



Yield and quality of leafy lettuce in response to nutrient solution composition and growing season

Carlo Fallovo ¹, Youssef Rousphael ², Mariateresa Cardarelli ¹, Elvira Rea ³, Alberto Battistelli ⁴ and Giuseppe Colla ^{1*}

¹Dipartimento di Geologia e Ingegneria Meccanica, Naturalistica e Idraulica per il Territorio, Università della Tuscia, 01100 Viterbo, Italy. ²Department of Crop Production, Faculty of Agricultural and Veterinary Sciences, Lebanese University, Dekwaneh-Al Maten, Lebanon. ³CRA-Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo, Via della Navicella 2-4, 00184 Roma, Italy. ⁴CNR-Istituto di Biologia Agro-ambientale e Forestale, Via Guglielmo Marconi, 05010 Porano, Italy. *e-mail: giucolla@unitus.it

Received 9 January 2009, accepted 12 April 2009.

Abstract

The aim of this study was to investigate the effect of macro-anion (NO_3^- , H_2PO_4^- , and SO_4^{2-}) and macro-cation (K^+ , Ca^{2+} and Mg^{2+}) proportions in the nutrient solution during two consecutive growing seasons (spring and summer) on yield and leaf quality (chlorophyll content, color parameters, carbohydrates and mineral composition) of *Lactuca sativa* L. var. *acephala* grown in a floating system. Marketable yield, shoot biomass and leaf area index were unaffected by nutrient solution composition. A high proportion of calcium in the nutrient solution increased the quality attributes in particular calcium, chlorophyll, glucose and fructose concentrations. Plants grown in the spring season exhibited a lower yield, growth (total dry biomass and leaf area index), leaf mineral content (N, K and Mg), total carotenoids and water use efficiency than those grown in the summer season but were influenced positively by some quality parameters (higher content of glucose and fructose and lower nitrate content). The results demonstrated, that the effect of growing season on leafy lettuce performance (yield and quality) was more pronounced than the effect of nutrient solution composition.

Key words: Growing season, floating system, leafy lettuce, nutrient solution composition, nutritional quality.

Introduction

Baby leaf vegetables (i.e. rocket, lamb's lettuce, headless lettuce, endive, escarole, water cress) are the subject of increased consumption and are mostly requested for mixed salads, both as fresh market products and ready-to-use vegetables. The nutritional quality of vegetables can be affected by many pre- and post-harvest factors ¹. Fertilization is one of the most practical and effective pre-harvest ways to control and improve yield and nutritional quality of crops for human consumption. In this perspective, the soilless culture represents an important tool, because it permits a precise control of plant nutrition ²⁻⁷. Several authors ⁸⁻¹⁰ have shown a significant influence of cationic and anionic ratio on yield and quality attributes of several vegetable crops. For instance, Fanasca *et al.* ¹⁰ have reported that a high proportion of K in the nutrient solution increased the quality attributes (fruit dry matter, total soluble solids content) and the lycopene content of tomato fruit, whereas a high proportion of Ca improved tomato fruit yield and reduced the incidence of blossom-end rot (BER). Similarly, Trudel and Ozburn ¹¹ showed a 40% increase in lycopene concentration when potassium concentration in the nutrient solution has been increased from 0 to 8 mM, whereas a 26% depression of β -carotene concentration has been observed. Nutrient solution composition also affects crop growth and yield. Soundy *et al.* ⁹ demonstrated that at least 15 mg L⁻¹ P, supplied via floating irrigation to a peat + vermiculite mix, was required to

produce lettuce plantlets with the highest fresh and dry shoot biomass. Soundy and Cantliffe ¹² have reported that shoot growth of lettuce plantlets increased as N concentration increased from 0 to 60 mg L⁻¹ in a floating irrigation system. Moreover, fresh and dry shoot weight of lettuce plantlets grown in a floating system were unaffected by applied K from 15 to 60 mg L⁻¹ ¹³. However, the former studies focused on the influence of nutrients on biometric characteristics of head lettuce transplants while there is a lack of information on the influence of nutrient solution composition on yield and quality of leafy lettuce (*Lactuca sativa* L. var. *acephala*) that is considered a primary component of fresh-cut (minimally processed) vegetables and it is one of the most important horticultural crops grown in a floating (hydroponic) system in Italy.

An optimal nutrient solution composition for vegetable crops in closed fertigation systems also depends on the environmental conditions. Unfortunately, most recommendations for the fertilization of vegetables do not take into account environmental conditions. Therefore, different temperatures and solar radiation conditions may be good treatments variable to look at possible interactive effects of environmental conditions and ionic proportions in the nutrient solution on plant growth, yield and quality.

The objective of this work was to assess the effect of macro-anion

(NO₃⁻, H₂PO₄⁻ and SO₄²⁻) and macro-cation (K⁺, Ca²⁺ and Mg²⁺) proportions in the nutrient solution during two consecutive growing seasons (spring and summer) on yield and leaf quality (chlorophyll content, colour parameters, carbohydrates and mineral composition) of *Lactuca sativa* L. var. *acephala* grown in a floating system.

