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Nutrient content and accumulation in mini lettuce as a function of fertigation management strategies

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ABSTRACT

Determining the amount of nutrients accumulated by crops is an important tool for defining the order in which nutrients are taken up and required. However, the order and amount of accumulated nutrients vary depending on the genetic characteristics of the cultivars, climatic conditions, fertilization, soil and management systems. The aim of this study was to evaluate the influence of irrigation management strategies and doses of nitrogen (N) fertigation on the content and accumulation of macro and micronutrients and the N use efficiency in mini romaine lettuce “Astorga” cultivated in a protected environment, in two consecutive crop cycles. The treatments consisted of three irrigation strategies: continuous irrigation (Cont), intermittent irrigation, with three fractions a day, at 1-hour intervals (Int1); intermittent irrigation, with three fractions a day, at 4-hour intervals (Int2) and two nitrogen doses (100 and 130 kg/ha N), in daily fertigation. Intermittent irrigation management strategies (Int1 and Int2) increased the contents and accumulation of dry matter and N, P, K, Ca, Mg, S, B, Fe, Mn and Zn. The dose of 130 kg/ha N only increased the content and accumulation of P and Mn in the first and second cycles, respectively. In general, lettuce accumulated macronutrients in the following decreasing order: K>N>Ca>P>Mg>S, whereas micronutrients followed the order Fe>Mn>Zn>B>Cu. The treatments Int1, Int2 and 100 kg/ha N optimized the N use efficiency.

Keywords: *Lactuca sativa*, fertilizer use efficiency, intermittent irrigation, pulse irrigation, nitrogen.

RESUMO

Teor e acúmulo de nutrientes em mini alface em função de estratégias de manejo de fertirrigação

A determinação da quantidade de nutrientes acumulados pelas culturas é uma ferramenta importante para definir a ordem em que os nutrientes são absorvidos e exigidos. Essas informações variam em função das características genéticas das cultivares, condições climáticas, adubação, solo e sistemas de manejo. O objetivo deste estudo foi avaliar a influência de estratégias de manejo de irrigação e doses de nitrogênio (N) em fertirrigação sobre o teor e acúmulo de macro e micronutrientes e a eficiência de uso do N em mini alface romana “Astorga” cultivada em ambiente protegido, em dois ciclos consecutivos de cultivo. Os tratamentos constaram da aplicação de três estratégias de irrigação: irrigação contínua (Cont), irrigação intermitente, com 3 fracionamentos no dia, em intervalos de 1h (Int1); irrigação intermitente, com 3 fracionamentos no dia, em intervalos de 4h (Int2) e duas doses de N (100 e 130 kg/ha de N), aplicadas em fertirrigação diária. As estratégias de manejo de irrigação intermitente (Int1 e Int2) aumentaram os teores e acúmulos de matéria seca e dos nutrientes N, P, K, Ca, Mg, S, B, Fe, Mn e Zn. A dose de 130 kg/ha de N aumentou apenas o teor e acúmulo de P e Mn no primeiro e segundo ciclos, respectivamente. De modo geral a alface acumulou os macronutrientes na seguinte ordem decrescente: K>N>Ca>P>Mg>S, enquanto os micronutrientes seguiram a ordem Fe>Mn>Zn>B>Cu. Os tratamentos Int1, Int2 e 100 kg/ha de N otimizaram a eficiência de uso do N.

Palavras-chave: *Lactuca sativa*, eficiência de uso de fertilizante, irrigação intermitente, irrigação por pulso, nitrogênio.

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Lettuce (*Lactuca sativa*) is a leafy vegetable with high nutritional and commercial value, standing out as the most produced leafy vegetable marketed in Brazil and worldwide. Growth, yield and nutrient accumulation, as well as post-harvest quality are affected by many factors, such as the levels and methods fertilizers are applied and, especially, by irrigation management strategies used, since this vegetable is very sensitive to stress caused by the decrease in soil water availability.

Intermittent fertigation is a management of water and nutrients which can maximize the nutrient absorption efficiency and accumulation by lettuce. This strategy consists of applying water and splitted fertilizers (fertilization cycles) during the day: application period, rest period and another application period. This cycle is repeated until the fertigation level is fully applied to the crop. Some studies show that this fertigation strategy can optimize the plant development, absorption and nutrient accumulation in vegetables, such as lettuce (Xu *et al*., 2004), green pepper (Silber *et al*., 2005) and coriander (Menezes *et al*., 2020a,b).

