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Response of Young Citrus Trees to NPK Fertilization Under Greenhouse and Field Conditions

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Abstract: Experiments were conducted with lemon (*Citrus limon* v. Eureka) and orange trees (*Citrus sinensis* cv. Maltese) to determine the optimum NPK fertilizer rates for young citrus trees in greenhouse and field conditions. Greenhouse nursery trees received 0-0-0, 0-25-50, 25-25-50, 50-25-50 or 100-25-50 mg L^{-1} of N-P_2O_5-K_2O via drip irrigation. The results showed that increased N rates improved leaf number, shoot length, total leaf area and stem diameter. The optimum tree growth was occurred with 50 and 100 mg N L^{-1}. Results also showed that the percentages of N in the leaves were increased in proportion to the amount of N added while the percentage of P and K were decreased. However, the concentrations of Ca, Mg, Fe, Mn and Zn were unaffected by N rates but the leaf Cu concentration increased significantly. Leaf Mg concentration was increased by the presence of K in the nutrition solution. Form this study, we recommended the use of 100 N-25 P_2O_5-50 K_2O mg L^{-1} for the good growth of nursery citrus plants in the absence of any possibility of deficiency or excessive accumulation of mineral elements. In the second experiment, two fertilizers mix 180-90-180 and 360-90-180 of N-P_2O_5-K_2O kg ha^{-1} were applied to young citrus tree in field condition. Increased the level of N had no effect on leaf number and stem diameter in Eureka lemon however shoot length and total leaf area were slightly increased and these increment in growth was higher in Maltese orange.

Key words: *Citrus sinensis, Citrus limon*, fertilization, plant growth, mineral nutrition, Tunisia

INTRODUCTION

Citrus is among the first fruit crop in Tunisia. The Maltese orange is the cultivar which one associates more with Tunisia which is the only producer and world export. It occupies 45% of the citrus planted area and nearly 97.5% of the total exported quantities of oranges according to the 2008 agricultural statistics. Despite the increasing demands of local consumption and export, there is a lack of information about the optimum integrated nutrient management response of young citrus trees. Fertilization programs for young trees are highly variable thus, different fertilizer formulations, frequencies and rates are used for container-grown and field-grown trees (Guazzelli *et al*., 1996.)

Adequate supply of Nitrogen (N), Phosphorus (P) and potassium (k) is important for citrus tree growth (Obreza *et al*., 2008). Nitrogen is the key component in mineral fertilizers applied to citrus groves; it has more influence on tree growth, appearance and fruit production/quality than any other element (Zekri and Obreza, 2003). In young trees, N fertilizers can favour vegetative growth and decrease flower induction (Menino *et al*., 2003). Excess nitrogen application enhances vegetative tree growth and may cause groundwater contamination if leached with excess irrigation and/or rainfall (Alva *et al*., 2006). However, it affects the absorption and distribution of practically all other elements (Zekri and Obreza, 2003). Potassium is necessary for basic physiological functions such as formation of sugars and starch, synthesis of proteins and cell division and growth (Obreza, 2003; Abbas and Fares, 2008). It is important in fruit formation and enhances fruit size, flavor and color.

Phosphorus is necessary for many life processes such as photosynthesis, synthesis and breakdown of carbohydrates and the transfer of energy within the plant (Zekri and Obreza, 2003). Fertilizer recommendations for young citrus trees include a range of rates for each of the first three years (Ferguson *et al*., 1995). Nitrogen nutrition

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for young trees is highly variable, as reflected by the optimum N fertilization rates reported for the first year in the field which range from 70-400 g N/tree/year whereas P and K are applied, respectively in amounts equivalent to 0.4 and 0.8 of N required by trees. According to Obreza *et al*. (2008), potassium should be applied at equal amount to N rate.

Leaf chemical analysis is commonly used as a diagnostic criterion for nutrient recommendation and fertilization of citrus groves (Quaggio *et al*., 1998). The interpretation of leaf tissue mineral analysis depends on the physiological stage of leaves that are sampled for analysis, leaf decontamination procedure and analytical methods (Hanlon *et al*., 1995).

Although, critical leaf concentration standards for almost all nutrients are well established for citrus orange (Hanlon *et al*., 1995; Alva *et al*., 2005) however, there is few research results related to Maltese orange or Eureka lemon leaf chemical analysis, furthermore in Tunisia there is a little knowledge concerning fertilizer recommendation in nursery greenhouse conditions. This research aimed to evaluate the effect of N rates on nutritional status and vegetative growth of trees in greenhouse and in field conditions to determine the optimum rates of N for maximum plant growth correlated to adequate levels of leaf nutrient concentrations and to select the more suitable NPK fertilizer mix for the young Maltese orange and Eureka lemon trees budded on Sour orange rootstock.

MATERIAL AND METHODS

Experiment 1: Young nursery citrus plants of lemon (*Citrus limon* v. Eureka) and orange (*Citrus sinensis* cv. Malti) on Sour orange (*Citrus aurantium* Osbeck) rootstock were grown in to 10 L plastic pots containing a medium composed of perlite and peat moss (v/v). The experiments were conducted in a greenhouse under controlled conditions with the following regimes: temperature: min/max 17/35°C; relative humidity: min/max 30/70%; photoperiod (14/10 h day/night). The plants of Eureka lemon and Maltese orange were divided into 5 groups for fertilizer treatments: (T0) 0-0-0; (T1) 0-25-50; (T2) 25-25-50; (T3) 50-25-50 and (T4) 100-25-50 mg L^{-1} of N-P_2O_5-K_2O. Deionised water was used in all nutrient solutions and NH_4NO_3, H_3PO_4 and K_2SO_4 are the NPK source of the experiments. In addition to the NPK nutriment the solution contained (mg L^{-1}) 120 Ca as $CaSO_4$ $2H_2O$; 40 Mg as $MgSO_4$, $7H_2O$; 0.5 Mn as $MnCl_2$ $4H_2O$; 0.02 Mo as $NaMoO_4$ $2H_2O$; 0.02 Cu as $CuSO_4$ $5H_2O$; 0.05 Zn as $ZnSO_4$, $7H_2O$; 0.5 B as BO_3 and 5 Fe as NaFeEDTA (Maust and Williamson, 1994). The treatments consist irrigation every second day with 500 mL of fertilized solutions. Ten trees from each treatment were selected for final measurements and data analysis. Initiation and cession of flushes were recorded during the experiments. Leaf number, shoot length, total leaf area and stem diameter were measured through three scion growth flushes. A portable leaf area meter (model LI-300; LI-COR) was used to determine the total leaf area of the shoot. Ten leaves per tree were collected on 19 May, 28 July and 29 September for nutrient analysis.

