UNRAVELING THE EFFECT OF ADVANCED HORTICULTURAL PRACTICES ON HUANGLONGBING-AFFECTED GRAPEFRUIT

By

DINESH PHUYAL

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2020

To my family

ACKNOWLEDGMENTS

I would like to extend my gratitude to those individuals whose assistance helped to make this thesis possible.

First of all, I would like to thank my academic advisor Dr. Rhuanito Soranz Ferrarezi for the research opportunity, his support, valuable suggestions, and motivations throughout my master's study and research. I could not imagine completion of this research project and developing it into thesis without his guidance and support.

I would also like to thank Dr. Kelly Morgan and Dr. Davie M. Kadyampakeni for serving as a committee member, for their excellent cooperation, motivation, and willingness to help, whenever asked for. Without their insightful comments, valuable suggestions and encouragement, it would not be possible for me to conduct this research. I also want to thank Dr. Thiago Assis Rodrigues Nogueira for his careful and detailed revision of this document and assistance in the fieldwork.

From the lab, I would also like to thank Taylor Meadows and Hernan Soto for all the help in fieldwork and data collection. I really appreciate the support from Randy Burton, Tom James, Cristina Gil, Dr. Arun Jani, Dr. Kayla Thomason, Andres Gonzalez, Clarence King, Dr. Guilherme Locatelli, Donald Davis, Dr. Gustavo Haddad, Dr. Xiongjie Lin, and Megan Eckman for assisting me in the fieldwork and all the fun that we had in both lab and field. I enjoyed the camaraderie with my colleagues Ricardo Lesmes, Natalia Macan, and Martin Zapien.

From the IRREC, I am grateful to the director Dr. Ronald Cave for facilitating resources that immensely helped my personal and professional development. I am thankful to Robin Koestoyo for the news release of my research and scholarships. I appreciate Velma Spencer for assisting me all the paperwork. I extend my sincere thank

to Patricia Prade for her friendship. Her guidance, support, and suggestion helped me a lot especially during my first month at IRREC.

I am thankful to the Horticultural science department especially Mr. Curtis Smyder for the academic advice. I am also thankful to Dr. Lorenzo Rossi and Dr. Mark Ritenour for their insightful suggestions and help during my research and study. Also, I am thankful to my friends in the Horticultural Science Department for their support and all the fun we had during our coursework.

I extend my special thanks to my family, who always supported and motivated me to move forward on my academic career. Finally, I am grateful to the UF/IFAS Citrus Initiative and USDA-APHIS-MAC Agreement # AP19PPQSandT00C116 for funding the project.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACP	Asian citrus psyllid
CRF	Controlled-release fertilizer
Ct value	Cycle threshold value
FAM	Foliar application of micronutrients
HLB	Huanglongbing
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
PCR	Polymerase chain reaction
PD	Planting density
UF/IFAS	University of Florida/Institute of food and agricultural science
USDA	United States Department of Agriculture
SR/LD	Single row low-density
SR/HD	Single row high-density
DR/HD	Double row high-density

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

UNRAVELING THE EFFECT OF ADVANCED HORTICULTURAL PRACTICES ON HUANGLONGBING-AFFECTED GRAPEFRUIT

By

Dinesh Phuyal

May 2020

Chair: Rhuanito Soranz Ferrarezi
Major: Horticultural Sciences

Advanced horticultural practices have been used in commercial citrus production to mitigate the negative effects of citrus greening disease or Huanglongbing (HLB). We set up a series of studies to understand how tree planting density, soil, and foliar nutrient application impact grapefruit canopy growth, fruit yield, and fruit quality under HLB epidemics in the Flatwoods soil. Two separate trials were conducted for two years using ‘Ray Ruby’ grapefruit (*Citrus paradisi*) on Kuharske citrange (*Citrus sinensis* × *Poncirus trifoliata*) planted in September 2013. In the first experiment (2017-2019), we tested three planting densities (300, 440, and 975 trees/ha) and two controlled-release fertilizer (CRF) blends in a split-plot design with four replications. In the second experiment (2018-2020), three planting densities, two CRF blends, and four foliar micronutrient application (a blend of B, Mn, and Zn at 0×, 1.5×, 3×, and 6× the UF/IFAS recommended rates) were tested in a split-split-plot design with four replications. On both experiments, we found that 975 trees/ha planting resulted in the greatest canopy volume, fruit yield, and yield of solids than the other treatments. The CRF blends with higher micronutrients increased canopy volume without aiding fruit yield. Foliar micronutrient application was negatively correlated with fruit yield. Our study shows

high-density planting is a promising way to increase fruit yield per unit area. Further, after two years, supplemental nutrient application is not beneficial to produce more grapefruit.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Overview of Citrus Production in Florida

Citrus is the most important fruit crop of the state of Florida, which produces 44% of overall United States citrus (USDA, 2019a). The Florida citrus industry is mainly based on processing orange for juice along with several other industrial byproducts such as citrus pulp, meal, molasses, and citrus oil (Court et al., 2018). In 2018/19 the total bearing acreage (land planted with citrus trees older than 3 years) was 400,900 acres, which is 3.5% lower than the previous year. The total citrus production was 3.4 million tons with 7% been for the fresh market and the remaining for the processing industry (USDA, 2019a). In 2016/17, the Florida industry reported employing approximately 50,030 people, with 32,860 jobs on citrus fruit production, 16,683 jobs on citrus juice and byproducts manufacturing, and 487 jobs on citrus fruit-packing houses for the fresh market (Court et al., 2018).

Huanglongbing Disease in Florida

Citrus greening was first reported in 1956 under the name Huanglongbing (HLB) (Chinese translation: yellow dragon disease or yellow shoot disease) by Lin Kung Hsiang (Lin, 1956). In 1995, the conference of “International Organization of Citrus Virologists” (IOCV) organized in Fujian, province of China, proposed the official name for the disease as ‘Huanglongbing’ and later was accepted for common use (Bove, 2006). This disease continued to spread, and by the end of the 20th century, HLB became endemic to the major citrus-producing parts of the world including China, Southeast Asia, and Southern Africa (Satranjoe, 2014). Intensive surveys performed by the Cooperative Agricultural Pest Survey (CAPS) found HLB symptoms for the first time

in South Miami-Dade County, Florida in August 2005. At that time, the U.S. Department of Agriculture (USDA) and the State of Florida's Division of Plant Industry (DPI) also confirmed HLB in Florida (Halbert et al., 2008). The disease was introduced and has been dispersed in Florida by the Asian citrus psyllid (ACP, *Diaphorina citri* Kuwayama). The weather conditions favored the vector multiplication and the dissemination of HLB, and by February 2009, all the citrus-producing counties in Florida were infected with the disease (Chung and Brlansky, 2005).

Impact of Huanglongbing in Florida

Citrus greening diseases or HLB has been a serious threat to the citrus production in Florida. It is estimated that virtually all mature trees in the commercial groves are infected by this insidious disease (Li et al., 2019). HLB-affected trees produce fewer fruit than healthy trees, with the fruit of inferior quality. When the infection rate increases, trees become unproductive and may eventually perish (Farnsworth et al., 2014). The citrus production in Florida has been decreasing rapidly after 2005, when the disease was reported in the U.S. (7.8 million tons of citrus produced in 2005/06), dropping to 2.2 million tons in 2017/18, a 72% decrease over 12 years. Florida citrus industry production reached 49.6 million boxes in 2017/2018, a 37% decrease in comparison to the previous season. The grapefruit yield in 2017/18 was 130 boxes/acre, 43% less than in 2016/17 (USDA, 2018).

Symptoms and Transmission of the HLB Disease

The disease is transmitted by the vectors *Trioza erytreae* (the African Citrus Psyllid, AfCP) and *Diaphorina citri* (the Asian Citrus Psyllid, ACP) (Bove, 2006). *Trioza erytreae* is known as two-spotted citrus psyllid because the nymphs bear a pair of spots on the base of the abdomen.

In the U.S. and its territories including Florida, the ACP is widely distributed (Mead and Fasulo, 2017). The body size of fully-grown ACP ranges from 3 to 4 mm. The body appears dusty due to coverage of whitish waxy secretion. They feed on citrus leaves or young shoots. The adult lays its body at 45° angle with the plant surface while feeding or resting on a plant. Adults can live for several months (Hall et al., 2013; Mead and Fasulo, 2017). Many factors influence the survival of the ACP such as humidity, temperature, and the host plant (Tsai and Liu, 2000; Nava et al., 2007). The ACP normally flies short distances as its wings are weakly developed. Therefore, for long-distance travel, multiple short distance flights are required (Kobori et al., 2011).

Female insects lay eggs in young leaves. Tsai and Liu (2000) reported that an adult female laid an average of 858 eggs on the grapefruit plant. The freshly deposited eggs are oval with a light yellow color that becomes bright orange having two distinct red eyespots after maturity (Hall et al., 2013). Egg gets anchored into the new flushes with the help of tapered stalk. First instar nymphs are 0.25 mm in size and can reach 1.77 mm during the 5^{th} instar stage. Growth and development of eggs and nymphs require young flushes (Hall et al., 2016; Mead and Fasulo, 2017). Therefore, the increase in the ACP population is correlated to the growth stages of the plant. In North America, infestation normally observed late spring through mid-summer although outbreaks can still occur in any season if young flushes are available (Hall et al., 2008)

The disease is caused by a phloem-limiting bacterium, *Candidatus* Liberibacter, which has three known species: *asiaticus*, *africanus*, and *americanus*. *Candidatus* Liberibacter asiaticus (*C*Las) is the prevailing species in the U.S. Since the pathogen distribution is uneven in the plant, infected branches serve as a source of inoculum.

Once the bacterium is acquired by the psyllid, it retains and transmits the disease throughout its lifecycle (Brlansky and Rogers, 2007). Besides psyllids, grafting is another method of disease transmission. But buds acquired from infected trees do not necessarily transmit the disease (Chung et al., 2005).

The bacterium is restricted to the phloem's sieve tubes (Li et al., 2019). There is significant damage to the roots before exhibiting symptoms in the leaves as the bacteria replicate and moves towards the roots right after being inoculated (Johnson et al., 2014). HLB causes loss of fibrous roots and depletion of starch in the roots (Etxeberria et al., 2009; Graham et al., 2013). Conversely, starch content in the leaves - particularly photosynthetic cells, vascular parenchyma, and sieve elements - are abnormally higher compared to non-HLB trees. The unbalanced partitioning of photoassimilates in above and belowground of HLB-affected trees causes poor tree health due to root starvation (Etxeberria et al., 2009; Gonzalez et al., 2012). In the early stages of HLB infection, yellow shoots commonly develop due to blotchy mottled leaves. Those leaves are thick and leathery, and the symptoms spread out to the whole canopy in later stages. In addition, infected trees show stunted growth, bear a few deformed, small-sized fruit having color development from the peduncular end (Bove, 2006).

Disease Management

Once the bacterium enters into the plant, there is no cure for the disease. Palliative horticultural practices have been used in commercial groves to alleviate the disease severity and maintain growers in business (Halbert and Manjunath, 2004). Some approaches include the elimination of inoculum (Xia et al., 2011), enhanced nutritional programs alone or combination with pesticides (Stansly et al., 2014; Morgan et al., 2016; Spyke et al., 2017; Vashisth and Grosser, 2018), use of tolerant scions and

rootstocks (Castle et al., 2019), application of hormones, antibiotics, thermotherapy treatment (Li et al., 2019), and production of citrus under protective screen houses (Ferrarezi et al., 2019).

To successfully manage this disease, it is imperative to prioritize long-term fruit yield by focusing on finding ways to produce quality fruit on infected trees while seeking a cure for the disease. Along those lines, improved horticultural practices such as increased tree planting density, the use of controlled-release fertilizer (CRF), and foliar micronutrient applications are viable options for Florida citrus growers.

Tree Planting Density

Tree density has become a critical consideration in new citrus plantings due to the potential for inducing increased yield/area, generating early gains, and maximizing the return of investment over the tree productive period (Whitney et al., 1984; Stover et al., 2008). In Florida, the greater proportion of the existing groves are planted at 358-370 trees/ha, while some newly planted groves are at 544-556 trees/ha (Trejo-Pech, 2018). Planting a new citrus grove with the tree density of Florida state's average is no longer profitable after the HLB epidemics (Singerman et al., 2018). With the spike in grove production costs, the increase of tree density planting is a global trend (Dalal et al., 2013). Studies show that high-density plantings result in increased yield/area despite of higher investment (Wheaton et al., 1991; Wheaton et al., 1995; Dalal et al., 2013; Singerman et al., 2018; Moreira et al., 2019). Rootstock selection (Castle et al., 2019) and tree vigor are also important factors for tree density determination (Zekri, 2000). Fruit quality is another significant attribute for both fresh and processing citrus production. Recent studies suggested that in HLB endemic sites with high-density planting resulted in lower cumulative HLB incidence probably due to the lower invading

psyllid to plant ratio. Additionally, removing those HLB symptomatic trees in high-density planting areas may reduce the proportion of HLB-affected trees resulting in greater profitability to the growers (Moreira, 2019). Since HLB-affected trees have higher production costs and lower fruit yield (Farnsworth et al., 2014), it is crucial to find the relationship between tree density with fruit yield and quality. There are no enough long-term studies yet to indicate the effect of tree density on grapefruit tree health, fruit yield, and fruit quality.

Challenges for Nutrient Application in Florida Sandy Soils

There are several methods of nutrient application depending upon the blend type and crop needs such as broadcasting, banding, topdressing, foliar feeding, and fertigation. In horticultural crops as well as in protective cultivation, foliar application and fertigation are commonly used (Malhotra, 2016). The application of water-soluble fertilizer by banding or broadcasting is known as dry granular fertilization. Application of dry granular fertilization can cause root damage in citrus due to the accumulation of salt in the root zone (Morgan et al., 2009a, Kadyampakeni et al., 2015). Foliar application of nutrients is further described in chapter 3. Fertigation refers to the application of soluble fertilizer with water using a micro-sprinkler or drip irrigation system. Fertigation has the advantage over other methods of fertilization such as continuous and efficient application of nutrients. Drip fertigation has advantages over sprinkler fertigation such as application of nutrients directly to the root zone and without having fertilizers contacting tree leaves (Kafkafi and Kant, 2005; Obreza and Boman, 2008; Kadyampakeni et al., 2014a). Studies have found that fertigation has advantages over conventional fertilization to have a better fruit yield in citrus (Schumann et al., 2003). However, fertigation method is not suitable for some nutrients such as NH^{4+}, Ca^{2+}, Cu^{2+},

Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, MoO_4^{2-}, Ni^{2+}, H_2PO^{4-}, HPO_4^{2-}, K^+, and Zn^{2+} because of their immobile nature in soil (Barker and Pilbeam, 2015). Further, the chemical reaction between fertilizer mineral can result in precipitate formation in the irrigation system resulting in emitter plugging, which can ultimately decrease the efficiency of nutrient application (Boman and Obreza, 2015)

The other challenge is, as indicated previously, HLB-affected trees have weak root architecture (Johnson et al., 2014) which reduces nutrient uptake from the soil. Once trees fail on absorbing all the applied nutrients, the unused nutrients leach or runoff to groundwater. The consequence is not only a decreased fertilization efficiency but also environmental pollution due to watershed contamination (Obreza and Sartain, 2010).

Application of Nutrients in the Soil as Controlled-release Fertilizer

A potential alternative to the water-soluble fertilizers is the use of controlled-release fertilizer (CRF) to slow down the nutrient release process. The CRF prills are generally surrounded by a semi-permeable membrane activated by soil moisture and temperature. When the outer membrane is wetted, it slowly releases the nutrients by creating micropores through the coating film, resulting in lower nutrient leaching potential and higher nutrient use efficiency (Obreza et al., 2006; Morgan et al., 2009b; Irfan et al., 2018). Irfan et al. (2018) elucidated the nutrient release mechanism of N through CRF in different steps (step a to d). Due to the chemical properties and physical interference, coated granules release the nutrients (Figure 1-1). Temperature plays an important role in the diffusion mechanism of polymer-coated CRF and thereby release of the nutrients (Du et al., 2006). Additionally, nutrient release is dependent on soil pH and water content. Further, granule radius, coating thickness, and diffusion coefficient of

the fertilizer granule plays an important role while releasing the nutrients (Irfan et al., 2018). The nutrient release period can vary from 3 to 16 months (Morgan et al., 2009a)

The application of CRF is advantageous over dry granular fertilization. Morgan et al. (2009b) reported that CRF in young citrus trees results in better tree growth and higher fruit yield. Additionally, the authors reported that dry granular fertilization can damage the citrus root system due to excessive accumulation of salt over a small area. The application of dry granular fertilization is labor-intensive due to multiple application requirements (Kadyampakeni et al., 2015). Application of CRF results in enhanced nutrient use efficiency and reduces the nutrient leaching potential particularly in crops grown in sandy soil with low water and nutrient holding capacity (Morgan et al., 2009b).

Application of Higher Rates of Micronutrients via CRF

The HLB-affected trees typically exhibits nutrient deficiency symptoms such as P, Ca, Mg, Fe, Mn, and Zn in the tree canopy regardless of the fertility status of a soil due to the loss of fibrous root which is responsible for nutrient uptake (Pustika et al., 2008; Morgan et al., 2016). The continuous supply of small doses of nutrients in the form of CRF can establish and preserve root function under HLB in citrus trees since HLB-affected trees typically exhibits smaller and weaker root architecture (Vashisth and Grosser, 2018). Additionally, the amount of nutrient application can be reduced by 10-20% in CRF to obtain the equivalent fruit yield with dry granular fertilizer (Vashisth, 2017). Even though the CRF is ideal for continuous growth and development of citrus trees in Florida soils, the question is whether the previously recommended nutrient form and rates are enough.

Application of Supplemental Foliar Micronutrients

Foliar application of micronutrients is generally important for perennial fruit crops especially when nutrient demand is high and soil supply is not enough to match uptake (Mengel, 2002). The foliar application alone or in combination with other products such as hormones has increased the fruit yield in citrus (Morgan et al., 2016; Al-Obeed et al., 2018). Foliar application of fertilizer has decreased the disease expression of HLB in mandarin trees (*Citrus nobilis* × *Citrus deliciosa*) irrespective of the soil condition, suggesting the importance of the foliar application of nutrients to extend tree life and reduce fruit yield losses (Pustika et al., 2008). A long-term foliar application of B, Mn, and Zn on HLB-affected 'Valencia' sweet orange (*Citrus sinensis*) on Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) rootstock has significantly increased the Ct (Cycle threshold) value (Shen et al., 2013). Foliar application of micronutrients can help to provide adequate nutrient supply to the plant (Mengel, 2002). Spraying of mobile or partially mobile nutrients in plants such as B, Mn, and Zn can instantly increase the foliar concentration in plants (Morgan et al., 2016). Additionally, foliar application of nutrients can enhance plant physiology (Li et al. 2014).

The current nutrient recommendation was made before the HLB condition. There are complex interactions between the amount of nutrient application with tree growth and the fruit yield of HLB-affected trees. The conventional approaches to correct nutrient deficiencies in citrus trees need to reappraised (Morgan et al., 2016).

Objectives and Hypothesis

The overall goal of this study is to understand how tree spacing, soil, and foliar fertilizer application can improve grapefruit plant health, canopy growth, fruit yield, and

fruit quality under high HLB pressure in Flatwoods soil. The specific objectives of our study were:

1. To evaluate the effect of tree planting density on HLB incidence, canopy volume, and trunk diameter along with finding an optimum planting density to have better fruit yield per unit area while improved fruit quality.

2. To verify the efficacy of a higher amount of micronutrient and Mg applied in the soil using CRFs and foliar micronutrient applications while evaluating the HLB incidence, canopy growth, fruit yield, fruit quality, and plant physiology.

3. To find optimum combination for foliar micronutrient application to mitigate plant disease, ameliorate canopy growth, increase fruit yield, improve fruit quality, enhance plant physiology, and improve plant nutrient concentration.

Two separate experiments were conducted to achieve those objectives. Two CRF blends and three planting density were tested in experiment 1 (Chapter 2), and two CRF blends, three planting density, and four foliar supplemental micronutrient application rates were tested in experiment 2 (Chapter 3).

We hypothesized that tree planting density and nutrient application are important to increase the fruit yield of HLB-affected grapefruit in field conditions. The specific hypotheses of our study are:

- High-density plantings can cause competition for resources such as solar radiation and fertilizers resulting in lower canopy volume and trunk diameter.
- Higher tree density planting results in more efficient land use and contributes to more fruit yield per area without compromising fruit quality.
- The HLB-affected tree requires continuous application of nutrients particularly micronutrients that help to mitigate plant disease, ameliorate canopy volume, increase trunk diameter, increase fruit yield, improve fruit quality, and enhance plant physiology under HLB epidemics in Flatwood soil.
- The HLB-affected tree has a weak root system that requires a supplemental foliar nutrient application to mitigate plant disease and ameliorate canopy growth, increase fruit yield, improve fruit quality, enhance plant physiology, and improve plant nutrient concentration.

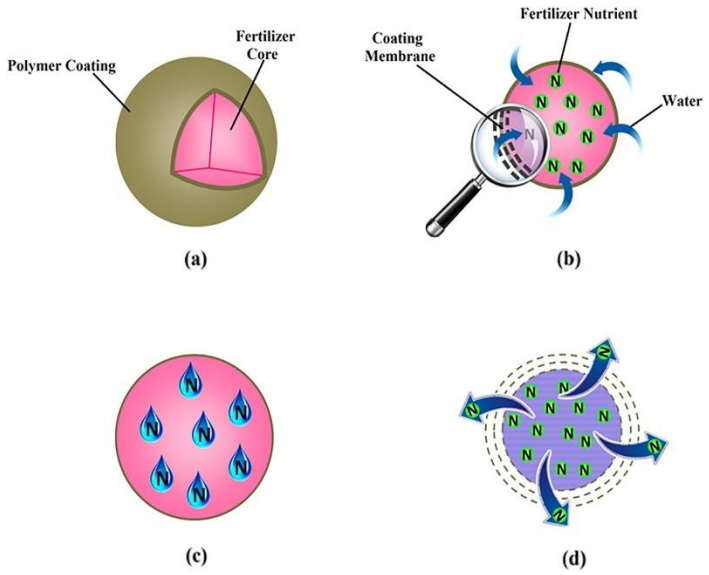

Figure 1-1. Mechanism of nutrient release in controlled-release fertilizers. Fertilizer core inside polymer coating (a), transport of water through the coating by diffusion (b), osmotic pressure generation (c), release of nutrient (d), release of N through membrane. Source: Irfan et al. (2018).

