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Nitrogen on growth and yield of lettuce plants grown under root confinement

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ABSTRACT

The objective of this research was to test the hypothesis that when lettuce plants grow under root confinement, development and yield is reduced and that such negative effects may be compensated by increasing nitrogen availability in the rooting media. The experiment was conducted between August 11st and September 23rd 2011. Lettuce transplants, cultivar Stella, bearing five leaves, were planted in pots using sand as substrate. Treatments were three root confinement levels and five N concentrations in the nutrient solution, in a 3x5 factorial randomized experimental design with four replications. Root confinements were simulated by pot sizes of 2.5 dm^3 (no confinement, the control); 1.0 dm^3 (moderate) and 0.4 dm^3 (severe). Nitrogen concentrations were: 5.55 (C1), 8.05 (C2), 10.55 (C3), 13.05 (C4) and 15.55 (C5) mmol/L. Interactions were observed between confinement levels and N concentrations. Plants grown under severe root confinement supplied by the nutrient solution at the concentration of 10.55 mmol/L of N reached leaf growth similar to those under no root confinement at 5.55 mmol/L of N. Increasing the N concentration in the nutrient solution enhanced shoot growth but decreased root growth. In lettuce plants grown under root confinement, plant growth is reduced and it is not compensated by increasing N fertilization rates. In both horticultural conventional or soilless production systems, managing practices for stimulate root growth has to be considered to maximize lettuce plant growth and nitrogen use efficiency.

Keywords: *Lactuca sativa*, fertilization, horticulture, mineral nutrition.

RESUMO

Nitrogênio no crescimento e produtividade de plantas de alface cultivadas sob confinamento radicular

A pesquisa teve por objetivo testar a hipótese de que sob condições de confinamento das raízes em um volume limitado, os efeitos negativos no crescimento e produtividade da planta de alface podem ser compensados aumentando a disponibilidade de N no meio radicular. O experimento foi realizado entre 11 de agosto e 23 de setembro de 2011. Mudas de alface da cultivar Stella com cinco folhas foram plantadas em vasos empregando areia como substrato. Os tratamentos foram constituídos por três níveis de confinamento do sistema radicular e cinco concentrações de nitrogênio na solução nutritiva, em esquema fatorial 3x5, em parcelas subdivididas, inteiramente casualizado com quatro repetições e 22 plantas por sub-parcela. O confinamento das raízes foi simulado usando tamanhos de vasos de 2,5 dm^3 (sem confinamento, controle); 1,0 dm^3 (moderado) e 0,4 dm^3 (severa). As concentrações de nitrogênio foram: 5,55 (C1), 8,05 (C2), 10,55 (C3), 13,05 (C4) e 15,55 (C5) mmol/L. Houve interação entre os dois fatores, de forma que plantas sob confinamento severo das raízes na concentração de 10,55 mmol/L de N apresentaram crescimento de folhas similar àquelas sem confinamento (controle) na dose de 5,55 mmol/L de N. O aumento da concentração de N favoreceu o crescimento da parte aérea da planta, porém, reduziu o crescimento das raízes. Em plantas de alface cultivadas em condições de confinamento radicular, o seu crescimento é reduzido e não é compensado pelo aumento da concentração de nitrogênio. Tanto no sistema de produção convencional ou fora do solo, práticas de manejo para estimular o crescimento de raízes devem ser consideradas para maximizar o crescimento das plantas de alface e a eficiência de uso do nitrogênio.

Palavras-chave: *Lactuca sativa*, adubação, horticultura, nutrição mineral.

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Raised beds made by rotary cultivators are the main soil preparing methods used for growing horticultural crops. Fertilizers and irrigation water are added in the upper layer, either at planting or during the cropping period. When polyethylene mulching is used, water and nutrients are applied by fertigation on the soil surface and percolation and salt lixiviation is reduced or avoided (Andrade Junior *et al*., 2005; Monteiro *et al*., 2008). When no-till soil planting methods are used, organic matter and fertilizers are retained on the soil surface (Bertol *et al*., 2011). In soilless systems, plants grow in restricted volumes of rooting media, like pots, bags or NFT-gullies. The main objective of soil preparing methods and soilless systems is to create favorable conditions for root growth, water absorption and nutrient uptake.