Experiment 2: A field experiment was conducted in a private orchard farm in the North East of Tunisia on 1 year old Eureka lemon and Maltese orange trees budded on Sour orange rootstock planted at 6.0×4.0 m spacing with 417 trees ha^{-1}. The soil is sandy loam at 0-40 cm depth and sandy clay loam at 40-80 cm depth of the experimental site of Eureka lemon. For the Maltese orange, the soil of the experimental site is sandy. The soil of the region is alkaline in nature and has adequate content of calcium and low levels of nitrogen, phosphorus and potassium. The experiment ran from the beginning of March to the beginning of November and the trees were divided into 2 groups each of which received 180N - $90P_2O_5$ - $180K_2O$ or 360N - $90P_2O_5$ - $180K_2O$ kg ha^{-1}. Fertilizer treatments were applied through a drip irrigation system consisted of two drip lines which delivered about 0.048 m^3 h^{-1} at 0.1 MPa for each tree. Nitrogen, phosphorus and potassium were applied as ammonium nitrate, phosphoric acid and potassium sulphate, respectively. Fertigation was used from March-October except during the rainy days. Weekly plant growth measurements were made from 7 April until 5 October. Shoot length was determined by measuring four shoots per growth flush per tree at harvest time. Leaf number per shoot, total leaf area per shoot and stem diameter were also determined for each tree.

Sampling of 2-3 month old leaves of spring, summer and autumn flush leaves were sampled following the procedure described by Obreza (1992). Approximately, 25 leaves were sampled from each of the middle two trees within each plot and pooled into 1 sample per plot. The leaves were washed with deionised water, oven-dried at 70°C for 72 h and then ground to pass a 0.5 mm sieve and analysed for N by a micro-Kjeldahl method. Sub-samples were ashed at 550°C, digested with HCl and analysed for P, K, Ca, Mg, Mn, Zn, Fe and Cu. Phosphorus was determined by UV-vis spectrophotometry and the other nutrients by ICP spectrometry.

RESULTS AND DISCUSSION

Greenhouse studies: The presence of PK in the nutrition solution increased the growth parameters (leaf number, shoot length, total leaf area and stem diameter) in the both potted young citrus plants (Table 1). However, this increment was higher with the addition of nitrogen in the nutrition solution. For example, the total surface area of spring shoot was increased by 85.3 and 86.8% with T2 (NPK) and only by 28.5 and 23.4% with T1 (PK) as compared with control (T0) in Eureka lemon and Maltese orange, respectively.

In agreement with the findings of Lu *et al.* (2004) indicating that the length of the spring shoot of young citrus tree was decreased by 21.3% in the absence of nitrogen in the nutrition solution and only by 0-6, 1% when the plants was treated without potassium and phosphorus, respectively. On the other hand, the citrus plant growth increased linearly with increasing N rates (Table 1).

Doubling the rate of N from 25-50 mg L^{-1} was associated with the higher increment in plant growth for example, the shoot length was increased by 26.3-29.3% in this range of treatments and only by 9.5-13.1% when doubling the N rate from 50-100 mg L^{-1} in the different growth periods for Eureka lemon.

The same trends was occurred with the different growth parameters in the Maltese orange cultivar thus, the leaf number was increased by 33.8-54.5% with increasing N rates from 25-50 mg L^{-1}, however they raised only by 11.7-16.8% when doubling N rate from 50-100 mg L^{-1} in the different growth periods for Maltese orange. In the other hand, the shoot length and total leaf area was significantly different among N treatments coupled with growth periods in lemon. The higher percent of increase in shoot length (145.6%) and leaf area (134.2%) was occurred at the summer and autumn growth period (Table 1). In contrast, the results show no significant deference between N treatments coupled with growth periods in the leaf number, shoot length and stem diameter in Maltese orange.

The positive effect of N on the growth parameters has also been reported in nursery orange plants (Maust and Williamson, 1994; Guazzelli *et al.*, 1996; De Campos Bernardi *et al.*, 2000). Nitrogen is one of the most important nutrients for citrus tree growth generally required in greater amounts than any other plant nutrient to maximize tree growth and development (Thompson *et al.*, 2002). Indeed, nitrogen is a major constituent of proteins and therefore, plays an important role on plant metabolism and growth.

Furthermore, the highly positive significant correlation between the N fertilizer rates and growth parameter (data not shown) in the one hand and between leaf N concentration and growth parameters confirmed the major role of nitrogen in the growth of young citrus trees. However, the P and K fertilizer rates had no profound effect on young citrus tree growth during the first 3 years of plantation (Obreza, 2001). On the other hand, the relative lower increase in the growth parameters observed at 100 mg N L^{-1} as compared with 50 mg N L^{-1} may be caused by the presence of critical N concentration. Maust and Williamson (1994) indicated that critical N concentration for the dry weight accumulation in Hamlin orange was 15-19 mg L^{-1}. Table 2 showed that leaf mineral content was

Table 1: The effect of fertilizer treatments (T0, 0-0-0; T1, 0-25-50; T2, 25-25-50; T3, 50-25-50; T4, 100-25-50 mg L^{-1} of N-P_2O_5-K_2O) on leaf number, shoot length, total leaf area per shoot and stem diameter in spring, summer and autumn growth period of Eureka lemon and Maltese orange grown under greenhouse conditions

