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INFLUENCE OF VARIOUS PHOSPHORUS AND POTASSIUM RATES ON JUICE VITAMIN C, β-CAROTENE, LYCOPENE AND SUGAR CONCENTRATIONS OF FLAME GRAPEFRUIT

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Abstract. **Flame grapefruit trees on Swingle citrumelo rootstock were planted in 1998. Phosphorus fertilizer was applied at 0, 48 (optimal), and 96 (high) kg P/ha in 1999-2000 to establish a range of soil-test P, and none was applied since Spring 2000. Potassium fertilizer was applied every year since planting at 0, 186 (optimal), and 372 (high) kg K/ha. The P and K treatments were applied in all factorial combinations. Nitrogen and other cultural practices followed the recommendations (www.CREC.IFAS.UFL.EDU) for citrus production. Fruit were harvested in January 2003 and 2004 equally from all sides of the tree from 5 blocks totaling 25 trees. Harvested fruit were hand-juiced and assayed for vitamin C, pigments, and sugar by an HPLC system. The results indicated that vitamin C and total sugar (sum of glucose, fructose, and sucrose) was higher in year 2004 than 2003 fruits. The highest vitamin C and sugar levels were in the treatment block without P and optimal K (0 × 186) while the lowest was found in the optimal P and non-K (48 × 0) block among the five treatments. This trend was reflected in sucrose concentration, but not in fructose and glucose concentrations in fruit juice. In turn, β-carotene and lycopene were the highest in the optimal P and non-K (48 × 0) treatment during year 2003 and 2004, respectively. No significant difference was found among the rest of treatments. The study demonstrated that high level of P or K does not increase the fruit vitamin C, pigments, and sugar concentrations. Since Flame grapefruit is characterized and perceived by consumers as being rich in antioxidant compounds, and high in nutritional value, consideration should be given to using only the optimal fertilization rates. Increasing P and K fertilization rates does not increase the fruit internal nutritional value of the desired and healthful compounds found in Flame grapefruit.**

It is well known that phosphorus (P) and potassium (K) influence citrus fruit internal and external quality. Nagy (1980) reported that potassium affects fruit quality more so than fruit yield. Generally, a high K fertilization rate increases vitamin C in lemons, oranges, and grapefruit (Embleton and Jones, 1966; Reitz and Koo, 1960; Smith and Rasmussen, 1960). High K fertilization rate also increases fruit juice and juice acid concentration in grapefruit (Smith and Rasmussen, 1960). Research has confirmed that K nutrition greatly influences the fruit's external quality characteristics, such as fruit size, color, peel thickness, and disorder incidence (Quaggio et al., 2000; Rodriguez et al., 2000). However, P is seldom a factor in fruit quality (Agustí, 1999). Tucker et al. (1995) summarized P and K effects on fruit quality and reported that P application increases fruit sugar/acid ratio by reducing grapefruit acid levels, increasing number of green fruit, and reducing peel thickness. K application decreases fruit juice content, solids, ratio and juice color, while increasing acid content and fruit size/thickness. P and K fertilization rate is the key factor influencing fruit quality. The best P and K management for citrus has been developed for young citrus trees by Obreza (2001); and for bearing citrus trees by Tucker et al. (1995).

Lycopene, sugar, and vitamin C are key nutritional attributes in grapefruit. The changes of vitamin C and sugar concentrations in citrus products have been studied in the past with regard to the effects of processing technology, package, and storage conditions (Nagy and Attaway, 1980). Also, lycopene and β-carotene in grapefruit have been evaluated by Lee (2000). A search of the literature reveals that there are limited reports regarding the influence of P and K fertilization rate on Flame grapefruit vitamin C, lycopene, and sugar concentrations. Recently, the demand for pigmented grapefruit such as 'Flame' has increased because of its lycopene content, which has been found to have health benefits. The objective of the current study is to investigate the influence of P and K fertilization rates on fruit lycopene, vitamin C, and sugar concentrations in Flame grapefruit.