Materials and Methods

Location, experimental design and crop management: Two experiments were conducted in two consecutive growing seasons: spring season (Experiment 1) and summer season (Experiment 2) in a polyethylene 200 m² greenhouse situated at the experimental farm of Tuscia University, central Italy (latitude 42°25'N, longitude 12°08'E, altitude 310 m). Inside the greenhouse, ventilation was provided automatically when the air temperature exceeded 26°C, light was provided only by natural solar radiation. The following climate data inside the greenhouse was determined: dry and wet bulb air temperature by means of wire resistance thermometers in aspirated boxes, solar radiation by means of a pyranometer (CM11 Kipp and Zonen, Netherlands). All measurements were collected on a data logger system (CR10X, Campbell Scientific, Inc., UK), the sensors were scanned every minute and the 30 min average values were recorded.

Seeds of lettuce (*Lactuca sativa* L. var. *acephala* cv. 'Green Salad Bowl', SAIS seed company, Cesena, Italy) were sown on 24 March 2007 (Experiment 1) and on 31 May 2007 (Experiment 2) into a floating raft growing system, consisting of polystyrene plug trays floating in plastic tanks with a constant volume (65 L) of stagnant nutrient solution, which was continuously aerated with an air compressor in order to maintain the oxygen content above 6.0 mg L⁻¹. The planting density was 1857 plants m⁻², as used commercially for similar leafy vegetables in floating systems.

In both growing seasons (Experiments 1 and 2), a randomized complete-block design with three replicates was used to compare six nutrient solution having the same total concentration of

nutrients (40 mequiv L⁻¹) but different proportions among macro-anions (NO₃⁻, SO₄²⁻ and H₂PO₄⁻) and macro-cations (K⁺, Ca²⁺ and Mg²⁺). For both anionic and cationic groups, the nutrient formulations were defined by a high proportion of one nutrient and an equally low proportion of the others giving the same anion:cation ratio (1) (Fig. 1). In all anionic treatments the concentrations of K⁺, Ca²⁺ and Mg²⁺ (mequiv L⁻¹) were 10.20, 5.04 and 4.76, respectively. In all cationic treatments, the concentrations of NO₃⁻, SO₄²⁻ and H₂PO₄⁻ (mequiv L⁻¹) were 14.96, 4.02 and 1.02, respectively. Each experimental unit consisted of 0.147 m² (273 plants) container filled with 65 L of aerated nutrient solution. In all treatments, the micronutrient concentrations (μmequiv L⁻¹) were Fe, 40.0; Mn, 18.0; Cu, 3.0; Zn, 6.0; B, 60.0 and Mo, 1.8. The pH of the nutrient solution for all treatments was 6.0±0.5. Demineralized water was used in the preparation of all nutrient solutions.

Measurements and analysis: During both growing cycles, the water used by the crop was monitored in all treatments. The amount of water used was recorded by a flowmeter. Water use efficiency for harvested yield was calculated as the marketable fresh weight divided by the evapotranspiration losses. In Experiments 1 and 2, lettuce were harvested on 17 April and 21 June, respectively, at the same physiological age, expressed as the standard accumulation of growing-degree (base-temperature of 6°C; ceiling temperature of 30°C) days after sowing, which was in the range of 380-385 degree-days. Fifty plants per plot were separated into shoots to determine marketable fresh yield and roots, and their tissues were dried in a forced-air oven at 80°C for 72 h for biomass determination. Root to shoot ratio was calculated by dividing root dry weight by the shoot dry weight. Leaf areas (LA) were measured on 8 plants per treatment using an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). Leaf area index (LAI) was computed as the ratio of green leaf area divided by the ground area.

In both experiments, dried leaf tissues were ground separately in a Wiley mill to pass through a 20 mesh screen, then 0.5 g of the dried plant tissues were analyzed for the following macro and micronutrients: N, P, K, Ca, Mg, Fe, Cu, Mn and Zn. Nitrogen concentration in the plant tissues was determined after mineralization with sulfuric acid by "regular Kjeldahl method"¹⁴. P, K, Ca and Mg concentrations were determined by dry ashing at 400°C for 24 h, dissolving the ash in 1:25 HCl and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP Iris, Thermo Optek, Milano, Italy)¹⁵.