High fertilization rates have been currently used as a strategy to reach high yield and quality of horticultural crops. In Brazilian field grown lettuce crops, nitrogen rates have been applied between 100 and 130 kg/ha from mineral fertilizers plus 40 to 60 t/ha of farmyard manure (Filgueira, 2008; Trani *et al*., 2014). Six to eight cropping cycles may be reached during the year. The amount of total N supplied can reach 1040 kg/ha/year and estimated uptake quantities by the crop are about 165 to 320 kg/ha/year (Beninni *et al.*, 2005; Grangeiro *et al.*, 2006). In soilless systems like NFT, nutrient solutions at high nitrogen concentrations have been used in order to avoid nutrient deficiencies for crop growth and yield (Helber Junior *et al*., 2008; Silva *et al.*, 2008;) and they are discarded after the growing period. Nowadays, there has been an increased awareness about the negative effects of wasting fertilizers in the environment and such practice must be reviewed. In the Rio Grande do Sul and Santa Catarina States the nitrogen fertilization rates are based on the content of organic matter present in the soil, what permit greater control of fertilization (CQFS-RS, 2004).

It has been demonstrated in the literature that roots grow to search for water and nutrients by physical and/or physiological processes (Seginer *et al*., 2004; Le Bot *et al.*, 2009). Active root hairs on growing root tips are essential for nutrient uptake (Capon, 2010). The theoretical approach that has been used to explain the equilibrium between shoot and root growth is based on carbon and nitrogen concentrations in the plant (Seginer *et al.*, 2004). During growth and development, carbon concentration increases as a consequence of photosynthesis and N concentration decreases due to its assimilation. The lack of N increases the labile carbon concentration which is allocated to roots for growing new root hairs. The rate of N uptake is increased by new root hairs until a new equilibrium between C and N concentration is attained in the plant (Seginer *et al.*, 2004; Grechi *et al.*, 2007). When root growth is restricted, the number of root hairs may also be reduced and water absorption and nutrient uptake might be impaired. As a consequence, leaf area, shoot and root growth and crop yield might also be reduced (Costa *et al.*, 2012).

In natural ecosystems, plants are able to grow under low soil nutrient concentrations. It has been reported in the literature that soilless tomato plants can store N quantities higher than those required for growth and yield, suggesting that fertilizer rates supplied to this crop might be reduced (Le Bot *et al.*, 2001). Nevertheless, similar results were not reported for leafy vegetables during its vegetative growth phase. Plants growing in the soil are submitted to strong variations in water and nutrient availability which can also affect root growth and nutrient uptake more severely than in soilless conditions. Most of results reporting restrictions in root growth and its effects on shoot growth and plant development concern the production of transplants in small containers or tray cells in nurseries (Trani *et al*., 2004, Costa *et al.*, 2012). In horticultural crops during the post-transplant period, such results are scarce in the literature.

The main objective of this research was (i) to test the hypothesis that when lettuce plants grow under root confinement its growth, development and yield are reduced and (ii) such reduction may be compensated by increasing nitrogen availability in the rooting media.

MATERIAL AND METHODS

The experiment was carried out between August 11st and September 23rd 2011, inside a polyethylene greenhouse at Departamento de Fitotecnia, UFSM. The climate of this location is subtropical wet, Cfa formula according to the Köppen system. Average air temperatures and global solar radiation were, respectively, 15.4°C and 9.34 MJ/m^2/day in August, 17.3°C and 12.62 MJ/m^2/day in September, 19.9°C and 14.85 MJ/m^2/day in October.

