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Comparison of Controlled Release Fertilizer (CRF) for Newly Planted Sweet Orange Trees under Huanglongbing Prevalent Conditions

Tripti Vashisth* and Jude Grosser
Department of Horticultural Sciences, Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA

Abstract

The Huanglongbing (HLB) endemic has negatively impacted the Florida citrus industry. Currently, 80% to 90% of groves are affected by HLB, and there is yet to be a cure. Over the last few years, several preliminary studies have suggested that a constant supply of nutrients, preferably, all year round, is beneficial for HLB affected trees. These trees tend to look healthier, and produce more yield as compared to trees that do not receive optimal nutritional care. For decades, the use of controlled release fertilizer (CRF) has been demonstrated to be the most efficient strategy that provides constant nutrition to plants all year-round. However, citrus growers are confronted with a variety of CRF options without adequate decision-making information to make the most informed choice with limited budget. Our study was aimed at providing guidance to citrus farmers with regards to the CRF formulations that would be effective under HLB prevalent conditions. We compared five different CRF formulations that are popular among growers, and from three different manufacturers. We tested these formulations on the growth and development of a new plant of *Citrus sinensis* cultivar, Valquarius sweet orange, on rootstock US-897 under prevalent HLB conditions. The trial was carried out over the span of 4 years and, yields were measured in the fourth year. Using the five different CRF formulations, we found that there were no statistical differences in the yield and fruit quality. However, yields from all treatments were exceptionally high for a 4-year-old tree grown under high disease pressure. Overall, all the CRF formulations yielded good production and fruit quality. All the tested CRF formulations were similar in performance. Therefore, focus should be on constant supply of nutrition in managing groves under HLB prevalent conditions irrespective of manufacturer. Hence, the cost of fertilizer can potentially be a deciding factor for growers in selecting the CRF product for their grove fertilization program.

Keywords: Citrus; Citrus Greening; HLB; Fertilizer; Nutrition; New establishment

Introduction

In the last 12 years, the production of Florida oranges has dropped from 242 million boxes (40.8 kg=1 box) in the pre-hurricane, pre-huanglongbing (HLB) 2003-2004 season to approximately 68 million boxes in the 2016-2017 season [1]. The dramatic reduction in yield can be attributed to several reasons, including the loss of citrus area in the state, and diseases such as citrus canker and HLB. In fact, HLB is now recognized as the main reason for the decline in citrus yields. HLB is a bacterial disease caused by the fastidious, phloem-restricted bacterium *Candidatus* Liberibacter asiaticus (CLas), transmitted by the Asian psyllid (*Diaphorina citri* Kuwayama). Once CLas is transmitted to the citrus fruit, the phloem plugging can be observed [2]. In CLas-infected trees, there is a disruption of vascular function, loss of roots, and an alteration in mineral nutrition which results in the stunting of plants, fruit growth and eventual death of the tree [3,4]. The typical symptoms of HLB in a tree include reduced plant height, leaf yellowing, stain spotting, and chlorotic leaf patterns that resemble those caused by zinc and iron deficiencies [2]. There is currently no cure for HLB, which leaves citrus growers with the daunting choice of either replanting a new grove, or managing their mature groves. Currently, the control of psyllids and removal of trees (inoculum) are the only recommendations for the management of citrus groves in Florida. As a result, in the absence of curative treatment options, and HLB-resistant cultivars, new plantings are still susceptible to infection and may decline in both quality and quantity before they reach maturity.