CHAPTER 2
TREE DENSITY AND SOIL MICRONUTRIENT APPLICATION ON GRAPEFRUIT AFFECTED BY HUANGLONGBING

Justification

High-density tree plantings have been used for years to obtain greater fruit yield/area in commercial citrus groves. Studies regarding the use of high-density plantings date from the 1980s - before citrus greening or HLB epidemics (Castle, 1980). At the time, results were not promising due to the lack of technical and financial advantages. Wheaton et al. (1991) showed high-density planting of sweet oranges (*Citrus sinensis*) and grapefruit (*Citrus paradisi*) quickly induce fruit bearing with no fruit yield increment and difficulty in harvesting operations. Wheaton et al. (1995) also observed similar results in mature citrus groves, with high-density plantings increasing the fruit yield in early stages without compromising fruit quality, but no fruit yield response on trees with 9-13 years. The authors suggested planting densities ranging from 350 to 1000 trees/ha were for Florida soil conditions.

After the HLB discovery in Florida, several approaches have been tried to fight the disease such as elimination of HLB-affected trees (Bove, 2006). The first approach practically failed mainly because infected trees can remain asymptomatic for 2 years, and the elimination of symptomatic tree may not remove all the diseased trees. If a single grower refused to eradicate diseased trees, that tree may serve as an inoculum for the nearby area. And failing to timely discover and remove a single tree may give opportunity for the Asian citrus psyllid (ACP, *Diaphorina citri*) to spread the disease across the entire grove (Spreen et al., 2014).

The disease management strategies such as controlling the insect vector and improving plant health by nutritional treatment to extend survival have sharply increased

the production costs due to HLB along with other exotic diseases such as citrus canker (caused by the bacterium *Xanthomonas axonopodis*) and citrus black spot (a fungal disease caused by *Guignardia citricarpa*). The grove caretaking of HLB-affected trees has higher production costs compared to healthy trees due to extensive use of foliar sprays to maintain production and tree health and use of insecticides to control ACP (Singerman and Burani-Arouca, 2017; Tansey et al., 2017; Singerman et al., 2018).

Keeping the disease inoculum resulted in rapid spread of HLB across Florida. In just a few years the disease became endemic. Several groves turned unprofitable over time, and the number of abandoned groves surpassed newly established groves (Singerman et al., 2018). In recent years, the citrus industry has been diminishing both at the grower and industry level. In 2018/19 bearing area of citrus in Florida covers about 162,238 ha which is 3.5% less than the previous year. Grapefruit alone covers 10,254 ha, which was 17% less than the previous year (USDA, 2019a). Until a cure is found, the establishment of new groves should be done using high-density plantings to maximize fruit yield (Schumann et al., 2012; Singerman et al., 2018)

Some recent studies of high-density planting showed promising results. Dalal et al. (2013) compared ‘Kinnow’ mandarin (*Citrus nobilis* × *Citrus deliciosa*) under three different densities and observed the greatest fruit yield per area in high-density plantings (550 trees/ha) despite the fact fruit yield per plant was not significantly different. Moreira et al. (2019) tested different tree densities of sweet orange ranging from 220 to 714 trees/ha of 3- to 13-year-old trees and noticed higher density plantings resulted in lower HLB incidence probably due to the lower psyllid to plant ratio.

Profitability associated with new groves depends on several factors such as initial investment, grove maintenance, fruit yield, and return of investment period (Spreen and Zansler, 2016). Singerman et al. (2018) showed that high-density planting is a profitable way to manage HLB disease even though it requires a high initial investment. The authors assessed cost and benefit analysis under three different densities: 358, 544, and 749 trees/ha, and found that in the current scenario, groves with Florida's traditional density of 358 trees/ha (Spreen and Zansler, 2016) is no longer profitable. Plantings with 544 and 749 trees/ha are profitable and could result in a payback period 4 years earlier than the low-density planting. The authors noted that seeking for an early economic return of investment by establishing high-density planting to maximize fruit yield per area is a wise way to manage the HLB due to the reduced lifespan of declining citrus groves in Florida.

The main challenges for grove establishment in Florida's Flatwoods soils are the shallow water table and poor drainage because of lower elevation, flat surface, and a hard soil layer below the root zone. Such edaphic conditions can create waterlogging issues for citrus trees. Citrus roots are limited to a depth of about 0.3 m, and the construction of raised beds of 0.5 to 1 m deep with longitudinal ditches can facilitate the drainage without harming the root system (Boman, 1994; Boman and Obreza, 2018; Kadyampakeni et al., 2014b). Another important consideration is the adjustment of bed width to allow easy access of agricultural machineries such as tractors, haulers, and trucks. The standard width on Florida groves is a 2.5 m driving middle for a 2.13 m wide tractor (Schumann et al., 2012). One of the limitations of such a restricted bed dimension is to obtain high-density planting by planting trees closer within the row.

Another important consideration while establishing the grove is the rootstock selection. Several studies have concluded that rootstocks should be a prime consideration while establishing a citrus grove because it affects scion vigor, fruit yield, fruit size, fruit quality, and pest tolerance (Wheaton et al., 1995; Zekri, 2000; Albrecht et al., 2019, Castle et al., 2019). Castle (2010) categorized rootstock selection based on long-term observation into three groups: 1) replacement rootstock, 2) alternative rootstock, and 3) special-purpose rootstock. Kuharske (*Citrus sinensis* × *Poncirus trifoliata*) citrange is considered as special-purpose rootstock because of its resistant nature against burring nematode, a problem in some areas of citrus-producing groves in Florida (Beeson et al., 2017; Castle et al., 2019). In addition to this, several studies reported Kuharske has high tolerance capacity against HLB and develops low pathogen titer (Folimonova et al., 2009; Bowman et al., 2016; Stover et al., 2016). Because of those specific reasons, we selected Kuharske in our study.

The use of controlled-release fertilizer (CRF) in citrus results in higher fruit yield and better fruit quality (Zekri and Koo, 1992; Obreza et al., 2006; Kadyampakeni et al., 2015; Morgan et al., 2018). Vashisth and Grosser (2018) also showed that CRF can result in greater fruit yields by constantly supplying nutrients to sweet orange trees affected by HLB. In addition to increasing fruit yield, CRF improves the nitrogen (N) fertilization efficiency and decreases the N leaching potential (Dou and Alva, 1998; Obreza et al., 1999; Obreza et al., 2006; Obreza and Morgan, 2008; Spyke et al., 2017).

The CRF slowly releases nutrients to the soil, reducing potential losses by leaching or volatilization and creating a nutrient reservoir (Spyke et al., 2017). The HLB-affected trees tend to show deficiency of micronutrients such as iron (Fe), manganese

(Mn), and zinc (Zn) in both roots and leaves compared to healthy trees (Spyke et al., 2017; Zambon et al., 2019). Several approaches have been developed such as foliar sprays to correct nutrient deficiencies in roots. Because of the low mobility of some ions, those approaches become unproductive leaving CRF application as a best way to synchronize the nutrient release according to the plant demand (Zambon et al., 2019). Elevating the soil micronutrients in citrus affected by HLB is beneficial to increase root mass and tree growth (Spyke et al., 2017). Foliar application of micronutrients is costly (Tansey et al., 2017) and challenging in Florida due to weather conditions. Very few studies have evaluated the application of micronutrients to the soil in the form of CRF in grapefruit. A recent study by Zambon et al. (2019) showed that soil applications of overdoses of Mn improved vascular function of HLB-affected sweet orange trees indicating tree health improvement.

It is necessary to evaluate the efficacy of soil application of elevated rates of micronutrients to monitor the performance of grapefruit in Florida's Flatwoods soil.

We hypothesized that tree density and soil micronutrient applications can increase the fruit yield of HLB-affected grapefruit in field conditions. The specific objectives of our study were: 1) to assess the best tree planting spacing (low-density single row, high-density single row, and high density staggered) for better fruit yield and tree growth; 2) to compare the soil application of two different fertilizer blends {(16N-1.31P-16.6K) and [(12N-1.31P-7.47K) with higher micronutrients]} for optimum grapefruit fruit yield.

Materials and Methods

Study Site

The study was conducted at the University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) Indian River Research and Education Center in Fort Pierce, Florida (27°26'0.8.2" N, 80°26'4.3.2" W, and altitude of 8 m) (Figure 2-1). Weather data was monitored by the Florida Automated Weather Network (FAWN) system utilizing the St. Lucie West weather station (Figure 2-2).

Plant Material

'Ray Ruby' grapefruit (*Citrus paradisi*) on Kuharske citrange rootstock was used as planting material. Trees were purchased from a commercial citrus nursery (Brite Leaf, Lake Panasoffkee, FL) and planted in September 2013.

Experimental Design and Treatments

The experiment was conducted in a split-plot design with four replicates (Table 2-1). We tested three planting densities {single row low-density (SR/LD) (4.5 × 7 m, 300 trees/ha), single row high-density (SR/HD) (3 × 7 m, 440 trees/ha), and double-row high-density staggered in diamond setting (DR/HD) [(2.7 × 1.5 × 1 m) × 6 m, 975 trees/ha]} and two CRF blends [16-3-20 (16N-1.31P-16.6K) with 81% of N and 50% of K as CRF with iron as chelates and all other micronutrients as sulfates and 12-3-9 (12N-1.31P-7.47K) with 100% of N and P and 95% of K as CRF with iron as chelates and all other micronutrients as sulfur-coated products] (both from Harrell's, Lakeland, FL). Details about the nutrient composition in both blends are displayed in Table 2-2. Both blends had a 4-month release period and were applied in February, July, and October. The CRF amount applied was calculated based on the N recommendations for grapefruit in Florida (180 kg ha^{-1} of N) as outlined by Obreza and Morgan (2008). The

CRF amount/tree was calculated by assuming the total of 358 trees/ha which is close to the current state's average (Singerman et al., 2018). Each tree received the same amount of nutrients despite of the planting density. The fertilizer was applied manually under the dripline avoiding row middles in the single row spacing as recommended by Obreza and Morgan (2008). For DR/HD, fertilizer was applied around tree canopy area.

The study was conducted on 3-year-old trees for two seasons: 2017/18 and 2018/19. The overall experimental area was 2.13 ha consisting of eight raised beds with two rows each (15.25 m wide × 175 m long). There were 24 experimental units in equal size having dimensions of 15.24 m width and 58.23 m length. The total number of trees in each plot was 26, 38 and 84 in SR/LD, SR/HD, and DR/HD, respectively. Each plot consisted of two beds where one tree at each end of the bed was kept as a border tree.

Soil Type and Grove Layout

Trees were planted on a Pineda soil series, classified as loamy, siliceous, active hyperthermic Arenic Glossaqualfs (USDA, 2020). The land profile present natural slopes ranging from 0 to 2%, and poorly drained soil. This soil has 96% sand, 2.5% silt, and 1.5% clay with 0.5–2.0% organic matter (Obreza and Morgan, 2008).

We used micro-sprinkler irrigation due to the possibility of stress in the plant in the period of low rainfall (Boman and Obreza, 2018) and the requirement of frequent irrigation of smaller amount of water in HLB affected trees (Kadyampakeni et al., 2014a). One 40.5-liter/hour micro-sprinkler (40 blue nozzle size; Bowsmith, Exeter, CA) was installed/tree in the treatments with 300 and 440 trees/ha, and one micro-sprinkler was installed per two trees in 975 trees/ha. The irrigation controller (HI10000Pro; Hanna Instruments, Woonsocket, RI) was programmed to irrigate the trees twice a day. The amount of water application was adjusted based on the equation derived using the

canopy area, the class A pan evaporation in inches/day, and the pan evaporation factor as suggested by Wright (2000).

The grove construction involved parallel beds consisting of two rows of trees planted in each bed oriented north-south to have better interception of sunlight from all directions. The drain ditches were 15.24 m apart to facilitate water drainage. We achieved 440 trees/ha by planting trees 3 × 7 m (plant to plant × row to row distance). Spacing wider than necessary result in fewer plants per area and thus the fruit yield per unit area decrease. Tree spacing determination should be based on several factors such as climate, soil, scion, rootstock, and expected lifespan of the grove (Vincent et al., 2019). For high-density grove, we came to an approach that involves the formation of double-row configurations. In this planting system, the two rows are separated at a distance of 91.44 cm only. Trees were arranged in triangular fashion so that the herbicide can be sprayed without obstruction from adjacent trees on either side. In our experiment, the high-density double-row staggered in diamond setting [(2.7 × 1.5 × 1 m) × 6 m] resulted in 975 trees/ha.

The grove maintenance and caretaking followed standard practices (Vincent et al., 2019).

Concentration of *C*Las DNA on Plant Leaf Tissue

Leaf samples were collected in April for the determination of the concentration of *C*Las DNA on plant leaf tissue. Symptomatic, fully expanded, and hardened matured leaves were collected with the leaf petiole attached. The samples were collected from the branches with mottled leaves. Eight random trees were chosen per plot excluding border trees to collect leaf samples. The collected leaf samples were placed in a re-sealable zip-lock plastic bag and carefully placed in a cooler with ice cubes to prevent

heat damage. To avoid direct contact between the samples bag and ice cubes, a jute bag was placed as a separation layer. The samples were analyzed by real-time quantitative polymerase chain reaction (PCR) (Li et al., 2006).

Tree Growth

Tree growth was measured twice a year: after spring flush (June/July) and before fruit harvesting (December). Only data from December is presented on each year since it reflects the largest tree size in a growing season. For the measurements, eight trees were selected randomly in each plot excluding the border trees. Trunk diameter was measured at 8 cm above the graft union in a North-South direction using a digital caliper. Tree height was measured from the soil surface perpendicular to the tree. Tallest leaf or branch was measured using the meter stick (Model No. 98024; Seco Manufacturing, Mound City, IL). Canopy diameter was measured parallel (North-South direction) and perpendicular (East-West direction) of a canopy to the tree row. Canopy volume was calculated by using a geometric prolate spheroid: $[(4/3)\ (\pi)\ (\text{tree height}/2)\ (\text{average canopy diameter})^2]$ (Obreza and Rouse, 1993).

Fruit Number, Size and Yield

For the determination of fruit number and size, fruit from each plot (Eight trees from each plot were randomly selected excluding the border trees) were sorted out by passing through an optical sorter (Autoline; Reedley, CA). Tiny, diseased, and damaged fruit were discarded. The sorter was programmed to categorize fruit size into the following count categories: >21 fruit/carton (> 123 mm), 27 (123–117 mm), 32 (117–111 mm), 36 (111–105 mm), 40 (105–100 mm), 48 (100–95 mm), 56 (95–90 mm), 64 (90–84 mm), < 64 (< 84 mm). The fruit diameter is calculated as: (average diameter in each fruit count category × the number of fruit on that category) ÷ total fruit count.

To determine fruit yield, fruit from an individual tree was hand picked, collected using a fruit harvesting bag and placed in a container. Fruit was weighed using a portable scale (D51P60HR1; Ohaus, Los Angeles, CA). The total fruit yield per area was calculated by multiplying the total weight of fruit/tree and the total number of area.

Fruit Quality and Yield of solids

For the fruit quality determination, 20 uniform size fruit ranging from 90–95 mm diameter were selected. Fruit were weighed and passed through a juice press (model 2720; Brown International Corporation, Covina, CA). Juice volume and juice weight were recorded. The soluble solids content of the juice was measured using a refractometer (HI96801; Hanna Instruments, Woonsocket, RI). A pipette was used to pour two to three drops of juice in the clean glass measuring surface of the refractometer. The acidity of the fruit was measured by using an automatic potentiometric titrator (HI931; Hanna Instruments, Woonsocket, RI). For titration, 25 mL of sample juice was diluted with deionized water to make 50 mL. Later the solution was titrated using a solution of sodium hydroxide 1N. Fruit quality is also expressed in ratio calculated as soluble solids content divided by titratable acidity.

Obreza et al. (1999) reported the gross dollar return per orange tree calculation by using pound solids yield and a seasonal average price fruit that goes to concentrated juice. Pound solids or juice yield is considered an important indication of internal fruit quality to processing plants (Wardowski et al., 1995). Even though grapefruit is not produced specifically for canning, the fruit that does not meet the quality standards for the fresh market goes to processing. Further, the juice yield was calculated and expressed as solids yield/ha of fruit calculated by multiplying (juice percentage in fruit ÷ 100), (soluble solids content ÷ 100) and fruit yield (kg ha^{-1}) (Ferrarezi et al., 2019).

Leaf Nutrient Concentration

Leaf samples were collected in August for the determination of leaf nutrient concentration. Fully expanded and hardened matured leaves were collected with the leaf petiole avoiding immature, diseased, insect-damaged, mechanically injured or dead leaves (Obreza and Morgan, 2008). Eight random trees were chosen excluding border trees to collect leaf samples. The samples were preserved in a cooler during the sampling period to prevent from heat damage. The samples were acid-washed prior to analysis. The samples were placed in an oven at 80 ^{o}C and left overnight to get dry. The dried samples from each plot were ground to pass a 1-mm mesh screen (Wiley Laboratory Mill Model 4 3375-E10; Thomas Scientific, Swedesboro, NJ). Five grams of leaf samples were analyzed using the dry-ashing method, and assessed by inductively coupled plasma atomic emission spectroscopy (ICP–AES) at the Water laboratory (Camilla, GA) to determine the concentration of P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn. Leaf N concentration was measured by macro dry combustion using a LECO CNS-2000 analyzer (LECO Corporation, St. Joseph, MI).

Soil Nutrient Concentration

Samples were collected before fertilization in February. The samples were collected 60 cm away from the trunk in the area wetted by the micro-sprinkler for maximum root activity by following the methods recommended by Obreza and Morgan (2008). One 20-cm deep soil core/tree in 15 different locations/plot was taken. The subsamples were thoroughly mixed in a nonmetal bucket to get a composite representative sample. The samples were analyzed by Mehlich 3 acid extraction (Obreza and Morgan, 2008) to determine extractable P, K, Ca, and Mg along with S, B, Cu, Fe, Mn, and Zn using 0.2 M CH_3COOH, 0.015 M NH_4F, 0.013 M HNO_3, 0.001 M

EDTA, and 0.25 M NH_4NO_3. For soil pH determination, 25 g of homogeneous soil samples were mixed with 25 mL of deionized water. The solution was stirred, and the measurement was taken following the methods outlined by Schofield et al. (1955).

Statistical Analysis

Data were analyzed by year for two consecutive seasons (2017/18 and 2018/19). Generalized linear mixed model was used to analyze error variance, with treatments entered as fixed effect and block as a random effect. The data were checked for assumptions of the linear model. Log transformation and square root transformation was executed as needed. Data were submitted to analysis of variance by the F-test and, when significant, multiple comparisons were assessed by Tukey's posthoc honest significant difference test ($P \leq 0.05$). Statistics were performed using the software SAS 9.4 (SAS Institute, Cary, NC).

Result and Discussion

Concentration of *C*Las DNA on Plant Leaf Tissue

HLB was present during all the experimental period, with visible disease symptoms such as loss of foliage, fruit drop, and tree death. Ct values of *C*Las DNA were lower than 32 in all the treatments during the experimental period with no statistical differences among treatments (Table 2-3). There was a significant interaction between planting density and the CRF blends ($P = 0.0013$) in 2018/19 but the Ct value was also lower than 32. Values lower than 32 are considered positive in the PCR test indicating all the sampled trees were affected by HLB (Albrecht and Bowman, 2011; Shin and van Bruggen, 2018).

Our study shows no effect of planting density on Ct value of *C*Las DNA. Recently Moreira et al. (2019) observed high-density resulted in lower HLB incidence in sweet

oranges, indicating those trees might have an advantage caused by a lower psyllid to plant ratio compared to low-density planting. High-density planting could benefit diluting the psyllid population resulting in lower disease incidence. However, that was not the case with grapefruit on this study.

Few literature sources are available about the effect of higher than recommended micronutrient rates on Ct value of HLB affected grapefruit. Recently, Zambon et al. (2019) showed that the ground application of 4× recommended rate of Mn increased the Ct value of HLB affected 'Vernia' sweet orange. The authors suggested that elevated application of Mn could be toxic to the *C*Las but not to the tree. The same effect was not observed when the Mn was applied together with B. The potential cause of no effect of micronutrients can be explained by the fact that HLB results in loss of fibrous roots which ultimately results in lower nutrient absorption.

Tree Growth

Canopy volume was significantly affected by planting density ($P < 0.0001$). With the increase in planting density, canopy volume decreased (Table 2-4). In 2017/18, canopy volume was 28% lower in DR/HD planting than the canopy volume of the other treatments. In 2018/19, canopy volume in DR/HD planting was 28% and 33% lower than SR/HD and SR/LD planting. Similarly, trunk diameter was also affected by planting density ($P < 0.0001$). With an increase in planting density, trunk diameter decreased (Table 2-4). In 2017/18, trunk diameter was 11% lower in DR/HD planting than the trunk diameter of other treatments. In 2018/19, trunk diameter in DR/HD planting was 13% and 17% lower than SR/HD and SR/LD planting densities, respectively.

Our study indicates planting density affects tree growth. This is expected since the competition for water, fertilizer, and solar radiation is aggravated by high-density

planting. Previous studies support that canopy volume decreases with an increase in planting densities. A study conducted by Zaman and Schumann (2005) measuring tree volume of 'Valencia' sweet orange manually and using an ultrasonic device indicated greater canopy growth in widely spaced trees.

Canopy volume was 10% higher in 2017/18 and 9% higher in 2018/19 using 12N-1.31P-7.47K blend with similar trunk diameter (Table 2-4). Statistical analysis showed no interaction between planting and CRF blends ($P > 0.05$).

Fruit Number, Size and Yield

The fruit yield was 30% lower in 2018/19 compared to 2017/18 due to Hurricane Irma which hit Florida as a Category 4 storm in September 2017. Extensive damages during and after hurricanes are expected. Ferrarezi et al. (2020) showed the impact of hurricanes and tropical storms in citrus production due to fruit damage and fruit drop along with deterioration of the health of grove for several seasons by spreading pests and diseases.

In 2017/18, DR/HD planting resulted in the and lowest fruit yield/tree ($P = 0.0214$) and eight less fruit than SR/LD ($P = 0.0322$). In the following year, fruit yield was similar in all the planting densities ($P > 0.05$). The DR/HD produced the greatest fruit yield (9968 kg ha^{-1}). The SR/HD and SR/LD produced 43% and 62% less fruit than DR/HD (Table 2-5). In 2018/19, DR/HD produced the highest fruit yield (6886 kg ha^{-1}). The SR/LD produced 62% lower fruit yield than DR/HD (Table 2-5).