The color parameters L*, a* and b* [in the CIELAB-System: the L* component represents lightness; the a* component represents values from green (-) to red (+) and the b* component represents values from blue (-) to yellow (+)] were evaluated on leaves from marketable plants using a portable colorimeter (Minolta Chroma Meter CR-200, Minolta Camera Co. Ltd.,

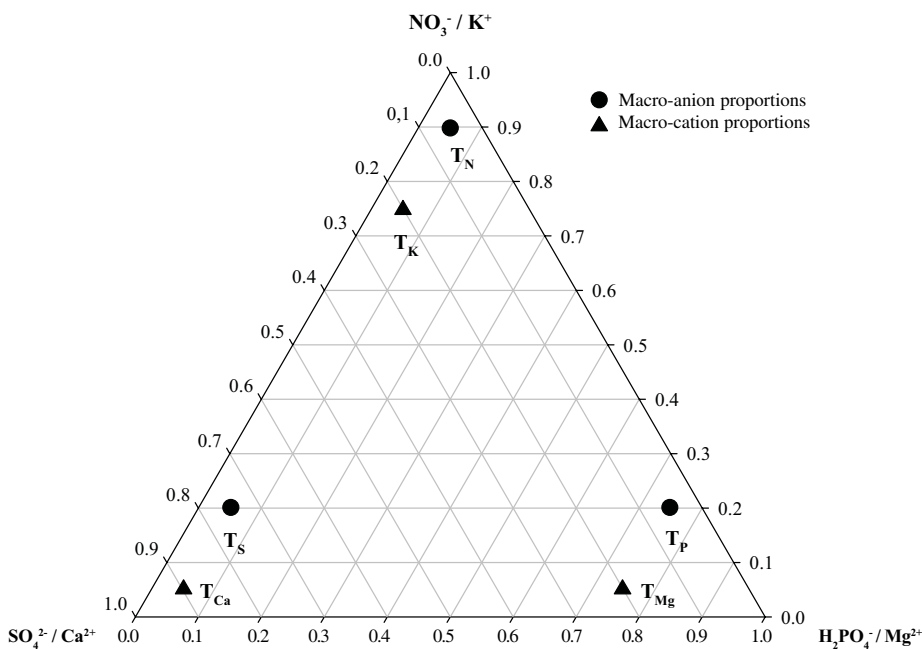


Figure 1. Nutrient proportions in the nutrient formulation treatments. T_N, T_P, T_S, T_K, T_{Ca}, T_{Mg} are nutrient formulations with increased proportion of NO₃⁻, H₂PO₄⁻, SO₄²⁻, K⁺, Ca²⁺, Mg²⁺, respectively.

Osaka, Japan). For the chlorophyll and carotenoids analyses, leaf discs were taken from 24 plants per replicate. The total chlorophyll and carotenoid contents were determined by UV-VIS spectrophotometry (Beckman DU-50 spectrophotometer; Beckman Instruments, Inc., Fullerton, CA). The absorbance of the solution was measured at 470, 647 and 664 nm. Formulae and extinction coefficients used for the determination of chlorophyllous pigments (total chlorophyll and carotenoids) were described by Lichtenhaler and Wellburn ¹⁶.

In both experiments, 16 lettuce plants per experimental unit were harvested, frozen and stored in liquid nitrogen for quality analysis. The frozen samples were ground to a fine powder with a mortar and pestle under liquid nitrogen. Spare powder was freeze-dried and used for measurements of soluble carbohydrates, starch, nitrate and protein contents.

Soluble carbohydrate (glucose, fructose and sucrose) and starch were determined by spectrophotometric coupled enzymatic assays as described by Jones *et al.* ¹⁷ including the modification of Antognozzi *et al.* ¹⁸. The nitrate concentration was measured with enzymatic assay ¹⁹, the assay was performed with a dual wavelength (340–400 nm) spectrophotometer (Thermo Spectronic-HELIOS, UK). Finally, quantification of proteins was performed according to the principle of protein-dye binding ²⁰.

Statistical analysis: All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Combined analysis of variance was performed using season as a fixed variable ²¹. Duncan's multiple range test was performed at $P = 0.05$ on each of the significant variables measured.

Results

Differences between the cropping seasons in daily solar radiation (R_g), mean air temperature (T_a) and relative humidity (RH) inside the greenhouse were observed. During the spring season, the daily R_g , T_a and RH ranged from 6.6 to 20.9 MJ m⁻², from 16.2 to 22.7°C and from 48 to 75%, respectively. Moreover, during the summer season, the daily R_g , T_a and RH ranged from 5.4 to 23.6 MJ m⁻², from 19.2 to 29.6°C and from 48 to 82%, respectively.

Since no significant interaction was recorded between the growing season and the nutrient solution composition for growth,

yield and quality parameters, the main effects of growing season and nutrient solution composition were separately reported.

Marketable yield, total dry biomass, leaf area index (LAI) and the root-to-shoot ratio (R/S) of lettuce were highly influenced by the growing season but not by the nutrient solution composition (Table 1). When averaged over nutrient solution composition, the marketable yield, total dry biomass and LAI were significantly higher by 77, 33 and 44%, respectively, in the summer season in comparison to the spring season, while an opposite trend was observed for the R/S ratio with the highest values recorded in the spring season in comparison to the summer cropping season (Table 1).

