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NITROGEN, PHOSPHORUS AND POTASSIUM FERTILIZATION INTERACTIONS ON THE PHOTOSYNTHESIS OF CONTAINERIZED CITRUS NURSERY TREES

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☐ *The objective of this research was to evaluate the nitrogen, phosphorus and potassium (NPK) fertilizer effect on the development of 'Valencia' sweet orange nursery trees budded on Rangpur lime rootstocks in a greenhouse using containers. The experiment was a complete (1/5) 5^3 factorial randomized blocks design. Treatments comprised five concentrations (g per plant) of N (1.25; 6.25; 11.25; 16.25; 21.25); K (0.42; 3.75; 6.22; 9.34; 12.45); and P (0.19; 0.89; 1.59; 2.29; 2.99). Carbon dioxide assimilation rate (A), transpiration rate (E), stomata conductance (g_s), and internal carbon dioxide (CO_2) concentration (Ci) and water use efficiency (WUE). Reducing sugar, sucrose and starch were evaluated. Response functions were adjusted and nutrient rates for maximum yield were presented. The results indicated that the high levels of N (> 16.25 g per plant) interferes negatively on photosynthesis. Potassium at intermediate fertilization levels (from 3.75 to 6.22 g per plant) had positive effects on photosynthesis. But P had little interference on photosynthesis. Carbohydrates levels were not related to nutrients fertilized.*

Keywords: gas exchanges, leaf nutrients, carbohydrates levels, fertilizer

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INTRODUCTION

Photosynthesis is a series of linked processes: interception of the incident light and its conversion into chemical energy as (nicotinamide adenine dinucleotide phosphate) NADPH and adenosine triphosphate (ATP), broadcasting of carbon dioxide (CO_2) to the chloroplasts and triose phosphate reduction, the synthesis of the starch in the chloroplasts or sucrose in the cytoplasm and the carrier of the photosynthates to other plant sites (Moorby and Besford, 1983). The main limitations to the photosynthetical process are the light intensity and the CO_2 content. However, the mineral nutrients affect the whole process, and many of them interfere in more than one step (Natr, 1972; Barker, 1979; Longstreth and Nobel, 1980; Moorby and Besford, 1983).

Leaf nitrogen is related to chlorophyll levels, with carbon dioxide assimilation and transpiration rates (Longstreth and Nobel, 1980), besides of carboxylation efficiency (Syvertsen, 1984). These positive correlation between leaf nitrogen and net photosynthesis are due the high nitrogen levels associated to the enzyme responsible for the carboxylation process, the RUBISCO (Ribulose - 1,5 – biphosphate carboxylase/oxygenase). According to Marschner (1995), 20 to 30% of total nitrogen in the leaves of C3-plants are present as RUBISCO.

Syvertsen (1987) related the nitrogen leaf levels with the photosynthetic capacity of orange and pomelo seedlings. Leaf nitrogen was positively correlated with the net CO_2 assimilation, water use efficiency, chlorophyll levels and carboxylation efficiency, but negatively correlated with the compensation point of CO_2.

The results about phosphorus effects on net photosynthesis are not clearly related in the literature (Barker, 1979, Natr, 1972). Syvertsen (1987) also did not find relationship between the phosphorus levels in leaves and photosynthetic capacity of orange and pomelo seedlings.

Phosphorus fixes the C in chloroplasts, since the final product of the Calvin Cycle is the conversion of three molecules of CO_2 into one triose-phosphate. Chloroplasts need inorganic P supply for the maintenance of the rates of C fixation, because there is a stechiometric ratio between P use and triose phosphate production. Then in cytoplasm, the triose-P is transformed into sucrose (Sivak and Walker, 1986). Severe P deficiencies affects the photosynthates transport out of leaves, leading to a starch accumulation (Barker, 1979).