Root confinement was simulated by three pot sizes with volumes of 2.5 dm^3 (no confinement, the control); 1.0 dm^3 (moderate) and 0.4 dm^3 (severe). Washed sand was used as rooting media in a closed soilless system (Godoi *et al*., 2009). Sand physical characteristics were 0.00-0.03 m gauge, 1.6 kg/dm^3 bulk density and 0.243 L/dm^3 maximum water retention capacity. Pots were placed over 0.80 m height benches and fertigated by drip irrigation five times a day for 15 min, at a flow rate of 1.3 L/h. Nutrient solutions were prepared in five 300 L fiberglass tanks and supplied to plants by a submersible pump and drip tubings.

Different nitrogen availability levels were supplied to plants by means of a nutrient solution. Total nitrogen concentrations were, in mmol/L, 5.55 (C1), 8.05 (C2), 10.55 (C3), 13.05 (C4) and 15.55 (C5). Concentrations of macronutrients were, in mmol/L, 6.0 K^+; 4.0 H_2PO_4; 2.0 Ca^{+2}, 1.0 Mg^{+2} and micronutrients, in mg/L, 0.03 Mo; 0.26 B; 0.06 Cu; 0.50 Mn; 0.22 Zn and 1.0 Fe. Fertilizers were potassium nitrate, calcium nitrate, ammonium nitrate, mono ammonium phosphate, magnesium sulphate, potassium sulphate.

The electrical conductivities (EC) were 0.92; 1.02; 1.12; 1.30 and 1.36 dS/m, respectively, and the pH was maintained in the range between 5.5 and 6.5, by addition of NaOH or H_2SO_4 1 mol/L aliquots whenever necessary. A fraction of nutrient solution prepared at concentrations indicated above was added when its volume inside the respective reservoir reached 50% of the initial volume. A factorial 3x5 split-plot randomized experimental design was used, nitrogen concentrations in main plots and root confinement in sub-plots, with four replications and twelve plants per sub-plot.

Planting was done on August 11st, using only one 5-leave commercial lettuce transplant, cultivar Stella, per pot. The experiment was ended on September 23rd 2011. Six plants per treatment were harvested, excluding the border ones, and shoot and roots were separated. Number of leaves, the diameter and length of stem and shoot fresh mass were determined at harvesting. Root and shoot dry mass were determined after drying in a ventilated oven at 65°C until constant mass was recorded. Root density was calculated by the ratio between root dry mass per united volume of substrate. Dry mass of all plant organs was finely ground and N concentration determined in the laboratory by the Kjeldahl method.

Data were submitted to variance analysis using the Statistica Software®, at 5% probability (F-test). The significance of differences among discrete variables means was determined by the Tukey's test at 5% probability and the quantitative ones by polynomial regression.

RESULTS AND DISCUSSION

Significant interactions for shoot growth and tissue nitrogen concentration were found. When the effect of N concentration in the nutrient solution and the confinement levels were compared, shoot dry mass decreased only on plants in severe confinement (Figure 1A). When the lowest and the highest N concentrations in the nutrient solution were compared on plants grown in severe and no confinement, the highest N concentration increased growth in both conditions, but it was not effective to compensate the growth reduction in severe confinement (Figure 2B). Plants under severe confinement for root growth and at the C3 concentration (10.55 mmol/L) reached similar growth ($p<0.5$) to plants under no confinement at the lowest N (C1= 5.55 mmol/L) concentration. Thus, at the lowest N concentration, similar growth of C3 plants was obtained by increasing 6.25 times the volume of pots (no confinement).