		Eureka lemon			Maltese orange		
Parameters	Treatments	Spring shoot	Summer shoot	Autumn shoot	Spring shoot	Summer shoot	Autumn shoot
Leaf number	T0	5.5±0.4^{a}	5.2±0.5^{a}	5.6±0.4^{b}	2.8±0.3^{a}	3.0±0.3^{a}	3.1±0.2^{b}
	T1	7.2±0.6^{b}	6.8±0.4^{a}	7.0±0.5^{a}	3.5±0.3^{a}	3.7±0.4^{a}	4.0±0.3^{b}
	T2	10.7±0.8^{a}	9.7±0.7^{a}	10.2±0.6^{b}	5.2±0.4^{a}	5.5±0.4^{a}	6.2±0.4^{b}
	T3	12.7±0.8^{b}	11.8±0.8^{a}	12.2±0.8^{a}	7.7±0.5^{a}	8.5±0.6^{a}	8.3±0.5^{b}
	T3	14.2±1.2^{a}	13.5 ±1.1^{a}	14.0±1.0^{b}	9.0±0.8^{a}	9.5±0.7^{a}	9.5±0.7^{b}
Shoot length (cm)	T0	5.6±0.4^{a}	5.3±0.4^{b}	5.5±0.3^{a}	3.2±0.2^{a}	3.3±0.2^{a}	3.4±0.3^{b}
	T1	7.5±0.5^{a}	6.8±0.3^{b}	6.9±0.4^{a}	4.1±0.2^{a}	4.2±0.3^{a}	4.4±0.3^{b}
	T2	9.6±0.8^{a}	9.2±0.7^{b}	9.1±0.6^{a}	6.0±0.4^{a}	6.3±0.5^{a}	6.8±0.4^{b}
	T3	12.2±0.7^{a}	11.9±0.7^{b}	11.5±0.8^{a}	7.9±0.5^{a}	8.2±0.6^{a}	8.4±0.7^{b}
	T4	13.8±0.9^{a}	13.4±1.2^{a}	12.6±0.9^{b}	9.5±0.7^{a}	9.3±0.6^{a}	9.7±0.7^{b}
Total leaf area (cm^2)	T0	861±13^{a}	843±18^{b}	834±17^{b}	341±12^{a}	343±11^{b}	332±15^{b}
	T1	1107±15^{a}	1079±16^{a}	1083±19^{a}	421±15^{a}	442±13^{b}	456±12^{b}
	T2	1596±18^{a}	1621±19^{b}	1552±14^{b}	637±13^{a}	630±16^{b}	609±18^{b}
	T3	1908±16^{a}	1840±27^{a}	1831±24^{a}	802±15^{a}	836±12^{b}	843±17^{b}
	T4	2090±22^{a}	2110±28^{a}	2084±19^{a}	952±17^{a}	971±17^{b}	1006±21^{b}
Stem diameter (mm)	T0	2.13±0.14^{a}	2.12±0.11^{b}	2.14±0.09^{a}	2.07±0.09^{a}	2.05±0.08^{b}	2.06±0.07^{a}
	T1	2.15±0.15^{a}	2.13±0.10^{a}	2.14±0.08^{a}	2.06±0.09^{a}	2.06±0.07^{b}	2.07±0.07^{a}
	T2	2.31±0.12^{a}	2.31±0.11^{b}	2.34±0.14^{a}	2.21±0.08^{a}	2.19±0.12^{b}	2.21±0.10^{a}
	T3	2.49±0.16^{a}	2.48±0.14^{a}	2.53±0.13^{a}	2.32±0.14^{a}	2.32±0.12^{b}	2.35±0.15^{a}
	T4	2.60±0.15^{b}	2.59±0.14^{b}	2.63±0.15^{b}	2.41±0.13^{a}	2.39±0.16^{b}	2.42±0.15^{a}

Data are means values±SE of 4 measurements. Values in each column with the same letter are not significantly different (p = 0.05) as described by Duncan's test

Table 2: The effect of fertilizer treatments (T0, 0-0-0; T1, 0-25-50; T2, 25-25-50; T3, 50-25-50; T4, 100-25-50 mg L^{-1} of N-P_2O_5-K_2O) on leaf N, P, K, Ca, Mg, Fe, Mn, Zn and Cu concentrations in spring, summer and autumn growth period of Eureka lemon and Maltese orange grown under greenhouse conditions