Several authors showed a decrease of photosynthesis and an increase in respiration rate in potassium (K) deficiency. This nutrient regulates the stomatal movement and the CO_2 transfer rates in the leaves. Potassium deficiency leads to a decrease of photophosphorylation. The transport of photosynthesis products is also inhibited by the deficiency of K, causing an interruption in the conversion of intermediary products into sucrose

and a consequent accumulation of soluble carbohydrates, besides sugars reducers. Potassium accumulation reduces the osmotic potential, leading to a reduction in the respiration rate. Potassium is activator of numerous enzymes and, therefore it is requested in numerous metabolic processes (Barker, 1979, Natr, 1972; Huber, 1985).

Kriedman (1968, 1971) described some important characteristics on the photosynthetic activity of citrus leaves that in the last 30 years were still not very clear. These characteristics include the relatively low rates of CO_2 assimilation, stomata conductance and mesophyll conductance, recurrent oscillations on A and E with periodicity of 20 and 40 minutes. Syvertsen and Lloyd (1994) presented several mechanisms of citrus plant response to the environment, based on water and of C flows.

The objective of this work was to evaluate the effect of the nitrogen, phosphorus and potassium (NPK) fertilization on the photosynthesis and carbohydrates content of Rangpur lime rootstock and 'Valencia' orange budded on Rangpur lime nursery trees.

MATERIALS AND METHODS

'Valencia' sweet orange nursery trees (*Citrus sinensis*) budded on Rangpur lime rootstocks (*Citrus limonia*) were grown in insect-proof screenhouse without light, temperature or humidty control. The commercial growing media Plantmax® (Pinus bark, vermiculite and perlite mixture) was used. The substrate showed the chemical characteristics: P (resin) 48 mg dm^{-3}; organic matter, 731.7 g dm^{-3}; pH (CaCl$_2$) 5.2; K, 2.8 mmol$_c$ dm^{-3}; calcium (Ca), 18.8 mmol$_c$ dm^{-3}; magnesium (Mg), 19.5 mmol$_c$ dm^{-3}; hydrogen plus aluminum (H+Al), 27.2 mmol$_c$ dm^{-3}; cation exchange capacity, 68.3 mmol$_c$ dm^{-3}; basis saturation, 60.2%; copper (Cu), 0.4 mg dm^{-3}; iron (Fe), 13.6 mg dm^{-3}; manganese (Mn), 17.1 mg dm^{-3} and zinc (Zn) 1.4 mg dm^{-3}.

The experiment was a $(1/5)$ 5^3 factorial randomized block design (Conagin and Jorge, 1982). Treatments comprised five concentrations (g per plant) of N (1.25; 6.25; 11.25; 16.25; and 21.25); K (0.42; 3.75; 6.22; 9.34; and 12.45); and P (0.19; 0.89; 1.59; 2.29; and 2.99). Nitrogen, P, and K sources were ammonium nitrate (NH$_4$NO$_3$), potassium chloride (KCl) and super triple phosphate, respectively. Nitrogen and K fertilizers were applied in a water solution supplied every week. The total amount of N and K fertilizer were split as: 37.5% to rootstocks and rest to the grafted plants. All P fertilizer was supplied by mixing it to the growing media before the rootstocks transplanting. Physiological parameters in Rangpur lime rootstock and 'Valencia' sweet orange were evaluated 120 days after transplant and 180 days after budding.

The following parameters were measured: net CO_2 assimilation (A; μmol CO_2 m^{-2} s^{-1}), stomata conductance (g; mmol water (H$_2$O) m^{-2} s^{-1}), water

use efficiency (WUE), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), and internal CO_2 concentration (Ci; μmol CO_2 mol^{-1} air). Water use efficiency was determinate by A E^{-1} ratio. A portable photosynthetic analyzer system based on infrared radiation (PPS LI-6200 Licor, Inc. Lincon, NE, USA) was used to physiological measurements. The evaluations were done in intact nodetached youngest fully expanded leaf of the rootstocks and grafted plants, in a chamber with controlled radiation (700 μmol photons m^{-2} s^{-1}), temperature (25°C) and external CO_2 concentration (350 μmol CO_2 mol^{-1} air).

Leaf diagnosis for macronutrients was done in the same leaf (full youngest expanded leaf - FYEL), sampled after measurements. Following Sarruge and Haag (1974), in a sulfuric acid extract the concentration of N in leaves were determined by Kjeldahl method. Phosphorus and K were determined in the nitro-perchloric acid extract, respectively by colorimetric and flame-photometric methods.