Several studies have suggested that high-density planting of citrus produces greater fruit yield per unit area by increasing the ground coverage and optimum utilization of the resources without compromising fruit quality (Wheaton et al., 1991; Wheaton et al., 1995; Dalal et al., 2013; Singerman et al., 2018; Moreira et al., 2019).

High-density planting is a practical way to increase fruit yield per unit area in HLB prevalent condition. This is the case because the trees affected with HLB requires smaller space due to lower canopy volume (Bowman et al., 2016) and lesser fibrous roots (Graham et al., 2013) compared to healthy trees. Thus, HLB affected trees planted in high-density planting can easily get access to sunlight, nutrients, and soil moisture.

In 2017/18 season, 16N-1.31P-16.6K blend induced 9 more fruit/tree, 30% higher yield/tree, 32% more fruit yield per area There was no interaction between CRF blends and planting densities (Table 2-5).

The high micronutrient rates applied did not increase the fruit and solids yields, indicating nutritional treatments may not contribute to higher fruit yield in grapefruit affected with HLB.

Prior studies concluded that canopy volume is directly correlated to fruit yield. Levy et al. (1978) observed that increase in canopy volume result in grapefruit fruit yield possibly due to increase in the photosynthetic area. Our study showed no relationship between canopy growth and fruit yield in grapefruit affected by HLB (Tables 2-4 and 2-5).

Fruit Quality and Yield of solids

The soluble solids content were affected by treatments only in 2017/18. Fruit produced under DR/HD planting density induced 6% higher soluble solids than SR/LD. Fruit acidity was affected by planting density only in 2018/19. Fruit from DR/HD planting was 16% more acidic (titratable acidity 1.16 mg 100 mL^{-1}) than other treatments. In 2017/18, DR/HD yielded the greatest yield of solids (360 kg ha^{-1}). SR/HD and SR/LD produced 46% and 65% less yield of solids than DR/HD planting. In 2018/19, DR/HD

yielded the greatest yield of solids (245 kg ha^{-1}). The SR/LD produced 65% lower yield of solids than DR/HD planting (Table 2-6).

We observed that grapefruit grown under high-density planting resulted in deposition of more sugar in the fruit. It could be because of competition for water use, which increases fruit acidity and deposition of more sugars probably due to modifications in fruit water content and osmotic adjustment. Although the role of abiotic stresses in the synthesis of soluble sugar in citrus is not clear, the stress might influence the production of hormones such as abscisic acid and ethylene, which can alter the sugar sensing mechanism (Rosa et al., 2009). García-Tejero et al. (2010) suggested that water stress in sweet orange influences organoleptic fruit parameters, such as total soluble solids and total acidity with very low influence in morphological variables such as fruit weight, equatorial diameter or peel thickness. Hutton and Loveys (2011) suggested that reduction in water input due to partial root zone drying in navel orange resulted in significant reductions in the juice percent and an increase in percent acid in the experimental period without changing the sugar content.

Statistical analysis between the different CRF blends was significant for soluble solids content (P = 0.0038) only in 2017/18 (Table 2-6). The use of 16N-1.31P-16.6K resulted in greater soluble solids content and greater yield of solids than 12N-1.31P-7.47K. Juice obtained from 16N-1.31P-16.6K induced 5% higher soluble solids than 12N-1.31P-7.47K possibly because the 16N-1.31P-16.6K contained higher K levels (225 kg ha^{-1}) than 12N-1.31P-7.47K (135 kg ha^{-1}). Obreza (2003) also observed the application of 225 kg ha^{-1} of K in grapefruit resulted in higher soluble solids content and

a higher number of fruit. The following year (2018/19) had no effect of treatments on the number of fruit and fruit quality.

As an industry-standard, the higher the ratio, the greater is the fruit grade. The USDA classifies three grades of grapefruit based on its quality 1) U.S. Grade A, 2) U.S. Grade B, and 3) Substandard. Grade ‘A’ and ‘B’ are further classified into two different categories: unsweetened and sweetened based on soluble solids: acid ratio. The minimum ratio unsweetened for grade B is 7:1 and the maximum ratio sweetened for grade A is 14:1 (USDA, 2012). The ratio and remained the same with the application of the different treatments.

Leaf Nutrient Concentration

Leaf nutrient concentration varied by year as the tree grew (Tables 2-7 and 2-8). Planting density did not change the nutrient concentration ($P > 0.05$).

In 2017/18, 16N-1.31P-16.6K resulted in higher leaf K and P concentration. In 2018/19, 16N-1.31P-16.6K resulted in higher leaf K concentration and lower concentration of Ca, Fe, Mn, and S in the leaf. The leaf B concentration was higher in trees treated with 12N-1.31P-7.47K in both 2017/18 and 2018/19.

In 2017/18, 16N-1.31P-16.6K resulted in higher leaf P concentration. That could be due to the interaction of soil pH and plant nutrients since 16N-1.31P-16.6K resulted in pH values close to the neutral than 12N-1.31P-7.47K blend which could have facilitated plant to uptake more P. The concentration of B was higher in trees treated with 12N-1.31P-7.47K blend during the experimental period since this blend contained more B. Kadyampakeni (2019) also observed a linear increase in leaf nutrient concentration during fall with an increase in B application rate.

The interpretation based on Koo et al. (1984) showed in 2017/18 that Mn and Zn were deficient in plant leaf in some treatments (< 25 mg kg^{-1}). Additionally, in 2018/19, B was above sufficiency range in plant tissue (> 120 mg kg^{-1}) except at 16N-1.31P-16.6K blend. Further, the concentration of Cu was higher range (> 20 mg kg^{-1}) all the time due to the frequent application of copper-based fungicides to control citrus canker (*Xanthomonas axonopodis)*.

Soil Nutrient Concentration

Planting densities did not change the soil nutrient concentration overtime. Furthermore, there was no interaction between planting density and CRF blends (Tables 2-9, 2-10).

The use of 16N-1.31P-16.6K blend increased soil pH, Mg, and soil B concentration but a decrease in Mn concentration. Cation exchange capacity was similar for both treatments.

The higher soil pH in 16N-1.31P-16.6K blend is possibly due to the presence of more base forming cation K^+. Our study obtained similar results as described by McCauley et al. (2009) that soil pH can be greatly influenced by base- and acid-forming ions in the soil. Further, the soil pH was in the optimum range for nutrient availability (Obreza and Morgan, 2008). Even though, the soil was calciferous in our research area; the S nutrient in the fertilizer blends could have lower pH value. Shober et al. (2018) suggested that the elemental application of S can temporarily lower soil pH in alkaline soil.

Using the soil test interpretation outlined by Obreza and Morgan (2008), the Mehlich 3 acid extraction of our soil sample showed a high level of Ca (> 200 mg kg^{-1}), optimum level of P (17–29 mg kg^{-1}), and low Mg (< 25 mg kg^{-1}) in the sampled soil. The

concentration of B was higher in soil at 16N-1.31P-16.6K blend possibly due to higher leaf uptake at 12N-1.31P-7.47K blend resulting in less residue in soil (Tables 2-8 and 2-10). We also observed greater Mg concentration in soils at 16N-1.31P-16.6K possibly due to interaction of pH and nutrient as 16N-1.31P-16.6K resulted in higher pH which could have increased the Mg availability in the soil.

Conclusions

Our study shows high-density planting produced more fruit with more solids per unit area. Fruit produced from high-density planting tends to increase the soluble solids content to more acidic fruit. Planting density can also affect tree growth. High-density planting resulted in smaller tree trunk diameter and lower canopy volume.

The use of a blend with higher sulfur-coated micronutrient did not change the Ct value or HLB incidence on grapefruit. The increased micronutrient application resulted in higher canopy growth without contributing to fruit quality.

In summary, high-density planting is a good strategy for grapefruit production under severe HLB conditions. However, the cost for weed control and long-term effects of high-density planting will need to be accessed for a commercial recommendation.

Further nutritional studies using other rootstocks are necessary to expand the understanding of enhanced nutritional treatments on HLB-affected grapefruit.

Table 2-1. Brief description of the experimental design.

Treatment design	Factor and level
Main plot	CRF application in the soil 1. (16-3-20) 16N-1.31P-16.6K with 81% of N and 50% of K as CRF with iron as chelates and all other micronutrients as sulfates 2. (12-3-9) 12N-1.31P-7.47K with 100% of N and P, and 95% of K as CRF with iron as chelates and all other micronutrients as sulfur-coated products at higher rate
Subplot	Planting density 1. Single row low-density (SR/LD): 300 trees/ha 2. Single row high-density (SR/HD): 440 trees/ha 3. Double-row high-density (DR/HD): 975 trees/ha

Table 2-2. The nutrient composition and amount applied in two different controlled-release fertilizer (CRF) blends.

	16-3-20 (16N-1.31P-16.6K)		12-3-9 (12N-1.31P-7.47K)	
	Nutrient (%)	Amount (kg ha^{-1})	Nutrient (%)	Amount (kg ha^{-1})
N	16	180	12	180
P_2O_5	3	34	3	45
K_2O	20	225	9	135
Ca	1	11	2	30
Mg	1.50	16	2.30	35
S	6.50	73	14.90	223
B	0.06	0.67	0.07	1.09
Cu	0	0	0.04	0.60
Fe	0.53	5.88	1.04	15.58
Mn	1.59	17.82	1.27	18.90
Mo	0	0	0.01	0.09
Zn	0.53	5.88	0.49	7.32

Table 2-3. Cycle threshold (Ct) value of *C*Las DNA as a function of controlled-release fertilizer (CRF) and tree planting density (PD) in 2017/18 and 2018/19.

Treatment	Ct value of *C*Las DNA	
	2017/18	2018/19
CRF[z]		
12-3-9	25.98 ± 1.60[x]	24.70 ± 0.38
16-3-20	25.45 ± 1.05	25.45 ± 0.41
PD[y]		
SR/LD	27.69 ± 2.23	25.10 ± 0.49
SR/HD	23.88 ± 0.55	25.34 ± 0.61
DR/HD	25.58 ± 1.55	24.79 ± 0.42
Sources of variation	*Probability (P) value*	
CRF	0.7930 [NS]	0.1148 [NS]
PD	0.2534 [NS]	0.5445 [NS]
CRF*PD	0.2146 [NS]	0.0013**

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value [NS] = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-4. Trunk diameter and canopy volume as a function of controlled-release fertilizer (CRF) and planting density (PD) in 2017/18 and 2018/19.

Treatment	Trunk diameter (mm)		Canopy volume (m^3)	
	2017/18	2018/19	2017/18	2018/19
CRF[z]				
12-3-9	75.61 ± 0.82[x]	82.50 ± 0.49	6.74 ± 0.18 a	8.25 ± 0.13 a
16-3-20	75.45 ± 0.71	81.50 ± 0.48	6.09 ± 0.15 b	7.52 ± 0.12 b
PD[y]				
SR/LD	81.45 ± 0.77 a	88.85 ± 0.51 a	7.16 ± 0.19 a	9.19 ± 0.16 a
SR/HD	78.50 ± 0.65 a	84.87 ± 0.46 b	7.03 ± 0.20 a	8.55 ± 0.14 b
DR/HD	66.63 ± 0.80 b	73.33 ± 0.50 c	5.05 ± 0.17 b	6.13 ± 0.12 c
Sources of variation			*Probability (P) value*	
CRF	0.8535 [NS]	0.0815 [NS]	0.0026**	<0.0001***
PD	<0.0001***	<0.0001***	<0.0001***	<0.0001***
CRF*PD	0.0982 [NS]	0.5726 [NS]	0.1261 [NS]	0.4100 [NS]

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 32) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value [NS] = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-5. Total number of fruit, fruit diameter, fruit yield/tree, and fruit yield/area as a function of controlled-release fertilizer (CRF) and tree planting density (PD) in 2017/18 and 2018/19.

Treatment	Total number of fruit (No.)		Fruit diameter (mm)		Fruit yield/tree (kg)		Fruit yield/area (kg ha^{-1})	
	2017/18	2017/18	2017/18	2017/18	2017/18	2017/18	2017/18	2017/18
CRF[z]								
12-3-9	25.42 ± 1.63 b[x]	27.71 ± 2.54	100.90 ± 1.15	96.53 ± 0.65	10.67 ± 0.60 b	9.56 ± 0.90	5588.03 ± 368.27 b	4973.00 ± 515.38
16-3-20	34.24 ± 1.84 a	23 ± 2.08	102.90 ± 0.43	96.03 ± 2.14	13.55 ± 0.73 a	7.52 ± 0.69	7367.82 ± 522.63 a	4093.44 ± 507.27
PD[y]								
SR/LD	34.02 ± 2.29 a	27.78 ± 3.10	102.75 ± 2.29	97.27 ± 3.10	12.90 ± 0.78 ab	9.04 ± 1.04	3782.95 ± 231.59 c	2651.67 ± 304.75 b
SR/HD	29.61 ± 2.42 ab	27.81 ± 2.95	102.28 ± 2.42	97.12 ± 0.79	13.19 ± 1 a	9.47 ± 1.10	5654.64 ± 429.58 b	4061.69 ± 469.91 ab
DR/HD	26 ± 1.74 b	20.44 ± 2.37	100.66 ± 1.74	94.46 ± 3.19	10.27 ± 0.65 b	7.10 ± 0.79	9967.98 ± 633.74 a	6886.30 ± 770.91 a
Sources of variation	*Probability (P) value*							
CRF	0.0004	0.1484 ^{NS}	0.1082 ^{NS}	0.8235 ^{NS}	0.0026***	0.0759 ^{NS}	0.0006***	0.1718 ^{NS}
PD	0.0322 ^{NS}	0.1074 ^{NS}	0.3494 ^{NS}	0.5159 ^{NS}	0.0214*	0.1965 ^{NS}	<0.0001***	<0.0001***
CRF*PD	0.6181 ^{NS}	0.4392 ^{NS}	0.3064 ^{NS}	0.2620 ^{NS}	0.5317 ^{NS}	0.6685 ^{NS}	0.0570 ^{NS}	0.8929 ^{NS}

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 32) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value ^{NS} = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-6. Soluble solids content, acidity, ratio, and yield of solids as a function of controlled-release fertilizer (CRF) and planting density (PD) in 2017/18 and 2018/19.

Treatment	Soluble solids content (%)		Acidity (g/100mL)		Ratio		Yield of solids (kg ha^{-1})	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
CRF[z]								
12-3-9	7.68 ± 0.06 b	8.07 ± 0.17	0.99 ± 0.01	1.10 ± 0.02	7.82 ± 0.13	7.40 ± 0.09	188.24 ± 17.49 b	187.43 ± 22.84
16-3-20	8.08 ± 0.08 a	8.04 ± 0.15	1.06 ± 0.02	1.04 ± 0.02	7.7 ± 0.14	7.73 ± 0.15	265.55 ± 28.37 a	146.04 ± 23.00
PD[y]								
SR/LD	7.67 ± 0.09 b	7.59 ± 0.15	1.05 ± 0.02	1.00 ± 0.02 b	7.36 ± 0.16	7.56 ± 0.1	125.76 ± 9.32 c	103.90 ± 12.15 b
SR/HD	7.83 ± 0.11 ab	8.03 ± 0.12	1 ± 0.02	1.02 ± 0.01 b	7.84 ± 0.06	7.88 ± 0.15	194.92 ± 13.51 b	150.88 ± 17.79 ab
DR/HD	8.14 ± 0.06 a	8.55 ± 0.23	1 ± 0.03	1.18 ± 0.02 a	8.08 ± 0.2	7.26 ± 0.16	360.00 ± 24.52 a	245.42 ± 36.92 a
Sources of variation	*Probability (P) value*							
CRF	0.0038**	0.9361 NS	0.0567 NS	0.0846 NS	0.6390 NS	0.1731 NS	0.0013***	0.3412 NS
PD	0.0177**	0.0610 NS	0.5558 NS	0.0001***	0.0945 NS	0.1262 NS	<0.0001***	0.0401*
CRF*PD	0.4239 NS	0.9978 NS	0.6254 NS	0.9162 NS	0.9100 NS	0.9205 NS	0.0546 NS	0.9677 NS

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 32) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value NS = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-7. Leaf macronutrient concentration as a function of controlled-release fertilizer (CRF) and planting density (PD) in 2017/18 and 2018/19.

Treatment	N (mg kg^{-1})		P (mg kg^{-1})		K (mg kg^{-1})		Ca (mg kg^{-1})		Mg (mg kg^{-1})		S (mg kg^{-1})	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
CRF[z]												
12-3-9	2.79 ± 0.04[x]	2.80 ± 0.06	0.19 ± 0.003 b	0.17 ± 0.004	1.46 ± 0.02 b	1.16 ± 0.06 b	3.00 ± 0.04	4.87 ± 0.06 a	0.34 ± 0.004	0.34 ± 0.006	0.32 ± 0.005	0.40 ± 0.01 a
16-3-20	2.81 ± 0.02	2.83 ± 0.05	0.20 ± 0.002 a	0.18 ± 0.005	1.65 ± 0.05 a	1.42 ± 0.06 a	2.88 ± 0.07	4.25 ± 0.10 b	0.33 ± 0.006	0.33 ± 0.02	0.33 ± 0.005	0.37 ± 0.01 b
PD[y]												
SR/LD	2.85 ± 0.01	2.80 ± 0.07	0.20 ± 0.004	0.17 ± 0.006	1.59 ± 0.07	1.16 ± 0.09	2.90 ± 0.03	4.58 ± 0.16	0.33 ± 0.007	0.34 ± 0.01	0.31 ± 0.005	0.40 ± 0.01
SR/HD	2.80 ± 0.02	2.79 ± 0.06	0.20 ± 0.002	0.18 ± 0.007	1.56 ± 0.05	1.32 ± 0.06	2.96 ± 0.08	4.62 ± 0.14	0.34 ± 0.007	0.34 ± 0.01	0.32 ± 0.007	0.38 ± 0.008
DR/HD	2.75 ± 0.05	2.86 ± 0.08	0.20 ± 0.004	0.18 ± 0.006	1.52 ± 0.05	1.37 ± 0.04	2.95 ± 0.10	4.48 ± 0.16	0.34 ± 0.007	0.34 ± 0.02	0.33 ± 0.006	0.37 ± 0.02
Sources of variation	*Probability (P) value*											
CRF	0.6050 NS	0.7200 NS	0.0047	0.0990 NS	0.0041**	0.0056**	0.1575 NS	0.0001** *	0.9131 NS	0.6845 NS	0.2273 NS	0.0306*
PD	0.0980 NS	0.7888 NS	0.0843 NS	0.4560 NS	0.6247 NS	0.1168 NS	0.7916 NS	0.6457 NS	0.3173 NS	0.9669 NS	0.2021 NS	0.424 NS
CRF*PD	0.4886 NS	0.9899 NS	0.5345 NS	0.2046 NS	0.5907 NS	0.2345 NS	0.1692 NS	0.9123 NS	0.5405 NS	0.9751 NS	0.2367 NS	0.2565 NS

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value NS = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-8. Leaf micronutrient concentration a function of controlled-release fertilizer (CRF) and tree planting density (PD) in 2017/18 and 2018/19.

Treatment	B (mg kg^{-1})		Cu (mg kg^{-1})		Fe (mg kg^{-1})		Mn (mg kg^{-1})		Zn (mg kg^{-1})	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
CRF[z]										
12-3-9	92.49 ± 2.90 a[x]	138.43 ± 3.20 a	174.78 ± 23.23	255.59 ± 25.70	86.11 ± 3.87	98.78 ± 4.20 a	23.49 ± 0.68	55.91 ± 4.16 a	21.54 ± 1.64	42.01 ± 3.42
16-3-20	75.2 ± 3.40 b	106.95 ± 5.50 b	148.53 ± 10.74	241.34 ± 19.16	85.62 ± 4.13	75.08 ± 5 b	24.29 ± 1.04	39.63 ± 6 b	28.78 ± 3.69	35.84 ± 4.54
PD[y]										
SR/LD	84.85 ± 6.19	122.54 ± 8.70	164.25 ± 9.54	252.32 ± 33.47	87.85 ± 4.07	87.97 ± 10.30	22.57 ± 1.11	56.76 ± 7.19	23.13 ± 3.50	42.81 ± 5.89
SR/HD	83.41 ± 3.39	122.64 ± 6.20	148.33 ± 10.34	250.30 ± 25.30	86.35 ± 5.65	85.76 ± 3.78	23.51 ± 0.76	51.33 ± 6.09	24.72 ± 2.50	42.67 ± 4.47
DR/HD	83.28 ± 5.41	122.90 ± 9.35	172.39 ± 37.10	242.78 ± 26.01	83.40 ± 5.06	87.07 ± 6.22	25.6 ± 1.11	35.23 ± 5.17	27.62 ± 4.90	31.3 ± 3.62
Sources of variation	*Probability (P) value*									
CRF	0.0011**	0.0002***	0.2770 ^{NS}	0.6824 ^{NS}	0.9381 ^{NS}	0.0010**	0.5207 ^{NS}	0.0062**	0.1025 ^{NS}	0.9731 ^{NS}
PD	0.9505 ^{NS}	0.9990 ^{NS}	0.6998 ^{NS}	0.9717 ^{NS}	0.8382 ^{NS}	0.9554 ^{NS}	0.1455 ^{NS}	0.403 ^{NS}	0.6829 ^{NS}	0.8635 ^{NS}
CRF*PD	0.1491 ^{NS}	0.5564 ^{NS}	0.4031 ^{NS}	0.5567 ^{NS}	0.9903 ^{NS}	0.0441	0.7402 ^{NS}	0.5349 ^{NS}	0.4925 ^{NS}	0.5662 ^{NS}

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value ^{NS} = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-9. Soil pH, Cation exchange capacity (CEC), and soil macronutrient concentration as a function of controlled-release fertilizer (CRF) and tree planting density (PD) in 2018/19.