		Lemon shoots			Orange shoots		
Parameters	Treatments	Spring	Summer	Autumn	Spring	Summer	Autumn
N	T0	1.31±0.05^{d} (-)	1.32±0.06^{d} (-)	1.37±0.08^{d} (-)	1.35±0.07^{d} (-)	1.37±0.06^{d} (-)	1.33±0.06^{d} (-)
	T1	1.33±0.07^{d} (-)	1.30±0.05^{d} (-)	1.31±0.06^{d} (-)	1.37±0.08^{d} (-)	1.36±0.06^{d} (-)	1.37±0.07^{d} (-)
	T2	2.12±0.08^{c} (-)	1.99±0.09^{c} (-)	1.88±0.07^{c} (-)	2.03±0.09^{c} (-)	1.93±0.07^{c} (-)	1.90±0.08^{c} (-)
	T3	2.61±0.12^{b} (**)	2.67±0.12^{b} (**)	2.72±0.11^{b} (**)	2.66±0.09^{b} (**)	2.60±0.11^{b} (**)	2.63±0.09^{b} (**)
	T4	2.89±0.15^{a} (***)	3.06±0.13^{a} (+)	3.05±0.14^{a} (+)	3.00±0.12^{a} (***)	3.18±0.13^{a} (+)	3.16±0.09^{a} (+)
P	T0	0.130±0.01^{c} (**)	0.126±0.01^{d} (**)	0.131±0.01^{d} (**)	0.128±0.01^{d} (**)	0.130±0.01^{d} (**)	0.131±0.01^{d}(**)
	T1	0.165±0.01^{a} (**)	0.174±0.01^{a} (**)	0.168±0.01^{a} (**)	0.172±0.01^{a} (**)	0.173±0.01^{a} (**)	0.178±0.01^{a}(**)
	T2	0.159±0.01^{a} (**)	0.165±0.01^{b} (**)	0.166±0.01^{ab} (**)	0.167±0.01^{a} (**)	0.169±0.01^{a} (**)	0.180±0.01^{a}(***)
	T3	0.164±0.01^{a} (**)	0.170±0.01^{ab} (**)	0.160±0.01^{b} (**)	0.153±0.01^{b} (**)	0.159±0.01^{b} (**)	0.164±0.01^{b}(**)
	T4	0.138±0.01^{b} (**)	0.136±0.01^{c} (**)	0.139±0.01^{c} (**)	0.139±0.01^{c} (**)	0.137±0.01^{c} (**)	0.140±0.01^{c}(**)
K	T0	1.20±0.05^{d} (**)	1.10±0.06^{e} (*)	1.08±0.05^{e} (*)	1.11±0.07^{d} (*)	1.08±0.06^{e} (*)	1.06±0.07^{e} (*)
	T1	1.94±0.07^{a} (***)	2.06±0.09^{a} (***)	2.02±0.09^{a} (***)	2.38±0.10^{a} (***)	2.17±0.12^{b} (***)	2.15±0.13^{b}(***)
	T2	1.89±0.08^{a} (***)	1.73±0.08^{b} (***)	1.87±0.09^{b} (***)	2.20±0.09^{b} (***)	2.35±0.12^{a} (***)	2.32±0.12^{a}(***)
	T3	1.72±0.10^{b} (**)	1.61±0.08^{c} (**)	1.68±0.08^{c} (**)	1.61±0.11^{c} (**)	1.67±0.11^{c} (**)	1.62±0.09^{c} (**)
	T4	1.47±0.07^{c} (**)	1.22±0.05^{d} (**)	1.21±0.07^{d} (**)	1.52±0.09^{c} (**)	1.34±0.08^{d} (**)	1.23±0.07^{d} (**)
Ca	T0	3.48±0.34^{b} (**)	3.64±0.27^{b} (**)	3.37±0.23^{b} (**)	4.27±0.38^{a} (**)	3.91±0.31^{b} (**)	4.05±0.36^{ab}(**)
	T1	3.57±0.37^{b} (**)	3.70±0.29^{ab} (**)	3.43±0.28^{ab} (**)	4.42±0.35^{a} (**)	4.31±0.34^{a} (**)	3.70±0.33^{c} (**)
	T2	3.49±0.31^{b} (**)	3.65±0.33^{b} (**)	3.37±0.31^{b} (**)	3.96±0.27^{b} (**)	4.07±0.29^{b} (**)	3.83±0.32^{bc}(**)
	T3	3.54±0.29^{b} (**)	3.85±0.35^{a} (**)	3.30±0.24^{b} (**)	3.79±0.31^{b} (**)	4.10±0.32^{ab} (**)	4.25±0.38^{a} (**)
	T4	3.92±0.36^{a} (**)	3.82±0.31^{ab} (**)	3.63±0.37^{a} (**)	4.22±0.39^{a} (**)	4.32±0.40^{a} (**)	3.93±0.35^{bc}(**)
Mg	T0	0.441±0.02^{a} (**)	0.447±0.03^{a} (**)	0.432±0.03^{a} (**)	0.429±0.02^{a} (**)	0.420±0.03^{a} (**)	0.474±0.03^{a}(**)
	T1	0.401±0.03^{b} (**)	0.391±0.03^{bc} (**)	0.390±0.03^{b} (**)	0.387±0.03^{b} (**)	0.345±0.03^{bc} (**)	0.409±0.04^{bc}(**)
	T2	0.405±0.03^{b} (**)	0.412±0.04^{b} (**)	0.385±0.03^{b} (**)	0.368±0.03^{b} (**)	0.353±0.03^{b} (**)	0.418±0.04^{b}(**)
	T3	0.382±0.04^{b} (**)	0.378±0.04^{c} (**)	0.381±0.04^{b} (**)	0.371±0.04^{b} (**)	0.336±0.03^{c} (**)	0.391±0.04^{c}(**)
	T4	0.391±0.04^{b} (**)	0.393±0.05^{bc} (**)	0.379±0.03^{b} (**)	0.385±0.04^{b} (**)	0.347±0.03^{bc} (**)	0.425±0.04^{b}(**)
Fe	T0	86.2±7.3^{ab} (**)	75.7±6.8^{ab} (**)	84.5±7.8^{b} (**)	106.7±9.2^{a} (**)	89.2±7.9^{ab} (**)	100.2±8.5^{ab}(**)
	T1	92.0±7.1^{a} (**)	76.5±7.1^{ab} (**)	83.0±8.2^{b} (**)	96.1±8.8^{b} (**)	82.6±6.8^{b} (**)	104.0±8.6^{a} (**)
	T2	89.5±8.2^{ab} (**)	76.7±7.0^{ab} (**)	88.5±7.3^{a} (**)	104.5±8.7^{a} (**)	87.5±7.4^{ab} (**)	87.8±7.9^{c} (**)
	T3	91.0±7.7^{a} (**)	72.4±8.2^{b} (**)	90.7±8.8^{a} (**)	98.7±8.1^{b} (**)	91.7±7.5^{a} (**)	102.1±8.2^{a} (**)
	T4	81.5±6.9^{b} (**)	81.2±8.4^{a} (**)	75.2±6.7^{c} (**)	109.2±9.2^{a} (**)	93.8±8.4^{a} (**)	95.2±8.1^{bc}(**)Mn
	T0	63.7±4.8^{ab} (**)	72.5±5.9^{ab} (**)	74.7±6.3^{a} (**)	39.0±4.1^{a} (**)	55.2±4.3^{a} (**)	53.2±3.8^{b} (**)
	T1	60.5±4.6^{bc} (**)	68.1±5.5^{bc} (**)	78.8±6.7^{a} (**)	41.6±3.8^{a} (**)	57.8±3.9^{a} (**)	49.5±3.7^{b} (**)
	T2	57.5±4.0^{c} (**)	69.0±5.4^{abc} (**)	78.5±6.8^{a} (**)	42.0±3.9^{a} (**)	48.0±3.7^{b} (**)	58.7±3.9^{a} (**)
	T3	64.0±5.1^{ab} (**)	76.2±6.5^{a} (**)	75.5±7.0^{a} (**)	45.2±4.2^{a} (**)	50.1±4.1^{b} (**)	63.5±4.8^{a} (**)
	T4	67.1±5.2^{a} (**)	63.7±5.4^{c} (**)	71.2±6.5^{a} (**)	41.3±3.7^{a} (**)	60.2±4.5^{a} (**)	51.3±4.3^{b}(**)Zn
	T0	56.0±4.1^{ab} (**)	43.4±3.0^{c} (**)	44.6±3.3^{b} (**)	46.0±3.8^{ab} (**)	51.2±3.9^{b} (**)	42.2±3.5^{a} (**)
	T1	52.4±4.2^{b} (**)	40.6±2.7^{c} (**)	42.3±3.5^{b} (**)	41.8±3.6^{b} (**)	52.1±4.1^{b} (**)	39.0±3.4^{ab} (**)
	T2	51.7±4.4^{b} (**)	47.2±3.4^{b} (**)	53.0±4.1^{a} (**)	43.5±3.5^{ab} (**)	49.5±3.9^{b} (**)	34.2±3.0^{c} (**)
	T3	58.5±4.3^{a} (**)	54.7±3.8^{a} (**)	42.8±4.0^{b} (**)	47.2±4.1^{a} (**)	59.0±4.3^{a} (**)	40.1±3.5^{ab} (**)
	T4	51.5±3.9^{b} (**)	40.1±3.4^{c} (**)	47.1±4.2^{b} (**)	42.7±3.7^{ab} (**)	52.5±4.0^{b} (**)	40.9±3.3^{ab} (**
Cu	T0	9.4±0.08^{d} (**)	10.2±0.09^{d} (**)	8.4±0.07^{d} (**)	7.2±0.06^{d} (**)	7.9±0.05^{d} (**)	6.3±0.05^{d} (**)
	T1	9.8±0.07^{d} (**)	10.0±0.11^{d} (**)	8.3±0.08^{d} (**)	7.4±0.05^{d} (**)	8.0±0.05^{d} (**)	6.2±0.04^{d} (**)
	T2	11.2±0.08^{c} (**)	11.8±0.09^{c} (**)	9.2±0.07^{c} (**)	8.7±0.07^{c} (**)	9.6±0.06^{c} (**)	7.2±0.06^{c} (**)
	T3	15.7±0.13^{b} (**)	14.9±0.13^{b} (**)	12.6±0.09^{b} (**)	12.9±0.10^{b} (**)	12.0±0.08^{b} (**)	11.3±0.09^{b} (**)
	T4	18.2±0.15^{a} (***)	19.2±0.14^{a} (***)	17.1±0.16^{a} (***)	16.8±0.14^{a} (**)	16.7±0.13^{a} (**)	18.5±0.16^{a}(***)