Carbohydrates determination in plant tissues were analyzed in triplicate, with 30 mg of frozen and dry milled (40 mesh) leaves and roots. Extractions were done with 15 mL of boiling water in 2 min and centrifuged at 800 g for 2 min, as described by Eissensat and Duncan (1992). The supernatant was used in reduced sugar (RS) determination using arsenomolibidate according to Nelson (1944). Sucrose (Suc) was determinated using antrone plus sulfuric acid after the alkaline treatment as described by Van Handel (1968). Soluble and insoluble starch (St) were determined in supernatant and in the precipitate, respectively using amiloglucosidase after the correction of free glucose.

The variance analysis was undertaken for all parameters, as a function of nutrients levels and response functions were adjusted (Tables 1 and 2). The statistical analyses were carried out using the SAS statistical software (SAS Institute, Cary, NC, USA) and followed Pimentel-Gomes (1984). Functions were simplified, when 1 or 2 nutrients were considered significant (Table 3). Correlation (r) between photosynthetic parameters and nutrients levels in leaves were also undertaken.

RESULTS AND DISCUSSION

Table 3 shows the regression coefficients of the adjusted response functions for significant terms obtained for net CO_2 assimilation rate (A), transpiration (E), stomata conductance (g), and water use efficiency (WUE) in the youngest fully expanded leaves of Rangpur lime rootstocks and 'Valencia'/Rangpur lime as function of NPK fertilizer level. Carbohydrate levels were not affected by nutrient levels used in the fertilization.

There was an interaction between N and K fertilization on CO_2 assimilation (A) of Rangpur lime rootstock and 'Valencia' orange nursery tree. The

TABLE 1 Surface response equation for net CO_2 assimilation (A); stomata conductance (g); water use efficiency (WUE); transpiration rate (E) in the youngest fully expanded leaf of **Rangpur lime** rootstock and 'Valencia'/Rangpur lime as function of NPK fertilization levels

Variable	γ_0	N	N^2	P	P^2	**K**	K^2	NP	NK	**PK**	$F^{(1)}$
						Rangpur lime					
A	6.941	−1.135	0.052	−0.635	0.317	2.705	−0.486	0.273	0.03	−0.429	$N_L*K_{LQ}**$
G	0.149	−0.023	0.0009	0.105	−0.023	0.002	−0.002	−0.0009	0.006	−0.008	$*NK*$
Ci	254.51	5.258	0.048	32.378	−7.266	−32.853	5.108	−4.843	1.115	3.171	n.s.
E	0.003	−0.0002	0.000003	0.0006	−0.0001	0.0002	−0.00006	0.00004	0.00004	−0.0001	$NK*$
WUE	2387.5	−264.459	16.372	−520.333	151.793	802.221	−126.217	80.961	−19.156	−79.427	$K_{LQ}**$
						'Valencia'/Rangpur lime					
A	7.584	−0.418	0.005	−0.212	0.228	0.728	−0.054	0.116	0.006	−0.16	$N_L*K_{LQ}*$
G	0.167	−0.09	0.0002	0.083	−0.017	0.002	−0.0005	0.0001	0.0007	−0.003	$NK*$
Ci	244.38	2.257	0.05	17.776	−3.672	−5.319	0.271	−1.854	0.037	1.312	n.s.
E	0.003	−0.0001	0.000001	0.0004	−0.00007	0.00007	−0.000006	0.00002	0.000004	−0.00004	K_Q***
WUE	2499.8	−86.75	0.685	−198.898	75.633	203.135	−14.231	−0.307	33.866	−30.911	$K_{LQ}**$

[1] L and Q indicate linear and quadratic significant effects, respectively. N.S. indicates not significant.