Treatment	Soil pH	CEC (meq gm^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	S (mg kg^{-1})
CRF[z]							
12-3-9	6.00 ± 0.12 b[x]	3.03 ± 0.06	15.88 ± 1.06	20.29 ± 1.75	350.92 ± 20.37	19.29 ± 1.03 b	18.08 ± 2.40
16-3-20	6.38 ± 0.12 a	3.19 ± 0.26	13.67 ± 1.50	21.00 ± 1.46	422.13 ± 56.55	24.08 ± 1.20 a	13.75 ± 2.07
PD[y]							
SR/LD	6.00 ± 0.19	3.23 ± 0.36	16.44 ± 1.74	19.25 ± 1.51	403.06 ± 89.50	20.38 ± 1.42	15.19 ± 3.20
SR/HD	6.40 ± 0.12	3.04 ± 0.17	14.31 ± 1.33	21.25 ± 2.11	379.88 ± 28.36	23.25 ± 2.26	15.38 ± 2.73
DR/HD	6.17 ± 0.13	3.06 ± 0.06	13.56 ± 1.75	21.44 ± 2.28	376.63 ± 13.73	21.44 ± 0.79	17.19 ± 2.76
Sources of variation				*Probability (P) value*			
CRF	0.0268*	0.6867 [NS]	0.2848 [NS]	0.7487 [NS]	0.3303 [NS]	0.0387*	0.2279 [NS]
PD	0.1489 [NS]	0.9322 [NS]	0.4772 [NS]	0.7572 [NS]	0.9692 [NS]	0.3422 [NS]	0.8748 [NS]
CRF*PD	0.4637 [NS]	0.8046 [NS]	0.7412 [NS]	0.7000 [NS]	0.7102 [NS]	0.4946 [NS]	0.4139 [NS]

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value [NS] = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2-10. Soil micronutrient concentration a function of controlled-release fertilizer (CRF) and tree planting density (PD) in 2018/19.

Treatment	B (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
CRF[z]					
12-3-9	0.19 ± 0.01 b[x]	13.52 ± 0.56	8.33 ± 0.74	4.96 ± 0.42 a	9.81 ± 0.61
16-3-20	0.38 ± 0.07 a	14.07 ± 0.70	8.83 ± 0.84	2.42 ± 0.19 b	9.78 ± 0.91
PD[y]					
SR/LD	0.31 ± 0.11	13.95 ± 0.83	9.19 ± 0.85	4.13 ± 0.81	10.09 ± 1.16
SR/HD	0.25 ± 0.02	13.74 ± 0.48	9.75 ± 0.89	3.38± 0.43	9.31 ± 0.93
DR/HD	0.29 ± 0.05	13.69 ± 1.00	6.81 ± 0.88	3.56 ± 0.55	9.99 ± 0.76
Sources of variation			*Probability (P) value*		
CRF	0.0149*	0.5500 [NS]	0.6862 [NS]	0.0062**	0.9731 [NS]
PD	0.7541 [NS]	0.9800 [NS]	0.0757 [NS]	0.4030 [NS]	0.8635 [NS]
CRF*PD	0.2282 [NS]	0.9548 [NS]	0.6930 [NS]	0.5349 [NS]	0.5662 [NS]

[z]12N-1.31P-7.47K, and 16N-1.31P-16.6K

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at the $P < 0.05$ level by Tukey's test.

[w]Probability value [NS] = Nonsignificant at $P < 0.05$. *, **, and *** = Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

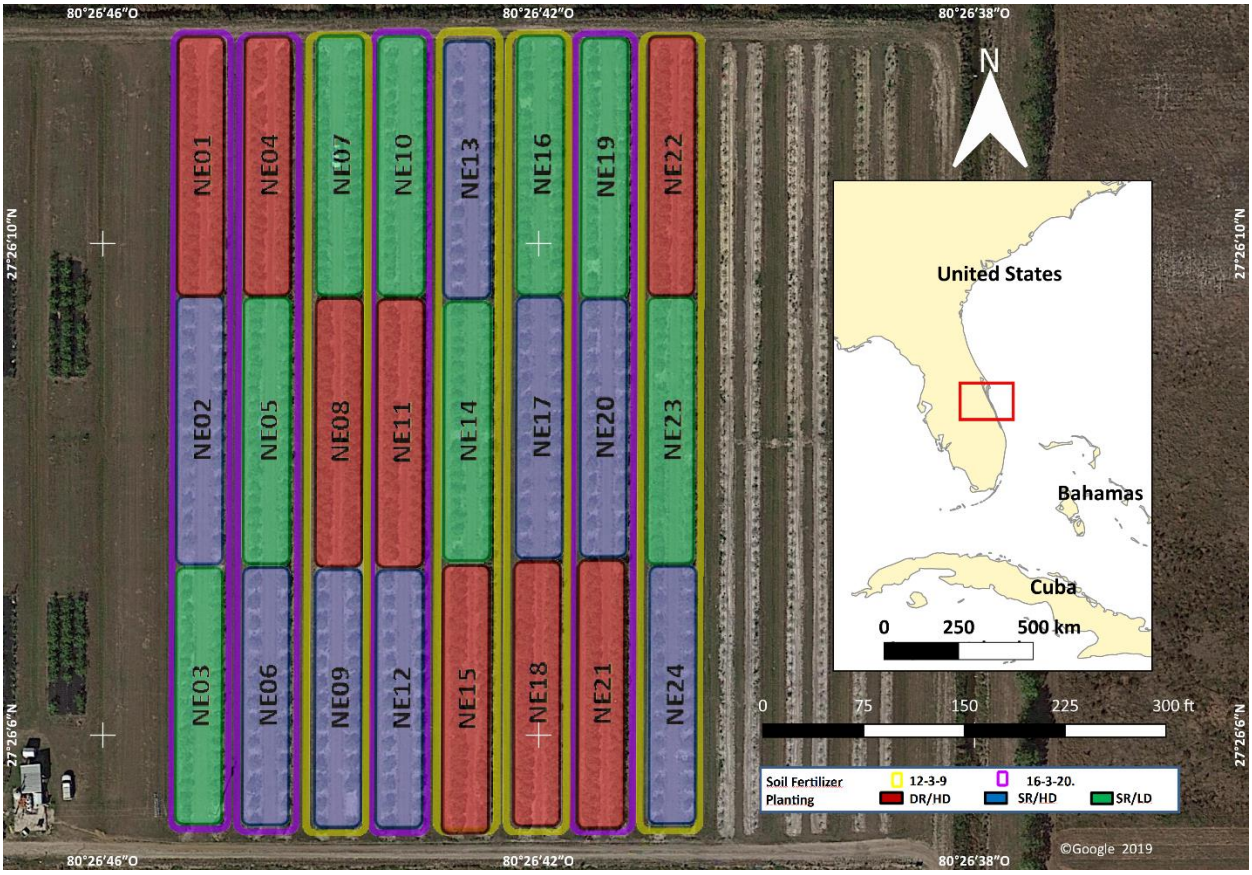

Figure 2-1. Overview of the study site at the UF/IFAS Indian River Research and Education Grove, Fort Pierce, FL with 24 experimental units. We tested two CRF blends [12-3-9 (12N-1.31P-7.47K) and 16-3-20 (16N-1.31P-16.6K)] ;and three planting densities [single row low-density (SR/LD), single row high-density (SR/HD), and double row high-density staggered in diamond setting (DR/HD)].

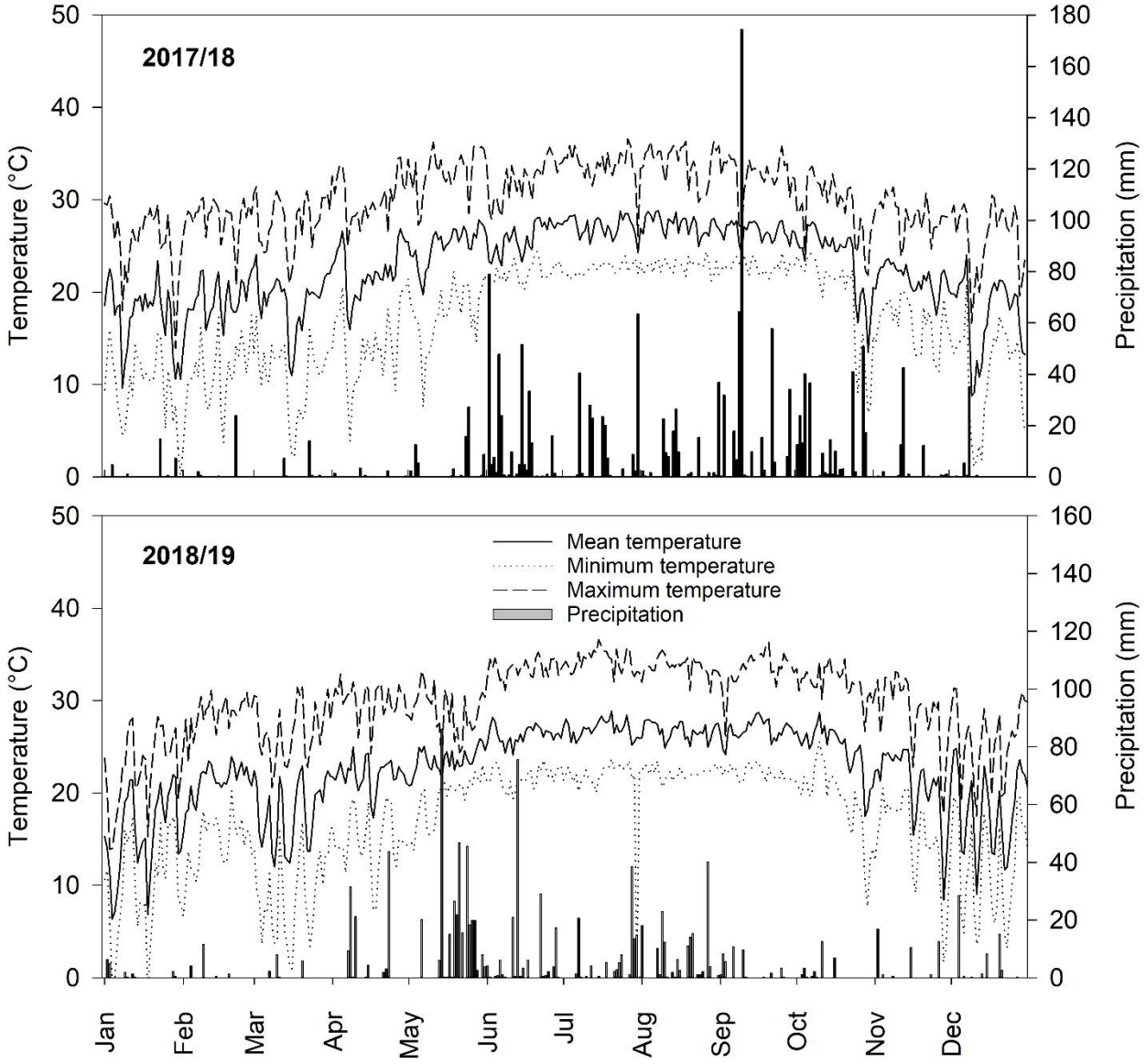

Figure 2-2. Temperature and precipitation data collected by Florida Automated Weather Network (FAWN) using St. Lucie West weather station in 2017/18 and 2018/19.

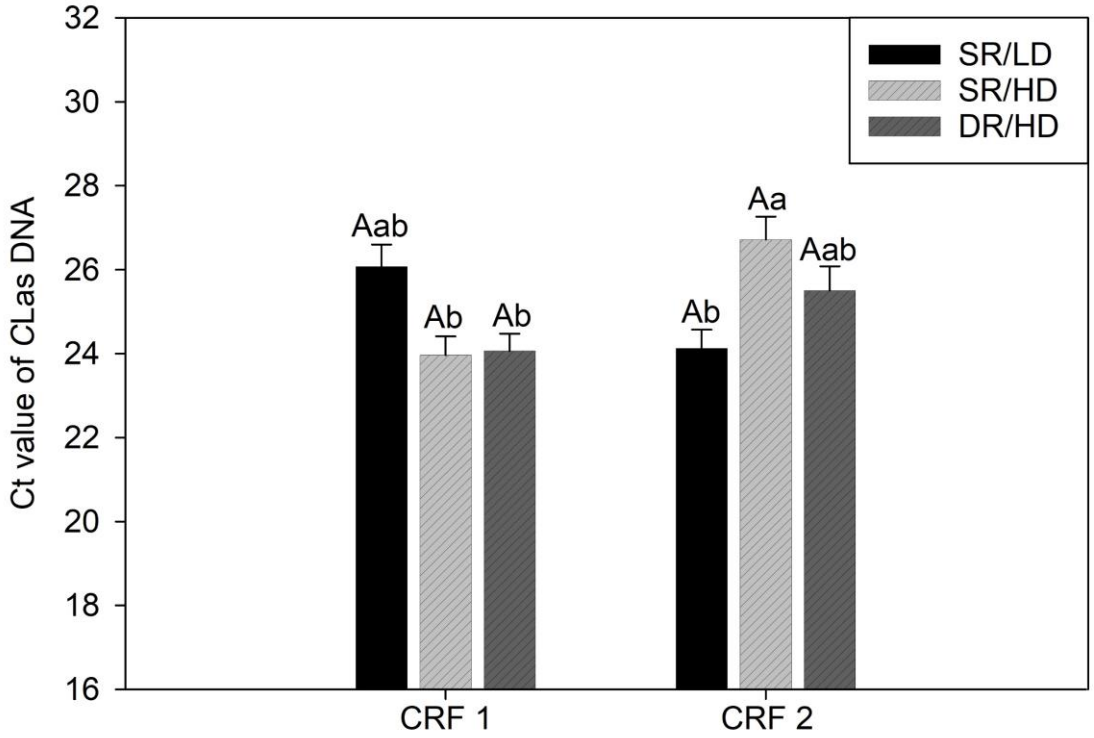

Figure 2-3. Cycle threshold (Ct) value of *Candidatus* Liberibacter asiaticus (*C*Las) DNA in plant leaf tissue under two controlled-release fertilizer (CRF) blends: CRF 1 (12N-1.31P-7.47K) and CRF 2 (16N-1.31P-16.6K); and three planting density [single row low-density(SR/LD), single row high-density (SR/HD), and double-row high-density staggered in diamond setting(DR/HD)]. Means ± standard errors followed by different letters (uppercase compare CRF and lowercase compare PD) are significantly different by Tukey's test at $P < 0.05$.

CHAPTER 3
GRAPEFRUIT TREE PLANTING DENSITY AND NUTRIENT APPLICATION IN HUANGLONGBING-AFFECTED GROVES

Justification

Growers have been using advanced horticultural practices and modification of grove design to cope with the disease after the epidemic of HLB in Florida. One practice is high-density planting to get early bearing and economic return. The other benefit of high-density planting is less disruption in fruit yield as the same number of trees progress to die compared to low density (Stover et al., 2008; Singerman et al., 2018). Moreira et al. (2019) also observed lower HLB incidence in high-density planting possibly due to lower psyllid to tree ratio that induced disease dilution.

Several studies concluded high-density planting results in greater fruit yield in mandarins (*Citrus nobilis* × *Citrus deliciosa*), sweet orange (*Citrus sinensis*), and limes (*Citrus aurantifolia*) (Dalal et al., 2013; Singerman et al., 2018; Moreira et al., 2019; Ladaniya et al., 2020). There are limited studies available about the effect of high-density plantings on grapefruit tree health, fruit yield, and fruit quality. It is imperative to understand the performance of high-density tree planting under the present HLB epidemics. The effect of advanced horticultural practices such as application of soil and foliar nutrients and tree planting on high-density under HLB conditions needs to be examined to allow profitable fruit yield and disease symptom mitigation.

Huanglongbing can affect tree physiology in citrus (Etxeberria et al., 2009; Gonzalez et al., 2012). Nwugo et al. (2013) found that HLB-affected grapefruit (*Citrus paradisi*) shows alteration of several proteins in leaves mainly due to deficiency of nutrients. The authors observed that downregulation of most of the protein associated with photosynthesis is correlated with a reduction of calcium (Ca), copper (Cu), iron

(Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) in leaf tissue. The changes in plant physiological processes associated with the downregulation of proteins imply vulnerable health conditions of HLB-affected trees. Conversely, nutrient management can improve plant physiology in HLB-affected citrus. Li et al. (2014) showed that a higher rate of Zn can increase the photosynthesis and stomatal conductance of HLB-affected grapefruit seedling under a hydroponic study. The authors concluded that elevated nutritional treatment can enhance the electron transfer rate resulting in greater photochemical quantum yield. Additionally, they concluded that the higher Zn concentration can increase the number of cuticle layers in plant leaf and result in better organization of epidermal cells inducing a higher number of stomata. Correspondingly, Yang et al. (2012) observed reduced CO_2 assimilation in Mg deficient pomelo (*Citrus grandis*) possibly due to the decreases in photosynthetic electron transport capacity.

Magnesium is an essential constituent of chlorophyll, is involved in photosynthesis and plays an important role as an activator of several enzymes, protein synthesis, carbohydrate partitioning and plant growth (Marschner, 2012). Deficiency or toxicity of Mg can influence a wide range of plant physiological functions such as photosynthesis, carbohydrate metabolism, and synthesis of nucleic acids (Huber and Jones, 2013; Zekri and Obreza, 2013). Deficiency of Mg in citrus results in the impairment of photosynthesis in leaves, alterations of gas exchange, and metabolites imbalance in plant parts (Tang and Chen, 2012; Li et al., 2017). Application of Mg in Mg-deficient soil can improve the quality of horticultural crop products, especially when the quality traits are dependent on photosynthesis and assimilate translocation process regulated by Mg within the plant system (Gerendás and Führs, 2013). Magnesium is

also an important mineral actively involves resistance mechanisms against bacterial and fungal diseases in plants (Huber and Jones, 2013).

The foliar application of nutrients is important during demanding stages such as flowering and fruit setting to induce higher fruit yield and enhance fruit quality characteristics. Also, when the soil condition is not preferable to nutrient uptake - such as low or high temperatures, low or excess soil moisture, pH, and salinity - foliar applications can fulfill the nutrient demand by directly supplying nutrients to the plants. Additionally, - compared to soil application - foliar application of nutrients can reduce nutrient accumulation in soil, runoff water, and surface water thereby helps to protect the environment (Lovatt, 1999; 2013).

The foliar application of micronutrients is important for citrus trees grown under calciferous soil since they typically show deficiency symptoms of some major micronutrients such as Fe and Zn. The high soil pH limits the uptake of micronutrients, and foliar application accelerates the process of nutrient acquisition to correct the deficiency (Ibrahim et al., 2007).

Several studies have shown that foliar application of micronutrient increases the leaf nutrient concentration. Labanauskas (1963) showed that Mn and Zn deficiency in grapefruit can be corrected by foliar spray of those nutrients. Boaretto et al. (1997) showed that foliar application of B increases the leaf nutrient concentration of sweet orange to an adequate range without affecting fruit yield and quality. In another experiment, Boaretto et al. (2002) showed that foliar application of Zn increases the leaf Zn concentration of sweet oranges to an adequate range without contributing to fruit yield and quality. In both experiments, authors noted that frequent spraying of

micronutrients increases soil nutrient concentration in the soil under the tree canopy possibly due to solution runoff to the soil (Boaretto et al.,1997; 2002). A recent study by Morgan et al. (2016) noted that spraying of B, Mn, and Zn increases the leaf nutrient concentration of 6-year-old ‘Valencia’ sweet orange on Swingle citrumelo rootstock (*Citrus paradisi* × *Poncirus trifoliata*).

The foliar application alone or combination with other substances such as hormones and insecticides has shown increased fruit yield in some types of citrus. Several authors noted higher fruit yield of ‘Valencia’ sweet orange bud-grafted on Swingle with the application of insecticides and foliar nutrients (Stansly et al., 2014; Tansey et al. 2017). Morgan et al. (2016) showed that foliar application containing three-times the recommended rate of Mn increased the leaf nutrient concentration as well as fruit yield in 6-year-old ‘Valencia’ on ‘Swingle’. Rouse et al. (2017) observed enhanced foliar nutrient application than the standard micronutrient increased the fruit yield of 16-year-old ‘Valencia’. Al-Obeed et al. (2018) showed that foliar application of B and Zn increases fruit yield and enhances fruit quality in ‘Kinnow’ mandarin trees (*Citrus reticulata*).

Foliar spray of a nutrient can mitigate the disease expression. Pustika et al. (2008) observed reduced disease symptoms on mandarin trees affected with HLB due to the foliar application of minerals. Shen et al. (2013) observed increased disease resistance, higher Ct value, and improved canopy size in ‘Valencia’ orange on Swingle by the long-term application of foliar nutrients along with salicylic acid and phosphite. Those studies imply that foliar application of nutrients can prolong the life of HLB-affected trees.

Balanced application of nutrients to correct deficiencies is an important tool to control biotic and abiotic stresses as well as improve the nutrient use efficiency (Zekri and Obreza, 2013). The efficiency of hormonal and nutritional treatment on HLB-affected trees has been a topic of debate and there is no clear conclusion on the efficiency of those substances to increase fruit yield and mitigate the disease symptoms (Gottwald et al., 2012; Morgan et al., 2016). There is limited information available about the compounding application of soil and foliar nutrients on HLB-affected grapefruit. Nevertheless, there is a high chance that nutrients – especially Mg and micronutrients – applied to grapefruit affected with HLB can increase the fruit yield as well as reduce the disease expression as a result of improved plant physiological parameters.

Materials and Methods

Study Site

The study was conducted on a large-scale field at the University of Florida's Institute of food and agricultural sciences (UF/IFAS) Indian River Research and Education Center in Fort Pierce, Florida (27°26'0.8.2" N, 80°26'4.3.2" W, and altitude of 10 m a.s.l) (Figure 3-1). Weather data were collected through the Florida Automated Weather Network (FAWN) utilizing the St. Lucie West weather station (Figure 3-2).

Soil moisture was collected during the experimental period using soil moisture sensors (TDT ACC-SEN-SDI; Acclima Inc, Meridian, ID) installed in the research field. The sensors were placed 0.6 m away from the main trunk, 15 cm below the soil surface, where the maximum root activity was observed. Square-point ditching shovel was used to excavate the soil and the probe was inserted horizontally (Philpot, 2008). The sensors were connected to a datalogger (CR 205; Campbell Scientific Inc, Logan, UT)

programmed to collect data every 15 minutes. The datalogger was connected to a rechargeable battery (6FM7; Toyo, Tonawanda, NY) and a solar panel (HY015-12P, Acopower, Walnut, CA) mounted on a pole. The data were averaged according to the treatments (Figure 3-3).

Plant Material

'Ray Ruby' grapefruit on Kuharske citrange (*Citrus sinensis*·× *Poncirus trifoliata*) rootstock was used as planting material. Trees were purchased from a commercial citrus nursery (Brite Leaf, Lake Panasoffkee, FL) and planted in September/2013.

Experimental Design and Treatments

The experiment was conducted in a split-split-plot design with four replications (Table 3-1). We tested three planting densities, two CRF blends applied by soil and four foliarly applied micronutrient rates.