Total chlorophyll, carotenoids, nitrate concentration and color parameters represented by a^* (greenness) and the b^* (yellowness) components were highly influenced by the growing season, whereas the effect of nutrient solution composition was less pronounced since only the total chlorophyll and the nitrate concentrations were affected by the ionic proportions in the nutrient solution (Table 2). Irrespective of nutrient solution composition, the total chlorophyll, total carotenoids and nitrate contents were significantly higher by 22, 60 and 19%, respectively, in the summer season in comparison with the spring season (Table 2), whereas an opposite trend was observed for the colour parameters, where the highest a^* and b^* values were recorded in the spring season, compared to those observed with the summer season (Table 2). Moreover, the highest total chlorophyll and nitrate concentrations were recorded on plants grown in nutrient solution with a high proportion of Ca (T_{Ca}) and N (T_N), respectively, whereas the lowest values were observed with T_P and T_{Mg} for total chlorophyll and with T_{Ca} for nitrate concentrations (Table 2).

No significant difference among treatments was observed for starch (avg. 0.8 mg g⁻¹ of fresh weight) and total protein concentrations (avg. 14.4 mg g⁻¹ of fresh weight). Sucrose was the predominant sugar in both seasons, while glucose and fructose were presented in lower quantities (Table 3). Concentrations of glucose, fructose, sucrose and total carbohydrates were highly influenced by the growing season. In the leafy lettuce harvested during the summer cropping season, the concentrations of glucose, fructose, sucrose and total carbohydrates were reduced by 16, 21,

Table 1. Main effects of growing season and nutrient solution composition on marketable yield, total dry biomass, leaf area index (LAI) and root-to-shoot ratio of lettuce plants.

Treatment	Marketable yield (kg m ⁻²)	Total dry biomass (g m ⁻²)	LAI	Root:shoot
Growing season				
Spring	2.2 b	160.8 b	7.8 b	0.23 a
Summer	3.9 a	214.5 a	11.2 a	0.16 b
Nutrient solution composition				
T_N	3.1	183.8	10.0	0.17
T_P	3.0	192.7	8.7	0.21
T_S	3.1	176.1	8.5	0.21
T_K	3.2	179.0	10.4	0.17
T_{Ca}	2.9	187.9	8.9	0.21
T_{Mg}	3.1	206.7	10.5	0.15
Significance				
Growing season (S)	***	***	***	**
Nutrient solution (N)	NS	NS	NS	NS
S x N	NS	NS	NS	NS

T_N , T_P , T_S , T_K , T_{Ca} , T_{Mg} are nutrient formulations with increased proportion of NO₃⁻, H₂PO₄⁻, SO₄²⁻, K⁺, Ca²⁺, Mg²⁺, respectively. NS, ** and *** non-significant or significant at $P \leq 0.01$ and 0.001, respectively. Means separated using Duncan's test at $P=0.05$.

Table 2. Main effects of growing season and nutrient solution composition on chlorophyll, carotenoids, nitrate contents and colour parameters of lettuce leaves.

Treatment	Total chlorophyll (mg kg ⁻¹ FW)	Total carotenoids (mg kg ⁻¹ FW)	Nitrate (mg kg ⁻¹ FW)	L*	a*	b*
Growing season						
Spring	558.9 b	99.5 b	1698 b	57.0	-13.6 b	41.0 a
Summer	679.3 a	159.6 a	2015 a	56.9	-16.4 a	39.5 b
Nutrient solution composition						
T _N	647.2 ab	128.2	2207 a	57.6	-14.9	40.9
T _P	553.1 b	115.1	1702 ab	57.4	-14.9	40.3
T _S	601.3 ab	153.2	1885 ab	55.9	-15.2	39.5
T _K	598.0 ab	126.2	2054 ab	57.2	-15.1	41.0
T _{Ca}	793.6 a	153.0	1592 b	56.8	-15.1	40.0
T _{Mg}	521.4 b	101.7	1699 ab	56.8	-14.9	40.0
Significance						
Growing season (S)	*	***	*	NS	***	**
Nutrient solution (N)	*	NS	*	NS	NS	NS
S x N	NS	NS	NS	NS	NS	NS

T_N, T_P, T_S, T_K, T_{Ca}, T_{Mg} are nutrient formulations with increased proportion of NO₃⁻, H₂PO₄⁻, SO₄²⁻, K⁺, Ca²⁺, Mg²⁺, respectively. NS, *, ** and *** non-significant or significant at P ≤ 0.05, 0.01 and 0.001, respectively. Means separated using Duncan's test at P=0.05.

Table 3. Main effects of growing season and nutrient solution composition on glucose, fructose, sucrose, starch, total carbohydrates and proteins of lettuce leaves.