TABLE 2 Surface response equation for reducers sugar (RS), sucrose (Suc) and starch (St) in the youngest fully expanded leaf and roots of Rangpur lime and 'Valencia'/Rangpur lime as a function of NPK fertilizer level

Variable	γ_0	N	N^2	P	P^2	K	K^2	NP	NK	PK	$F^{[1]}$
					Rangpur lime						
RS_{leaf}	1.462	0.192	−0.27	−0.049	0.225	−0.442	0.11	−0.0007	0.008	−0.198	n.s.
Suc_{leaf}	6.095	−0.279	0.026	−0.514	−0.231	1.032	−0.245	0.08	−0.015	0.292	n.s.
St_{leaf}	1.603	0.13	−0.003	0.089	0.015	−0.200	0.043	−0.057	−0.014	0.015	n.s.
RS_{root}	1.614	−0.075	0.002	−0.041	0.044	0.059	−0.005	0.027	0.011	−0.6	n.s.
Suc_{root}	2.539	0.115	−0.02	0.131	0.086	−0.293	0.07	0.014	0.011	−0.128	n.s.
St_{root}	4.797	−0.006	0.003	0.124	−0.211	0.177	−0.086	−0.043	0.017	0.204	n.s.
					'Valencia'/Rangpur lime						
RS_{leaf}	0.778	0.045	−0.002	−0.019	0.166	−0.076	0.009	−0.0003	0.009	−0.55	n.s.
Suc_{leaf}	4.03	−0.086	0.003	−0.114	−0.155	0.107	−0.01	0.009	−0.006	0.045	n.s.
St_{leaf}	0.918	0.0008	0.003	−0.014	0.008	−0.010	−0.003	−0.003	0.0008	0.0035	n.s.
RS_{root}	0.872	0.025	−0.002	−0.083	0.065	−0.038	0.003	−0.002	0.0013	−0.011	n.s.
Suc_{root}	1.309	−0.009	−0.0002	0.293	−0.012	−0.017	0.0009	−0.0026	0.0036	0.024	n.s.
St_{root}	1.999	−0.012	0.0009	−−0.004	−0.055	0.01	−0.002	−0.004	−0.0005	0.023	n.s.

(1) N.S. indicates not significant.

TABLE 3 Adjusted equation for net CO_2 assimilation (A); stomata conductance (G); water use efficiency (WUE); transpiration rate (E) in Rangpur lime rootstock and 'Valencia'/Rangpur lime nursery tree as function of NPK fertilization levels

Variable	Equation	R^2
	Rangpur lime	
A	$Y = 0.052N^2\text{-}1.083N\text{-}0.486K^2+2.623K+0.03NK+6.832$	0.263
G	$Y = 0.001N^2\text{-}0.023N\text{-}0.002K^2+0.001K+0.006NK+0.165$	0.129
E	$Y = \text{-}0.102P^2+0.572P\text{-}0.061K^2+0.266K\text{-}0.114PK+2.616$	0.193
WUE	$Y = 0.016N^2\text{-}0.249N\text{-}0.126K^2+0.787K\text{-}0.019KP+2.304$	0.278
	'Valencia'/Rangpur lime	
A	$Y = 0.005N^2\text{-}0.396N\text{-}0.054K^2+0.698K+0.006NK+7.5522$	0.245
G	$Y = 0.0002N^2\text{-}0.009N\text{-}0.0005K^2+0.001K+0.007NK+0.18$	0.124
E	$Y = \text{-}0.0059K^2+0.066K+2.857$	0.174
WUE	$Y = \text{-}0.0142K^2+0.197K+2.365$	0.363

highest N levels used in fertilization caused a decrease in A. The largest CO_2 assimilation were observed in the intermediary K levels and in the lowest N levels (0.47 and 1.25 g for plant). The positive role of N and K supply on this parameter were described by Longstreth and Nobel (1980). The maximum CO_2 assimilation rates observed (10 mmol m^{-2} s^{-1}) were similar to that found by other authors, measured under favorable conditions (Syvertsen, 1984; Medina and Machado, 1998).

