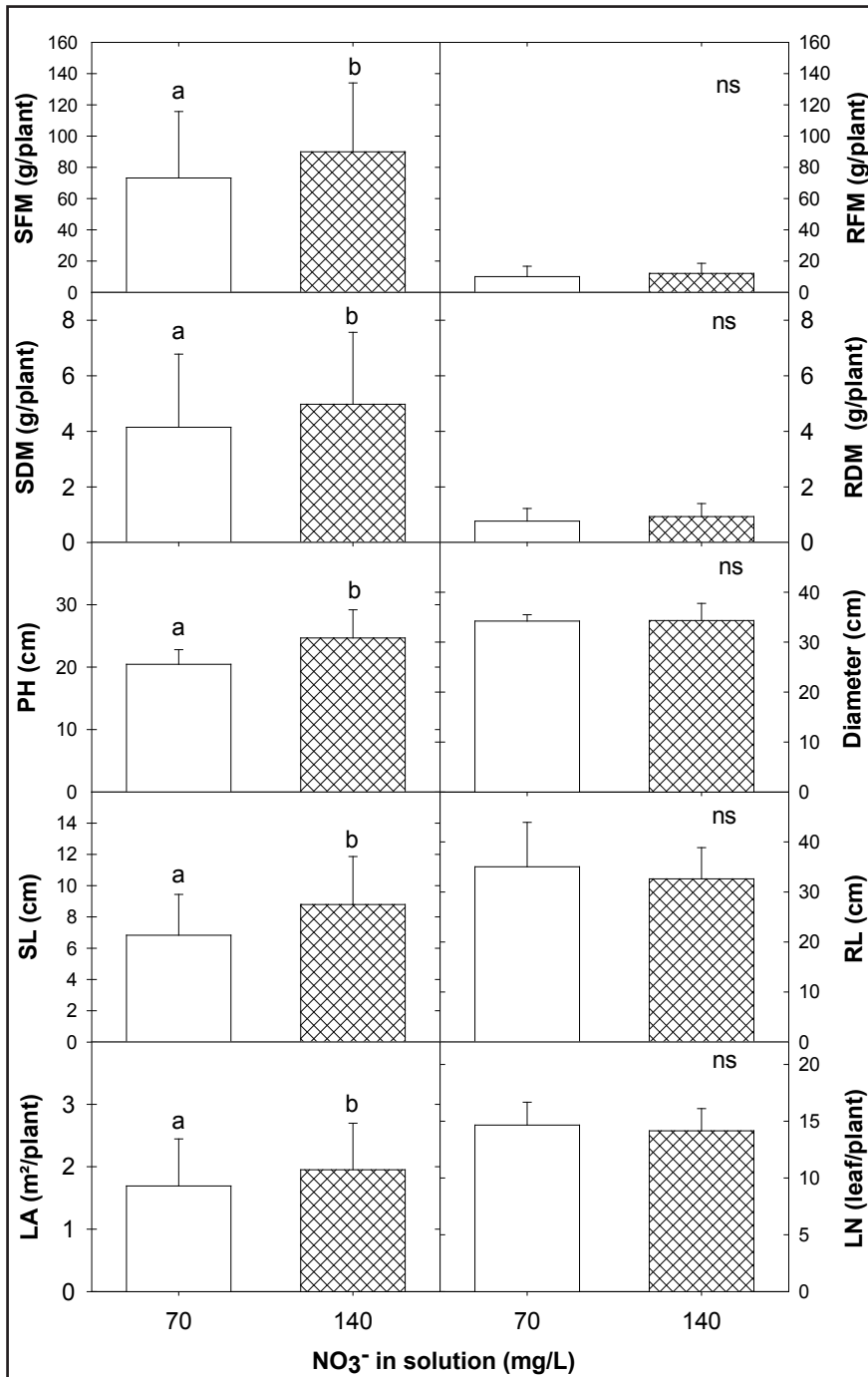


Figure 2. Morphological variables of hydroponic lettuce in function of the nitrate concentration in the nutrient solution: SFM (shoot fresh mass); RFM (root fresh mass); SDM (shoot dry mass); RDM (root dry mass); PH (plant height); SL (stem length); RL (root length); LA (leaf area); LN (number of leaves) (variáveis morfológicas da alface em cultivo hidropônico em função dos teores de nitrato na solução nutritiva. SFM (massa fresca da parte aérea); RFM (massa fresca da raiz); SDM (massa seca da parte aérea); RDM (massa seca da raiz); AP (altura da planta); SL (comprimento do caule); RL (comprimento de raiz); LA (área foliar); LN (número de folhas)). Alegre, UFES, 2009.