In past few years, there have been several reports on use of nutritional foliar sprays to extend the vigor and productivity of the HLB-affected trees [5]. In 2011, several growers were reported to use various enhanced nutritional programs and intensive management of mineral nutrition to maintain the productivity of HLB-affected trees [6]. Zhao et al. reported that in greenhouse CLas positive plants demonstrated a deficiency in phosphorus, however, with the application of phosphorous, the well-known symptoms of HLB were alleviated [7]. Similarly, in a recent greenhouse study, HLB-affected sweet orange leaves displayed lower concentrations of potassium, calcium, magnesium, copper, iron, zinc, manganese, and boron as compared to leaves from healthy trees [8]. Another study demonstrated that the application of three times the pre-HLB recommended concentrations of minerals like manganese, and zinc drastically improved the health of HLB-affected trees [8]. Our work has also demonstrated that both roots and leaves of HLB-affected trees are deficient in both secondary and minor nutrients (J.W. Grosser, unpublished data). Preliminary greenhouse studies suggest that higher than recommended rates of controlled release micronutrients, some in the form of sulfates, improve the density and health of feeder roots of Clas-infected 'Valencia' sweet orange ('UFR-3' rootstock) (J.W. Grosser, unpublished data). Overall, such research and field observations suggest that the presence of HLB upsets the nutritional balance of minerals in trees. Therefore, intensive nutrition management benefits HLB-affected trees. Furthermore, HLB-affected trees often suffer from a significant root loss, and the loss

***Corresponding author:** Tripti Vashisth, Department of Horticultural Sciences, Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA, Tel: 8639568846; E-mail: tvashisth@ufl.edu

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of root mass can lead to a reduction in the uptake of both water and nutrients from the soil [9]. It is therefore recommended that growers provide a constant and optimum supply of nutrients to provide trees with higher probability of absorbing nutrients and preventing nutrient deficiency related stress.

Mineral nutrition plays a vital role in the physiological processes such as growth and development of plants, and plant defense response. Many of the micronutrients act as the catalyst in the various physiological reactions that include housekeeping, plant hormone biosynthesis, regulation, and plant defense mechanisms. For example, K fertilizer is widely reported to decrease infestation from insects, which leads to a decrease in disease incidence in many host plants. One study demonstrates that the rate of rice borer infestation was the greatest when there was no supply of K, but decreased rapidly as the concentration of K was increased [10]. In this same study, they found that an increased concentration of K fertilizer significantly reduced the incidence of stem rot and aggregate sheath spot, all indications of disease. Furthermore, negative correlations were found between the percentage of K in leaf blades and the severity of disease. Field observations have shown that there is a positive correlation between sulfur-fertilization and enhanced resistance against fungal pathogens that lead to disease. In sum, their findings beg the question of whether sulfate availability could be a limiting and determining factor in the ability of plants to resist pathogenic infections [11,12]. Studies have shown that soil-supplied sulfur has a strong influence on plant resistance by directly stimulating biochemical processes in both primary and secondary metabolism [12,13]. Furthermore, many compounds that play a role in active defense against pathogens contain sulfur, including cysteine-rich antifungal peptides, phytoalexins, and glucosinolates (GSL) [14,15]. While the use of enhanced mineral nutrition alone cannot cure CLas-infected trees, studies point to the fact that the combined use of both a balanced nutrition and integrates pest management practices seem to hold great potential for the control and minimization of the impact of HLB in the short term, as long-term solutions are being investigated.

According to production cost analysis, fertilizers and foliar nutritional products constitute approximately 20% to 25% of the cost of total citrus production [16]. At present, very few scientific reports support the anecdotal evidence of the effect of mineral nutrition on HLB-affected trees. These reports are needed to formulate recommendations on effective fertilizer programs for HLB-affected citrus groves. The constant supply of balanced fertilizer seems to be promising for improving the growth and productivity of HLB-affected trees. As the majority of Florida growers are looking for strategies of a continuous supply of nutrients, CRF seems to be an easy and effective alternative for the continuous supply of nutrients. Furthermore, numerous studies have demonstrated that CRF is effective in improving the yield of crops as compared to conventional fertilizer. However, CRF are expensive products, which presents citrus growers with a budgeting constraint as they seek measures to improve yields from HLB-affected trees. As such, citrus growers are confronted with a variety of formulations and nutritional programs, and lack adequate and objective information to make an informed choice with regards to the most effective CRF options. Therefore, the aim of our research was to compare the popular formulations of CRF from different patented technologies, and examine their effect on growth, development, HLB incidence, and yield in a new planting of sweet orange trees. No comparisons were made between conventional fertilizer and CRF, as CRF has already been proven to be effective over conventional fertilizer in several studies [17,18].