There are other N effects on the photosynthesis besides its participation in the complexes of proteins and of chlorophyll in the chloroplasts. A decrease on carbon dioxide assimilation was observed with increasing N levels. This may be related to the N-fertilizer source used (e.g. ammonium nitrate - NH_4NO_3). Thus high levels of ammonium accumulated in cells may have caused severe damages such as proteolysis, structural and functional alterations of membranes, inhibition of the chlorophyll synthesis, chlorophyll destruction, reduction of the oxidative photophosphorylation, chloroplast structure degradation, and carbohydrate decrease (Barker, 1979; Gil and Gonzalo, 1993).

Ammonium accumulation in the plants inhibits glucose transport (Bassham et al., 1981). On the other hand nitrate (NO_3) up take and assimilation request energy and the use of reducer agents that would be used on CO_2 process and fixation (Barker, 1979). Syvertsen and Lloyd (1994) confirmed that the electronic transport can also diminish A.

Rangpur lime transpiration rate (E) was influenced by potassium and phosphorus fertilization. The highest value (3.51 mmol m^{-2} s^{-1} H_2O) was obtained in the lowest K level and highest P level (0.47 and 2.99 g for plant, respectively). In the nursery trees, the supply of K increased E until 3.04 mmol m^{-2} s^{-1} H_2O, which were obtained with 5.59 g by plant. Several reports exist in the literature showing the smallest transpiration rates of plants showing K deficiency (Huber, 1985). According to Sinclair and Allen

(1982), under normal conditions of citrus growth, transpiration is stabilized around 3.5 and 5 mmol m^{-2} s^{-1}.

Reductions in the photosynthesis due to moderate K deficiency are initially related to increases in the mesophyll resistance (Huber, 1985). Increases in the stomatal resistance are related with severe deficiencies of the nutrient, as demonstrated by Natr (1972) and Logstrech and Nobel (1980). The results for rootstocks and nursery trees showed the effect of N on stomatal conductance. Increasing K supply increased stomatal conductance, while the amounts of N had the opposite effect, decreasing this parameter.

Phosphorus had little influence in the photosynthesis as it only affected the rootstock transpiration. The role of this nutrient in the photosynthesis is variable and generally is smaller than the other macronutrients (Barker, 1979; Natr, 1972) and this was due to an increase of the mesophyll resistance to the CO_2 transfer (Natr, 1972).

Leaf epidermis reduces water vapor and CO_2 losses and also control the stomatal assimilation and transport. The function of stomata is to control the balance of losses water and carbon acquisition or biomass production. Stomata conductance measurement was relevant in this study, because this cultivation system aims the optimization of the water use and the plants were cultivated in containers with limited substrate volume for root growth.

Rootstocks showed interaction between the N and K supply. For nursery trees K supply increased WUE up to 6.9 g of K per plant showing 3,046 μmol mol^{-1}. Those results were expected due to macronutrients functions in photosynthesis (Barker, 1979; Huber, 1985, Marschner, 1995). The values of WUE are lower than those reported by Syvertsen (1985), whose maximum values were close to 8 μmol mol^{-1}.

A correlation study among the variables related to the photosynthesis was carried out. The high coefficients (r) indicate the existence of an interdependence among these factors. Leaf conductance may influence the photosynthesis directly interfering the available concentration of CO_2 for the carboxylation. Variations in the leaf resistance induced by the humidity variation or the stomatal opening were related with photosynthesis and transpiration of ' Washington Navel' and 'Valencia' leaves (Kriedemann, 1971). Figure 1 exhibits the quadratic relationship between A and g. The largest values (A = 10.0 and 10.7 μmol m^{-2} s^{-1} CO_2) were obtained with g in 0.3 mmol m^{-2} s^{-1} H_2O. Khairi and Hall (1976) also observed a narrow relationship between conductance and photosynthesis in orange tree leaves.