Materials and Methods

Plant materials. 'Flame' grapefruit trees on Swingle citrumelo rootstock were planted in 1998. Phosphorus fertilizer was applied at 0, 48, and 96 kg P/ha in 1999-2000 to establish a range of soil-test P, and none was applied since Spring 2000. Potassium fertilizer was applied every year since planting at 0, 186, and 372 kg K/ha. The P and K treatments were applied in all factorial combinations. Nitrogen and other cultural practices followed the recommendations (www.crec.ifas.ufl.edu) for citrus production. Fruit were harvested in January 2003 and 2004 equally from all sides of the tree from 5 blocks totaling 25 trees.

Analysis of juice Brix and acid. Degree Brix of juice was measured using a LEICA MARK II (Leica, Buffalo, NY, USA) table-model refractometer with temperature and acid corrections. Percent analysis citric acid was determined by titration with standard alkali using phenolphthalein indicator.

Measurement of fruit and juice color. Fruit color was measured in 10 fruit using a Minolta Colorimeter. Tristimulus val-

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ues (X, x, and y) were converted to Chroma (0 = lowest intensity, 100 = greatest intensity), and hue angle (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue) according to McGuire (1992). Fruit juice color was measured in a Macbeth Color-Eye 3100 spectrophotometer (Kollmorgh Instruments Corporation) according to Lee (2000).

Analysis of fruit vitamin C. Vitamin C was analyzed with an HPLC system coupled with an YMC ODS-AO guard column using a model spectra 200-uv/visible detector. Two milliliters of juice were mixed with 2 mL 2.4% (w/v) metaphosphoric acid, and centrifuged at 6500 rpm for 5 min at 5°C. After that, an aliquot of sample (0.5 mL) was transferred to a 10 mL volumetric flask and brought to volume with 2.5% (w/v) metaphosphoric acid. The sample was filtered through a 0.45 μm nylon syringe filter (Fisher Scientific, Pittsburgh, Pa.) prior to injection.

Analysis of juice sugar concentration. Fruit juice sugar concentration was analyzed by an HPLC system using a Mac-Mod Analytical NH_2 column coupled with a refractive index detector (Hewlett Packard, Calif.). Approximately 6 mL juice were centrifuged for 5 min at 6500 rpm. Three milliliters of juice were passed through a water C_{18} Sep-Pack cartridge that had been conditioned by rinsing with 3 mL of methanol followed by rinsing with two individual rinses of 3 mL deionized water. The first 1.5 mL of juice passed through the cartridge was discarded and the remainder of the juice was collected. Ten-μL samples were injected into the HPLC system automatically.

Analysis of juice pigments. Approximately 150 mL juice was homogenized for 1 min using an Omni mixer/homogenizer. Homogenization causes air to be introduced during the homogenization process, therefore, juice was degassed in a sonicator under vacuum to remove air. In a darkened room, a centrifuge tube containing 5 mL of extraction solvent (hexane/acetone/ethyl alcohol, 50:25:25) and 2 mL of homogenized juice was centrifuged at 6500 rpm for 5 min at 5°C in a refrigerated centrifuge. The top hexane layer containing the pigments was pipetted into an autosample vial for injection. Carotenoids were analyzed by injecting 10 μL aliquots of sample onto a YMC C30 column, 4.6 × 150 mm, 5 μm, with inline filter. Carotenoids were eluted by gradient analyses and monitored at 450 nm.

Statistical analysis. Study was organized in a completely randomized block design. Data were analyzed by ANOVA using PlotIt (Scientific Programming Enterprises, Haslett, Mich.). Means were separated by Duncan's new multiple range test at $P \leq 0.05$.

Results

Color. Fruit color parameters (L, C, and H) are clearly lower in the treatments of optimal P * low K (48 * 0 kg/ha) than the rest of the treatments (P * K: 48 * 186, 48 * 372, or 96 × 372) in the 2002-03 season. In the 2003-04 season, the fruit also developed better color at low P and optimal K treatment (29 × 186 kg/ha) in comparison to the rest of treatments. This trend is only occurred in juice color in the term of hue Angle. In most cases, the differences among the rest of treatments are not statistically significant (Tables 1 and 2).