Data are means values±SE of four measurements. Values in each column with the same letter are not significantly different (p = 0.05) as described by Duncan's test. (-): Deficient; (*): Low; (**): Optimum; (***): High; (+): Excess levels according to the guidelines for interpretation of leaf analysis (Hanlon *et al*., 1995)

significantly affected by different N treatments in both growth periods. In this concern, the leaf N concentrations in Eureka lemon and Maltese orange increased linearly with N rates. In T3 (2N-1P_2O_5-2K_2O analysis), N concentration of leaves in the both citrus plants were within the range of optimal concentrations as suggested by the guidelines for interpretation of leaf analysis (Hanlon *et al*., 1995).

However, it was considered as deficient in N when subjected to T2 (1N1$P_2O_5$2K_2O analysis). This decrease on leaf N concentration may be related to the higher P fertilization applied in this treatment. These results are in agreement with those obtained by many investigators such as and Thompson and White (2004). They reported that leaf nitrogen content of citrus tress was positively correlated with increasing rates of nitrogen applications. The 4N1$P_2O_5$2K_2O analysis (T4) showed a higher leaf N concentration in spring shoot and then an excessive accumulation of N in summer and autumn shoots.

Leaf P concentration was affected by N supply even though, the latter was responsible for the change on this variable. The maximum P concentration was observed at

T1 (PK), T2 (25 mg N L^{-1} and T3 (50 mg N L^{-1}) (Table 2). A negative relationship was observed between N fertilizer rates and leaf P concentration in the both citrus plants. Mattos *et al*. (2006) reported an antagonism between N and P those two at high N fertilizer rates in young citrus trees. In contrast, Sabbah *et al*. (1997) shows that leaf P content was not affected significantly by N rates in orange trees. During the growing season, leaf P concentrations in all N treatments of both citrus studies plants were within the critical tissue P range of 12-16% (Hanlon *et al*., 1995). Concerning leaf K, the nutrient concentration varied as a function of the interaction between N and K fertilization. The results show a negative correlation between N fertilizer rates and leaf K concentration in the both citrus plants (Table 2). The maximum foliar level found was reached at T1 (0N-25P_2O_5-50K_2O mg L^{-1}) and T2 (25N-25P_2O_5-50K_2O mg L^{-1}), these values are considered to be higher according to Hanlon *et al*. (1995). In agreement with the findings of De Campos Bernardi *et al*. (2000) observed for seedlings, 4 months after transplant that maximum leaf K was occurred with application of N and K rates = 0.47 and 4.67 g $plant^{+}$, respectively.

At the highest N level T4 (100 mg L^{-1}), leaf K concentration was decreased significantly in Eureka lemon and Maltese orange while remaining within the optimum levels sufficient for adequate development for young citrus trees (Table 2). This decreased leaf K concentrations with increasing fertilization rates my be considered a dilution effect cause by the vigorous growth induced by nitrogen application similar results were occurred on citrus tress by Sabbah *et al*. (1997) and Wassel *et al*. (2007). According to Robert *et al*. (1999), as fertilizer N/K_2O ratio increased, leaf K concentration decreased. The adequate foliar range of K proposed for young citrus trees was observed essentially with the 2N1$P_2O_5$2K_2O analysis. Leaf Mg concentration decreased by 9.1-17.8% in the shoots of Maltese orange and Eureka lemon at T1 (PK) as compared with control. This decrease may be resulted from the competitive uptake between K and Mg as reported by Ruschel ***et al.*** (2004). In Contrast, leaf Ca, Mg, Fe, Mn and Zn concentrations were unaffected by increasing N rate and their values were at acceptable levels (Table 2). However, increasing rates of N application resulted in a significant increase in leaf copper content. Higher content of Cu was observed at the highest N rate (100 mg L^{-1}) in the deferent growth periods of Eureka lemon and at autumn growth period of Maltese orange. These findings were in agreement with those obtained by Sabbah *et al*. (1997) who found that soil application of nitrogen fertilizers to citrus trees increased copper in the leaves to toxic levels. Based on norms defined by Panero, the N/K ratio indicates a probable nitrogen deficiency in the leaf of both citrus plants grown in control condition or fertilized with 0N1$P_2O_5$2K_2O (T1) and 1N1$P_2O_5$2K_2O (T2) analysis at the deferent growth periods (Table 3).