Figure 1B shows a positive and direct correlation between CO_2 assimilation and transpiration rate. Gama-Rodrigues et al. (1995) found the same tendencies in cacao nursery trees. In the containerized citrus nursery trees production due to restricted volume for root growth, it would be desirable that the high rates of net photosynthesis were accompanied by a regulation on water losses, to avoid water deficit. Even so, the water losses regulation are obtained by stomatal closing that also reduces the photosynthesis
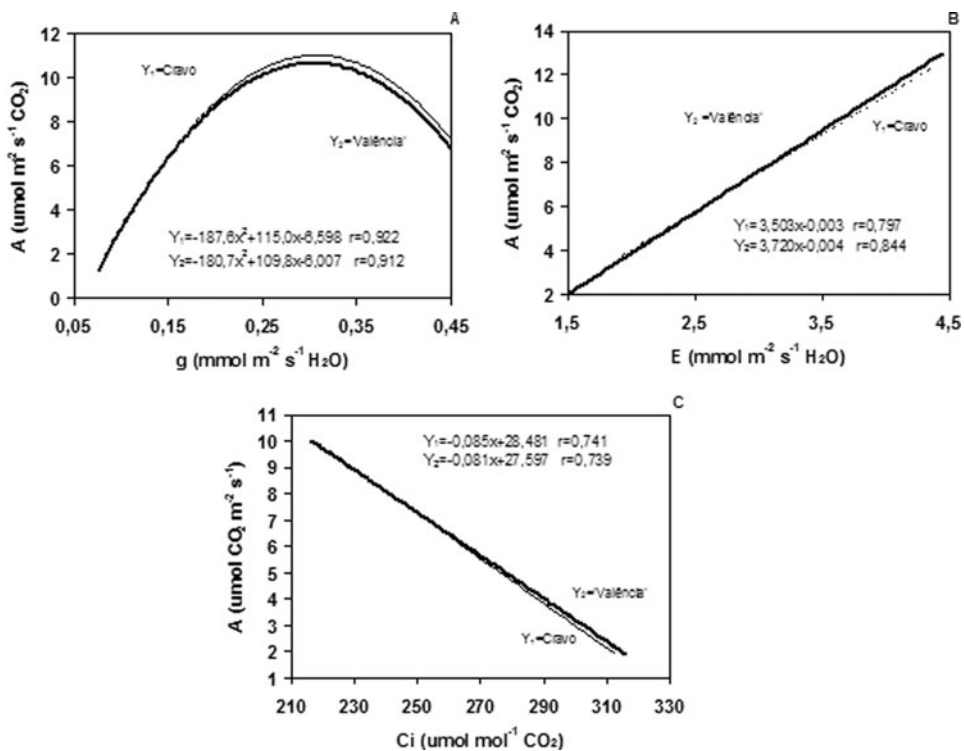
FIGURE 1 Relation of CO_2 assimilation - A with A) stomata conductance - g; B) transpiration rate - E; and C) CO_2 internal concentration - Ci.

(Kriedmann, 1971; Khairi and Hall, 1976). An alternative would be to increase the non-stomatal aspects of the photosynthesis, through the increase of the protein production (Khairi and Hall, 1976).

Carbon dioxide internal concentration indicates which factors are limiting the assimilation of CO_2. When Ci decreases, it indicates that the limitations are related to stomatal movement, and when Ci increases the limitations are related to the leaf mesophyll (Long and Halgren, 1987). Results showed in the Figure 1C, indicate the inverse relationship between A and Ci. This negative relationship suggests that there are no limitations to the stomatal operation and a decrease in RUBISCO activity occurred, leading to a regeneration reduction. One factor that can also limit the CO_2 assimilation is the RUBISCO activity (Syvertsen and Lloyd, 1994). Assimilation of CO_2 was probably limited by this factor, leading to a smaller supply of RuBp for carboxylation and, in agreement with Farquhar et al. (1980). This can be the reason for the decrease in the CO_2 assimilation with the increasing amounts of N.

The correlation among N, P and K levels in the leaves of the rootstocks and the nursery trees with the parameters of the photosynthesis were established. Considering all the N and K amounts in the leaves of the Rangpur

lime no adequate correlation was found, probably due to high nutrient up-take and high fertilization levels used in this study.

CONCLUSIONS

The results indicated that the high levels of N (>16.25 g per plant) interferes negatively on photosynthesis. Potassium at intermediate fertilization levels (from 3.75 to 6.22 g per plant) had positive effects on photosynthesis. But P had little interference on photosynthesis. Carbohydrates levels were not related to nutrients fertilized.

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