Carotene. In contrast to fruit color results, the lycopene concentration in fruit is higher in the 2 year study in the treatments of 48 P × 0 K (OPT P × non-K) and 0 P × 186 K (non-P × OPT K) kg·ha^{-1} than the other three treatments as described above. Surprisingly, lycopene concentrations were significantly lower in optimal and high P and K combinations (Table 2). β-carotene concentration in juice was 6 times lower than the lycopene concentration which showed no significant difference among the treatments except 48 P × non-K (OPT P × 0 K) kg/ha treatment.

Table 1. Influence of P and K fertilization rate on Flame grapefruit color 5 d after harvesting.[z]

	N × K rate[y] (kg·ha^{-1})	Lightness	Chroma	Hue angle
		2002-2003 season		
1 OPT P OPT K	(48 × 186)	72.1 c[x]	53.8 c	89.2 bc
2 OPT P Low K	(48 × 0)	67.8 a	46.9 a	78.2 a
3 OPT P High K	(48 × 372)	71.9 bc	52.3 bc	90.6 c
4 Low P OPT K	(0 × 186)	70.7 bc	49.8 ab	85.9 b
5 High P OPT K	(96 × 372)	69.9 b	50.7 b	85.5 b
		2003-2004 season		
1 OPT P OPT K	(48 × 186)	69.3 b	49.0 b	82.6 cd
2 OPT P Low K	(48 × 0)	67.4 a	48.0 a	79.5 ab
3 OPT P High K	(48 × 372)	70.8 c	50.9 d	85.5 e
4 Low P OPT K	(0 × 186)	67.9 a	48.6 ab	78.4 a
5 High P OPT K	(96 × 372)	69.8 bc	49.1 bc	81.1 bc

[z]10 fruit were each measured along the equator at four locations at 90° angle apart. Total of 40 measurements were made.
[y](1 kg·ha^{-1} = 0.892 lb/acre).
[x]Column means followed by the same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

Vitamin C. In parallel to the previous color and lycopene results, P × K application at (148 × 0 kg·ha^{-1}) (OPT P × non-K) significantly reduced the vitamin C concentration in comparison to the other four treatments. In contrast, non P × optimal K fertilization (0 × 186 kg·ha^{-1}) enhanced the juice vitamin C concentration more than OPT P × non-K rate (48 × 93 kg·ha^{-1}) in 2 years studies (Table 3). The current studies also indicated a similar trend of vitamin C and juice internal Brix. However, vitamin C was negatively correlated to juice acid in 2002-03, while a positive relationship was seen in 2003-04 season (Table 3).

Sugar. Juice internal sugar concentrations were significantly different among the P × K treatments. Similar to the vitamin C concentrations, the optimal P × non-K fertilization rate at 48 × 0 kg·ha^{-1} resulted in the lowest total sugar, while the non-P × optimal K at 186 treatment had the highest internal total sugar concentration. These results were clearly reflected in the juice sucrose concentrations. Fructose and glucose concentrations are higher in P × K rate of 48 × 0 or 0 × 186 kg·ha^{-1}, which is similar to the results of juice lycopene analysis (Tables 2 and 4).

Discussion

Generally, grapefruit juice is well-known for reducing strokes, some cancers, and lowering cholesterol, perhaps because of the high antioxidant levels in grapefruit and grapefruit juice (Nagy and Attaway, 1980). Some new grapefruit cultivars ('Flame', 'Rio Red', 'Ray Ruby', and 'Star Ruby') were released a decade ago because of the demand for red grapefruit types and reported health benefits associated with the red pigments (Dou et al., 2004; Nagy and Attaway, 1980; Saunt, 2000). Limited research has been done on these highly pigmented grapefruit cultivars. The present study confirms