Differences were recorded in shoot growth variables of plants grown under severe and no confinement (Figures 2A and 2B). Root growth did not differ significantly among confinement levels but did among nitrogen concentrations in the nutrient solution (Figure 1B). Fresh mass of leaves, which is an important commercial characteristic of lettuce plants, was 27.6% reduced on plants under severe confinement. By

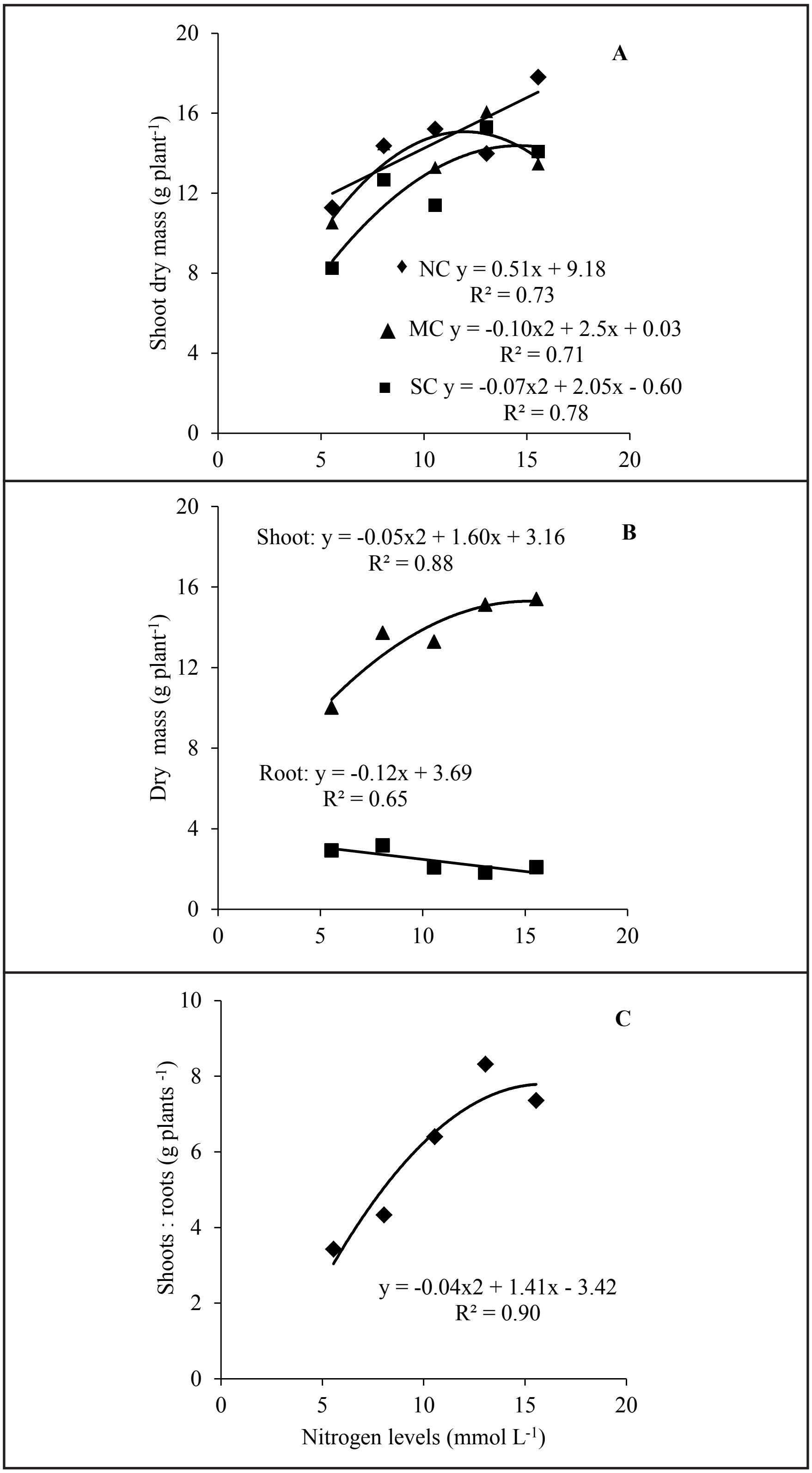

Figure 1. (A) Shoot dry mass of lettuce plants grown under no confinement (♦), moderate (▲) and severe (■) confinement of roots and nitrogen concentration from 5.55 to 15.55 mmol/L in the nutrient solution; (B) shoot (▲) and roots (■) dry mass, and (C) shoots:roots ratio of lettuce plants grown under nitrogen concentration from 5.55 to 15.55 mmol/L in the nutrient solution {(A) Massa seca da parte aérea de plantas de alface cultivadas sem (♦), moderado (▲) e severo (■) confinamento das raízes sob concentrações de nitrogênio de 5,55 a 15,55 mmol/L em solução nutritiva; (B) massa seca de parte aérea (▲) e raízes (■), e (C) relação parte aérea:raiz de plantas de alface cultivadas sob concentrações de nitrogênio de 5,55 a 15,55 mmol/L na solução nutritiva}. Santa Maria, UFSM, 2011.