Materials and Methods

Plant material

Nursery grown mid-season *Citrus sinensis* cultivar, Valquarius, on rootstock 'US-897' were planted in May 2011 at a commercial grove site near Arcadia, Fla. The soil type was sandy with pH ranging from 6.5 to 7.0. Trees were regularly irrigated and grown following commercial grove management practices, which included regular insect and pest control.

Fertilizer treatments

Five CRF formulations from the three leading commercial CRF manufacturers were tested. These manufacturers were: Florikote (American Horticultural Supply, Inc., CA), Harrell's (Harrell's Inc., FL), and Citriblend (ICL, OH). The five fertilizer programs were: (A) Florikote (14N–4P–10K), (B) Citriblend (17N–5P–12K), (C) Harrell's (13N–4P–9K), (D) Citriblend (18N–6P–11K), and (E) Harrell's (16N–5P–10K). The nitrogen in all the fertilizer formulations is a blend of coated ammonium nitrate and coated urea. Plants were fertilized according to the guidelines set by the University of Florida, Institute of Food and Agricultural Sciences guidelines for the fertilization of citrus [19]. The target rate of nitrogen for years 1 and 2 was 0.12 kg/tree, 0.23 kg/tree for year 3, and 0.35 kg/tree for year 4. The rate of nitrogen was the only parameter that was kept constant among all the different fertilizer programs. All the treatments were applied manually twice a year (early spring and late summer) - the product was placed in the wetted zone under tree canopy. The experiment was set up as a completely randomized block design with 20 trees in each block (n=6). Three trees per block were marked for data collection and examined for tree health, yield, and fruit quality data.

Huanglongbing foliar symptoms assessment and CLas quantification

Huanglongbing foliar assessment: Trained scouts visually performed HLB symptoms assessments each year. Scouts visited each tree and examined the leaves for symptoms of blotchy mottle. The leaves showing symptoms of blotchy mottle were sampled for confirmation of the presence of CLas DNA using real time quantitative polymerase chain reaction (qRT-PCR).

CLas confirmation: Leaf samples showing symptoms of HLB symptoms were collected from the field. The midribs of the leaves were excised and stored in -80°C until DNA extraction. DNA extraction was performed using DNeasy Plant Kits (Qiagen, Valencia, Calif.) and qRT-PCR was performed according to Li et al. [20] using *rpoB* [21]:

Forward – TGAGGAGAAACGATGGCAAAAGGC,

Reverse - GACATACCTGATCTCATTGAAGTTCAG,

Probe - TTGTGTTCAATGGTCTCGGGCG.

Leaf nutrient analysis: Thirty random leaves per block were collected from non-fruiting branches in July-August of each year. The collected leaves were washed using acidic soap, and then the leaves were dried for 48 h in a convection oven (Thermo Fisher Scientific, Waltham, Mass.), and ground to a fine powder. Ground leaves were sent to Water Ag Lab (Camilla, Ga.) to perform a standard leaf nutrient analysis (https://watersag.com/service/plant-analysis/).

Canopy volume measurement

Canopy volumes expressed as m^3 were calculated using a geometric prolate spheroid formula below:

$$[(4/3)\ (\pi)\ (H/2)\ (ACR)^2] \quad (1)$$

where π=3.14, H=tree height, and ACR=average canopy radius. ACR was calculated by dividing the tree diameter by 2 and calculating average radius. Tree diameter was measured in two directions - east to west (D1) and north to south (D2).