Table 2. Influence of P and K fertilization rate on juice color, β-carotene and lycopene of Flame grapefruit, day 3.[z]

	N × K rate[y] (kg·ha^{-1})	L	a	b	Hue	Chroma	β-carotene (mg/L)	Lycopene (mg/L)
					2002-2003 season			
1 OPT P OPT K	(48 × 186)	36.4 b[x]	5.6 a	5.6 ab	45.2 c	8.0 ab	1.58 a	9.6 ab
2 OPT P Low K	(48 × 0)	36.3 ab	5.9 c	5.6 ab	43.6 a	8.2 c	1.72 b	9.9 c
3 OPT P High K	(48 × 372)	36.1 a	5.7 b	5.5 a	43.4 ab	7.9 a	1.62 a	9.6 b
4 Low P OPT K	(0 × 186)	36.6 b	5.7 b	5.7 b	44.7 bc	8.1 abc	1.60 a	9.9 c
5 High P OPT K	(96 × 372)	36.5 b	5.6 a	5.9 c	46.5 d	8.1 bc	1.60 a	9.4 a
					2003-2004 season			
1 OPT P OPT K	(48 × 186)	34.3 a	5.1 a	5.0 a	44.3 c	7.2 a	1.88b	8.93 a
2 OPT P Low K	(48 × 0)	34.4 a	5.9 d	5.3 c	41.9 a	8.0 d	1.66 a	10.95 d
3 OPT P High K	(48 × 372)	35.1 b	5.4 b	5.1 b	43.5 b	7.5 b	2.03 b	9.42 b
4 Low P OPT K	(0 × 186)	34.2 a	5.5 bc	5.3 c	43.9 bc	7.7 bc	1.99 b	9.78 c
5 High P OPT K	(96 × 372)	35.3 b	5.2 a	5.0 a	43.9 bc	7.2 a	1.90 b	8.88 a

[z]10 fruit were each measured along the equator at four locations at 90° angle apart. Total of 40 measurements were made.
[y](1 kg·ha^{-1} = 0.892 lb/acre).
[x]Column means followed by the same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

Table 3. Influence of P and K fertilization rate on juice Brix, acid, and vitamin C concentrations of Flame grapefruit, day 3.[z]

	N × K rate[y] (kg·ha^{-1})	Vit. C (mg/100 mL)	Brix	Acids (%)
		2002-2003 season		
1 OPT P OPT K	(48 × 186)	32.9 c[x]	7.8 a	0.90 b
2 OPT P Low K	(48 × 0)	27.7 a	7.7 a	0.95 d
3 OPT P High K	(48 × 372)	32.1 b	8.0 b	0.94 c
4 Low P OPT K	(0 × 186)	36.9 e	8.1 b	0.85 a
5 High P OPT K	(96 × 372)	33.4 d	8.0 b	0.90 b
		2003-2004 season		
1 OPT P OPT K	(48 × 186)	38.0 b	8.5 b	0.82 a
2 OPT P Low K	(48 × 0)	32.4 a	8.0 a	0.86 c
3 OPT P High K	(48 × 372)	39.3 c	8.6 bc	0.84 b
4 Low P OPT K	(0 × 186)	40.0 c	8.9 d	0.91 d
5 High P OPT K	(96 × 372)	39.2 c	8.5 b	0.83 b

[z]10 fruit were juiced, Brix and acid were analyzed on the same day. Vitamin C was analyzed on the second day.
[y](1 kg·ha^{-1} = 0.892 lb/acre).
[x]Column means followed by the same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