SFM raised from 31.9 to 130.2, SDM from 1.6 to 7.8, RFM of 3.7 to 19.7 and RDM from 0.3 to 1.4 g/plant, as shown in Figure 1. Most greenhouses used by hydroponic farmers present incidence

of solar radiation below the values observed in this experiment, since plastic films, over time, accumulate dirt and loose transparency, reducing the incidence of solar radiation inside

greenhouses, reducing productivity as a consequence. Similar results, with lettuce shoot dry matter yield of 10.14, 7.69 and 3.29 g/plant were observed in an experiment with shading at zero, 18 and 50% respectively (Novo *et al.*, 2008). There was also a reduction in biomass production of shoots of lettuce under protected environment when shading increased from 10 to 90%, falling from 225.7 to 81.0 g/plant (Byrne *et al.*, 2002). The increase in fresh mass production showed a quadratic fit with lower elevation of the fresh weight by raising the PPF, while the dry weight gain followed a linear increase (Figure 1), showing that the difference between the models indicates greater water loss in plants without shading (PPF= 455 mol/m²/s). This can be verified by the surface temperature of leaves in plants without shading (Table 1), whose plants had temperatures from 1 to 2°C lower than more shaded plants, due to higher transpiration caused by early stomatal opening of these plants throughout the day, in relation to the shaded plants. On the other hand, the increasing linear dry phytomass production demonstrates that the treatments were not light-saturated yet when in the unshaded treatments, besides the plastic greenhouse and thermal reflective screen. Even though lettuce is a C3 plant, the saturation point for canopies appears to be close to 1000 μmol/m²/s (Frantz *et al.*, 2004). This shows the importance of exchanging greenhouse plastic film and using screens with little shading, thus avoiding crop productivity. Nitrate concentration in the shoot (fresh and dry mass basis) decreased along with increasing PPF with 70 mg L⁻¹ of N-NO₃⁻ nutrient solution (Figure 1). With 140 mg L⁻¹ of N-NO₃⁻ solution, shoot nitrate content reduced with increasing levels of PPF. The inflection of the curve would indicate a minimum level and a slight increase tending to stabilize the accumulation of nitrate. The maximum NO₃⁻ concentrations in shoot fresh mass were 792.8 mg kg⁻¹ and 966.3 mg kg⁻¹ when the supplies were 70 and 140 mg L⁻¹ and with PPF of 118 μmol/m²/s. These levels are below those established by the European Union, whose maximum permissible

levels vary from 3,500 to 4,500 mg N-NO₃⁻ kg⁻¹ fresh mass for the winter season and 2,500 mg of N-NO₃⁻ kg⁻¹ fresh mass for summer crops (Europe, 2009). These values are about 50% of the maximum value of 1,700 mg kg⁻¹ fresh weight observed in the lettuce cvs. Grandes Lagos, Regina, and Baba de Verão, in an experiment conducted during the autumn in Viçosa, Minas Gerais state, Brazil (Fernandes *et al.*, 2002) and similar to those observed in nutrient solution with 100 mg L⁻¹ of N-NO₃⁻ for the lettuce cvs. Brisa, Divina, and Prima, of 940.0, 844.5, and 719.8, respectively (Lastra *et al.*, 2009). Novo *et al.* (2008) growing lettuce in hydroponics, observed that shading of only 18% caused a build-up of nitrate above 3,000 mg kg⁻¹, but for us it is difficult to compare these results since the authors did not provide data of radiation incident (PPF). Ohse *et al.* (2009) observed in the cultivar Vera, in hydroponics without shading, an average shoot nitrate content of 80.22 mg kg⁻¹, a value below the lowest value observed in this experiment. This shows that in tropical environment under high PPF or protected environment with little shading, there is no accumulation of nitrate at levels of risk to human health. However, Miyazawa *et al.* (2001) reported that 50% of the samples of hydroponic lettuce marketed in Curitiba, Paraná state, contained between 6,000 and 9,000 mg kg⁻¹ of nitrate in dry mass basis. This range of values, if simulated by the model showed in Figure 1, would be below 700 mg kg⁻¹ of nitrate in fresh mass basis. Therefore, for consumption purposes, we suggest that nitrate must be analyzed in tissue on a fresh mass basis, thereby avoiding mistakes in interpretation, since the nitrate works as *osmoticum* and would provide water accumulation in plant tissue (Huang *et al.*, 2010).