The plant density treatments were: (1) single row low-density (SR/LD), 4.5 × 7 m, 300 trees/ha), (2) high-density single row (SR/HD), 3 × 7 m, 440 trees/ha, and (3) high-density double-row staggered in diamond setting (DR/HD), (2.7 ×1.5 ×1 m) × 6 m, 975 trees/ha.

In 2018/19, we tested two CRF blends [16-3-20 (16N-1.31P-16.6K) with 81% of N and 50% of K as CRF with iron as chelates and all other micronutrients as sulfates and 12-3-9 (12N-1.31P-7.47K) with 100% of N and P, and 95% K as CRF with iron as chelates and all other micronutrients as sulfur-coated products] as displayed in Table 2-2. In 2019/20, both CRF blends were adjusted, and we tested two slightly different blends [12-3-14 (12N-1.31P-11.62 K) with 70% of N as a CRF and other micronutrients as sulfates and 12-3-14 (12N-1.31P-11.62 K) with 2× Mg and 2.5× the recommended rates of micronutrients with 70% of N as a CRF and other micronutrients as sulfates]

displayed in Table 3-3. All the CRF blends were manufactured by a commercial fertilizer company (Harrell's; Lakeland, FL). The 4-month CRF blends were applied in February, July, and October. The CRF amount applied per area was calculated based on N recommendation for mature grapefruit trees in Florida as outlined by Obreza and Morgan (2008). The CRF amount per tree was calculated by assuming the total number of 358 trees/ha which is close to the current state's average (Singerman et al., 2018). Each tree received the same amount of fertilizer regardless of the planting density. In single row low density and single row high density, the fertilizer was applied manually under the dripline avoiding row middles as recommended by Obreza and Morgan (2008) and in double row high density, fertilizer was applied around tree canopy area.

Four treatments (0×, 1.5×, 3×, and 6×) were used as a foliar micronutrient (a blend of B, Mn, and Zn) application according to UF/IFAS recommendation (Obreza and Morgan, 2008). Application of foliar micronutrients were split three times/year and applied during the growth flushes period in March, May, and September as suggested by Morgan et al. (2016). We used commercial water-soluble chemicals {[manganese sulphate monohydrate ($MnSO_4.H_2O$) with 32% of Mn], zinc sulfate monohydrate [($ZnSO_4.H_2O$) with 36% of Zn], and disodium octaborate tetrahydrate [($Na_2B_8O_{13}.4H_2O$) with 21% of B]} to accelerate the process of nutrient application. Foliar treatment was applied when no rainfall forecasted within the next 24 h of application. The treatment was sprayed in a liquid form using water with the addition of an adjuvant (Induce; Helena Agri-enterprises, Collierville, TN). Treatments were applied using a commercial 100-gallon sprayer (MCCI100K43HR1M; Chemical Containers, Lake Wales, FL) at a constant pressure uniformly over the tree canopy.

Field Preparation and Grove Layout

The study started in 2018/19 on 5-year-old trees. The grove design and cultural practices followed were the same as indicated by Phuyal et al. (2020)[1]. There were 96 experimental units in equal size having dimensions of 15.24 m width and 29.12 m length. The total number of trees in each plot was 7, SR/LD, SR/HD, and DR/HD, respectively. One tree at each end of the plot was kept as a border tree.

The Concentration of *C*Las DNA in Plant Leaf Tissue

Eight random trees were chosen excluding border trees to collect leaf samples. The samples were analyzed by real-time quantitative polymerase chain reaction (qRT-PCR). The processes to collect and analyze leaf samples are explained in Phuyal et al. (2020)[1].

Tree Growth Measurement

Four trees were selected randomly in each plot excluding the border trees to access the canopy volume and trunk diameter measurement following Phuyal et al. (2020)[1].

Fruit Yield

Four trees from each plot were randomly selected excluding the border trees. Fruit yield/ tree, fruit yield per unit area, and fruit size were measured following Phuyal et al. (2020)[1].

[1] Material and Methods section on Chapter 2 of this thesis, to be submitted as a peer-reviewed, refereed manuscript (Phuyal, D., Nogueira, T. A. R., Jani, A. D., Kadyampakeni, D., Morgan, K., and Ferrarezi, R. S. 2020. Tree density and soil micronutrient application on grapefruit affected by Huanglongbing).

Fruit Size and Quality

The fruit acidity, soluble solids, ratio, and yield of solids was determined. The method to determine fruit size and fruit quality are available in Phuyal et al. (2020)[1].

Plant Photosynthesis Measurement

Net photosynthesis, stomatal conductance, intercellular CO_2, and transpiration rate were measured in April 2019 on two randomly selected trees per plot by using an infrared gas analyzer (LI-COR 6400XT; LI-COR Inc., Lincoln, NE). The measurements were taken one week after foliar spray. Two measurements were taken from 10 am to 2 pm on sunny days from each sampled tree from different branches. Leaves were selected from a sunlit portion of the canopy. Immature, diseased, insect-damaged, mechanically injured or dead leaves were avoided. Measurement was carried out in fully expanded mature citrus leaf as suggested by Iglesias et al. (2002) since the authors observed maximum photosynthesis. A constant flow rate of 500 μmol S^{-1}, the humidity of 50 to 80%, ambient reference CO_2 and photosynthetically active radiation with fast fan speed was adjusted in the leaf chamber of the gas analyzer. The leaf was clamped in the leaf chamber until the steady-state photosynthesis level was reached.

Leaf Sampling for Nutrient Analysis

Eight random trees were chosen excluding border trees to collect leaf samples. The leaf samples were analyzed for leaf nutrient concentration. The protocol to collect, preserve, and analyze leaf samples are available in Phuyal et al. (2020)[1].

Soil Sampling and Analysis

One 20-cm deep soil core/tree in 15 different locations/plot was taken to access the available soil nutrients, pH, and CEC. Analysis followed methods available in Phuyal et al. (2020)[1].

Statistical Analysis

Statistical analysis was performed using the software SAS 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed by year for two consecutive seasons. A generalized linear mixed model was used to analyze error variance where treatments were entered as fixed effect and block as a random effect. The data were checked for assumptions of the linear model. Log transformation and square root transformation was executed whenever necessary. To do a mean comparison, Tukey's multiple means comparison test was performed with probability (*P)* value of 5% ($P < 0.05$). Correlations were calculated to find the relationship between the amount of foliar application with the total number of fruit yield per tree and per area.

Result and Discussion

Ct value of *C*Las DNA

Planting density did not change the Ct value of *C*Las DNA (Table 3-4). The values were less than 32 indicating all the sampled trees were affected by HLB (Albrecht and Bowman, 2011; Gottwald et al., 2012; Shin and van Bruggen, 2018). The trees within different planting densities showed similar visual symptoms such as loss of foliage, fruit drop by almost >30% and even complete loss of two trees in the grove. Several authors have reported similar symptoms along with blotchy mottled leaves, deficiency of nutrients in plant parts, poor quality fruit, dead, and dying of twigs in HLB-affected citrus (Bové, 2004; Pustika et al., 2008; Gottwald et al., 2012; Morgan et al., 2016).

Our study also shows no different Ct values in trees receiving different CRF blends (Table 3-4). Zhang et al. (2016) observed negative effects of Zn application on 2-

year-old grapefruit seedlings. The authors concluded that Zn promotes the multiplication of bacteria which results in the decrease in Ct value. The soil supplemental application of micronutrients with elevated Mg were never tested before to examine how these nutrients change Ct value. The potential cause of no effect of micronutrient or elevated Mg in our experiment might be because the experimental period is not enough to see the differences.

We conducted our experiment for two consecutive seasons and observed no differences in Ct value due to foliar applied micronutrient (FAM) treatment. We observed no changes in disease symptoms among treatments. Gottwald et al. (2012) also observed no visual differences or disease expression among different micronutrient applications in HLB-affected ‘Valencia’ trees. It could be because the trial duration is not long enough and might need more time to observe reduction in bacterial DNA concentration among the treatments. Shen et al. (2013) observed an increase in Ct value after 3 years of nutritional treatment in HLB-affected ‘Valencia’ trees indicating that long-term foliar application is required to have a therapeutic effect. For the first two years, Shen et al. (2013) observed a negative effect of nutritional treatment on Ct value of HLB-affected ‘Valencia’ orange. The authors assumed the psyllids were more attracted to the trees receiving foliar treatments, which resulted in higher disease incidence and lower Ct value. Stansly et al. (2014) also noted that nutritional treatments can improve tree growth resulting in more opportunity for psyllid to feed on it which ultimately causes higher bacterial titer or lower Ct value. This result suggests that the FAM treatment could be counterproductive unless psyllid control measures are effective.

Tree Growth

The canopy volume and trunk diameter measurements are indicated in Table 3-5. Trunk diameter and canopy volume increased over time in all the treatments.

Canopy volume and trunk diameter were affected by planting density ($P < 0.0001$). In 2018/19, the DR/HD planting resulted in the lowest canopy volume (6.13 m^3), which was 31% lower than the canopy volume of other treatments. In 2019/20, the DR/HD planting resulted in the lowest canopy volume (6.14 m^3), which was 31% lower than SR/HD planting and 37% lower than SR/LD planting. In 2018/19, the DR/HD resulted in the smallest trunk diameter (73.33 mm), which was 13% smaller than SR/HD planting and 17% smaller than SR/LD planting. In 2019/20, the DR/HD planting resulted in the smallest trunk diameter (77.75 mm), which was 15% smaller than other treatments.

The decrease in trunk diameter and canopy volume with an increase in planting density is an expected outcome since high-density results in competition for resources such as water, mineral, and solar radiation. Zaman and Schumann (2005) also observed greater canopy growth in ‘Valencia’ sweet orange in widely spaced trees compared to other treatments.

CRF blends also affects the canopy volume ($P < 0.05$) although the trunk diameter remained statistically the same. CRF 1 resulted in 10% and 8% greater canopy volume than CRF 2 in 2018/19 and 2019/20, respectively (Table 3-5). Zekri and Obreza (2003) suggested that the calciferous soil having a high water table is naturally deficient in micronutrients. The authors noted that the deficiency of micronutrients particularly Mn and Fe can decrease the canopy growth. Our previous study (Phuyal et

al., 2020)[1] also confirms that soil application of elevated micronutrients by soil can improve canopy growth.

Canopy volume remained unaffected by the application of FAM. Rouse et al. (2017) also observed the foliar application nutrients including both macro and micronutrients did not change the tree volume in HLB-affected orange trees.

Fruit Yield

The fruit yield was 37% lower in 2018/19 compared to our previous study [Phuyal et al., (2020)[1] (Table 3-6)] possibly due to an increase in disease incidence along with long-term effects of hurricane Irma. In 2019/20, the fruit yield increased by 124% compared to 2018/19. The USDA forecast report also predicted that based on the favorable weather conditions in Florida in 2019/20, citrus production would increase in comparison to the previous year (USDA, 2019b).

In 2018/19, the greatest fruit yield was obtained with DR/HD (91% and 190% higher fruit yield than SR/HD and SR/LD). In 2019/20, DR/HD planting resulted in the lowest yield/tree (33% lower yield than other treatments), greatest fruit yield (43% and 136% higher than SD/HD and SR/LD), respectively.

Higher micronutrient application through CRF blends did not increase the fruit yield during 2018/19. The possible reason could be the reduction in fibrous roots in HLB-affected trees described by Graham et al. (2013) and Johnson et al. (2014), which did not have contributed to better nutrient uptake. An interaction was observed between CRF blends and planting density (P = 0.0179). CRF 1 blend in DR/HD planting yielded the greatest yield of solids compared to other treatments (Figure 3-4).

Foliar applied micronutrient showed a negative effect on fruit count (Table 3-6). The lower number of fruit in trees receiving foliar nutrients can be attributed to a large

fruit drop. Iglesias et al. (2003) explained that the fruit set is highly dependent on the nutritional status of the tree. In our study, because the FAM (B, Mn and Zn) were applied as a mixed solution it is difficult to decipher which specific micronutrient played a major role in fruit drop.

In 2018/19, FAM showed negative correlation on fruit yield/tree (r = -0.187, P = 0.0002) and fruit yield/area (r = -0.122, P = 0.0168). A similar trend was observed in 2019/20, fruit yield/tree (r = -0.22, $P < 0.0001$) and fruit yield/area (r = -0.176, P = 0.0005) with the foliar spray. The effect of foliar treatment on fruit yield of grapefruit is always a topic of controversy. Several studies suggest that foliar application alone or combination with substances such as hormones and insecticide has shown a positive result in a yield of HLB-affected citrus (Stansly et al., 2014; Morgan et al., 2016; Al-Obeed et al., 2018), while some studies indicated foliar application of nutrients is not effective to produce more fruit yield and improve fruit quality (Boaretto et al.,1997; Boaretto et al., 2002; Gottwald et al., 2012). The possible reason for lower fruit yield associated with foliar spray in our study is due to plant stress associated with the luxury application of micronutrients. Another explanation for lower yield with foliar treatment is, nutritional treatment could aggravate the plant health by attracting more psyllids resulting in more damage to the plant.

In 2019/20, an interaction between planting densities and FAM micronutrient applications observed in which SR/LD planting produced the largest size fruit compared to other treatments (Figure 3-6).

Fruit Quality and Yield of solids

In 2018/19, fruit acidity and soluble solids were affected by planting density (Table 3-7). The DR/HD planting fruit contained 5% more soluble solids than SR/LD.

Fruit from the DR/HD planting was 13% more acidic (titratable acidity 1.16 mg/100 mL) than the other treatments. The acidic nature of the juice dominated the soluble solids, inducing a lower ratio in the DR/HD planting. The DR/HD planting resulted in 7% lower ratio than the other treatments. In 2019/20, a similar trend was observed with acidity and soluble solids. DR/HD planting produced 5% higher solids and 6% acidity than other treatments. In 2018/19, DR/HD produced the greatest amount of yield of solids (232.53 kg ha^{-1}),130% more than the other treatments (Table 3-7). In 2019/20, DR/HD planting resulted greatest yield of solids (200% higher than other treatments). The overall yield of solids was higher in 2019/20 compared to 2018/19 possibly because the combination of higher juice, soluble solids, and fruit yield.

In 2018/19, the CRF blend did not change the fruit quality (Table 3-7). However, in 2019/20, CRF 1 produced higher soluble solids (10% more than CRF 2), higher ratio (7% more than CRF 2), and greatest yield of solids (86% higher than CRF 2).

In 2019/20, the foliar treatment increased the fruit acidity (P = 0.0101). The increase in fruit acidity with an increase in foliar rate could be because of the combination of higher K and Zn. Nasira et al. (2016) observed increased in fruit acidity of ‘Kinnow’ mandarin with the combined foliar application of K and Zn. In our study, we observed an increase in leaf K and Zn concentration with the application of higher rates of micronutrients, which could have induced more fruit acidity.

Plant Physiology

Photosynthesis and stomatal conductance were affected by planting density (Table 3-8). The DR/HD planting showed lower photosynthesis rates, 12% and 21% lower than SR/LD and SR/HD, respectively. Since photosynthesis is a physiological process which requires sunlight, high-density planting could have impaired plant

photosynthesis process. The SR/HD resulted in 16% higher stomatal conductance than DR/HD planting.

The CRF 1 increased photosynthesis (17%), intercellular CO_2 (10%), stomatal conductance (45%), and transpiration (46%) compared to CRF 2 (Table 3-8). Goldman (2010) noted that micronutrients are important for plant photosynthesis particularly Mn for chlorophyll formation and Zn itself is an important cofactor for enzyme carbonic anhydrase. To our knowledge, the effect of soil elevated micronutrient application on grapefruit plant physiology under field conditions has never tested before. We observed the elevated soil micronutrient can improve plant photosynthesis thereby plant canopy growth. (Table 3-8 and 3-5).

The increase in FAM rates showed a negative effect on net photosynthesis as well as stomatal conductance. lyas et al. (2015) observed an increase in net photosynthetic rate, stomatal conductance, and transpiration with the foliar application of B, Cu, and Zn. The authors also noted the increase in fruit yield and quality with the foliar treatment. The decrease in photosynthesis with an increase in the foliar micronutrient application in our study could be attributed to stress in the plant by luxurious nutrient supply (Table 3-11). Morgan and Connolly (2013) suggested that the accumulation of a higher level of micronutrients can damage plant cells by generating reactive oxygen species.

A significant interaction of intercellular CO_2 observed between tree planting density and CRFs blends. The CRF 1 resulted in better intercellular CO_2 in all the planting densities (Figure 3-8).

Enhanced nutritional treatment via foliar application may not provide additional benefits to the plant physiology. Conversely, higher nutrient (Mg and micronutrients) applications through soil showed the deciding factor to regulate the plant photosynthesis.

Leaf Nutrient Concentration

Planting density did not affect leaf nutrient concentration (Table 3-9 ,3-10,3-11 and 3-12).

The CRFs blends affected the leaf nutrient concentration. In 2018/19, CRF 1 caused increased leaf nutrient concentrations of B by 26%, Mn by 50%, Fe by 28% and Zn by 45% and decreased K by 15% in plant leaf tissue ($P < 0.05$). In 2019/20, CRF 1 resulted in increased leaf B by 14%, S by 8%, Cu by 19%, Fe by 9%, Mn by 30%, and Zn by 18% concentration but decreased leaf Mg by 3% in the plant leaf tissue ($P < 0.05$). This is an expected outcome since CRF 1 contains higher amount of micronutrients than CRF 2. In the first year (2018/19), CRF 1 blend had higher K and the Mg amount was elevated in the same blend that increases the leaf nutrient concentration of the corresponding element. It shows that grapefruit is highly responsive to the CRF.

The FAM significantly affected leaf nutrient concentration. In 2018/19, with an increase in foliar application rate, FAM showed a positive correlation of B ($r = 0.2731$, $P = 0.0071$), Mn ($r = 0.5916$, $P < 0.0001$), and Zn ($r = 0.5427$, $P < 0.0001$) in the plant leaf tissue. Further, in 2019/20, FAM showed a positive correlation of Mn ($r = 0.3584$, $P = 0.0003$) and Zn ($r = 0.2954$, $P = 0.0035$) in plant leaf tissue with the increment of foliar rate. In 2019/20, the FAM application increased the K concentration in plant leaf tissue, whereas decreased leaf Cu concentration ($P > 0.05$). Mengel and Kirkby (1980) noted

the synergistic interrelationship between K with B and Zn. It is most likely that B, Zn or combination of B and Zn could have facilitated more uptake of K with increase in FAM. The decrease in Cu concentration with increase in foliar rate is possible due to the antagonistic effect of Zn. Studies have shown the antagonistic effect of Zn and Cu nutrition on wheat (*Triticum aestivum)* and rice (*Oryza sativa*) (Chaudhry et al., 1973; Imtiaz et al., 2003).

In 2018/19, leaf S concentration showed an interaction between planting density and CRF blends, in which CRF 1 showed higher leaf S concentration (Figure 3-7). It could be due to the higher amount of S-coated fertilizer application in CRF 1 blend. In 2019/20, leaf B, Mn, and Zn concentration showed an interaction between planting and CRF blends (Figure 3-8).

Leaf nutrient concentration of most of the macronutrients such as N, P, Ca, and S decreases with the aging of the tree. Conversely, leaf nutrient concentration of micronutrients B, Mn, and Zn increases with the aging of trees while leaf nutrient concentration of other micronutrients such as Cu and Fe decreases.

According to the leaf nutrient interpretation by Koo et al. (1984), the concentration of Ca and N in the leaf was in an optimum range [Ca (3.0–4.9 g kg^{-1}) and N (2.5–2.7g kg^{-1})] for all the treatment applications. Leaf Cu concentration was in excess range (> 20 mg kg^{-1}) for all the treatments due to the frequent application of Cu containing fungicides to control citrus canker disease (*Xanthomonas axonopodis*). In 2018/19, the concentration of P and Fe in the leaf was also above optimum range [P (> 16 g kg^{-1}), and Fe (> 120 mg kg^{-1})] dropped to optimum range [P (0.12–0.16 g kg^{-1}) and Fe (60–120 mg kg^{-1})]. Further, leaf Mg concentration was in the optimum range (0.30–

0.49 g kg^{-1}) dropped to a low range (Mg < 0.30 g kg^{-1}) even we applied more Mg possibly due to HLB affect. In 2018/19, leaf Mn concentration was in the optimum range (25–100 mg kg^{-1}) for all the treatments except 6× foliar spray of recommended rate where it exceeded the optimum range. In 2019/20, leaf nutrient concentration of B, Mn, and Zn reached high levels (B concentration > 120 mg kg^{-1}, Mn and Zn concentration > 100 mg kg^{-1}).

As expected, our study shows that application of FAM in increased rates does increase leaf B, Mn, and Zn concentration. Based on Koo et al. (1984) interpretation, FAM application brought the plant into high nutrient level.

Soil Nutrient Concentration, pH, and CEC

Planting affected soil nutrient concentration only in 2019/20 (Tables 3-13, 3-14, and 3-15). The DR/HD resulted in higher concentration of K, Ca, Mg, S, and B in the soil but lower soil P concentration than other treatments. The higher availability of nutrients in the high-density planting could be due to less uptake by plant (Tables 3-13, 3-14, 3-15, 3-16).

The CRF blends resulted in changes in soil pH and nutrient availability (Tables 3-13, 3-14, 3-15 and 3-16). In 2018/19, CRF 2 (with lower micronutrient rates) increased soil pH by 6%, soil B by 100% and decreased Mn concentration by 55%. In 2019/20, the use of CRF 2 led to increase soil pH by 12%, B by 25%, Mg by 13%, and Zn by 19% and decreased S by 42% in the soil. Interestingly, despite the application of less B in the CRF 2 blend (0.67 kg/ha), B soil availability was higher (Tables 3-14). Goldberg et al. (2000) has reported the availability of B in the soil depends of several factors such as texture, moisture, temperature, and oxide content along with soil pH since low pH favors B uptake. Zhu et al. (2007) reported that the B concentration in soils is generally related

to the amount of B taken up by plants. In our study, CRF 1 blend resulted in a decrease in soil pH which could have facilitated B uptake by the trees. Consequently, soil samples from CRF 2 treatment showed higher B concentration in the soil even though we applied less B than CRF 1. Further, the increase in S content with CRF 1 is probably from the higher application of S-coated fertilizer.