Treatment	Glucose		Fructose		Sucrose		Starch		Total Carbohydrates		Proteins	
	(mg g ⁻¹ FW)											
Growing season												
Spring	3.1 a	2.5 bc	2.9 b	2.9 b	7.5	0.9	0.7	14.0	14.7	16.7 a	14.2	14.2
Summer	2.6 b	3.2 ab	3.1 ab	3.1 ab	7.7	0.8	0.9	14.9	14.6	12.6 b	14.6	14.6
Nutrient solution composition												
T _N	2.9 abc	2.9 abc	3.1 ab	3.1 ab	8.3	0.7	0.7	15.2	14.1	14.0	14.7	14.7
T _P	2.0 c	2.0 c	2.2 b	2.2 b	7.8	0.8	0.8	12.9	15.8	16.7	14.6	14.6
T _K	3.8 a	3.8 a	3.8 a	3.8 a	8.0	0.9	0.9	16.7	15.2	14.1	14.1	14.1
T _{Ca}	2.7 bc	2.7 bc	2.9 ab	2.9 ab	7.5	0.8	0.8	14.1	14.1	12.2	14.7	14.7
T _{Mg}												
Significance												
Growing season (S)	*	*	**	**	***	NS	NS	***	NS	***	NS	NS
Nutrient solution (N)	*	*	*	*	NS	NS	NS	NS	NS	NS	NS	NS
S x N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

T_N, T_P, T_S, T_K, T_{Ca}, T_{Mg} are nutrient formulations with increased proportion of NO₃⁻, H₂PO₄⁻, SO₄²⁻, K⁺, Ca²⁺, Mg²⁺, respectively. NS, *, ** and *** non-significant or significant at P ≤ 0.05, 0.01 and 0.001, respectively. Means separated using Duncan's test at P = 0.05.

Table 4. Main effects of growing season and nutrient solution composition on macronutrient composition of lettuce leaves.

Treatment	Macronutrients (g kg ⁻¹ DW)				
	N	P	K	Ca	Mg
Growing season					
Spring	46.2 b	5.5	58.6 b	8.9	4.8 b
Summer	48.0 a	5.9	71.1 a	9.4	5.5 a
Nutrient solution composition					
T _N	48.0 a	5.7	69.7 ab	9.0 b	4.7 b
T _P	46.7 b	6.1	65.1 abc	8.9 b	4.8 b
T _S	45.8 b	5.7	66.9 abc	9.2 b	4.5 b
T _K	47.3 ab	6.0	74.7 a	8.4 b	4.5 b
T _{Ca}	47.6 ab	5.4	53.7 c	11.3 a	5.3 b
T _{Mg}	47.3 ab	5.4	59.0 bc	8.0 b	7.0 a
Significance					
Growing season (S)	*	NS	**	NS	*
Nutrient solution (N)	*	NS	*	*	**
S x N	NS	NS	NS	NS	NS

T_N, T_P, T_S, T_K, T_{Ca}, T_{Mg} are nutrient formulations with increased proportion of NO₃⁻, H₂PO₄⁻, SO₄²⁻, K⁺, Ca²⁺, Mg²⁺, respectively. NS, * and ** non-significant or significant at $P \leq 0.05$ and 0.01, respectively. Means separated using Duncan's test at $P = 0.05$.

32 and 25%, respectively, compared to leafy lettuce picked during the spring season (Table 3). Moreover, irrespective of the growing season, the highest concentrations of glucose and fructose were recorded on leafy lettuce grown in nutrient solution with proportion of Ca (T_{Ca}).

The macroelements concentration of lettuce plants as a function of the growing season and nutrient solution composition are displayed in Table 4. The concentration of N, K and Mg were highly influenced by the growing season and nutrient solution concentration, whereas the Ca concentration was only affected by nutrient solution composition. Moreover, no significant difference among treatments was observed for P concentration (avg. 5.7 g kg⁻¹ dry weight). The highest concentration of N, K, Ca and Mg were recorded on plants with a high proportion of N, K, Ca and Mg, respectively (Table 4). Irrespective of the nutrient solution composition, the N, K and Mg concentrations in leafy lettuce recorded in the summer season were significantly higher by 4, 21 and 15%, respectively, than those harvested during the spring cropping season (Table 4).

Finally, at the end of the cultural cycle, the water uptake and the water use efficiency (WUE) was significantly ($P < 0.01$) affected by the growing season, but not by nutrient solution composition and nutrient solution x growing season interaction (data not shown). The water requirement of leafy lettuce in the summer season (93.0 L m⁻²) was significantly higher by 58% in comparison with those grown in the spring season (59.0 L m⁻²). To produce 1 g of lettuce dry biomass 18.2 L of nutrient solution was necessary in the summer season and 13.7 L in the spring season.

Discussion

In the current experiment, the nutrient solution composition did not significantly affect the marketable yield and the growth parameters of leafy lettuce. These results are consistent with the findings of Soundy *et al.*¹³ who observed that fresh and dry shoot weight, leaf area and relative growth rate of lettuce transplants using a flotation system were unaffected by applied K (15, 30, 45 or 60 g L⁻¹). Moreover, other researchers^{10,22} have reported a significant effect of nutrient proportion on yield and quality of chicory and tomato plants grown in soilless system. Explanations for this disagreement could be the variations between species in their sensitivity to ionic proportions; leafy lettuce is

characterized by a short growing cycle (25 and 22 days for spring and summer season, respectively) and consequently a low nutrient requirement leading to a scarce influence of nutrient proportions in the solution on marketable yield and growth.