Nitrogen (N) is the macronutrient lettuce plants absorb and accumulate in the greatest amount in the dry matter (Carvalho *et al*., 2018; Dalastra *et al*., 2020). This nutrient is essential for growing leafy vegetables, acting directly on photosynthesis, protein synthesis, enzymes and leaf chlorophyll, and when applied in appropriate amounts, it favors growth expressions, increases protein content, vitamins, accumulation of fresh and dry mass and the absorption and accumulation of other nutrients such as P, K, Ca, Fe, Mn and Zn (Awaad *et al*., 2016; El-Bassyouni, 2016; Zandvakili *et al*., 2019).

Determining the amount of nutrients accumulated by the crops is an important tool which can be used both to support fertilization programs and to define the order in which nutrients are absorbed and required by crops. However, the order and amount of the accumulated nutrients in dry matter of the plants vary among cultivars, weather conditions, soil, fertilizer and growing and management systems.

Intermittent management of daily fertigation increases the content and accumulation of macro and micronutrients and maximizes N use efficiency by lettuce, as it decreases the N dose to be applied. This study aimed to evaluate the influence of the strategies of irrigation management and N doses in fertigation on the accumulation content and of macro and micronutrients and N use efficiency on mini romaine lettuce grown in a protected environment.

MATERIAL AND METHODS

The experiments were carried out in two consecutive cycles in protected cultivation, at Faculdade de Ciências Agronômicas (FCA), Botucatu-SP, Brazil (22°51'03"S, 48°25'37"W, 786 m altitude). The first cycle (IC) was conducted from October 31 to December 5, 2019, and the second cycle (IIC) from December 13, 2019, to January 12, 2020.

The micrometeorological variables air temperature and relative humidity inside the greenhouse during the two cultivation cycles were monitored by a meteorological station. The readings were taken continuously every second and stored every 15 minutes in a Campbell Scientific datalogger CR10X.

The daily air temperature and relative humidity averages during the experiments were 21.57ºC and 78.47%, from 17.50 to 26.50°C and 67.69 to 94.04% in IC, respectively. In IIC the averages were 22.73°C and 81.26%, ranging from 19.62 to 24.81°C and 80.93 to 95.64%, respectively.

The experiments were carried out in a protected environment, simple arch type (metallic structure), 31 m long by 7 m wide, covered with low density polyethylene film, 150 μm thickness, 1.85 m height ceiling, 4.50 m high at the highest point. The sides, front and back were covered with 50%-shading black screens, measuring 1.85 m high.

The experimental design was in randomized blocks in a 3 x 2 factorial scheme, with four replicates, and treatments consisting of three daily irrigation management stategies: continuous irrigation (Cont: one single irrigation event); intermittent irrigation with three irrigations a day, at 1 h intervals, at 7, 8 and 9 am (Int1); and intermittent irrigation with three irrigations a day, at 4 h intervals, at 7 am, 11 am and 3 pm (Int2); associated with two nitrogen doses (100 and 130 kg/ha N) which corresponded to the minimum and maximum doses recommended by Raij *et al*. (1997). N topdressing was applied via daily fertigation according to irrigation management strategy. The irrigation started at 7 am and continued throughout the day, according to the treatments.

The experimental plots consisted of 2.0 m^2 seedbeds with three rows of twelve plants, totalizing 36 plants per plot. The useful plot consisted of ten plants in the central row.

The soil inside the greenhouse was classified as Dystroferric Red Nitosol sandy loam texture in 0-0.20-m layer (Santos *et al*., 2013). The soil was prepared using a rotary hoe and, according to the chemical analysis, liming was performed to raise base saturation (V) at 80% and correct acidity (Raij *et al*., 1997). Limestone was broadcast, at a rate of 1.60 t/ha and incorporated at a 0.20-m depth.

After a 30-day correction, soil presented the following chemical and physico-hydric characteristics in the 0-0.20 m layer: pH ($CaCl_2$) = 6.0; P (resin) = 50 mg/dm^3; base saturation (V) = 75%; organic matter = 19 g/dm^3; CTC= 76 mmol$_c$/dm^3; Ca^{2+}= 46 mmol$_c$/dm^3; Mg^{2+}= 10 mmol$_c$/dm^3; K^+= 1.8 mmol$_c$/dm^3; electrical conductivity = 362 μS/cm; Al^{3+}= 0.0 mmol$_c$/dm^3; S= 15 mg/dm^3; B= 0.31 mg/dm^3; Cu= 5.5 mg/dm^3; Fe= 19 mg/dm^3; Mn= 11.5 mg/dm^3; Zn= 7.0 mg/dm^3; and water-physical: sand = 634 g/kg; silt = 99 g/kg; clay =267 g/kg; soil density = 1.39 kg/dm^3; particle density = 1.98 g/cm^3; total porosity = 47%; field capacity humidity (θcc) = 0.1974 m^3/m^3; and moisture at the permanent wilting point (θpmp)= 0.1546 m^3/m^3.