that various P and K fertilization rates influence the concentrations of important nutrients in Flame grapefruit. Generally, a high P and K application does not increase fruit lycopene and juice color while this fertilizer application increases fruit sucrose concentration. The vitamin C concentration is the highest when trees are fertilized with non-P and optimal K (0 × 186 kg·ha^{-1}). In contrast, vitamin C concentration is the lowest at treatments with optimal P and non-K treatment (48 × 0 kg·ha^{-1}). Ahmed et al. (1988) reported an increase of vitamin C by N × P × K applications in lime, while Mann and Sandhu (1988) found a vitamin C increase by K, but not by P application in Kinnow Mandarin. There are many reports regarding fertilization rates on lemon, lime, and mandarins, while only found few papers have addressed the effects of P and K on grapefruit quality. Bar-Akiva et al. (1968) found a positive relationship between P and fruit vitamin C content. The authors further reported improved color and decreased acid by P application; however, no results were reported on the influence of P × K rate on vitamin C concentration. Hipp and Shull (1976) did not find any N, P, and K effect on fruit Brix/acid ratio, and acidity.

Table 4. Influence of P and K fertilization rate on juice fructose, glucose, sucrose, inositol, total sugar and glucose/fructose ratio of Flame grapefruit, day 5 after harvesting.[z]

	N × K rate[y] (kg·ha^{-1})	Fructose (g/100 mL)	Glucose (g/100 mL)	Sucrose (g/100 mL)	Inositol (g/100 mL)	Total sugar (g/100 mL)
				2002-2003 season		
1 OPT P OPT K	(48 × 186)	1.56 a[x]	1.45 a	2.81 c	0.055 b	5.88 b
2 OPT P Low K	(48 × 0)	1.63 b	1.57 c	2.45 a	0.051 a	5.69 a
3 OPT P High K	(48 × 372)	1.60 ab	1.51 b	2.93 d	0.064 c	6.10 c
4 Low P OPT K	(0 × 186)	1.74 c	1.55 c	2.73 b	0.051 a	6.06 c
5 High P OPT K	(96 × 372)	1.57 a	1.49 b	2.88 cd	0.061 c	6.00 bc
				2003-2004 season		
1 OPT P OPT K	(48 × 186)	1.87 a	1.76 a	2.42 b	0.043 b	6.09 b
2 OPT P Low K	(48 × 0)	2.12 c	1.90 bc	1.67 a	0.042 ab	5.73 a
3 OPT P High K	(48 × 372)	2.05 b	1.88 b	2.68 c	0.041 a	6.65 d
4 Low P OPT K	(0 × 186)	2.15 c	1.98 c	2.53 bc	0.047 c	6.71 d
5 High P OPT K	(96 × 372)	2.01 b	1.86 b	2.43 b	0.048 c	6.35 c

[z]10 fruit were juiced and analyzed on day five.
[y](1 kg·ha^{-1} = 0.892 lb/acre).
[x]Column means followed by the same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

The current study demonstrates an effect of P and K on fruit internal Brix, acid, vitamin C, sugar, and lycopene concentrations, and color development. Optimal P and non-K resulted in the lowest Brix and high acids in 'Flame' grapefruit, while lowest acid was found in non-P and optimal K treatment. These results are also reflected in the vitamin C contents as discussed above. Fruit and juice color developed better in optimal P and non-K treatment. This color development is negatively correlated to juice vitamin C and total sugar concentrations (Tables 1-4). Bar-Akiva et al. (1968) reported a juice color improvement by P application. The current study is the only one we have found in literature to address the P and K combinations on juice vitamin C, sugar, and lycopene concentrations. Clearly, non-P and optimal K is the best treatment in increasing juice vitamin C and sugar concentration among the five treatments.

As mentioned above, juice color was better in optimal P and low K treatment, which is reflected in juice lycopene and β-carotene concentrations. Both, lycopene and β-carotene are negatively correlated (Table 2). Lee (2000) measured 90 red grapefruit juices and concluded that lycopene and CIE A* (a color parameter) are best correlated. This is in agreement with our studies (Table 2) in which lycopene and color are positively related. Lycopene is 6 times higher in juice than β-carotene. So lycopene is a color indicator in addition as a major antioxidant in grapefruit. There are similar trends in levels of juice sugar and Brix at five treatments. Interestingly, high fructose was found in non-P and optimal K while low sucrose was found in optimal P and non-K treatments.

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