The higher marketable yield of lettuce in the summer cropping season in comparison to the spring season was due to solar radiation conditions²³. The higher solar radiation due to the high level of natural light and long photoperiod was presumably responsible for the increased photosynthesis in the summer with respect to the spring season: the mean value of daily global radiation in the greenhouse was 19.1 MJ m⁻² in the summer versus 14.6 MJ m⁻² in the spring season. In line with the above findings, Roupheal and Colla⁵ reported a reduction of zucchini squash marketable yield grown hydroponically by 33% in the summer to fall compared to the spring to summer cropping seasons. Moreover, Marcellis²⁴ observed that shading cucumber plants grown in a glasshouse during an extended period reduced the yield by 60% in comparison to cucumber plants grown at 100% irradiance level.

In Europe, widespread evidence of nitrate accumulation in leafy vegetables led to the European Community developing limits on fresh nitrate concentration of lettuce²⁵. Both nitrate supply and light intensity are known to be critical factors in determining nitrate levels in leafy vegetables²⁵. In the present study, a high proportion of N in the nutrient solution caused a linear increase in nitrate contents, but the nitrate values in leaf tissues were never as high as the limit value of 4500 mg kg⁻¹ fresh weight imposed by the European Community. The high correlation between nitrogen application rates and crop nitrate is in close agreement with the results of several earlier studies with lettuce grown in soil under both field²⁶ and greenhouse²⁷ condition. Moreover, low light levels are associated with nitrate accumulation in some leafy vegetables. For example Chadjaa *et al.*²⁸ and Gaurdeau *et al.*²⁹ reported that a reduction in the light level was associated with reduced nitrate reductase activity and increased nitrate accumulation in lettuce and spinach. Nitrate is favoured as an osmoticum at low light levels, replacing the energy-expensive carbohydrate to maintain turgor pressure in lettuce^{30,31}. However, Cantliffe³² observed little effect of light above irradiance levels of about 450-900 μmol m⁻² s⁻¹ on spinach leaf nitrate concentration. Similarly, Parks *et al.*³³ showed that the shoot nitrate concentration of Swiss chard was primarily affected by nitrogen supply and not

by light level because the light conditions exceeded the critical level ($\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$) required to increase leaf nitrate. They also observed that higher growth and greater nitrate accumulation of Swiss chard occurred in the spring experiment, compared with the winter experiment, and this was influenced by a higher range of temperatures. Finally, Laurie and Stewart³⁴ showed that high temperatures stimulated growth and did not affect root activity of chickpea but reduced shoot nitrate reductase activity, potentially leading to nitrate accumulation. In the current study the higher nitrate concentration in the summer growing season could be associated to a higher range of temperatures. Average minimum to maximum temperatures of 19.3–29.6°C were obtained for the summer experiment compared with 16.2–22.7°C for the spring experiment.

From a nutritional point of view, a high proportion of calcium in the nutrient solution increased the quality attributes in particular calcium, glucose and fructose concentrations. Calcium plays an essential role in plant development and overall plant health because it is a structural component of cell wall and it is necessary for cell growth and division. In lettuce increasing of calcium in the leaf tissues can increase photosynthetic capacity and also chlorophyll synthesis^{35,36}. This might lead to an increasing of primary product of photosynthesis such as glucose and fructose. In leafy vegetables, these soluble sugars have an influence on taste. Lettuce can be an excellent dietary source of calcium and is a good alternative for individuals with a diet low in dairy products. Hence, increasing calcium, glucose and fructose contents in lettuce could further improve their nutritional benefits, which is most likely to be of value to consumers. Moreover, the effect of the growing season significantly affected the quality parameters of leafy lettuce. In comparison to the summer season, our results indicate that during the spring season an improvement of glucose, fructose, sucrose and total carbohydrates was observed. The above findings are in line with those reported on tomatoes by Islam and Khan³⁷, who observed a reduction of sugar concentration during the warm season due to a lower activity level of sucrolytic enzymes as compared with the cool season.

The N, K, Ca and Mg concentrations in the leafy lettuce were positively affected by using nutrient solutions with high proportion of N, K, Ca and Mg, respectively. Our results are in line with a previous study on butterhead lettuce, where the uptake of Ca and K was linearly related to the high proportion of Ca and K in the nutrient solution³⁸. In comparison to the spring season, our results indicate that during the summer season higher concentrations of K and Mg were observed, which is interesting from a nutritional point of view because fruits and vegetables usually contribute to 35 and 24%, respectively, of the total K and Mg to the dietary intake of humans³⁹. The highest macronutrient content (N, K and Mg) of leafy lettuce in summer vs. spring season was mainly related to the total plant biomass. The total plant uptake of N, K and Mg in greenhouses is usually enhanced by stronger natural radiation or supplemental light⁴.

Finally, the daily water use of leafy lettuce in the spring season was lower in comparison to the summer season due to the reduced evaporative demand of the environment (lower global radiation and air temperature). Our results are in line with those recorded by Roupael and Colla^{4,5}, who observed that the transpiration rates of hydroponically grown zucchini squash were positively correlated with solar radiation and temperature. Moreover, the

lower water use efficiency (WUE) observed in the summer in comparison to the spring season may also be due to the high radiation and temperature that may have reduced the rate of photosynthesis and increased respiratory losses⁴⁰. A previous study⁴¹ on greenhouse rose crop showed that WUE was inversely proportional to solar radiation and to vapor pressure deficit.