Fertilizations using nitrogen (N), phosphorus (P) and potassium (K) were manually carried out in seedbeds, using the soil chemical analysis and recommendation proposed by Raij *et al*. (1997). In each of the two consecutive cycles, we applied, 7 days before seedling planting, 40, 300 and 100 kg/ha N, P_2O_5 and K_2O in urea sources (45% N), single superphosphate (18% P_2O_5) and potassium chloride (60% K_2O), respectively. Foundation nitrogen fertilization corresponded to rates of 40 kg/ha N, of the recommended dose. The seedbeds were built with the aid of a hoe, measuring 0.80 x 2.50 x 0.30 m wide, long and high, respectively, and spaced 0.50 m from each other.

We used pelleted mini green romaine lettuce seeds Astorga Rijk Zwaan, since this cultivar has become increasingly popular in the market. This is a new variety, adapted to protected cultivation, showing small size and can be grown throughout the year. The seeds were sown in 200-cell thin polystyrene plastic trays, using commercial substrate Plantmax Hortaliças™. The seedlings were kept in a protected environment and transplanted into seedbeds at 30 days after sowing, showing 3 to 5 definitive leaves, spacing 0.20 x 0.20 m between lines and plants, respectively.

N doses as topdressing were applied in urea form, via daily fertigation, according to irrigation management strategies. The doses were splitted in equivalent amounts throughout the growing cycles, totalizing 17 fertigations per cycle. Fertigations began at 10 days and finished at 26 days after transplanting (DAT), in both cultivation cycles, injecting solutions directly by the pumps.

A drip irrigation system was used. The system consisted of two centrifugal pumps (Eletroplas Model ICS-50AB), 1/2 hp power and 1.8 m^3/h flow rate. A water tank was installed for each pump (500 L) in order to dilute the nitrogen doses, a disc filter (120 mesh), a manometer to control pressure and three main lines of 25 mm nominal diameter (DN), with solenoid valves (NaanDanJain model S390-2W-4 24VAC) at the beginning of each main line to allow differentiation of irrigation management strategies.

The lateral lines of the irrigation system were polyethylene drip tubes NaanDanJain (AmnonDrip PC CNL) measuring 17 mm nominal diameter (DN), 2.5 m long, with self-compensating, non-draining integrated emitters, spaced 0.20 m, 2.0 L/h nominal flow rate, at an operating pressure of 1 bar, water use efficiency of 92.50%. A drip tube for two planting lines in each seedbed was used.

Irrigation was scheduled using a prototype controller, Microchip PIC 16F628a microcontroller, with an eight-digit numerical display to establish the exact operating times of each solenoid valve, coupled to a keyboard which established the operator interface with the microcontroller. Five relay drives were installed in the controller, establishing the microcontroller connections with the two pumps (a relay drive/pump) and the solenoid valves (one relay drive/two valves), activating a solenoid valve per pump synchronously.

The water for irrigation was from an artesian well and classified as C1S1, indicating no risks to salinization or soil sodification. Irrigation daily management was carried out using the reference evapotranspiration (ETo) outside the greenhouse, obtained by the Penman-Monteith method adapted by FAO (Allen *et al*., 2006). Daily data of ETo were collected in a meteorological station, of Faculdade de Ciências Agronômicas (FCA) in Botucatu-SP, located 100 meters from the greenhouse.

Crop coefficients (Kc) used were obtained by Bastos *et al*. (1996), considering 0.52 for phase I (1 – 10 DAT); 0.80 for phase II (11 – 25 DAT); and 0.92 for phase III (26 DAT – up to harvest). Irrigation strategies were adopted at 7 DAT in the two cycles, and finished at 35 DAT in the 1st cycle and at 30 DAT in the 2nd cycle. The water depths applied after transplantation were 96.28 and 86.08 mm in IC and IIC, respectively.