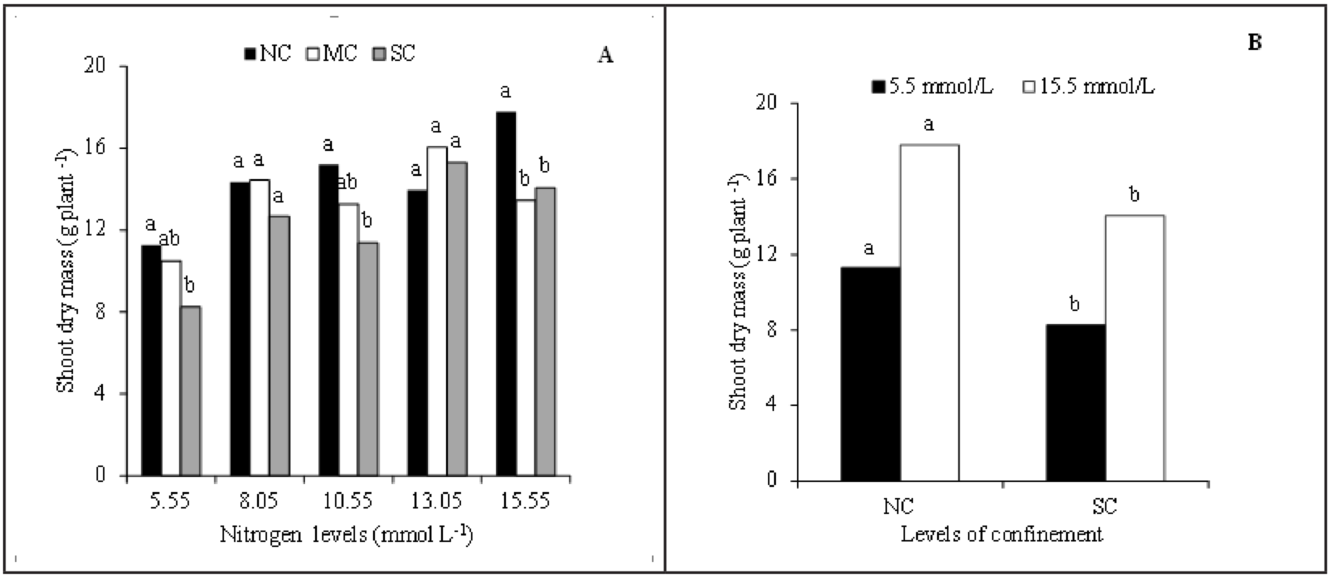

Figure 2. Shoot dry mass of lettuce plants grown under root no confinement (NC), moderate (MC) and severe (SC) root confinement and nitrogen concentrations from 5.55 to 15.55 mmol/L in the nutrient solution, figure (A). Shoots dry mass of the lettuce plants under no confinement (NC) and severe confinement (SC) of roots on low and high nitrogen availability in the nutrient solution, figure (B). [1]Columns followed by same letters in each concentration in figure A and in the same confinement in figure B, do not differ by Tukey's test at 5% of probability {massa seca parte aérea de plantas de alface sem (NC), moderado (MC) e severo (SC) confinamento de raízes sob concentrações de nitrogênio de 5,55 a 15,55 mmol/L na solução nutritiva, figura (A). Massa seca de parte aérea de plantas de alface cultivadas sem (NC) e com severo confinamento (SC) das raízes sob baixa e alta disponibilidade de nitrogênio na solução nutritiva, figura (B). ([1]colunas seguidas de mesma letra na concentração na figura A e no mesmo confinamento na Figura B, não diferem pelo teste de Tukey a 5% de probabilidade)}. Santa Maria, UFSM, 2011.