Conclusions

To summarize, we can conclude that marketable yield, shoot biomass and leaf area index were unaffected by nutrient solution composition. A high proportion of calcium in the nutrient solution increased the quality attributes, in particular calcium, chlorophyll, glucose and fructose concentrations. The results also indicate that the effect of growing season on yield and quality of leafy lettuce was significant and more pronounced than the effect of nutrient solution composition. Plants grown in the spring season exhibited a lower yield, growth (total dry biomass and leaf area index) and mineral composition (N, K, and Mg) than those grown in the summer season but offered the highest fruit quality (higher content of glucose and fructose and lower nitrate content).

References

- ¹Kader, A.A. 2008. Flavor quality of fruits and vegetables. *J. Sci. Food Agric.* **88**:1863-1868.
- ²Roupael, Y., Colla, G., Battistelli, A., Moscatello, S., Proietti, S. and Rea, E. 2004. Yield, water requirement, nutrient uptake and fruit quality of zucchini squash grown in soil and closed soilless culture. *J. Hort. Sci. Biotechnol.* **79**:423-430.
- ³Roupael, Y., Cardarelli, M., Rea, E., Battistelli, A. and Colla, G. 2006. Comparison of the subirrigation and drip-irrigation system for greenhouse zucchini squash production using saline and non-saline nutrient solutions. *Agric. Water Manage.* **82**:99-117.
- ⁴Roupael, Y., Cardarelli, M., Rea, E. and Colla, G. 2008. The influence of irrigation system and nutrient solution concentration on potted geranium production under various conditions of radiation and temperature. *Sci. Hort.* **118**:328-337.
- ⁵Roupael, Y. and Colla, G. 2005. Growth, yield, fruit quality and nutrient uptake of hydroponically cultivated zucchini squash as affected by irrigation systems and growing seasons. *Sci. Hort.* **105**:177-195.
- ⁶Roupael, Y. and Colla, G. 2005. Radiation and water use efficiencies of greenhouse zucchini squash in relation to different climate parameters. *Europ. J. Agron.* **23**:183-194.
- ⁷Roupael, Y. and Colla, G. 2009. The influence of drip-irrigation or subirrigation on zucchini squash grown in closed-loop substrate culture with high and low nutrient solution concentrations. *HortScience* **44** (In Press).
- ⁸Paiva, E.A.S., Sampaio, R.A. and Martinez, H.E.P. 1998. Composition and quality of tomato fruit cultivated in nutrient solutions containing different calcium concentrations. *J. Plant Nutr.* **21**:2653-2661.
- ⁹Soundy, P., Cantliffe, D.J., Hochmuth, G.J. and Stofella, P.J. 2001. Nutrient requirements for lettuce transplants using a floatation irrigation system. I. Phosphorus. *HortScience* **36**:1066-1070.
- ¹⁰Fanasca, S., Colla, G., Maiani G., Venneria, E., Roupael, Y., Azzini, E. and Saccardo F. 2006. Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition. *J. Agric. Food Chem.* **54**:4319-4325.
- ¹¹Trudel, M.J. and Ozbun, J.L. 1971. Influence of potassium on carotenoids content of tomato fruit. *J. Amer. Soc. Hortic. Sci.* **96**:763-765.
- ¹²Soundy, P. and Cantliffe, D.J. 2001. Improving lettuce transplant quality in response to nitrogen nutrition in a floating production system. *Proc. Fla. State Hort. Soc.* **114**:294-303.
- ¹³Soundy, P., Cantliffe, D.J., Hochmuth, G.J. and Stofella, P.J. 2001. Nutrient requirements for lettuce transplants using a floatation irrigation