The N/P ratio showed a probable nitrogen deficiency in the leaf of both citrus plants treated with T0 and T1 and a probable phosphorus deficiency at T4 in the summer shoot of Maltese orange. In addition, the N/K ratio indicates a probable potassium deficiency in the leaf of both citrus plants fertilized with 4N1$P_2O_5$2K_2O analysis at different growth periods. However, in the 2N1$P_2O_5$2K_2O

Table 3: The effect of fertilizer treatments (T0, 0-0-0; T1, 0-25-50; T2, 25-25-50; T3, 50-25-50; T4, 100-25-50 mg L^{-1} of N-P_2O_5-K_2O) on N/K, N/P, K/Mg and Fe/Mn ratios in the leaves of Eureka lemon and Maltese orange grown under greenhouse conditions

		Eureka lemon shoots			Maltese orange shoots		
Parameters	Treatments	Spring	Summer	Autumn	Spring	Summer	Autumn
N/K	T0	1.08 ± 0.08^{c} (-N)	1.19 ± 0.09^{c} (-N)	1.26 ± 0.09^{c} (-N)	1.21 ± 0.10^{c} (-N)	1.27 ± 0.09^{c} (-N)	1.24 ± 0.08^{c}(-N)
	T1	0.68 ± 0.05^{d} (-N)	0.63 ± 0.04^{d} (-N)	0.65 ± 0.05^{e} (-N)	0.57 ± 0.05^{e} (-N)	0.63 ± 0.04^{e} (-N)	0.64 ± 0.04^{e}(-N)
	T2	1.12 ± 0.08^{c} (-N)	1.14 ± 0.08^{c} (-N)	1.01 ± 0.07^{d} (-N)	0.92 ± 0.08^{d} (-N)	0.82 ± 0.05^{d} (-N)	0.83 ± 0.07^{d}(-N)
	T3	1.52 ± 0.12^{b}	1.65 ± 0.13^{b}	1.62 ± 0.12^{b}	1.65 ± 0.14^{b}	1.57 ± 0.11^{b}	1.62 ± 0.14^{b}
	T4	2.12 ± 0.17^{a}	2.52 ± 0.19^{a} (-K)	2.51 ± 0.15^{a} (-K)	1.97 ± 0.17^{a}	2.37 ± 0.18^{a} (-K)	2.54 ± 0.19^{a}(-K)
N/P	T0	10.0 ± 0.85^{c} (-N)	10.4 ± 0.91^{c} (-N)	10.3 ± 0.80^{d} (-N)	10.5 ± 0.74^{d} (-N)	10.5 ± 0.67^{d} (-N)	10.1 ± 0.82^{c}(-N)
	T1	9.2 ± 0.87^{d} (-N)	8.7 ± 0.72^{d} (-N)	8.6 ± 0.65^{e} (-N)	9.1 ± 0.72^{e} (-N)	8.8 ± 0.60^{e} (-N)	8.6 ± 0.71^{d}(-N)
	T2	13.3 ± 1.09^{c}	12.0 ± 0.98^{c}	11.3 ± 0.76^{c}	12.1 ± 0.95^{c}	11.4 ± 0.93^{c}	10.5 ± 0.74^{cd}
	T3	15.9 ± 1.21^{b}	15.7 ± 1.31^{b}	16.9 ± 1.28^{b}	17.3 ± 1.32^{b}	16.3 ± 1.45^{b}	15.9 ± 1.08^{b}
	T4	20.9 ± 1.52^{a}	22.6 ± 1.84^{a}	21.9 ± 1.70^{a}	21.5 ± 1.61^{a}	23.2 ± 1.96^{a} (-P)	22.6 ± 1.83^{a}
K/Mg	T0	2.72 ± 0.15^{d}	2.41 ± 0.19^{d}	2.51 ± 0.17^{e}	2.62 ± 0.18^{d}	2.57 ± 0.15^{d}	2.35 ± 0.21^{d}
	T1	4.85 ± 0.33^{a}	5.26 ± 0.41^{a}	5.19 ± 0.28^{a}	6.15 ± 0.37^{a}	6.30 ± 0.21^{a}	5.28 ± 0.35^{a}
	T2	4.71 ± 0.28^{ab}	4.21 ± 0.29^{b}	4.85 ± 0.27^{b}	6.01 ± 0.32^{a}	6.66 ± 0.29^{a}	5.54 ± 0.36^{a}
	T3	4.50 ± 0.34^{b}	4.28 ± 0.35^{b}	4.40 ± 0.33^{c}	4.34 ± 0.35^{b}	4.81 ± 0.25^{b}	4.15 ± 0.29^{b}
	T4	3.48 ± 0.27^{c}	3.09 ± 0.32^{c}	3.21 ± 0.24^{d}	3.96 ± 0.27^{c}	3.99 ± 0.31^{c}	2.92 ± 0.20^{c}
Fe/Mn	T0	1.35 ± 0.07^{c}	1.04 ± 0.06^{bc}	1.13 ± 0.06^{ab}	2.73 ± 0.15^{a}	1.61 ± 0.09^{ab}	1.87 ± 0.12^{ab}
	T1	1.52 ± 0.12^{ab}	1.12 ± 0.07^{b}	1.05 ± 0.05^{b}	2.31 ± 0.16^{ab}	1.42 ± 0.11^{b}	2.10 ± 0.14^{a}
	T2	1.56 ± 0.09^{a}	1.11 ± 0.08^{b}	1.13 ± 0.05^{ab}	2.53 ± 0.18^{ab}	1.83 ± 0.12^{a}	1.62 ± 0.09^{b}
	T3	1.42 ± 0.12^{bc}	0.94 ± 0.06^{c}	1.21 ± 0.06^{a}	2.22 ± 0.17^{b}	1.84 ± 0.11^{a}	1.61 ± 0.09^{b}
	T4	1.21 ± 0.08^{d}	1.28 ± 0.07^{a}	1.06 ± 0.05^{b}	2.65 ± 0.21^{ab}	1.55 ± 0.12^{b}	1.75 ± 0.08^{ab}

Data are means values±SE of four measurements. Values in each column with the same letter are not significantly different ($p = 0.05$) as described by Duncan's test