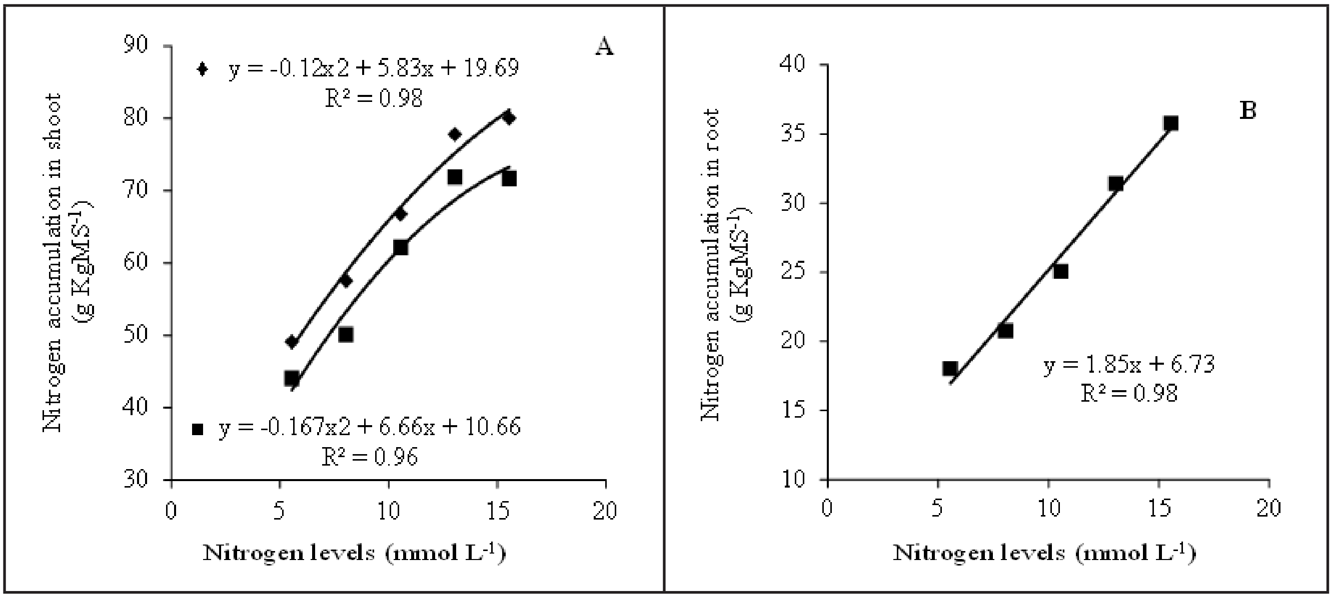

Figure 3. Nitrogen accumulation in shoots of lettuce plants under no confinement (♦) and severe confinement (■) and nitrogen concentration from 5.55 to 15.55 mmol/L in the nutrient solution, figure (A). Nitrogen accumulation in roots of lettuce plants and nitrogen concentration from 5.55 to 15.55 mmol/L in the nutrient solution, figure (B) {acúmulo de nitrogênio na parte aérea de plantas de alface sem confinamento (♦) e confinamento severo (■), sob concentração de nitrogênio de 5,55 a 15,55 mmol/L na solução nutritiva, figura (A). Acúmulo de nitrogênio nas raízes de plantas de alface sob concentração de nitrogênio de 5,55 a 15,55 mmol/L na solução nutritiva, figura (B)}. Santa Maria, UFSM, 2011.

effect of N concentration, shoot dry mass increased polinomially, while root dry mass decreased linearly (Figure 1B). Differences between C1 (5.55 mmol/L) and C5 (15.55 mmol/L) were 34% for leaves and 58% for the stem, while roots decreased 29%.