- system II. Potassium. *HortScience* **36**:1015-1170.
- ¹⁴Bremner, J.M. 1965. Total nitrogen. In Black, C.A., Evans, D.D., White, I.L., Ensminger, L.E. and Clark, F.E. (eds). *Methods of Soil Analysis*. Agron. Monograph 9 (Part 2), pp. 1149–1178.
- ¹⁵Karla, Y.P. 1998. *Handbook of Reference Methods for Plant Analysis*. CRC Press, Boca Raton, FL, pp. 165–170.
- ¹⁶Lichtenhaler, K.H. and Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 60 3rd Meeting Liverpool, 11, pp. 591–592.
- ¹⁷Jones, M.G.K., Outlaw, W.H. and Lowry, O.H. 1977. Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues. *Plant Physiol.* **60**:379–383.
- ¹⁸Antognozzi, E., Battistelli, A., Famiani, F., Moscatello, S., Stanica, F. and Tombesi, A. 1996. Influence of CPPU on carbohydrate accumulation and metabolism in fruits of *Actinidia deliciosa* (A. Chev.). *Sci. Hort.* **65**:37–47.
- ¹⁹Beutler, H.O., Wurst, B. and Fisher, S. 1986. Eine neue Methode zur enzymatischen Bestimmung von Nitrat in Lebensmitteln. *Dtsch. Lebensmitt. Rundsch.* **82**:283–289.
- ²⁰Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72**:248-254.
- ²¹Gomez, K.A. and Gomez, A.A. 1983. Comparison between treatments means. In Gomez, K. A. and Gomez, A. A. (eds). *Statistical Procedures for Agricultural Research*. 2nd edn. John Wiley & Sons, NY, USA, pp. 187-240.
- ²²De Rijck, G. and Schrevens, E. 1998. Multifactorial optimisation of the nutrient solution for hydroponically grown chicory plants. *Sci. Hort.* **76**:149-159.
- ²³Adams, P. 2002. Nutritional control in hydroponics. In Savvas, D. and Passam, H.C. (eds). *Hydroponic Production of Vegetables and Ornamentals*. Embryo Publications, Athens, Greece, pp. 211–261.
- ²⁴Marcelis, L.F.M. 1993. Fruit growth and biomass allocation to the fruits in cucumber effect of irradiance. *Sci. Hort.* **54**:123–130.
- ²⁵Santamaria, P. 2006. Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Sci. Food Agric.* **86**:10-17.
- ²⁶Sørensen, J.N., Johansen, A.S. and Poulsen, N. 1994. Influence of growth conditions on the value of crisphead lettuce. 1. Marketable and nutritional quality as affected by nitrogen supply, cultivar and plant age. *Plant Foods Human Nutr.* **46**:1-11.
- ²⁷McCall, D. and Willumsen, J. 1998. Effects of nitrate, ammonium and chloride application on the yield and nitrate content of soil-grown lettuce. *J. Hort. Sci. Biotechnol.* **73**:698-703.
- ²⁸Chadja, H., Vezina, L.P. and Gosselin, A. 1999. Effect of supplementary lighting on growth and primary nitrogen metabolism of greenhouse lamb's lettuce and spinach. *Can. J. Plant Sci.* **79**:421–426.
- ²⁹Gaudreau, L., Charbonneau, J., Vezina, L.P. and Gosselin, A. 1995. Effects of photoperiod and photosynthetic photon flux on nitrate content and nitrate reductase activity in greenhouse-grown lettuce. *J. Plant Nutr.* **18**:437–453.
- ³⁰Blom-Zandstra, M. and Lampe, J.E.M. 1985. The role of nitrate in the osmoregulation of lettuce (*Lactuca sativa* L.) grown under different light intensities. *J. Exp. Bot.* **36**:1043-1052.
- ³¹Buwalda, F. and Warmenhoven, M. 1999. Growth-limiting phosphate nutrition suppresses nitrate accumulation in greenhouse lettuce. *J. Exp. Bot.* **50**:813–821.
- ³²Cantliffe, D.J. 1972. Nitrate accumulation in spinach grown under different light intensities. *J. Amer. Soc. Hort. Sci.* **97**:152-154.
- ³³Parks, S.E., Huett, D.O., Campbell, L.C. and Spohr, L.J. 2008 Nitrate and nitrite in Australian leafy vegetables. *Aust. J. Agric. Res.* **59**:632-638.
- ³⁴Laurie, S. and Stewart, G.R. 1993. Effects of nitrogen supply and high temperature on the growth and physiology of the chickpea. *Plant Cell Environ.* **16**: 609-621.
- ³⁵Reiss, C. and Beale, S.I. 1996. External calcium requirements for light induction of chlorophyll accumulation and its enhancement by red light and cytokinin pretreatments in excised etiolated cucumber cotyledon. *Planta* **196**:635-641.
- ³⁶Marschner, H. 1998. Function of mineral nutrients. In *Mineral Nutrition of Higher Plants*. Academic Press Inc., San Diego, CA, pp. 229-396.
- ³⁷Islam, M.S. and Khan, S. 2001. Seasonal fluctuations of carbohydrate accumulation and metabolism of three tomato (*Lycopersicon esculentum* Mill.) cultivars grown at seven sowing times. *J. Hort. Sci. Biotechnol.* **76**:764–770.
- ³⁸Voogt, W. 1988. K/Ca ratios with butterhead lettuce grown in recirculating water. In Proc. 7th Internat. Congr. Soilles Culture, Flevohof, ISOSC, Wageningen, pp. 469-481.
- ³⁹Levander, O.A. 1990. Fruit and vegetable contributions to dietary mineral intake in human health and disease. *HortScience* **25**:1486–1488.
- ⁴⁰Craufurd, P.Q., Wheeler, T.R., Ellis, R.H., Summerfield, R.J. and Williams, J.H. 1999. Effect of temperature on water-use efficiency, carbon isotope discrimination, and specific leaf area in Peanut. *Crop Sci.* **39**:136–142.
- ⁴¹Duchain, M.C., Baille, M. and Baille, A. 1995. Water use efficiency and nutrient consumption of a greenhouse rose crop grown in rockwool. *Acta Hort.* **408**:129–135.