Table 4: The effect of fertilizer treatments (T1, 180-90-180; T2, 360-90-180 kg ha^{-1} of N-P_2O_5-K_2O) on leaf number, shoot length, total leaf area per shoot and stem diameter in spring, summer and autumn growth period of Eureka lemon and Maltese orange grown under field conditions

		Eureka lemon			Maltese orange		
Parameters	Treatments	Spring shoot	Summer shoot	Autumn shoot	Spring shoot	Summer shoot	Autumn shoot
Leaf number	T1	15.2±1.2^a	14.5±0.8^a	17.2±1.3^a	8.2±0.7^b	10.2±0.9^b	18.4±1.5^b
	T2	15.7±1.1^a	15.5 ±0.9^a	17.5±1.4^a	9.5±0.7^a	11.5±0.8^a	20.5±1.6^a
Shoot length (cm)	T1	15.6±0.9^b	14.2±1.1^b	18.7±1.5^a	8.2±0.6^b	9.0±0.7^b	20.1±1.4^b
	T2	16.7±1.2^a	15.6±1.0^a	19.2±1.6^a	9.6±0.8^a	10.9±0.8^a	23.1±1.6^a
Total leaf area (cm^2)	T1	2901±21^b	2013±15^b	3314±24^b	954±10^b	1055±16^b	2194±21^b
	T2	3173±23^a	2244±19^a	3547±22^a	1075±12^a	1280±13^a	2491±24^a
Stem diameter (mm)	T1	2.83±0.1^a	2.89±0.2^a	3.03±0.2^b	2.62±0.1^a	2.63±0.1^a	2.72±0.2^a
	T2	2.87±0.2^a	2.91±0.2^a	3.06±0.2^a	2.63±0.1^a	2.64±0.2^a	2.73±0.2^a

Data are means values±SE of four measurements. Values in each column with the same letter are not significantly different (p = 0.05) as described by Duncan's test

analysis the deferent ratios between nutritional elements does not present any probable nutrient deficiency in the leaf of Eureka lemon and Maltese orange which is in favor for the use of this fertilization base analyses for the best growth of young citrus trees. In addition, the K/Mg and Fe/Mn ratios were acceptable for all N treatments in the both citrus plants and no probability of such element deficiency is detected (Table 3).

Field studies: The increasing rate of N from 180-360 kg ha^{-1} was not associated with such increment of plant growth as observed in potted plants. Thus, no significant effect of increased N fertilizer rate was observed on tree leaf number in lemon and stem diameter in both citrus plants (Table 4). In addition only a slightly increase in the other growth parameters was occurred in both citrus trees with increasing N fertilizer rate and the higher raise was observed in Maltese orange as compared with Eureka lemon. For example, the total leaf area per shoot was increased by 7-11.4% when doubling the N rate from 180-360 Kg ha^{-1} in the deferent growth periods in Eureka lemon however these increase reached 12.6-21.3% in Maltese orange trees. The difference in total foliar area was attributed essentially to the presence of larger leaves than more leaves per shoot in lemon trees. These results are in harmony with those obtained by Guazzelli *et al.* (1996) reported that trunk diameter, tree height or shoot number or length was not consistently affected by N rates in young orange trees grown in field conditions.

Menino *et al.* (2003) showed that N treatment did not significantly affect tree height, trunk circumference or canopy height. From theses results, it appeared that nutrient reserve in the young trees at the time of planting, depending on the nursery fertilization program could contribute to nutrient requirement of the trees during the early years in the field. Although in most cases, nutrient reserves in nursery grown trees contribute to nutrient requirement of the trees during the early years (Alva *et al.*, 2008). As observed in potted citrus plants, increasing nitrogen rates resulted in a significant increase in leaf nitrogen content whereas K and P tended to decrease with increasing nitrogen rates but it remains within the range of optimal concentrations as suggested by the guidelines for interpretation of leaf analysis (Hanlon *et al.*, 1995) (Table 5). Thus, leaf P and K concentrations showed that the trees maintained adequate P and K throughout the experiment. In the fertilizer mixture 180N-90P_2O_5-180 K_2O kg ha^{-1}, N concentration for the both citrus trees were within the optimum ranges but it was in excess in the treatment T2. In addition, the N/K and N/P radios show a probable deficiency in K and P, respectively in Maltese orange fertilized with T2.

However, there are no symptoms of deficiency of these nutrients in the leaves of plants and the relative diminution of these nutriment was compensated by an adequate supply of N. On the other hand, the apparent efficiency of N occurred in the summer growth period is said passive and it was compensated by an adequate supply of K. Leaf Ca, Mg and micronutrient (Fe, Mn, Zn and Cu) were unaffected by the different N treatments (Table 5).

On the basis of leaf nutrient guidelines for citrus trees (Hanlon *et al.*, 1995), the concentrations of these nutriments were within the optimum ranges and no probability of such element deficiency was detected. The result for this part of studies indicate that the fertilizer mixture 180N-90P_2O_5-180 K_2O kg ha^{-1} (T1) was more suitable for the growth of young Maltese orange and Eureka lemon trees as compared with 360N- 90P_2O_5-180 K_2O kg ha^{-1} (T2). Thus, only a slightly increase in plant growth was occurred with T2 but was accompanied by excessive accumulation of N in the leaves and decreased leaf P and K concentrations. In agreement with the findings of Obreza *et al.* (2008) which the annual N rate applied to 2-3 years old orange trees should not exceed 225 kg ha^{-1} and that K fertilizer should be applied at a K_2O rate equal to the N rate. For P fertilizer, they recommended the application of P_2O_5 rate equal to 50% of

Table 5: The effect of fertilizer treatments (T0, 0-0-0; T1, 0-25-50; T2, 25-25-50; T3, 50-25-50; T4, 100-25-50 mg L^{-1} of N-P_2O_5-K_2O) on leaf N, P, K, Ca, Mg, Fe, Mn, Zn and Cu concentrations and on N/K, N/P, K/Mg and Fe/Mn ratios in the leaves of Eureka lemon and Maltese orange grown under field conditions