In roots, nitrate accumulation on both fresh and dry mass basis followed a quadratic model for the solutions with 70 and 140 mg L⁻¹ of N-NO₃⁻ (Figure 1). The increase of the PPF (455 μmol/m²/s) promoted the reduction of nitrate concentration in the roots, due to the greater availability of photosynthates for assimilation and reducing power

for nitrate reductase. However, when the plants were intensively shaded, with PPF= 118 μmol/m²/s, there was a marked decrease in nitrate concentration, indicating restrictions on nitrate uptake, causing depletion of nitrate in the root to keep its flow towards the shoot. The absorption of nitrate is dependent on light, which can change the ability of low-affinity carriers to absorb nitrate. This suggests an energy-dependent absorption process (Peuker & Jeschke, 1998), because the influx of nitrate to the roots is an active process, driven by the gradient of H⁺ (Forde, 2002). In contrast, above 118 μmol/m²/s, the accumulation of nitrate in the roots increases, but does not follow linearly the increase of PPF, a pattern opposite to that observed in the shoot. Thus, the root seems to have a buffering effect preventing the accumulation of nitrate in the shoot to levels harmful to plant growth. This effect has been suggested in rhizome of *Paspalum notatum* (Fernandes *et al.*, 1985), and stem of lettuce in hydroponics (Cometti *et al.*, 2004). This mechanism has been explained as a feedback regulation by products of the assimilation of N uptaken (Forde, 2002).

Lettuce shoot phytomass (SFM) was significantly higher in 140 mg L⁻¹ N-nitrate solution (Figure 2). Root fresh and dry mass (RFM and RDM), diameter of the plant, length of roots (RL) and number of leaves (LN) showed no significant differences between the treatments of nitrate in the nutrient solution. These results indicate that the reduction of nitrate in the nutrient solution may decrease lettuce canopy productivity. Therefore, concentrations lower than 140 mg L⁻¹ N-NO₃⁻ are not recommended. Despite the increased production of phytomass by plants grown in solution with 140 mg L⁻¹ of N-NO₃⁻, there was no effect in the number of leaves, showing that higher nitrogen was responsible for leaf expansion, as observed for LA. Lettuce cultivars grown in various concentrations of N in nutrient solution did not show difference for leaf number (Lastra *et al.*, 2009).

Nitrate levels in the nutrient solution had significant influence on nitrate accumulation in shoot and

roots, reaching the highest values with 140 mg L⁻¹ of nitrate (Figure 1). The NO₃⁻ dissolved in water facilitates the root uptake, which means there is an absorption of amounts above the plant's ability to reduce NO₃⁻ to NH₄⁺, thus accumulating the surplus in the plant tissue (Miyazawa *et al.*, 2001). Thus, larger amounts of NO₃⁻ in the nutrient solution cause greater root uptake. The low-affinity ion transporters involved in N uptake at concentrations above 1.0 mmol L⁻¹ (Peuker & Jeschke, 1998), gain importance in hydroponics, and thus the increase of 5 to 10 mmol L⁻¹ carried out in this trial may explain the difference in accumulation of nitrate in the shoot of lettuce. This mechanism of N-NO₃⁻ uptake is induced by external nitrate concentration and regulated by feedback by the products of N assimilation (Forde, 2002).

In summary, the reduction of light led to the increase of nitrate accumulation in the leaves, but even at low levels of photosynthetic photon flux, 118 mol μmol/m²/s, there was not nitrate accumulation above the European Union parameters. The maximum concentration of nitrate found in this study (966.3 mg kg⁻¹ fresh matter at PPF = 118 μmol/m²/s and 140 mg L⁻¹ de N-NO₃⁻ in nutrient solution) does not represent risk for human consumption of hydroponics lettuce in tropical environment conditions. Decreasing nitrate in the nutrient solution from 140 to 70 mg L⁻¹ led to reduction of nitrate accumulation on leaves, but also decreased shoot phytomass production, as well as leaf area.

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