Fruit yield and quality

Fruit were hand harvested by commercially trained harvesters when the total soluble solids (TSS) to titratable acidity (TA) ratio of fruit reached commercial harvest standards. Fruit were harvested from individual trees in March 2015. The number of fruit, total yield (kg), and fruit size (inch) were recorded. A subset of 10 fruit per tree was collected to extract juice and measure TSS, TA, pound of solids, weight, and color. Pound of solid is defined as the amount of soluble solids (sugars and acid) contained in one box of citrus fruit.

Potential return for yield

The potential return on yield was calculated to demonstrate the revenue that can be generated when using any of the tested CRF formulations. This information can be useful for growers when budgeting for the cost of fertilization in the total cost of production. In the calculation, the cost of production, pick, and haul was not considered, as this can vary from grower to grower and also depends on the acreage involved. The potential return per acre was calculated by considering pound of solids per box and box yield per acre. The average number of boxes for an acre was calculated by multiplying the average number of boxes per tree in a treatment and the number of trees per acre. Currently, the number of trees per acre can range anywhere from 140 to 280. Therefore, 215 was used as an average for per acre [1]. The average price per pound per solid in 2015 was \$1.92 [1]. The calculated returns for a treatment (\$)=((no. boxes per tree × pound of solid) × 215) × \$1.92.

Statistical analysis

All statistical analyses were performed using the SigmaPlot (version 11; Systat Software, San Jose, CA) software. Data were compared using two-way analysis of variance (ANOVA) with an alpha significance level, α=0.05. The mean separation among treatments with significant differences was performed using Tukey's honest significance test (HSD) at p=0.05.

Results and Discussion

From the time of planting to conclusion of the experiments, the trees were surveyed annually for any symptoms of HLB symptoms. In 2014, 9% of the planted trees in the trial were positive for symptoms of HLB. Additionally, HLB positive trees were found in all CRF treatment groups, which ranged from 4% to 19% across all groups. In 2015, the number of HLB positive trees increased considerably, with an average of 78% HLB symptomatic trees out of all the trees in the trial. Here, the HLB symptomatic trees ranged from 55% to 95% among the different CRF treatment groups. Overall, by the end of the experiment, only 55% and 61% trees in CRF treatment groups C and E were HLB positive, however, the rest of treatment groups had more than 80% HLB positive trees. According to cumulative ranking for HLB positive trees in year 2014 and 2015, treatments A, E, and C were the best performing.

There was a large variability in all the measured parameters observed across all blocks and in all treatment groups. However, a large variability is often typical in HLB-affected trees. We found no statistical differences at 95% confidence interval (CI) in canopy volume at the end of the trial, and among different treatment groups (Figure 1). An analysis of the leaf nutrient demonstrated that all the nutrients were present in optimum to high range among the different treatment groups. The ranges are as per University of Florida/Institute of Food and Agricultural Sciences recommendations [19]. Except for Manganese, our analysis revealed that nutrients were not statistically significantly different across the different treatment groups. The concentration of manganese was found to be significantly higher (p<0.05) in fertilizer D plant leaves as compared to fertilizer A and C plants. Overall, these results suggest that all the five tested fertilizer formulations are sufficient in providing the required mineral nutrients to the aboveground portions of the trees.

There were no significant differences (95% CI) in the yield or fruit produced from each treatment (Figure 2). All the treatments yielded more than 50 kg fruit per tree. Yield ranged from 53 to 72 kg per tree, which is equivalent to 1.2 to 1.8 box of fruit (Table 1). A box of fruit is common terminology in commercial citrus production where one box is equivalent to 40.8 kg of orange fruit in Florida [1]. According to USDA [1] and Savage [22], under central Florida conditions, the average yield for a 4-year old orange tree in either early-, mid-, or late-season is 0.7 box per tree. In a previous enhanced fertilizer trial in 1993, an average yield of 21 kg per tree (0.5 box per tree) from 2.5-year-old 'Hamlim' oranges (*C. sinensis*) was considered exceptional and indicative of vigorous growth [23]. However, since 2005, due to HLB prevalent conditions, there is a dramatic reduction of yield per acre. According to a survey conducted by Singerman, in 2014-2015, 300 boxes per acre generated marginal profit using optimal grove production practices [16]. The current spacing trend for Florida citrus industry is 20 feet × 10 feet, which results in approximately 215 trees per acre. Average yield in this trial extrapolates to 268 to 387 boxes per acre for all the fertilizer treatments. As such, yields from 4-year-old trees seem promising and noteworthy. Our results suggest that optimal plant nutrition is the key, irrespective of the kind of CRF. The