There were interactions among confinement levels and nitrogen concentration in the nutrient solution for the accumulation of nitrogen in shoot tissues. Higher accumulation was recorded in plants grown at the highest N concentration in the nutrient solution, but it was lower on severe confined plants (Figure 3A). In roots, any significant differences were recorded for this variable by effect of confinement levels, but it increased linearly by effect of N concentration in the nutrient solution (Figure 3B).

The shoot:root ratio was affected by N concentration in the nutrient solution, increasing 53% (Figure 1C). Results herein agree with the theoretical approach of shoot:root ratio in plants which state that N is the key element on root growth regulation (Seginer *et al.*, 2004). It has also been reported by Broadley *et al*. (2000) that hydroponicaly grown lettuce plants can use carbon assimilates to explore a greater volume of rooting media, minimizing the negative effects of low N availability on shoot growth. Their conclusions were supported by present results, because root growth was enhanced at the lowest N concentration in the nutrient solution while shoot growth was higher at the highest concentration (Figure 1B).

Root growth did not differ among confinement levels, but root density per plot did. It was 6.19, 2.45 and 0.93 g/dm^3 in severe, moderate and no confined plants, respectively. The higher the root density the lower will be the water retention capacity in the rooting media (Trani *et al*., 2004; Costa *et al.*, 2012). In small substrate or soil volumes porosity may be reduced by roots growing among particles, lowering the water retention capacity. In such condition, water availability to plants can shut down quickly during the diurnal phase of the day and growth be depressed by water stress.

As a consequence, it may not be discarded that reduction of growth on severe confined plants was due to diurnal variations in the water container capacity. It has been demonstrated in the literature that water absorption and nutrient uptake are independent plant processes (Barker & Pilbeam, 2007). While water is mainly absorbed as a consequence of the transpiration stream, nitrogen uptake is associated to carbon assimilation from photosynthesis and can be stored in the plant (Masclaux-Daubresse *et al.*, 2010) for further assimilation. The impact of diurnal variations in water availability would be stronger in the plant water flux. This hypothesis is corroborated by data of Figure 3A, showing that nitrogen accumulation increased in both severe and no confined plants. However, the same figure highlights that although root (Figure 3B) and shoot (Figure 3A) N accumulation was enhanced by its concentration in the nutrient solution, it was not able to compensate for the root confinement. It can be concluded that growth in confined plants was reduced by other plant physical and/or physiological processes than nutrient uptake.

Water absorption is a physical process depending mainly on solar radiation, air saturation deficit and salinity in the rooting media (Katsoulas & Kittas, 2011). Low growth of lettuce plants under salinity has been reported in the literature (Soares et al., 2007; Helber Junior et al., 2008). It is unlike that a salinity effect has been at the origin of variations in plant growth of the present experiment, because the EC of the nutrient solution was maintained between 0.92 dS/m (C1= 15.55 mmol/L) and 1.36 dS/m (C5= 5.55 mmol/L). It was within the 1.2-2.5 dS/m range reported in the literature for soilless cultivation of this crop (Helber Junior et al., 2008; Magalhães et al., 2010).

Agronomical implications can be drawn from present results. In lettuce plants grown under root confinement, plant growth is reduced and it is not compensated by increasing N fertilization rates. In both horticultural conventional or soilless production systems managing practices for stimulate root growth has to be considered to maximize lettuce plant growth and nitrogen use efficiency.

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