Plants were harvested at 35 and 30 DAT in IC and IIC, respectively, considering as harvest point the commercial standard and the maximum vegetative development, no signs of flowering. Three plants of each plot were collected and dried in an oven at 65ºC, with forced air circulations until reaching constant mass in order to obtain accumulation of the shoot dry matter mass (AMMS). The dried samples were weighed with the aid of a 0.01 g digital scale.

The dried samples were taken to the Laboratório de Solos e Recursos Naturais of Faculdade de Ciências Agronômicas (UNESP-FCA) and the macronutrient contents were determined: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), and micronutrients: boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). N content was determined by Kjeldahl method, P and B through colorimetric method, K by flame photometry and Ca, Mg, S, Cu, Fe, Mn and Zn through atomic absorption spectrophotometry.

Using these chemical analyses, the authors determined N, P, K, Ca, Mg and S contents in g/kg of dry mass (MS), and B, Cu, Fe, Mn and Zn contents in mg/kg of MS. The nutrient accumulation in shoot part of the plants was calculated by multiplying the content of each nutrient by the AMMS of the samples. Nitrogen use efficiency (EUN) was calculated by the ratio: EUN (%) = (amount of N absorbed/amount of N applied) x 100 (Pereira *et al*., 2017).

Data were submitted to the variance analysis at 0.05 significance. When significant effect was noticed, qualitative analysis of average comparison by Tukey's test at 0.05 significance was performed. Statistical analyses were performed using SAS v. 9.4 software (SAS Institute, Cary, Carolina do NC, EUA).

RESULTS AND DISCUSSION

Intermittent irrigation strategies Int1 and Int2 provided significant increases in contents of N, P, Ca and Mg in IC. In IIC, these strategies also increased the contents of N, P, K and S (Table 1).

The highest contents of macronutrients observed using the intermittent irrigation strategies are related to a better soil water availability in the surface layer during the day, due to a decrease in soil water movement in the vertical direction, reducing water percolation rates (Abdelraouf *et al*., 2019) and fewer losses of nutrients by leaching. Moreover, higher daily irrigation frequency decreases the soil water tension (Xu *et al*., 2004), increases water content in plant tissues, optimizes stomatal opening process and nutrient uptake (N, Ca, Mg and S) by mass flow generated by the transpiration stream (Oliveira *et al*., 2010; McMurtrie & Näsholm, 2018; Baslam *et al*., 2021; Xing *et al*., 2021).

Combination between intermittent irrigation strategies and phosphate and potassium fertilizations used only on foundations in the toopsoil layer (0 - 0.20 m) in the two growing cycles, shows the greatest amounts of P and K uptaken in the presence of higher soil moisture content throughout the day in this layer, favoring greater contact of the lettuce root system with the ions present in the soil solution, optimizing the extraction by the roots.

According to Oliveira *et al*. (2010), a greater contact of K and P in soil with roots occurs by diffusion and the extraction of these elements from the soil solution is closely related to soil moisture which acts as a transport of ions to the roots. For Xu *et al*. (2004), increasing daily irrigation frequency limits the soil moisture depletion in the root zone of plants, optimizes nutrient uptake via transpiration and reduces the significance of diffusion in the nutrient-root contact in the soil. These authors observed an increase of P concentration in leaves and roots of lettuce Iceberg with the use of intermittent daily irrigation management.

A decrease in K contents in lettuce leaves in all treatments in IIC was noticed (Table 1), probably due to the competition between K^+ and NH_4^+ ions in soil promoted by an increase of N fertilization, since the increase in N levels increases the amount of NH_4^+ NO_3^- ions, the ways N is uptaken by plants (Buragohain *et al*., 2019; Baslam *et al*., 2021). This decrease in K contents in IIC may also have favored a greater uptake of Ca and Mg by lettuce plants, since these nutrients show an antagonistic relationship with K (Menezes *et al*., 2020b).

N, P, K, Ca, Mg and S are essential nutrients for the development and growth of plants. These nutrients take part in numerous compounds and perform vital functions for plants, acting in the synthesis of amino acids, proteins, DNA, RNA, control of stomatal and enzymatic processes, photosynthesis, respiration, chlorophyll synthesis, control of hormonal, growth, division and cell differentiation mechanisms, directly reflecting the gain in mass accumulation of the plant dry matter (Carstensen *et al*., 2018; Hasanuzzaman *et al*., 2018; Samborska *et al*., 2019; Zhang *et al*., 2020; Baslam *et al*., 2021 Xing *et al*., 2021).