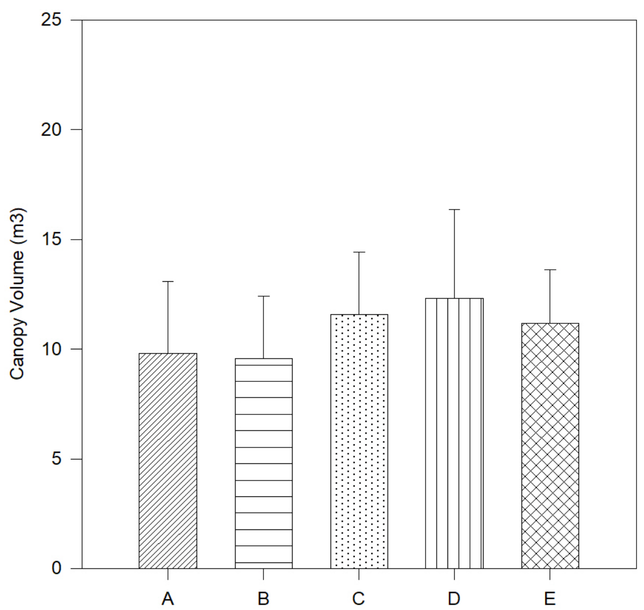

Figure 1: Canopy volume in (m³) of 4-year-old 'Valquarius' on 'US-897' sweet orange trees grown on five fertilizer treatments. The five fertilizer programs were: (A) Florikote (14N–4P–10K), (B) Citriblend (17N–5P–12K), (C) Harrell's (13N–4P–9K), (D) Citriblend (18N–6P–11K), and (E) Harrell's (16N–5P–10K).

consistent supply of balanced nutrients can aid in achieving good yield even under the HLB prevalent condition.

A decrease in fruit size is a well-known symptom of HLB. Spann and Oswalt [24] reported that approximately 40% more of fruits that were considered small were harvested from HLB affected trees compared to healthy trees. In another survey conducted by Vashisth et al. (unpublished data), under HLB prevalent conditions, fruit size continued to decrease considerably in 'Hamlin' and 'Valencia'.

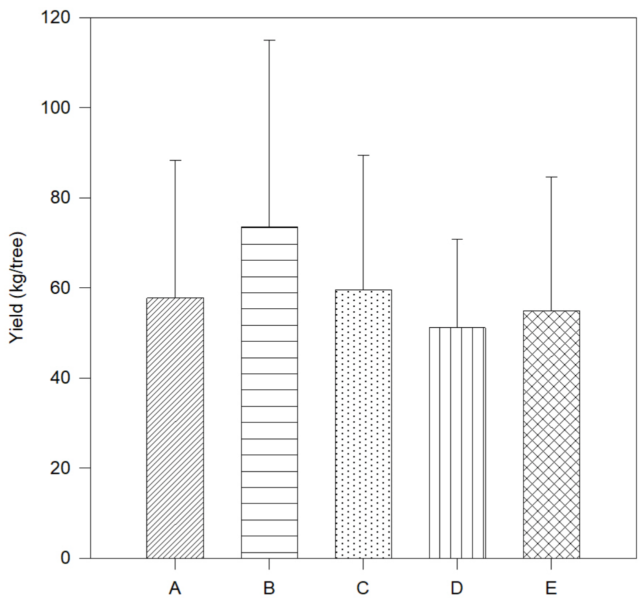

Figure 2: Average fruit yield (kg) from 4-year-old 'Valquarius' on 'US-897' sweet orange trees grown on five fertilizer treatments. The five fertilizer programs were: (A) Florikote (14N–4P–10K), (B) Citriblend (17N–5P–12K), (C) Harrell's (13N–4P–9K), (D) Citriblend (18N–6P–11K), and (E) Harrell's (16N–5P–10K).