The application of higher amount of Mg via CRF 1 decreased the Mg concentration in soil (Table 3-13). However, the leaf nutrient analysis shows higher leaf Mg concentration in the samples collected from CRF 2 applied trees. In the year 2019/20, the application of higher amount of Mg via CRF 1 shows 11% lower Mg concentration in soil (Table 3-13). Several studies suggested that Mg is highly mobile element in soil and shows its maximum availability in the range of soil pH 7 to 8 (Troug 1947; Gransee and Führs 2013). Prior studies showed that with a small increase in soil pH, Mg availability drastically reduced in the soil (Sumner et al., 1978; Haileset et al.,1997). In our study, CRF 1 resulted in medium acidic soil pH (5.63), which was 11% lower than CRF 2. The lower pH in CRF 1 could have decreased the Mg availability in soil.

Both CRF blends resulted in optimum soil pH for most of the nutrient availability for citrus as suggested by (Obreza and Morgan, 2008). Using the soil test interpretation outlined by Obreza and Morgan (2008), in 2018/19, the Mehlich 3 acid extraction showed a medium level of P and low Mg concentration in soil [P (17–29 mg kg^{-1}) and Mg (25–33 mg kg^{-1})]. Using the same guidelines, as a comparison, in 2019/20, the concentration of P exceeded the medium range (≥ 30 mg kg^{-1}) and the Mg concentration reached between medium (25–33 mg kg^{-1}) and high range (> 33 mg kg^{-1}).

In both seasons, soil Ca concentration was in the sufficient range (> 200 mg kg^{-1}) because of the calciferous nature of the soil.

In 2019/20, foliar application of micronutrients affected the soil nutrient concentration only for Mn and Zn (Table 3-14). The increase in soil concentration of these nutrients with FAM spray of the corresponding nutrient is possibly due to the spray solution that might have leached to the soil. Boaretto et al. (2002) also observed a similar result with a foliar spray of Zn on oranges trees. However, the concentration of B was not affected by the foliar micronutrient application.

Conclusions

The planting density did not affect Ct value. High-density planting resulted in smaller trunk diameter and canopy volume. Despite lower yield/tree in 2019/20, high-density planting induced the greatest yield/area among all the treatments. Further, the fruit produced from high-density planting had higher soluble solids and was more acidic.

The CRF blend with higher Mg and micronutrients did not result in change of Ct value as well. Though fruit yield remained the same, higher micronutrient application improved plant physiology and canopy volume without affecting fruit quality.

Foliar micronutrient applications did not affect Ct value, canopy volume, and trunk diameter. The fruit quality remained the same with the foliar micronutrient application. Fruit yield, photosynthesis, and stomatal conductance decreased with the increase in foliar micronutrient application. Application of foliar micronutrient increased both soil and leaf Mn and Zn concentration along with leaf B in 2019/20.

Our study shows that supplemental nutrient application is not beneficial for grapefruit trees affected by HLB over a 2-year period. However, our data shows that

high-density plantings result in improved yield. Cost for weed control and cultural field management expenses along with long term yield response should be determined before making a recommendation for a grower. Additionally, future recommendation is to access the nutritional treatment on other HLB-tolerant rootstock and continue with psyllid control measures.

Table 3-1. Brief description of the experimental design.

Experimental design	Factor and level
Main plot	CRF application in the soil 1. CRF 1 blends[z] 2. CRF 2 blends[y]
Subplot	Three plant densities 1. Single row low density (SR/LD): 300 trees/ha 2. Single row high density (SR/HD): 440 trees/ha 3. Double row high density (DR/HD): 975 trees/ha
Sub-subplot	Four foliar treatments 1. No supplemental nutrients applied 2. 1.5 × the UF/IFAS recommended doses of B, Mn, and Zn 3. 3 × the UF/IFAS recommended doses of B, Mn, and Zn 4. 6 × the UF/IFAS recommended doses of B, Mn, and Zn

[z]CRF 1: In 2018/19 = 12N-1.31P-7.47K with 100% of N, 100% of P and 95% of K as CRF with iron as chelates and all other micronutrients as sulfur-coated products at higher rate ; and in 2019/20 =12N-1.31P-11.62K with 2× Mg and 2.5× micronutrients
[y]CRF 2: In 2018/19 = 16N-1.31P-16.6K with 81% of N and 50% of K as CRF with iron as chelates and all other micronutrients as sulfates; and 2019/20 = 12N-1.31P-11.62 K with 1× micronutrients the recommended rate.

Table 3-2. The amount of micronutrients (a blend of B, Mn, and Zn) applied as a foliar treatment in 2018/19 and 2019/20, according to recommended rates (Obreza and Morgan, 2008).

Treatment	B	Mn	Zn
	kg ha^{-1}		
0.0×	0.00	0.00	0.00
1.5×	0.42	6.73	8.41
3.0×	0.84	13.45	16.81
6.0×	1.68	26.90	33.63

Table 3-3. The nutrient composition and amount applied in two different controlled-release fertilizer (CRF) blends.

	CRF 1		CRF 2	
	Nutrient (%)	Amount (kg ha^{-1})	Nutrient (%)	Amount (kg ha^{-1})
N	12	180	12	180
P_2O_5	3	44.82	3	44.82
K_2O	14	209.17	14	209.17
Ca	1	14.94	1	14.94
Mg	2.41	36.07	1.21	18.08
S	15.25	227.83	13.01	194.34
B	0.11	1.69	0.05	0.67
Cu	0.10	1.49	0.04	0.60
Fe	0.99	14.72	0.40	5.89
Mn	1.41	21.04	0.56	8.41
Mo	0.02	0.22	0	0
Zn	0.99	14.72	0.40	5.89

Table 3-4. Cycle threshold (Ct) value of *C*Las DNA as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments	Ct value of *C*Las DNA (unitless)	
	2018/19	2019/20
CRF[z]		
CRF 1	24.95 ± 0.24[w]	29.88 ± 0.17
CRF 2	24.94 ± 0.26	29.96 ± 0.16
PD[y]		
SR/LD	25.12 ± 0.39	29.83 ± 0.22
SR/HD	24.91 ± 0.27	30.10 ± 0.20
DR/HD	24.82 ± 0.28	29.82 ± 0.19
FAM[x]		
0.0×	25.07 ± 0.34	29.98 ± 0.22
1.5×	25.13 ± 0.41	30.01 ± 0.24
3.0×	24.64 ± 0.34	30.03 ± 0.25
6.0×	24.96 ± 0.34	29.66 ± 0.24
Sources of variation	*Probability (P) value*	
CRF	0.8074 [NS]	0.7386 [NS]
PD	0.7750 [NS]	0.5991 [NS]
CRF*PD	0.2074 [NS]	0.7559 [NS]
FAM	0.5642 [NS]	0.6952 [NS]
CRF*FAM	0.0266 [NS]	0.6676 [NS]
PD*FAM	0.7381 [NS]	0.9123 [NS]
CRF*PD*FAM	0.2790 [NS]	0.6151 [NS]

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-5. Trunk diameter and canopy volume as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments		Trunk diameter (mm)		Canopy volume(m^3)	
		2018/19	2019/20	2018/19	2019/20
CRF[z]					
	CRF 1	82.82 ± 1.16[w]	87.69 ± 1.25	8.33 ± 0.28 a	8.63 ± 0.30 a
	CRF 2	81.86 ± 1.08	86.49 ± 1.33	7.59 ± 0.25 b	7.98 ± 0.32 b
PD[y]					
	SR/LD	88.81 ± 0.79 a	93.26 ± 1.22 a	9.18 ± 0.28 a	9.83 ± 0.32 a
	SR/HD	84.88 ± 0.68 b	90.26 ± 0.95 a	8.56 ± 0.23 a	8.93 ± 0.26 b
	DR/HD	73.33 ± 0.65 c	77.75 ± 0.92 b	6.13 ± 0.20 b	6.14 ± 0.19 c
FAM[x]					
	0.0×	82.31 ± 1.69	87.63 ± 1.99	8.01 ± 0.44	8.59 ± 0.58
	1.5×	81.42 ± 1.48	85.60 ± 2.00	7.75 ± 0.35	7.85 ± 0.41
	3.0×	82.64 ± 1.69	88.17 ± 1.56	7.95 ± 0.36	8.31 ± 0.32
	6.0×	83.01 ± 1.53	86.96 ± 1.77	8.12 ± 0.39	8.45 ± 0.43
Sources of variation		*Probability (P) value*			
CRF		0.2471 [NS]	0.3328 [NS]	0.0045**	0.0264**
PD		<0.0001***	<0.0001***	<0.0001***	<0.0001***
CRF*PD		0.7509 [NS]	0.6151 [NS]	0.6832 [NS]	0.7307 [NS]
FAM		0.5725 [NS]	0.4834 [NS]	0.7417 [NS]	0.2963 [NS]
CRF*FAM		0.1234 [NS]	0.2496 [NS]	0.1456 [NS]	0.5397 [NS]
PD*FAM		0.7020 [NS]	0.4442 [NS]	0.2670 [NS]	0.1042 [NS]
CRF*PD*FAM		0.7031 [NS]	0.8255 [NS]	0.3854 [NS]	0.1446 [NS]

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 16) followed by different lowercase letters are significantly different at P < 0.05 by Tukey's test.

[NS] = Not significant. ** and *** = Significant at P < 0.01 and P < 0.001, respectively.

Table 3-6. Total no. of fruit, fruit diameter, yield/tree, and fruit yield per unit area as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments		Total # fruit (Nu)[w]		Fruit diameter (mm)		Yield/tree (Kg)		Fruit yield (kg ha^{-1})	
		2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]									
	CRF 1	21.9 ± 1.11	38.71 ± 2.31 b	96.46 ± 0.60	98.39 ± 0.28	7.60 ± 0.40[w]	16.97 ± 0.87	4189.76 ± 270.08	8634.24 ± 463.90
	CRF 2	22.31 ± 0.94	48.06 ± 1.92 a	96.08 ± 0.77	98.89 ± 0.25	7.41 ± 0.31	18.53 ± 0.69	3994.16 ± 212.45	9724.25 ± 456.64
PD[y]									
	SR/LD	22.52 ± 1.32	47.16 ± 2.61 a	95.82 ± 0.83	100.16 ± 0.35 a	7.72 ± 0.48	18.69 ± 0.95 a	2263.83 ± 142.00 c	5482.64 ± 279.57 c
	SR/HD	23.94 ± 1.32	51.43 ± 2.93 a	97.16 ± 0.37	99.03 ± 0.26 b	8.01 ± 0.44	21.18 ± 1.06 a	3436.16 ± 189.41 b	9080.97 ± 455.94 b
	DR/HD	19.95 ± 1.12	31.56 ± 1.95 b	95.82 ± 1.15	96.73 ± 0.27 c	6.78 ± 0.37	13.37 ± 0.70 b	6575.89 ± 363.70 a	12974.12 ± 675.11 a
FAM[x]									
	0.0×	25.34 ± 1.65 a	54.24 ± 3.9 a	96.28 ± 1.11	98.87 ± 0.39 ab	8.54 ± 0.57 a	21.96 ± 1.40 a	4533.22 ± 362.48 a	11002.44 ± 763.59 a
	1.5×	22.8 ± 1.33 ab	42.71 ± 2.98 b	97.01 ± 0.44	97.81 ± 0.39 b	7.78 ± 0.51 ab	17.39 ±1.07 b	4248.30 ± 339.13 ab	8991.36 ± 609.61 ab
	3.0×	22.6 ± 1.51 ab	42.05 ± 2.6 b	96.24 ± 1.12	98.69 ± 0.33 ab	7.59 ± 0.50 ab	17.33 ± 0.98 b	4187.28 ± 374.52 ab	9140.85 ± 636.23 ab
	6.0×	17.78 ± 1.22 b	34.53 ± 2.07 b	95.52 ± 1.08	99.18 ± 0.36 a	6.11 ± 0.40 b	14.32 ± 0.75 b	3399.05 ± 284.75 b	7582.33 ± 542.77 b
Sources of variation		*Probability (P) value*							
CRF		0.8034 NS	0.0009***	0.7923 NS	0.1366 NS	0.6988 NS	0.1276 NS	0.5025 NS	0.0549 NS
PD		0.0740 NS	<.0001***	0.4366 NS	<.0001***	0.1111 NS	<.0001***	<0.0001***	<.0001***
CRF *PD		0.7927 NS	0.1611 NS	0.1788 NS	0.0744 NS	0.8330 NS	0.6611 NS	0.6594 NS	0.2566 NS
FAM		0.0028**	<.0001***	0.7637 NS	0.0259*	0.0068**	<.0001***	0.0410*	0.0004***
CRF*FAM		0.1893 NS	0.3871 NS	0.4823 NS	0.2702 NS	0.1659 NS	0.1750 NS	0.5540 NS	0.4185 NS
PD*FAM		0.6544 NS	0.3759 NS	0.5761 NS	0.0479*	0.8485 NS	0.3745 NS	0.9640 NS	0.9825 NS
CRF*PD*FAM		0.5917 NS	0.1505 NS	0.7606 NS	0.1130 NS	0.7375 NS	0.2790 NS	0.8839 NS	0.7656 NS

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 16) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.
[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-7. Soluble solids content, acidity, ratio, and yield of solids as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments		Soluble solid content (%)		Acidity (g/100 mL)		Ratio		Yield of solids (kg ha^{-1})	
		2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]									
	CRF 1	7.96 ± 0.10 [w]	8.31 ± 0.11 a	1.07 ± 0.02	0.89 ± 0.01	7.51 ± 0.10	9.33 ± 0.15 a	159.72 ± 16.68	885.62 ± 100.57 a
	CRF 2	7.74 ± 0.12	7.52 ± 0.14 b	1.08 ± 0.01	0.87 ± 0.01	7.21 ± 0.12	8.72 ± 0.24 b	130.55 ± 12.57	476.34 ± 73.57 b
PD[y]									
	SR/LD	7.67 ± 0.10 b	7.63 ± 0.17 b	1.03 ± 0.02 b	0.86 ± 0.02 b	7.62 ± 0.11 a	8.93 ± 0.27	85.79 ± 7.03 b	343.20 ± 30.98 b
	SR/HD	7.77 ± 0.11 ab	7.9 ± 0.16 ab	1.02 ± 0.01 b	0.87 ± 0.01 b	7.46 ± 0.11 a	9.14 ± 0.25	117.08 ± 9.17 b	472.60 ± 50.81 b
	DR/HD	8.11 ± 0.18 a	8.22 ± 0.17 a	1.16 ± 0.01 a	0.92 ± 0.01 a	6.99 ± 0.15 b	8.99 ± 0.23	232.53 ± 22.24 a	1227.14 ± 145.33 a
FAM[x]									
	0.0x	8.05 ± 0.16	7.98 ± 0.22	1.07 ± 0.02	0.87 ± 0.01 b	7.57 ± 0.12	9.22 ± 0.26	166.73 ± 22.83	683.14 ± 125.97
	1.5x	7.80 ± 0.13	8 ± 0.2	1.07 ± 0.02	0.86 ± 0.02 b	7.32 ± 0.12	9.46 ± 0.35	150.46 ± 19.68	663.23 ± 116.45
	3.0x	7.60 ± 0.18	7.8 ± 0.2	1.08 ± 0.02	0.89 ± 0.01 ab	7.12 ± 0.21	8.84 ± 0.25	142.47 ± 23.75	662.11 ± 123.44
	6.0x	7.94 ± 0.15	7.88 ± 0.19	1.07 ± 0.02	0.92 ± 0.01 a	7.43 ± 0.13	8.57 ± 0.25	120.89 ± 17.35	715.43 ± 161.23
Sources of variation		*Probability (P) value*							
CRF		0.3717 [NS]	<.0001***	0.6378 [NS]	0.1557 [NS]	0.1190 [NS]	0.0415*	0.0958 [NS]	0.0001***
PD		0.0223*	0.0471*	<0.0001***	0.0039**	0.0007***	0.8327 [NS]	<0.0001***	<.0001***
CRF*PD		0.0518 [NS]	0.8720 [NS]	0.3092 [NS]	0.3012 [NS]	0.4726 [NS]	0.5769 [NS]	0.1333 [NS]	0.0179*
FAM		0.1093 [NS]	0.8694 [NS]	0.9802 [NS]	0.0101*	0.1132 [NS]	0.1587 [NS]	0.3103 [NS]	0.9806 [NS]
CRF*FAM		0.2116 [NS]	0.9813 [NS]	0.1130 [NS]	0.9123 [NS]	0.0359 [NS]	0.9271 [NS]	0.8982 [NS]	0.9420 [NS]
PD*FAM		0.1281 [NS]	0.9532 [NS]	0.4238 [NS]	0.2992 [NS]	0.6506 [NS]	0.9050 [NS]	0.9943 [NS]	0.9980 [NS]
CRF *PD*FAM		0.3459 [NS]	0.7158 [NS]	0.4925 [NS]	0.8556 [NS]	0.6591 [NS]	0.6649 [NS]	0.8161 [NS]	0.9953 [NS]

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 16) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.
[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-8. Assimilation rate, intercellular CO_2, stomatal conductance, and transpiration rate as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) on 2018/19 and 2019/20.

Treatments	Assimilation rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Intercellular CO_2 ($\mu mol\ CO_2\ mol^{-1}$)	Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$)	Transpiration rate ($mmol\ H_2O\ m^{-2}\ s^{-1}$)
CRF[z]				
CRF 1	11.24 ± 0.23 a[w]	264.36 ± 2.55 a	0.16 ± 0.01 a	2.67 ± 0.08 a
CRF 2	9.61 ± 0.26 b	238.97 ± 2.51 b	0.11 ± 0.01 b	1.83 ± 0.07 b
PD[y]				
SR/LD	10.46 ± 0.31 b	248.41 ± 3.45	0.14 ± 0.01 ab	2.20 ± 0.09
SR/HD	11.62 ± 0.26 a	258.09 ± 3.55	0.15 ± 0.01 a	2.39 ± 0.11
DR/HD	9.19 ± 0.30 c	248.49 ± 2.77	0.12 ± 0.01 b	2.16 ± 0.09
FAM[x]				
0.0 x	11.34 ± 0.38 a	254.57 ± 3.59 ab	0.16 ± 0.01 a	2.41 ± 0.11
1.5 x	10.24 ± 0.33 ab	244.4 ± 3.39 b	0.13 ± 0.01 b	2.20 ± 0.11
3.0 x	9.83 ± 0.37 ab	250 ± 4.12 ab	0.13 ± 0.01 b	2.16 ± 0.11
6.0 x	10.28 ± 0.30 b	257.67 ± 3.98 a	0.13 ± 0.01 b	2.23 ± 0.13
Sources of variation		*Probability (P) value*		
CRF	<0.0001***	<0.0001***	<0.0001***	<0.0001***
PD	<0.0001***	0.3310 [NS]	<0.0001***	0.1576 [NS]
CRF*PD	0.4320 [NS]	0.0227*	0.8767 [NS]	0.3134 [NS]
FAM	0.0084**	0.0408*	0.009**	0.3347 [NS]
CRF*FAM	0.3391 [NS]	0.1463 [NS]	0.4172 [NS]	0.7182 [NS]
PD*FAM	0.1252 [NS]	0.3009 [NS]	0.0874 [NS]	0.1166 [NS]
CRF*PD*FAM	0.7413 [NS]	0.0632 [NS]	0.3502 [NS]	0.0595 [NS]

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.
[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-9. Leaf macronutrient concentration as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments	N (g kg^{-1})		P (g kg^{-1})		K (g kg^{-1})	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]						
CRF 1	2.83 ± 0.03[w]	2.73 ± 0.02	0.18 ± 0.0025	0.15 ± 0.0013	1.22 ± 0.03 b	0.98 ± 0.01
CRF 2	2.84 ± 0.03	2.76 ± 0.03	0.18 ± 0.0022	0.15 ± 0.0009	1.44 ± 0.03 a	1.02 ± 0.02
PD[y]						
SR/LD	2.80 ± 0.03	2.74 ± 0.04	0.18 ± 0.0029	0.15 ± 0.0015	1.28 ± 0.04	1.01 ± 0.02
SR/HD	2.81 ± 0.03	2.76 ± 0.03	0.18 ± 0.0026	0.15 ± 0.0013	1.32 ± 0.03	0.99 ± 0.02
DR/HD	2.89 ± 0.04	2.73 ± 0.02	0.19 ± 0.0032	0.15 ± 0.0014	1.40 ± 0.04	1.00 ± 0.03
FAM[x]						
0.0×	2.81 ± 0.04	2.75 ± 0.03	0.18 ± 0.0038	0.15 ± 0.0017	1.29 ± 0.05	0.95 ± 0.02 b
1.5×	2.86 ± 0.04	2.71 ± 0.02	0.18 ± 0.0025	0.15 ± 0.0016	1.32 ± 0.04	0.96 ± 0.02 b
3.0×	2.85 ± 0.04	2.72 ± 0.03	0.18 ± 0.0033	0.15 ± 0.0015	1.34 ± 0.04	1.03 ± 0.03 ab
6.0×	2.82 ± 0.04	2.80 ± 0.04	0.18 ± 0.0037	0.16 ± 0.0017	1.39 ± 0.04	1.08 ± 0.02 a
Sources of variation			*Probability (P) value*			
CRF	0.7442 [NS]	0.4660 [NS]	0.0676 [NS]	0.4735 [NS]	0.0266*	0.1004 [NS]
PD	0.1736 [NS]	0.8375 [NS]	0.2618 [NS]	0.9041 [NS]	0.3457 [NS]	0.6684 [NS]
CRF*PD	0.7380 [NS]	0.1285 [NS]	0.5602 [NS]	0.8785 [NS]	0.3014 [NS]	0.0931 [NS]
FAM	0.8398 [NS]	0.1933 [NS]	0.5102 [NS]	0.2270 [NS]	0.9402 [NS]	0.0002***
CRF*FAM	0.9785 [NS]	0.6393 [NS]	0.5658 [NS]	0.8059 [NS]	0.7580 [NS]	0.3175 [NS]
PD*FAM	0.9822 [NS]	0.8805 [NS]	0.8638 [NS]	0.5929 [NS]	0.4914 [NS]	0.8903 [NS]
CRF*PD*FAM	0.9556 [NS]	0.9389 [NS]	0.5918 [NS]	0.8379 [NS]	0.0666 [NS]	0.3310 [NS]