		Lemon shoots			Orange shoots		
Parameters	Treatments	Spring	Summer	Autumn	Spring	Summer	Autumn
N	T1	2.88±0.16 (**)	1.79±0.11[b] (*)	2.66±0.18[b] (**)	2.67±0.19[b] (**)	3.14±0.17[b] (**)	2.90±0.16[b] (**)
	T2	3.27±0.21[a] (+)	3.15±0.19[a] (+)	3.18±0.17[a] (+)	3.36±0.21[a] (+)	3.43±0.22[a] (+)	3.49±0.19[a] (+)
P	T1	0.170±0.01[a] (**)	0.155±0.01[a] (**)	0.171±0.01[a] (**)	0.165±0.01[a] (**)	0.155±0.01[a] (**)	0.150±0.01[a] (**)
	T2	0.163±0.01[b] (**)	0.157±0.01[a] (*)	0.164±0.01[b] (**)	0.145±0.01[b] (**)	0.136±0.01[b] (**)	0.131±0.01[b] (**)
K	T1	1.85±0.07[a] (***)	1.64±0.08[a] (**)	1.73±0.06[a] (**)	1.92±0.09[a] (***)	1.69±0.07[a] (**)	1.71±0.07[a] (**)
	T2	1.70±0.08[b] (**)	1.59±0.06[a] (**)	1.66±0.09[b] (**)	1.39±0.07[b] (**)	1.30±0.06[b] (**)	1.40±0.06[b] (**)
Ca	T1	2.01±0.15[b] (*)	2.44±0.12[a] (*)	2.04±0.09[a] (*)	2.77±0.14[a] (*)	2.13±0.14[a] (*)	2.01±0.11[b] (*)
	T2	2.12±0.13[a] (*)	2.18±0.14[b] (*)	1.96±0.09[b] (*)	2.41±0.12[b] (*)	2.24±0.15[a] (*)	2.18±0.14[a] (*)
Mg	T1	0.246±0.02[b] (*)	0.287±0.02[b] (*)	0.282±0.01[a] (*)	0.328±0.02[a] (**)	0.282±0.01[a] (*)	0.274±0.01[b] (**)
	T2	0.257±0.01[a] (*)	0.309±0.02[a] (**)	0.271±0.01[b] (*)	0.292±0.02[b] (*)	0.268±0.01[b] (*)	0.290±0.02[a] (**)
Fe	T1	110.1±5.2[b] (**)	219.0±8.1[a] (***)	243.0±9.8[a] (***)	98.3±6.1[a] (**)	289.3±7.9[b] (***)	230.1±9.8[a] (***)
	T2	144.0±7.6[a] (***)	215.7 ±9.4[a] (***)	227.1±7.3[b] (***)	87.5±6.5[b] (**)	297.5 ±8.4a (***)	213.0±8.7[b] (***)
Mn	T1	22.7±1.4[b] (*)	69.0±3.5[a] (**)	60.5±4.8[b] (**)	28.1±3.1[a] (**)	68.0±3.8[b] (**)	74.2±4.9[b] (**)
	T2	34.1±2.9[a] (**)	51.2±3.6[b] (**)	75.0±4.9[a] (**)	26.5±2.5[a] (**)	73.1±4.4[a] (**)	83.5±5.3[a] (**)
Zn	T1	58.7±3.2[a] (**)	44.7±2.8[b] (**)	49.1±3.7[b] (**)	45.2±3.2[a] (**)	34.6±2.5[b] (**)	20.8±1.8[b] (*)
	T2	52.6±3.4[b] (**)	49.5±2.5[a] (**)	56.7±4.3[a] (**)	39.3±2.9[b] (**)	37.5±2.4[a] (**)	33.1±2.3[a] (**)
Cu	T1	7.3±0.4[b] (**)	12.2±0.6[b] (**)	10.5±0.8[b] (**)	7.2±0.6[b] (**)	11.6±0.8[b] (**)	9.2±0.07[b] (**)
	T2	12.2±0.5[a] (**)	14.8±0.7[a] (**)	15.5±1.3[a] (**)	14.1±1.2[a] (**)	13.1±0.9[a] (**)	16.8±1.4[a] (**)
N/K	T1	1.55±0.11[b]	1.08±0.07[b] (-N)	1.53±0.09[b]	1.39±0.06[b]	1.85±0.12[b]	1.69±0.13[b]
	T2	1.92±0.15[a]	1.97±0.12[a]	1.91±0.11[a]	2.41±0.17[a] (-K)	2.63±0.16[a] (-K)	2.48±0.15[a] (-K)
N/P	T1	16.9±1.1[b]	11.5±0.8[b]	15.4±1.4[b]	16.1±1.2[b]	20.2±1.5[b]	19.3±1.6[b]
	T2	20.0±1.2[a]	20.0±1.5[a]	19.4±1.3[a]	23.1±1.7[a] (-P)	25.1±1.7[a] (-P)	26.9±1.9[a] (-P)
K/Mg	T1	7.51±0.5[a]	5.72±0.4[a]	6.16±0.5[a]	5.86±0.4[a]	6.00±0.4[a]	6.24±0.5[a]
	T2	6.60±0.5[b]	5.14±0.3[b]	6.09±0.4[a]	4.77±0.3[b]	4.84±0.3[b]	4.85±0.4[b]
Fe/Mn	T1	4.85±0.3[a]	3.14±0.3[b]	4.01±0.3[a]	3.50±0.2[a]	4.27±0.3[a]	3.10±0.2[a]
	T2	4.24±0.3[b]	4.23±0.3[a]	3.03±0.2[b]	3.31±0.2[a]	3.32±0.2[b]	2.55±0.2[b]

Data are means values±SE of four measurements. Values in each column with the same letter are not significantly different ($p = 0.05$) as described by Duncan's test (-): Deficient; (*): Low; (**): Optimum; (***): High; (+): Excess levels according to the guidelines for interpretation of leaf analysis (Hanlon *et al*., 1995)

the N rate. Therefore, if the annual N application rate was more than enough to maintain tree growth and high leaf N concentrations, it caused higher amount of N removal by the crop and a greater impact on N budgets. The giving results of this experiment in field condition confirmed the conclusions from the study of potted nursery citrus plants that the recommended fertilizer analysis for the better growth of young citrus plants was 2N-1P_2O_5-2 K_2O.

CONCLUSION

Similar results were detected concerning the effect of the amount of N applied on leaf nutriments as observed in the greenhouse study. From the economical point of view, using 180N-90P_2O_5-180K_2O kg ha^{-1} is recommended.

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