	Number of fruit [mean ± SD]	Size [mean ± SD (mm)]	Boxes per tree[x] [mean ± SD]
A (Florikote; 14N–4P–10K)	91.1 ± 91	90.8 ± 4.9 b	1.42 ± 0.7
B (Citriblend; 17N–5P–12K)	178.1 ± 111	93.1 ± 2.9 ab	1.80 ± 1.0
C (Harrell's; 13N–4P–9K)	139.7 ± 82	94.1 ± 2.8 ab	1.46 ± 0.7
D (Citriblend; 18N–6P–11K)	113.8 ± 48	94.6 ± 2.8 a	1.25 ± 0.5
E (Harrell's; 16N–5P–10K).	127.1 ± 72	93.7 ± 1.8 ab	1.35 ± 0.7

Table 1: Total number of fruit, fruit diameter (inch), and boxes per tree (calculated from yield) of harvested fruit from 4-year-old 'Valquarius' on 'US-897' sweet orange trees grown on five fertilizer treatments.

[x]A box of fruit is common terminology in commercial citrus production where one box is equivalent to 40.8 kg of orange fruit in Florida [1].

	TSS [mean ± SD (%)]	Titratable Acid [mean ± SD (% citric acid)]	TSS/TA [mean ± SD]
A (Florikote; 14N–4P–10K)	11.6 ± 0.5	0.6 ± 0.02	18.4 ± 0.7
B (Citriblend; 17N–5P–12K)	11.2 ± 1.6	0.7 ± 0.07	17.2 ± 0.6
C (Harrell's; 13N–4P–9K)	11.3 ± 0.3	0.6 ± 0.02	18.2 ± 0.2
D (Citriblend; 18N–6P–11K)	11.0 ± 0.03	0.6 ± 0.01	18.7 ± 0.2
E (Harrell's; 16N–5P–10K)	11.2 ± 0.1	0.6 ± 0	17.4 ± 0.2

Table 2: Total soluble solids (TSS), titratable acidity (TA), and TSS/TA of juice extracted from harvested fruits from 4-year-old 'Valquarius' on 'US-897' sweet orange trees grown on five fertilizer treatments. The five fertilizer programs were: (A) Florikote (14N–4P–10K), (B) Citriblend (17N–5P–12K), (C) Harrell's (13N–4P–9K), (D) Citriblend (18N–6P–11K), and (E) Harrell's (16N–5P–10K).

Florida growers have been struggling due to small sized fruit for fresh market intended fruit as large fruit size tends to get better revenue and fewer fruit are needed to fill a box. Interestingly, in our experiments, treatment D produced a significantly larger fruit in size as compared to fertilizer A ($p<0.05$) (Table 1). The fruit size for all the treatment groups were considerably good and ranged from 91 to 96 mm. In Vashisth et al. (unpublished data), in their 2-year survey of fruit size at multiple commercial citrus groves, they found that on average the fruit size was about 58 mm. Therefore, in current study, our results of an average fruit size of 91 mm can be considered good and desirable.

We found no significant differences in TSS, TA, and TSS/TA ratio in the juice extracted from the fruit harvested from the different fertilizer treatment groups (Table 2). In addition, no significant differences were observed in the weight of juice extracted from the fruit harvested from the different fertilizer treatment groups. The mean TSS and TA for across all the treatments groups were 11.2 and 0.6, respectively. Past work from Spann and Danyluk [25] reported the average TSS and TA of juice from HLB positive trees were 8.9 and 0.75, respectively. Our results suggest that the good fruit quality observed even under high disease pressure can be attributed to the continuous supply of nutrients by CRF, regardless of the formulation of CRF, as opposed to a particular of CRF.