The results found in this study corroborate the ones reported by El-Mogy *et al*. (2012) who verified an increase in contents of N, P and K in tissues of green beans using the intermittent irrigation strategy. Menezes *et al*. (2020b) also noticed significant increases in contents of N, P, K and S in coriander using intermittent fertigation. Silber *et al*. (2005) also verified an increase in leaf contents of K, Ca and Mg in green pepper with intermittent fertigation. On the other hand, Xu *et al*. (2004) did not verify significant responses considering leaf contents of N, NO_3^-, K, Ca and Mg in lettuce using intermittent daily irrigation.

Except P content in IC, the other leaf macronutrients were not influenced by N doses application in fertigation (Table 1). In this productive cycle, the dose of 130 kg/ha N provided a significant increase of 6.31% in P content in relation to the dose of 100 kg/ha N. Despite not having a significant effect, it was possible to notice that the increase in N in fertigation also benefited the P content

Table 1. Macronutrient contents in mini romaine lettuce in relation to irrigation strategies and N doses in two crop cycles. Botucatu, UNESP, 2020.

Strategy	First cycle (IC)					
	N	P	K	Ca	Mg	S
	(g/kg of dry matter)					
Cont	33.87 b	4.30 b	50.50	15.87 b	3.77 b	2.10
Int1	37.25 a	5.31 a	62.00	18.62 a	4.21 a	2.38
Int2	38.00 a	5.12 a	60.87	17.62 a	4.06 a	2.35
N (kg/ha)						
100	36.08	4.75 b	55.16	17.50	4.00	2.32
130	36.66	5.07 a	60.41	17.25	4.03	2.23
	Second cycle (IIC)					
Cont	34.25 b	3.93 b	28.37 b	18.12	3.92	1.60 b
Int1	35.50 a	5.52 a	39.00 a	20.25	4.42	1.85 a
Int2	35.87 a	5.06 a	40.12 a	20.12	4.36	1.98 a
N (kg/ha)						
100	34.83	4.78	35.08	19.58	4.10	1.79
130	35.58	4.90	36.58	19.41	4.36	1.86

Averages followed by different letters in columns differ from each other by Tukey test ($p<0.05$).

Table 2. Micronutrient contents in mini romaine lettuce in relation to irrigation strategies and N doses in two crop cycles. Botucatu, UNESP, 2020.

Strategy	First cycle (IC)				
	B	Cu	Fe	Mn	Zn
	(mg/kg of dry matter)				
Cont	25.00 b	14.12	1276.62 b	107.37	32.62 b
Int1	32.75 a	14.00	1998.62 a	120.12	52.75 a
Int2	35.75 a	13.62	2174.00 a	123.37	49.12 ab
N (kg/ha)					
100	32.50 a	14.41	1931.08 a	115.83	49.08
130	29.83 b	13.41	1701.75 b	118.08	45.25
	Second cycle (IIC)				
Cont	20.75 b	10.62	1307.87 b	107.50 b	35.62
Int1	33.00 a	11.75	1664.50 ab	153.87 a	50.62
Int2	31.37 a	10.50	1936.50 a	149.50 a	47.25
N (kg/ha)					
100	27.58	11.16	1510.66	123.50 b	41.16
130	29.16	10.75	1761.91	150.41 a	47.83

Averages followed by different letters in columns differ from each other by Tukey test ($p<0.05$).

in the IIC.

A greater P uptake noticed with the application of 130 kg/ha N may be due to the synergistic effect between these nutrients (Rietra *et al*., 2017). P is a macronutrient which acts in the growth and hormonal control of plants, and is also a fundamental component of ATP, NADPH, nucleic acids, in the storage and transfer of energy and in the activation of enzymes, directly affecting crop production (Carstensen *et al*., 2018). We highlight that N application to soil as urea source, in addition to increasing N availability, slightly reduces the pH, and increases soil P availability (Awaad *et al*., 2016).

An increase of P content of leaves in relation to a greater soil N availability was also observed by Awaad *et al*. (2016), El-Bassyouni (2016) and Taha *et al*. (2017) in lettuce cultivars Dark green, Oviation and Balady, respectively. However, Zandvakili *et al*. (2019) did not verify effects of N doses (urea) on P leaf contents of lettuce cultivars Iceberg, Romaine, Butterhead and Loose Leaf.