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-10. The concentration of Ca, Mg, and S in the leaf as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments	Ca (g kg^{-1})		Mg (g kg^{-1})		S (g kg^{-1})	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]						
CRF 1	4.79 ± 0.03 a[w]	3.20 ± 0.03	0.34 ± 0.0034	0.26 ± 0.0027 b	0.4 ± 0.0046 a	0.31 ± 0.0038 a
CRF 2	4.35 ± 0.05 b	3.24 ± 0.04	0.34 ± 0.01	0.27 ± 0.0026 a	0.37 ± 0.01 b	0.29 ± 0.0032 b
PD[y]						
SR/LD	4.57 ± 0.06	3.20 ± 0.04	0.34 ± 0.01	0.26 ± 0.0033	0.39 ± 0.01	0.30 ± 0.0047
SR/HD	4.59 ± 0.04	3.26 ± 0.03	0.33 ± 0.0043	0.27 ± 0.0028	0.38 ± 0.0036	0.29 ± 0.003
DR/HD	4.55 ± 0.07	3.20 ± 0.06	0.34 ± 0.01	0.27 ± 0.0035	0.39 ± 0.01	0.30 ± 0.01
FAM[x]						
0.0×	4.56 ± 0.09	3.29 ± 0.05	0.34 ± 0.01	0.27 ± 0.0032	0.38 ± 0.01	0.30 ± 0.0042
1.5×	4.57 ± 0.07	3.26 ± 0.04	0.34 ± 0.01	0.27 ± 0.0039	0.38 ± 0.01	0.30 ± 0.0046
3.0×	4.60 ± 0.07	3.23 ± 0.05	0.34 ± 0.01	0.26 ± 0.0039	0.39 ± 0.01	0.30 ± 0.01
6.0×	4.55 ± 0.07	3.10 ± 0.05	0.34 ± 0.01	0.26 ± 0.0034	0.39 ± 0.01	0.29 ± 0.01
Sources of variation	*Probability (P) value*					
CRF	<0.0001***	0.4917 ^{NS}	0.5057 ^{NS}	0.0137*	0.0003***	0.0002***
PD	0.8940 ^{NS}	0.4996 ^{NS}	0.7471 ^{NS}	0.7036 ^{NS}	0.3681 ^{NS}	0.2735 ^{NS}
CRF*PD	0.8248 ^{NS}	0.9699 ^{NS}	0.4664 ^{NS}	0.6134 ^{NS}	0.0179*	0.1581 ^{NS}
FAM	0.9505 ^{NS}	0.0569 ^{NS}	0.9986 ^{NS}	0.1037 ^{NS}	0.9243 ^{NS}	0.4624 ^{NS}
CRF*FAM	0.4572 ^{NS}	0.6017 ^{NS}	0.6831 ^{NS}	0.4115 ^{NS}	0.3958 ^{NS}	0.7837 ^{NS}
PD*FAM	0.6489 ^{NS}	0.7508 ^{NS}	0.9216 ^{NS}	0.9418 ^{NS}	0.6939 ^{NS}	0.1921 ^{NS}
CRF*PD*FAM	0.9309 ^{NS}	0.7483 ^{NS}	0.9222 ^{NS}	0.5330 ^{NS}	0.9156 ^{NS}	0.3096 ^{NS}

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

^{NS} = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-11. The concentration of B, Mn, and Zn in the leaf as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments	B (mg kg^{-1})		Mn (mg kg^{-1})		Zn (mg kg^{-1})	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]						
CRF 1	148.42 ± 3.07 a[w]	166.48 ± 5.06 a	93.04 ± 6.36 a	181.46 ± 8.49 a	74 ± 6.16 a	162.44 ± 8.21 a
CRF 2	117.13 ± 3.27 b	145.02 ± 3.35 b	62.14 ± 4.93 b	139.67 ± 7.67 b	51.74 ± 3.64 b	137.79 ± 7.99 b
PD[y]						
SR/LD	130.84 ± 4.38	143.84 ± 4.30 a	83.03 ± 7.70	137.97 ± 8.31 b	66.66 ± 6.65	133.69 ± 8.59 b
SR/HD	129.90 ± 3.82	158.47 ± 4.77 ab	67.16 ± 7.31	170.75 ± 10.25 a	68.17 ± 6.84	163.38 ± 9.56 a
DR/HD	137.59 ± 5.87	164.94 ± 6.80 b	82.58 ± 7.24	172.97 ± 11.87 a	53.77 ± 5.79	153.28 ± 11.56 ab
FAM[x]						
0.0 x	122.69 ± 4.51 b	156.08 ± 7.40	47.77 ± 3.92 c	113 ± 9.26 b	38.92 ± 2.85 c	100.29 ± 9.15 b
1.5 x	128.16 ± 4.26 ab	154.17 ± 4.69	62.71 ± 4.07 bc	172 ± 9.09 b	50.22 ± 2.90 bc	170.92 ± 9.05 a
3.0 x	138.23 ± 5.14 ab	159.63 ± 7.11	86.13 ± 7.06 b	175.71 ± 12.64 b	71.22 ± 7.38 ab	168.83 ± 11.67 a
6.0 x	142.01 ± 7.02 a	153.13 ± 6.49	113.76 ± 10.77 a	181.54 ± 12.22 a	91.11 ± 9.50 a	160.42 ± 10.71 a
Sources of variation			*Probability (P) value*			
CRF	<0.0001***	0.0004***	<0.0001***	<0.0001***	0.0006***	0.1876 NS
PD	0.3126 NS	0.0128*	0.0793 NS	0.0036**	0.1210 NS	0.0249*
CRF*PD	0.2822 NS	0.0113*	0.4136 NS	0.0108*	0.8434 NS	0.0205*
FAM	0.0109*	0.8657 NS	<0.0001***	<0.0001***	<0.0001***	<0.0001***
CRF*FAM	0.5301 NS	0.7029 NS	0.1297 NS	0.2850 NS	0.1606 NS	0.4331 NS
PD*FAM	0.8271 NS	0.2604 NS	0.9894 NS	0.0951 NS	0.9988 NS	0.1471 NS
CRF*PD*FAM	0.9831 NS	0.8921 NS	0.9872 NS	0.9907 NS	0.9672 NS	0.9702 NS

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

NS = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-12. The concentration of Cu and Fe in the leaf as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments	Cu (mg kg^{-1})		Fe (mg kg^{-1})	
	2018/19	2019/20	2018/19	2019/20
CRF[z]				
CRF 1	248.03 ± 12.71[w]	208.67 ± 8.27 a	99.95 ± 3.01 a	61.25 ± 1.24 a
CRF 2	242.80 ± 9.26	174.81 ± 7.84 b	77.43 ± 2.49 b	56.02 ± 1.05 b
PD[y]				
SR/LD	243.87 ± 16.29	188.50 ± 9.22	92.24 ± 4.56	58.41 ± 1.78
SR/HD	253.70 ± 11.46	197.41 ± 10.93	88.98 ± 3.93	56.44 ± 1.31
DR/HD	238.67 ± 12.76	189.31 ± 10.82	84.85 ± 3.14	61.06 ± 1.21
FAM[x]				
0.0 x	248.46 ± 15.75	216.25 ± 11.86 a	86.93 ± 4.02	61.33 ± 1.64
1.5 x	248.83 ± 14.92	209.63 ± 11.29 a	87.74 ± 3.74	57.08 ± 1.45
3.0 x	251.71 ± 16.81	182 ± 11.26 ab	91.01 ± 4.69	57.71 ± 1.94
6.0 x	232.66 ± 15.80	159.08 ± 9.76 b	89.08 ± 5.64	58.42 ± 1.73
Sources of variation		*Probability (P) value*		
CRF	0.7636 ^{NS}	0.0041**	<0.0001***	0.0010***
PD	0.7727 ^{NS}	0.7805 ^{NS}	0.3429 ^{NS}	0.0526 ^{NS}
CRF*PD	0.3326 ^{NS}	0.9399 ^{NS}	0.1323 ^{NS}	0.2350 ^{NS}
FAM	0.8625 ^{NS}	0.0022**	0.9036 ^{NS}	0.2195 ^{NS}
CRF*FAM	0.8395 ^{NS}	0.8505 ^{NS}	0.7514 ^{NS}	0.7724 ^{NS}
PD*FAM	0.9956 ^{NS}	0.9804 ^{NS}	0.9293 ^{NS}	0.0637 ^{NS}
CRF*PD*FAM	0.9977 ^{NS}	0.7590 ^{NS}	0.6628 ^{NS}	0.6804 ^{NS}

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

^{NS} = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 3-13. Soil macronutrient concentration as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatments	P (mg kg^{-1})		K (mg kg^{-1})		Ca (mg kg^{-1})		Mg (mg kg^{-1})		S (mg kg^{-1})	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]										
CRF 1	15.88 ± 1.06[w]	35.77 ± 1.14	20.29 ± 1.75	41.75 ± 1.25	350.92 ± 20.37	429.76 ± 17.82 b	19.29 ± 1.03	36.28 ± 1.13 b	18.08 ± 2.40	18.27 ± 0.78 a
CRF 2	13.67 ± 1.50	36.20 ± 0.89	21.00 ± 1.46	42.09 ± 1.57	422.13 ± 56.55	521.35 ± 21.59 a	24.08 ± 1.20	41.16 ± 1.22 a	13.75 ± 2.07	10.60 ± 0.44 b
PD[y]										
SR/LD	16.44 ± 1.74	34.39 ± 1.28 a	19.25 ± 1.51	38.84 ± 1.40 b	403.06 ± 89.50	386.64 ± 24.8 b	20.38 ± 1.42	32.75 ± 1.13 b	15.19 ± 3.20	12.94 ± 0.99 b
SR/HD	14.31 ± 1.33	39.75 ± 1.00 a	21.25 ± 2.11	40.77 ± 1.59 ab	379.88 ± 28.36	512.55 ± 23.93 a	23.25 ± 2.26	41.30 ± 1.55 a	15.38 ± 2.73	14.50 ± 0.90 ab
DR/HD	13.56 ± 1.75	33.81 ± 1.21 b	21.44 ± 2.28	46.16 ± 1.93 a	376.63 ± 13.73	527.48 ± 20.31 a	21.44 ± 0.79	42.11 ± 1.21 a	17.19 ± 2.76	15.88 ± 1.16 a
FAM[x]										
0.0 x	N/A[v]	37.46 ± 1.48	N/A	43.58 ± 1.68	N/A	482.94 ± 21.76	N/A	40.06 ± 1.42	N/A	15.96 ± 1.46
1.5 x	N/A	35.69 ± 1.40	N/A	43.23 ± 2.15	N/A	491.35 ± 35.67	N/A	40.00 ± 2.15	N/A	14.96 ± 1.20
3.0 x	N/A	35.08 ± 1.39	N/A	40.48 ± 1.72	N/A	484.50 ± 36.17	N/A	37.94 ± 1.87	N/A	13.46 ± 0.87
6.0 x	N/A	35.71 ± 1.54	N/A	40.40 ± 2.36	N/A	443.44 ± 21.28	N/A	36.88 ± 1.38	N/A	13.38 ± 1.15
Sources of variation	*Probability (P) value*									
CRF	0.2848 NS	0.7640 NS	0.7487 NS	0.8569 NS	0.3303 NS	0.0005***	0.0387 NS	0.0011**	0.2279 NS	<0.0001***
PD	0.4772 NS	0.0015**	0.7572 NS	0.0070**	0.9692 NS	<0.0001***	0.3422 NS	<0.0001***	0.8748 NS	0.0244*
CRF*PD	0.7412 NS	0.3286 NS	0.7000 NS	0.4731 NS	0.7102 NS	0.1925 NS	0.4946 NS	0.2644 NS	0.4139 NS	0.8648 NS

Table 3-13. Continued

Sources of variation	*Probability (P) value*									
FAM	N/A	0.6681 NS	N/A	0.4866 NS	N/A	0.5224 NS	N/A	0.3151 NS	N/A	0.1059 NS
CRF*FAM	N/A	0.3308 NS	N/A	0.2289 NS	N/A	0.7157 NS	N/A	0.9310 NS	N/A	0.1054 NS
PD*FAM	N/A	0.7523 NS	N/A	0.6414 NS	N/A	0.1450 NS	N/A	0.1160 NS	N/A	0.4183 NS
CRF*PD*FAM	N/A	0.9563 NS	N/A	0.1694 NS	N/A	0.7328 NS	N/A	0.9427 NS	N/A	0.6175 NS

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

[v]N/A= Value not available

Table 3-14. The concentration of B, Mn, and Zn in the soil as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatment	B (mg kg^{-1})		Mn (mg kg^{-1})		Zn (mg kg^{-1})	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
CRF[z]						
CRF 1	0.19 ± 0.01 b[w]	0.39 ± 0.01 b	4.96 ± 0.42 a	16.48 ± 0.87	9.81 ± 0.61	15.11 ± 0.66 b
CRF 2	0.38 ± 0.07 a	0.49 ± 0.02 a	2.42 ± 0.19 b	18.83 ± 1.30	9.78 ± 0.91	17.97 ± 0.95 a
PD[y]						
SR/LD	0.31 ± 0.11	0.35 ± 0.02 b	4.13 ± 0.81	16.13 ± 1.46	10.09 ± 1.16	14.59 ± 0.84 b
SR/HD	0.25 ± 0.02	0.50 ± 0.03 a	3.38± 0.43	19.17 ± 1.43	9.31 ± 0.93	18.07 ± 1.25 a
DR/HD	0.29 ± 0.05	0.47 ± 0.02 a	3.56 ± 0.55	17.67 ± 1.16	9.99 ± 0.76	16.97 ± 0.86 ab
FAM[x]						
0.0 x	N/A[v]	0.44 ± 0.02	N/A	14.85 ± 1.07 b	N/A	14.31 ± 0.88 b
1.5 x	N/A	0.46 ± 0.03	N/A	17.27 ± 1.38 ab	N/A	16.44 ± 1.27 ab
3.0 x	N/A	0.43 ± 0.03	N/A	17.29 ± 1.61 ab	N/A	16.58 ± 1.01 ab
6.0 x	N/A	0.44 ± 0.04	N/A	21.21 ± 1.91 a	N/A	18.85 ± 1.39 a
Sources of variation			*Probability (P) value*			
CRF	0.0149*	<0.0001***	0.0062**	0.1253 ^{NS}	0.9731 ^{NS}	0.0125*
PD	0.7541 ^{NS}	<0.0001***	0.4030 ^{NS}	0.2675 ^{NS}	0.8635 ^{NS}	0.0396*
CRF*PD	0.2282 ^{NS}	0.4408 ^{NS}	0.5349 ^{NS}	0.1640 ^{NS}	0.5662 ^{NS}	0.5887 ^{NS}
FAM	N/A	0.8750 ^{NS}	N/A	0.0360 *	N/A	0.0487 *
CRF*FAM	N/A	0.8598 ^{NS}	N/A	0.8275 ^{NS}	N/A	0.7866 ^{NS}
PD*FAM	N/A	0.7894 ^{NS}	N/A	0.2097 ^{NS}	N/A	0.1290 ^{NS}
CRF*PD*FAM	N/A	0.9499 ^{NS}	N/A	0.8856 ^{NS}	N/A	0.9818 ^{NS}

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

[v]N/A= Value not available

Table 3-15. The concentration of Cu and Fe in the soil as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

Treatment	Cu (mg kg^{-1})		Fe (mg kg^{-1})	
	2018/19	2019/20	2018/19	2019/20
CRF[z]				
CRF 1	13.52 ± 0.56[w]	29.22 ± 0.85	8.33 ± 0.74	81.56 ± 3.80
CRF 2	14.07 ± 0.70	30.56 ± 0.97	8.83 ± 0.84	83.58 ± 2.89
PD[y]				
SR/LD	13.95 ± 0.83	29.87 ± 0.82	9.19 ± 0.85	79.36 ± 3.88 b
SR/HD	13.74 ± 0.48	30.25 ± 1.12	9.75 ± 0.89	95.28 ± 3.61 a
DR/HD	13.69 ± 1.00	29.55 ± 1.38	6.81 ± 0.88	73.08 ± 3.93 b
FAM[x]				
0.0 x	N/A[v]	30.46 ± 1.00	N/A	86.13 ± 5.18
1.5 x	N/A	31.11 ± 1.55	N/A	83.52 ± 4.84
3.0 x	N/A	29.83 ± 1.45	N/A	79.02 ± 3.85
6.0 x	N/A	28.16 ± 1.08	N/A	81.63 ± 5.20
Sources of variation			*Probability (P) value*	
CRF	0.5500 [NS]	0.3335 [NS]	0.6862 [NS]	0.6689 [NS]
PD	0.9800 [NS]	0.9157 [NS]	0.0757 [NS]	0.0008***
CRF*PD	0.9548 [NS]	0.2469 [NS]	0.6930 [NS]	0.8129 [NS]
FAM	N/A	0.4685 [NS]	N/A	0.7485 [NS]
CRF*FAM	N/A	0.9609 [NS]	N/A	0.4993 [NS]
PD*FAM	N/A	0.6814 [NS]	N/A	0.8763 [NS]
CRF*PD*FAM	N/A	0.9285 [NS]	N/A	0.8508 [NS]

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

[NS] = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

[v]N/A= Value not available

Table 3-16. Soil pH and cation exchange capacity (CEC) as a function of controlled-release fertilizer (CRF), planting density (PD), and foliar-applied micronutrient (FAM) in 2018/19 and 2019/20.

	Soil pH		CEC (meq g^{-1})	
	2018/19	2019/20	2018/19	2019/20
CRF[z]				
CRF 1	6.00 ± 0.12 b[w]	5.63 ± 0.08 b	3.03 ± 0.06	4.11 ± 0.10
CRF 2	6.38 ± 0.12 a	6.36 ± 0.07 a	3.19 ± 0.26	4.13 ± 0.11
PD[y]				
SR/LD	6.00 ± 0.19	5.58 ± 0.11 b	3.23 ± 0.36	3.89 ± 0.12
SR/HD	6.40 ± 0.12	6.22 ± 0.10 a	3.04 ± 0.17	4.21 ± 0.14
DR/HD	6.18 ± 0.13	6.19 ± 0.09 ab	3.06 ± 0.06	4.26 ± 0.12
FAM[x]				
0.0 x	N/A[v]	6.00 ± 0.13	N/A	4.19 ± 0.12
1.5 x	N/A	5.98 ± 0.12	N/A	4.25 ± 0.18
3.0 x	N/A	6.03 ± 0.14	N/A	4.12 ± 0.16
6.0 x	N/A	5.97 ± 0.14	N/A	3.92 ± 0.10
Sources of variation		*Probability (P) value*		
CRF	0.0268*	<0.0001***	0.6867 NS	0.9129 NS
PD	0.1489 NS	<0.0001***	0.9322 NS	0.0903 NS
CRF*PD	0.4637 NS	0.4802 NS	0.8046 NS	0.1162 NS
FAM	N/A	0.9621 NS	N/A	0.4436 NS
CRF*FAM	N/A	0.1029 NS	N/A	0.8970 NS
PD*FAM	N/A	0.1160 NS	N/A	0.5256 NS
CRF*PD*FAM	N/A	0.5766 NS	N/A	0.9160 NS

[z]In 2018/19, CRF 1 = (12N-1.31P-7.47K with higher amount of micronutrient as sulfur-coated products); and CRF 2= (16N-1.31P-16.6K with micronutrients as sulfates). In 2019/20, CRF 1 = 12-3-14 (12N-1.31P-11.62K) with 2× Mg and 2.5× micronutrients; and CRF 2 = 12-3-14 (12N-1.31P-11.62 K).

[y]SR/LD = single row low-density (300 trees/ha). SR/HD = high-density single row (440 trees/ha). DR/HD = high-density double row staggered in diamond setting (975 trees/ha).

[x]Foliar applied micronutrients using the recommended rates of B, Mn, and Zn per year.

[w]Means ± standard error (n = 4) followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's test.

NS = Not significant. ** and *** = Significant at $P < 0.01$ and $P < 0.001$, respectively.

[v]N/A= Value not available

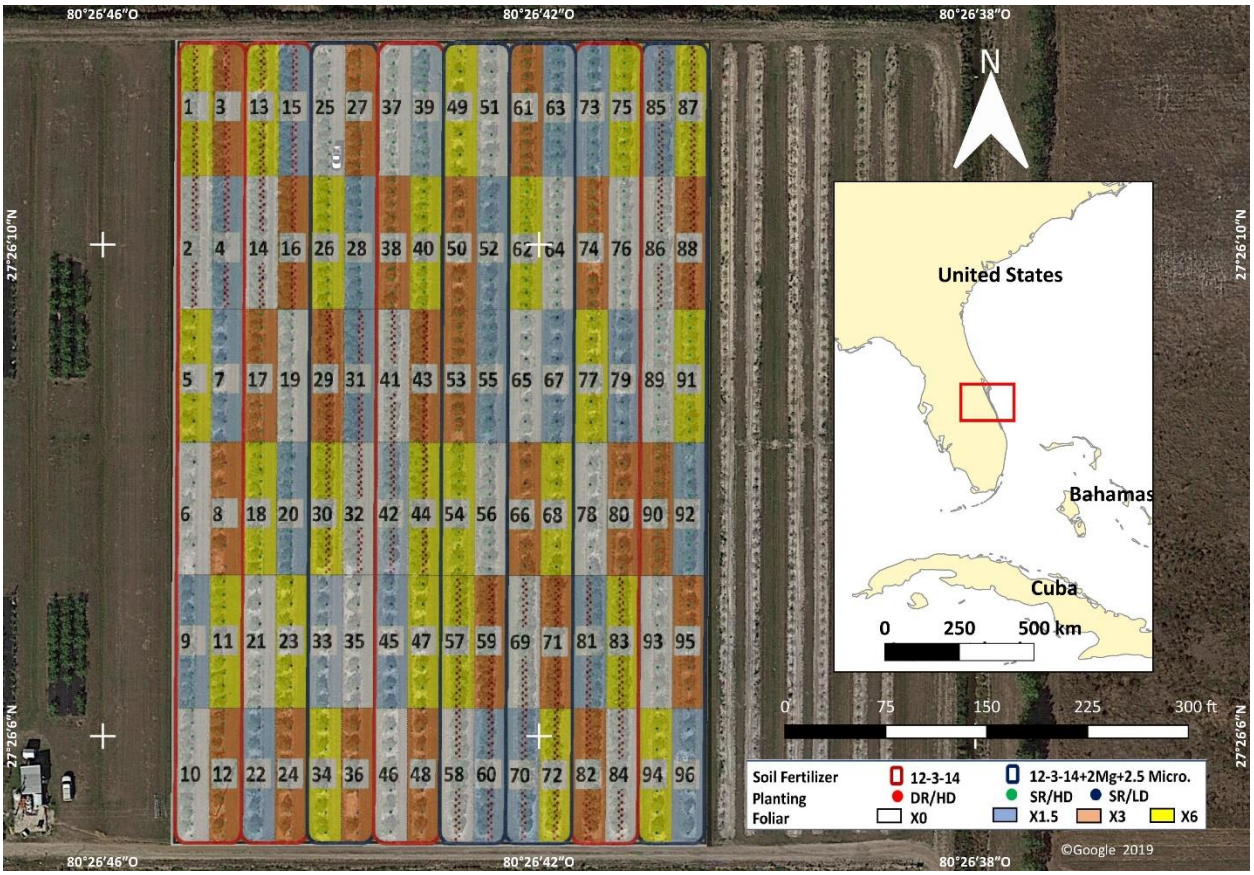

Figure 3-1. Study area at the UF/IFAS Indian River Research and Education Grove, Fort Pierce, FL, showing 96 experimental units. We tested two controlled-release fertilizer (CRF) blends {[12-3-9 (12N-1.31P-7.47K) with all micronutrients as sulfur-coated products at higher rates; and 16-3-20 (16N-1.31P-16.6K) with all micronutrients as sulfates] in 2018/19 and two CRF blends [12-3-14 (12N-1.31P-11.62 K) with 2× of Mg and 2.5× of recommended rates; and 12-3-14 (12N-1.31P-11.62 K) with the recommended rate for Mg and all micronutrients] in 2019/20}. Additionally, three planting density [single row low-density (SR/LD), single row high-density (SR/HD), and double row high-density staggered in diamond setting (DR/HD)] were tested. Four treatments (0×, 1.5×, 3×, and 6×) as a foliar micronutrient (a blend of B, Mn, and Zn) application according to recommended rates were used/year.