Our data demonstrated that the average pound of solids per box ranged from 5.6 to 6.2 pound of solids for the treatments and was not statistically different among the different treatment groups. Growers selling their fruit for orange juice processing are often paid based on the pound of solids. In 2014-2015, the average price per pound of solid delivered-in for processed citrus was $1.84 [1]. Table 3 shows the potential return on the fruit from each treatment group based on the average pound of solids per box and the average, minimum, and maximum yield per treatment. Based on the calculated data shown in Table 3, the fruit yield produced from all five treatment groups would at least break even to the average standard cost of production, excluding pick and haul per acre basis [16] under current central Florida conditions. However, the cost of CRF should be considered in the cost of production; generally, CRF products are more expensive than regular fertilizer, which can change the cost of production significantly; therefore, a grower should make decisions based on input versus potential output while choosing a CRF.

Conclusion

In the present study, we did not find significant differences in the effectiveness of the commercial CRF fertilizer products tested. Our data demonstrates that the constant supply of nutrients applied in the form of CRF seems to be the key to successful fruit production amidst HLB prevalent conditions. Overall, compared to literature, our experimental data suggest that use of CRF can result in good yield and fruit quality fruit even under high disease pressure. HLB-affected plants have a compromised root system, therefore, a constant availability of nutrients in form of CRF can help in the efficient uptake of minerals by the roots in the ground, throughout the growing season. The results of this study provide evidence that a continuous delivery of required nutrients can restore and maintain root function as needed for profitable citriculture under endemic HLB conditions. The cost of fertilizer, which is especially high for CRF, is a deciding factor for a grower when choosing CRF products. Therefore, growers are encouraged to research the available cost and performance information before deciding on a program. Although some of the CRF products appeared to slow the CLas infection rate, none could prevent the spread of disease. The benefit

	Avg boxes[x] per tree	Avg pound of solids per box[y]	Calculated boxes per acre (215 trees per acre)	Calculated pounds of solids per acre	Price per pound solid (delivered-in for processed Citrus)	Calculated least, median, and high returns ($) (excluding production cost, pick and haul)[z]		
A	1.42 ± 0.7	6.0 ± 0.4	304	1,826	1.92	1,525	3,498	5,743
B	1.80 ± 1.0	6.0 ± 1.2	388	2,327	1.92	1,564	4,467	8,377
C	1.46 ± 0.7	6.1 ± 0.5	314	1,916	1.92	1,677	3,652	5,923
D	1.25 ± 0.5	5.6 ± 0.1	269	1,509	1.92	1,760	2,902	4,078
E	1.35 ± 0.7	6.2 ± 0.1	290	1,795	1.92	1,533	3,419	5,391

[x] A box of fruit is common terminology in commercial citrus production where one box is equivalent to 40.8 kg of orange fruit in Florida [1].
[y] Pound of solid is defined as the amount of soluble solids (sugars and acid) contained in 40.8 kg of citrus fruit.
[z] Calculated return.

Table 3: Calculated extrapolated revenue on the fruit harvested from 4-year-old 'Valquarius' on 'US-897' sweet orange trees grown on five fertilizer treatments. The price per pound of solid has been adapted USDA NASS (2017). The five fertilizer programs were: (A) Florikote (14N–4P–10K), (B) Citriblend (17N–5P–12K), (C) Harrell's (13N–4P–9K), (D) Citriblend (18N–6P–11K), and (E) Harrell's (16N–5P–10K).

of slow-release applied overdoses of micronutrients were not tested in this study. However, adequate and balanced nutrition provided by all the tested CRF products helped trees in producing acceptable yields of quality fruit.

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