The average values for macronutrient contents in all treatments applied in this study are within the range considered adequate by Raij *et al*. (1997) N (30-50 g/kg); P (4.0-7.0 g/kg); K (50-80 g/kg); Ca (15-25 g/kg); Mg (4.0-6.0 g/kg) and S (1.5-2.5 g/kg), except K in IIC. The decreasing order in the levels of macronutrients observed in lettuce plants was: K>N>Ca>P>Mg>S. We highlight that K was taken up at the highest rate by the lettuce explored in this study. These studies are in accordance with the ones reported by Xu *et al*. (2004) who verified that K was taken up at the highest rate by the lettuce cultivar Iceberg. However, these data are different from the ones obtained by Dalastra *et al*. (2020) in which N was taken up at the highest rate by the cultivar Betty.

The contents of B, Fe and Zn were influenced by irrigation management strategies in IC. The irrigation strategies Int1 and Int2 provided significant increases in B, Fe and Zn contents. Greater variations in Zn contents were observed between the strategies Cont and Int1 in this cycle, though (Table 2). In IIC, the increases provided by the irrigation strategies Int1 and Int2 in the contents of B, Fe and Mn in relation to the strategy Cont, were 37.12 and 33.85%; 21.44 and 32.46% and 30.13 and 28.09%, respectively. However, the greatest differences in Fe contents occurred between the strategies Cont and Int2 (Table 2).

Intermittent irrigation management favors a greater supply of water and nutrients in the root part of the crops and results in a greater mass flow for transporting soil nutrients, such as B (Xu *et al*., 2004; Silber *et al*., 2005; Dridi *et al*., 2018). This greater water supply increases the nutrient-root contact by diffusion, since this transport contributes more than 99% to an uptake of Fe, Zn and Mn by the roots (Oliveira *et al*., 2010). The increase in foliar contents of Fe, Mn and Zn with the use of intermittent irrigation management was also observed in coriander plants by Menezes *et al*. (2020b). Silber *et al*. (2005) verified an increase in Mn contents in green pepper fruits in greater intermittence of daily irrigation.

Contents of B and Fe were influenced by N in IC, in which the application of 100 kg/ha N in fertigation increased significantly in 8.21 and 11.87% the contents of these micronutrients when compared with the highest dose (Table 2). This increase in B content can be related to the lower decrease in soil pH due to the lower dose of urea applied as a nitrogen fertilizer source, resulting in a better B availability in soil and consequently increasing the possibility of root extraction (Awaad *et al*., 2016; Bahia *et al*., 2019). In this context, the increase observed in Fe content may be related to a greater B uptake by the lettuce, since, according to literature, B influences in availability, uptake and translocation of nutrients such as P, K, Zn, Fe and Cu in plants (Shireen *et al*., 2018).

N doses significantly influenced Mn content in IIC. In this cycle, the dose of 130 kg/ha N increased Mn content in 17.89% in relation to the lowest dose (Table 2). This behavior was also observed in IC despite no difference was noticed between N dose averages (Table 2). This result can be explained by the reduction of pH in soil caused by the application of urea, favoring a greater availability and acquisition of Mn by the roots of the crop. Awaad *et al*. (2016) and Zandvakili *et al*. (2019) also verified an increase in Mn content in lettuce with an increase of N in soil.

B, Fe, Zn and Mn are essential for plants, and when present in adequate levels, they act in physiological processes such as photosynthesis, respiration, enzymatic regulation, cell wall formation, synthesis and transport of carbohydrates, hormonal metabolism, also favor resistance against disease and contribute to vegetative growth and crop yield (Shireen *et al*., 2018; Singh & Dwivedi, 2019).

Except Fe, the other micronutrient contents obtained in this study were close to the rate considered adequate by Raij *et al*. (1997) B (30-60 mg/kg); Cu (7.0-20 mg/kg); Fe (50-150 mg/kg); Mn (30-150 mg/kg) and Zn (30-100 mg/kg). We also highlight that Fe was the nutrient most taken up by lettuce in all treatments and that the plants did not show visual deficiency symptoms or excess of some nutrient, even in conditions of lower or higher contents. These results are in accordance with the ones found by Dalastra *et al*. (2020) in which Fe was the micronutrient most extracted and allocated in the shoot area of the lettuce cultivar Betty, with a much lower value than those described in this work, though.

Shoot dry mass accumulation (AMMS) was only influenced by irrigation strategies. In IC, the intermittent irrigation strategy Int2 provided greater gain in AMMS showing differences of 15.28 and 11.98% in relation to the strategies Cont and Int1, respectively. In IIC, intermittent irrigation strategies (Int1 and Int2) increased significantly the dry mass production in 15.28 and 11.98% in relation to the strategies Cont and Int1, respectively. In IIC, the intermittent irrigation strategies (Int1 and Int2) increased significantly dry mass production in 9.41and 13.22% in relation to the irrigation management Cont, respectively (Table 3).

The increase in AMMS in intermittent irrigation strategies is explained by the better daily water availability in the lettuce roots, which favors an increase in stomatal conductance and transpiration rate, and a greater absorption of nutrients, optimizing the net CO_2 assimilation and the vegetative growth (physiological data not shown). The gain in AMMS with the use of intermittent irrigation management was also observed in other crops such as green pepper (Silber *et al*., 2005) and coriander (Menezes *et al*., 2020a).

Macronutrient accumulation of dry matter was influenced by irrigation strategies in the two growing cycles. In IC, the strategy Int2 increased the accumulation of all the macronutrients when comparing with strategy Cont, whereas strategy Int1 increased only the accumulation of P, Ca and Mg. In IIC, the differences between the averages of strategies were more significant, and both Int1 and Int2 provided increases in accumulation of all the nutrients in relation to Cont strategy (Table 3). The increase in macronutrient accumulation is due to a greater growth rate and AMMS observed in intermittent irrigation

Table 3. Accumulation of dry matter (AMMS) and macronutrients in mini romaine lettuce in relation to irrigation management strategies and N doses in two crop cycles. Botucatu, UNESP, 2020.

Strategy	First cycle (IC)						
	AMMS	N	P	K	Ca	Mg	S
	(g/plant)						
Cont	8.48 b	0.287 b	0.036 b	0.435 b	0.134 b	0.031 b	0.018 b
Int1	8.81 b	0.328 ab	0.046 a	0.545 ab	0.163 a	0.037 a	0.021 ab
Int2	10.01 a	0.379 a	0.051 a	0.610 a	0.179 a	0.040 a	0.023 a
N (kg/ha)							
100	8.82	0.310	0.042 b	0.490	0.155	0.035	0.020
130	9.39	0.340	0.047 a	0.570	0.162	0.037	0.021
	Second cycle (IIC)						
Cont	8.27 b	0.283 b	0.033 b	0.233 b	0.151 b	0.032 b	0.013 b
Int1	9.13 a	0.324 a	0.050 a	0.354 a	0.185 a	0.040 a	0.016 a
Int2	9.53 a	0.341 a	0.048 a	0.380 a	0.191 a	0.041 a	0.018 a
N (kg/ha)							
100	9.17	0.320	0.043	0.322	0.180	0.037	0.016
130	8.79	0.312	0.043	0.323	0.171	0.038	0.016

Averages followed by different letters in columns differ from each other by Tukey test ($p<0.05$).

Table 4. Micronutrient accumulation and N use efficiency in mini romaine lettuce in relation to irrigation management strategies and N doses in two crop cycles. Botucatu, UNESP, 2020.

Strategy	First cycle (IC)					EUN (%)
	B	Cu	Fe	Mn	Zn	
	(mg/plant)					
Cont	0.212 c	0.120	10.722 c	0.913 b	0.337 b	30.09 b
Int1	0.286 b	0.123	17.660 b	1.061 ab	0.461 a	32.85 a
Int2	0.356 a	0.137	21.700 a	1.229 a	0.494 a	33.41 a
N (kg ha^{-1})						
100	0.288	0.128	17.314	1.026	0.434	36.07 a
130	0.289	0.125	16.074	1.109	0.428	28.16 b
	Second cycle (IIC)					
Cont	0.170 b	0.088 b	10.977 b	0.891 b	0.494 b	30.21 b
Int1	0.300 a	0.106 a	15.080 a	1.396 a	0.462 a	31.39 ab
Int2	0.298 a	0.102 a	18.441 a	1.422 a	0.453 a	31.63 a
N (kg ha^{-1})						
100	0.251	0.101	13.913	1.137 b	0.380	34.86 a
130	0.261	0.094	15.745	1.335 a	0.426	27.29 b

Averages followed by different letters in columns differ from each other by Tukey test ($p<0.05$).

strategies. These results corroborate the ones reported by Menezes *et al*. (2020a) who verified significant increases in accumulation of N, P, K and S in coriander using intermittent fertigation.

Only the accumulated amount of P in the dry matter was influenced by the N doses in IC. The dose of 130 kg/ha N increased in 10.61% P accumulation in relation to the lowest dose (Table 3). The greater vegetative growth and consequent gain in AMMS in relation to the increase in the N dose applied was followed by a greater P, showing absence of dilution effect and better proportion in the extraction/growth ratio.

In general, the macronutrients were accumulated in the following decreasing order: K>N >Ca>P>Mg>S. However, Cont irrigation strategy was the only one which provided an inversion between K and N in accumulation order in IIC. These results are similar to the ones reported by Carvalho *et al*. (2018) and Dalastra *et al*. (2020) who also verified that K and N were the most abundant nutrients in dry matter of lettuce.

The accumulation averages of N, P, K, Ca, Mg and S in dry matter obtained in this study are above the ones found by Dalastra *et al*. (2020), 0.160; 0.030; 0.120; 0.050; 0.030 and 0.010 g/plant in lettuce cultivar Betty, respectively. These differences in nutrient accumulation may be related to other factors which are not related to genetic traits considered in this study, growing season and ways of conducting the studies.

Intermittent irrigation strategies (Int1 and Int2) increased the accumulation of B, Fe and Zn significantly in relation to Cont in IC and only the strategy Int2 showed a different result for Mn. For B and Fe, the strategy Int2 also resulted in greater accumulations when compared with Int1. In IIC, the irrigation strategies Int1 and Int2 provided an increase in accumulation of all micronutrients in dry matter when compared with strategy Cont (Table 4).

A decrease in micronutrient accumulation with the use of Cont irrigation strategy showed plant growth restriction, with a reduction of AMMS in lettuce. This decrease in accumulation of the micronutrients Mn, Cu, Zn and Fe in dry matter with the use of the continuous irrigation management strategy was also reported by Menezes *et al*. (2020a) in coriander plants.

The dose of 100 kg/ha N reduced Mn accumulation in 14.83% when compared with the greatest dose in IIC (Table 4). The greatest vegetative growth and AMMS was followed by the lowest Mn accumulation in shoot part of the plant, highlighting slight dilution effect of this nutrient on dry matter and lower extraction/growth ratio.

In this study, the micronutrient accumulation followed this decreasing order: Fe>Mn>Zn>B>Cu. These results are in accordance with the ones found by Carvalho *et al*. (2018) and Dalastra *et al*. (2020) who observed that Fe was the most abundant nutrient in dry matter of lettuce. We highlight that except Zn, the accumulation average values of Fe, Mn, B and Cu in dry matter obtained in this study are above the ones reported by Dalastra *et al*. (2020), 0.86; 0.54; 0.55; 0.13 and 0.05 mg/plant, Fe, Mn, Zn, B and Cu, respectively. Considering these results, we can conclude that the differences in micronutrient accumulations are also related to genetics traits of the cultivars and the ways of conducting the experiments.

Irrigation strategies influenced significantly EUN in the two growing cycles. In IC, the intermittences Int1 and Int2 increased EUN, 8.4 and 9.9%, when comparing with strategy Cont (Table 4). In IIC, the increases were less significant, 3.75 and 4.48%, respectively, and no difference between strategies Int1 and Cont was noticed (Table 4). The higher EUN in intermittent irrigation strategies is possibly associated with the lower travel of NH4+ ions and especially $NO3^-$ with the soil moisture front to areas unexplored by the lettuce root system, optimizing the acquisition and application efficiency of this nutrient.

EUN was also significantly influenced by N doses in the two growing cycles. The treatment using 100 kg/ha N optimized EUN, 21.92 and 21.71%, in relation to a higher dose in IC and IIC, respectively (Table 4). These results show that an increase in N acquisition by lettuce was proportionally smaller than the amount of N added, reducing the efficiency of this production factor. The results found in this study are in accordance with the ones found by Pereira *et al*. (2017) who observed a decrease of EUN in lettuce grown with N doses above 112 kg/ha.

The intermittent irrigation management promotes nutrient acquisition and optimizes EUN by lettuce plants grown in a protected environment.

K and N were the macronutrients which were most extracted and accumulated in lettuce plants. The decreasing order of accumulation of nutrients by lettuce is N>Ca>P>Mg>S>Fe>Mn>Zn>B>Cu.

The doses of 100 and 130 kg/ha N did not show any significant differences in relation to content and accumulation of nutrients, the dose of 100 kg/ha seems to be more efficient in relation to the nitrogen use, though.

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