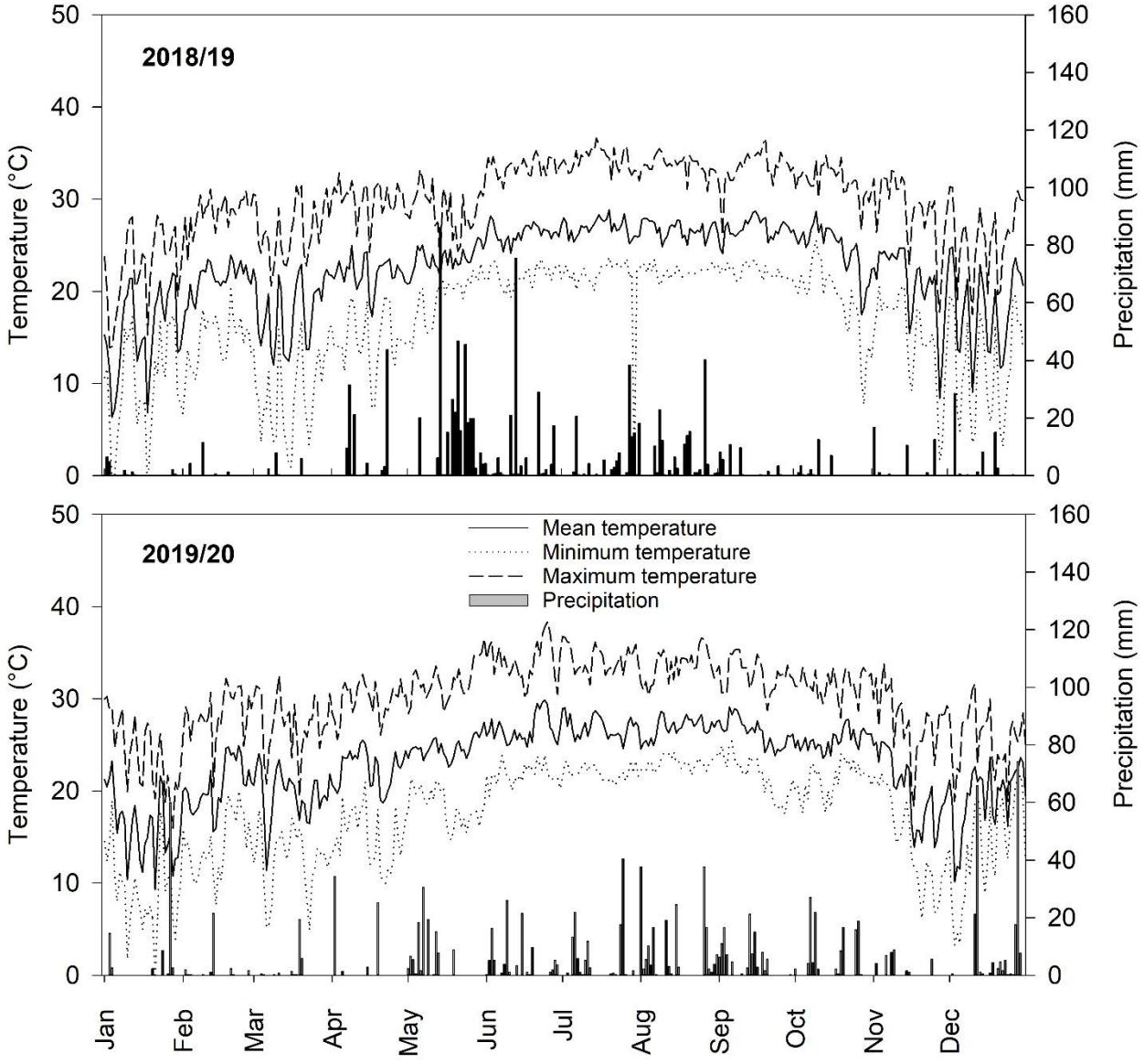

Figure 3-2. Monthly accumulated rainfall precipitation, mean, minimum, and maximum temperatures recorded in 2018/19 and 2019/20.

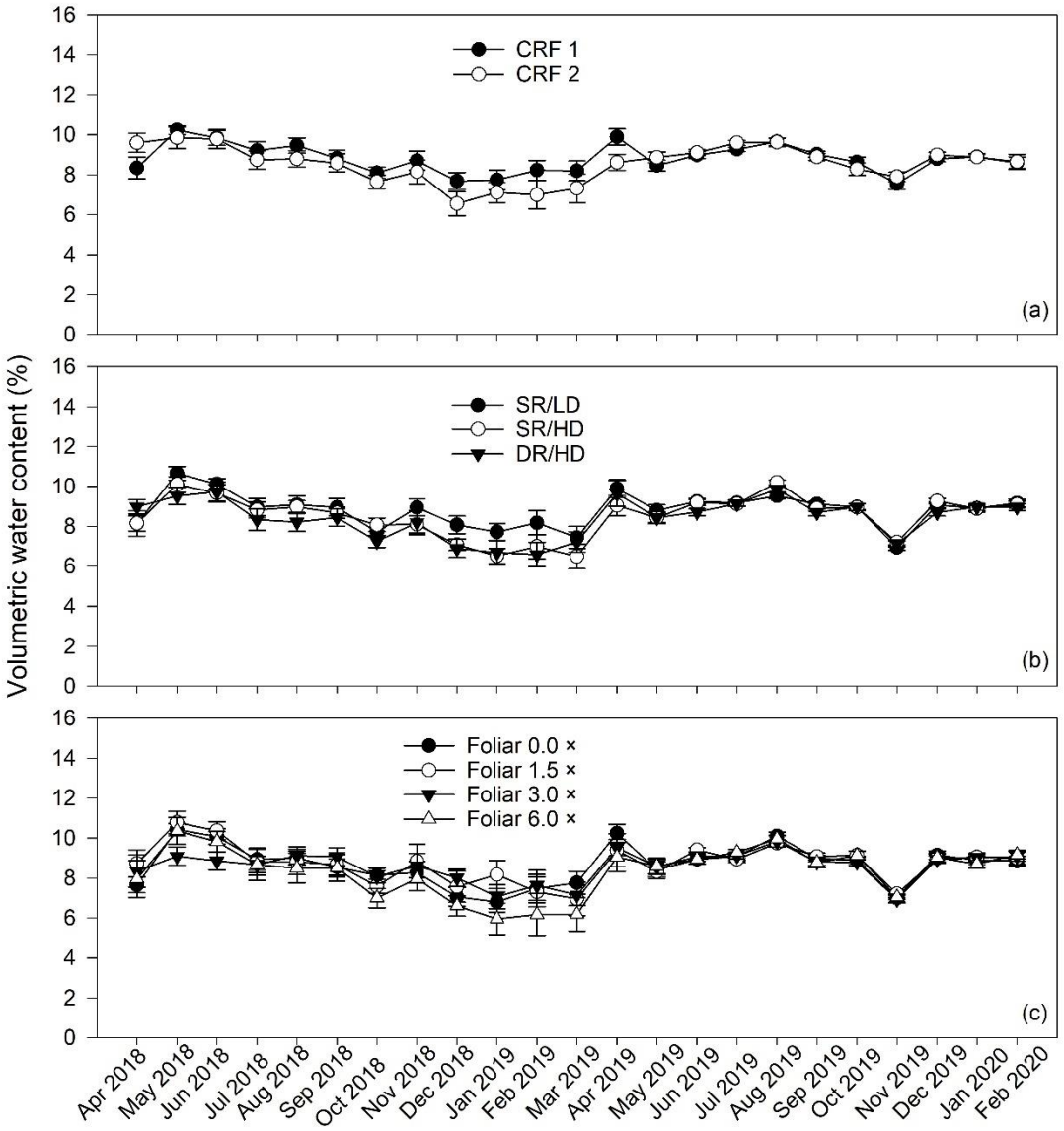

Figure 3-3. Volumetric water content reading during the experiment. Two different controlled-release fertilizer (CRF) blends [12-3-9 (12N-1.31P-7.47K) with all micronutrients as sulfur-coated products at higher rates; and 16-3-20 (16N-1.31P-16.6K) with all micronutrients as sulfates] in 2018/19 and two CRF blends [12-3-14 (12N-1.31P-11.62 K) with 2× of Mg and 2.5× of recommended rates; and 12-3-14 (12N-1.31P-11.62 K) with the recommended rate for Mg and all micronutrients] in 2019/20 (a), three planting density [single row low-density (SR/LD), single row high-density (SR/HD), and double row high-density staggered in diamond setting (DR/HD)] (b) and, four foliar treatments (0×, 1.5×, 3×, and 6×) of recommended rates of B, Mn and Zn (c). The vertical bar represents the standard error of the mean.

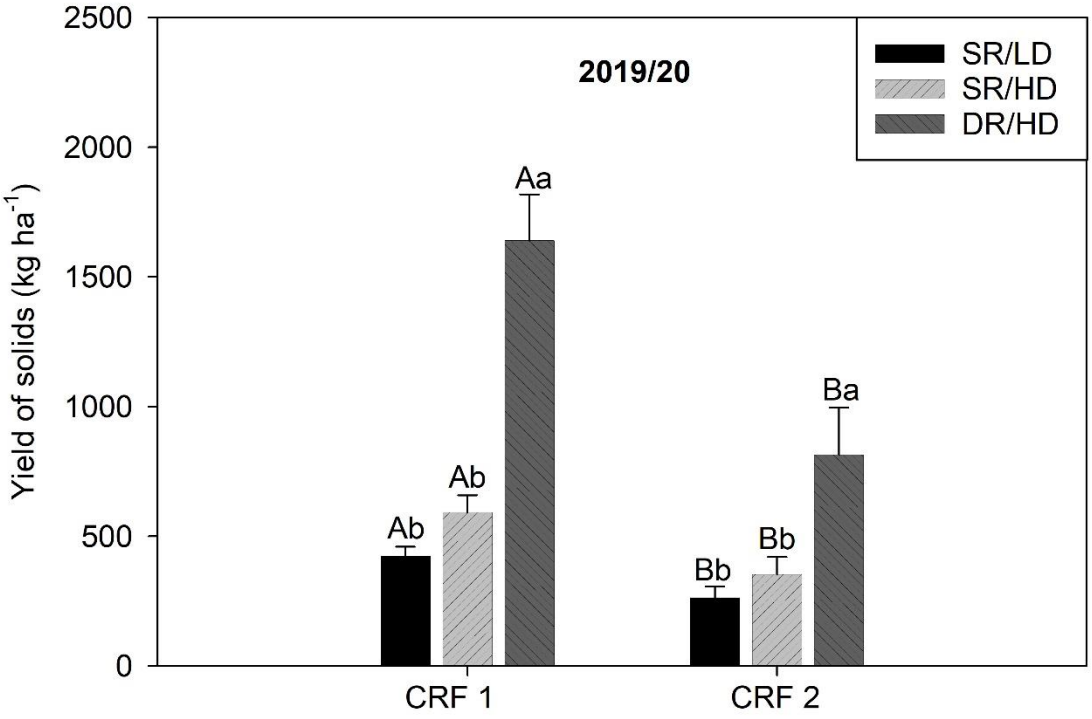

Figure 3-4. Yield of solids under two controlled-release fertilizers (CRF) blends [CRF 1 (12N-1.31P-7.47K with 2.5× micronutrients and 2× Mg) and CRF 2 (16N-1.31P-16.6K)]; and three planting density [single row low-density(SR/LD), single row high-density (SR/HD), and double-row high-density staggered in diamond setting(DR/HD)]. Means ± standard errors followed by different letters (uppercase compare CRF and lowercase compare PD) are significantly different by Tukey's test at $P < 0.05$.

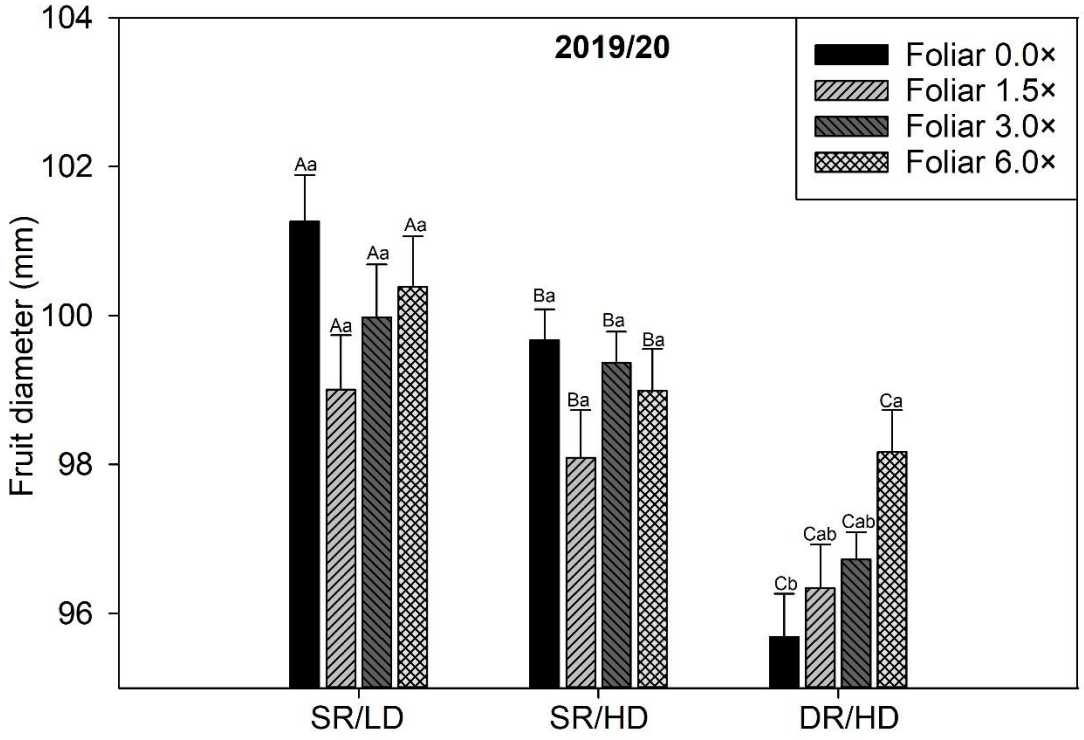

Figure 3-5. Fruit diameter under three planting density (PD) [single row low-density (SR/LD), single row high-density (SR/HD), and double-row high-density staggered in diamond setting (DR/HD)]; and four foliar-applied micronutrients (FAM) (0×, 1.5×, 3×, and 6×) of a blend of B, Mn, and Zn application according to recommended rates were used/year. Means ± standard errors followed by different letters (uppercase compare FAM and lowercase compare PD) are significantly different by Tukey's test at $P < 0.05$.

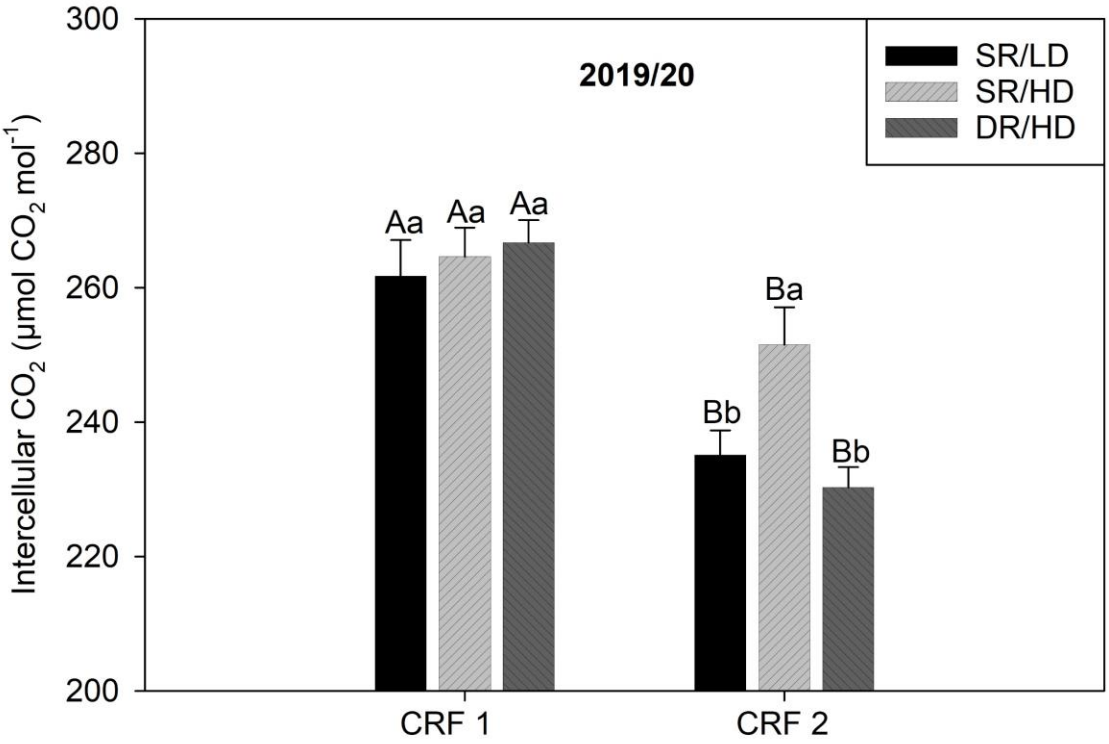

Figure 3-6. Intercellular CO_2 under two controlled-release fertilizer (CRF) blends [CRF 1 (12N-1.31P-7.47K with 2.5× micronutrients and 2× Mg) and CRF 2 (16N-1.31P-16.6K)]; and three planting density (PD) [single row low-density(SR/LD), single row high-density (SR/HD), and double-row high-density staggered in diamond setting(DR/HD)]. Means ± standard errors followed by different letters (uppercase compare CRF and lowercase compare PD) are significantly different by Tukey's test at $P < 0.05$.

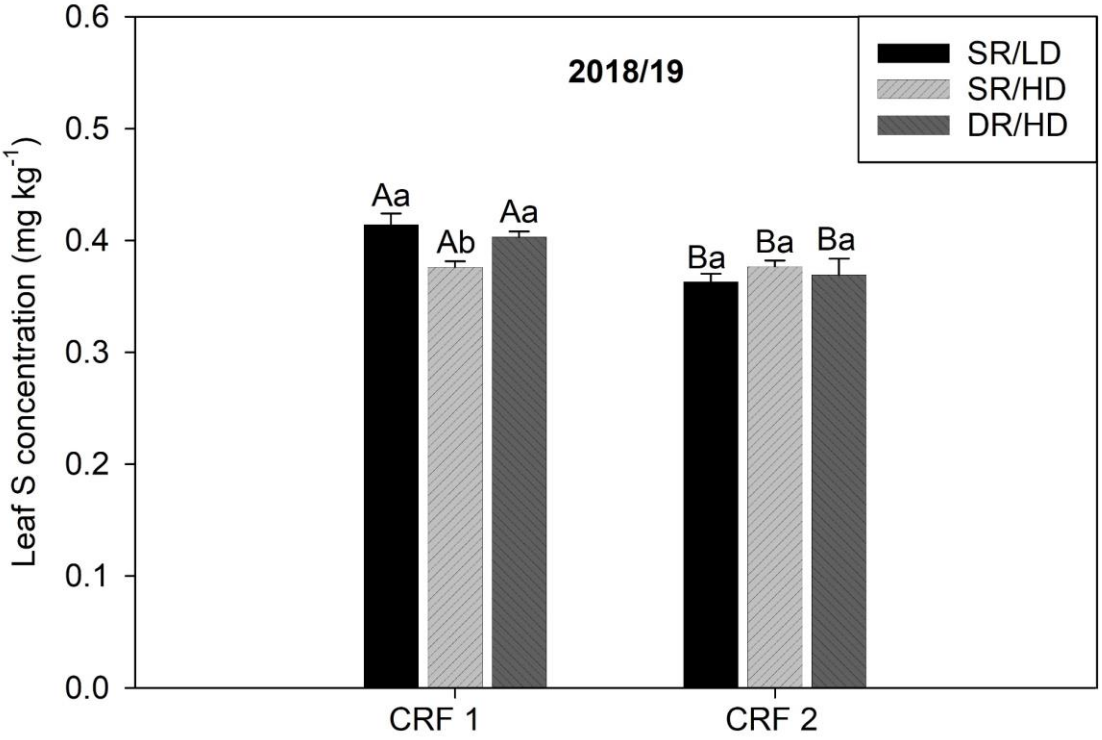

Figure 3-7. Leaf S concentration under two controlled-release fertilizers (CRF) blends [CRF 1 (12N-1.31P-7.47K with higher amount of micronutrient) and CRF 2 (16N-1.31P-16.6K)]; and three planting density (PD) [single row low-density (SR/LD), single row high-density (SR/HD), and double-row high-density staggered in diamond setting (DR/HD)]. Means ± standard errors followed by different letters (uppercase compare CRF and lowercase compare PD) are significantly different by Tukey's test at $P < 0.05$.

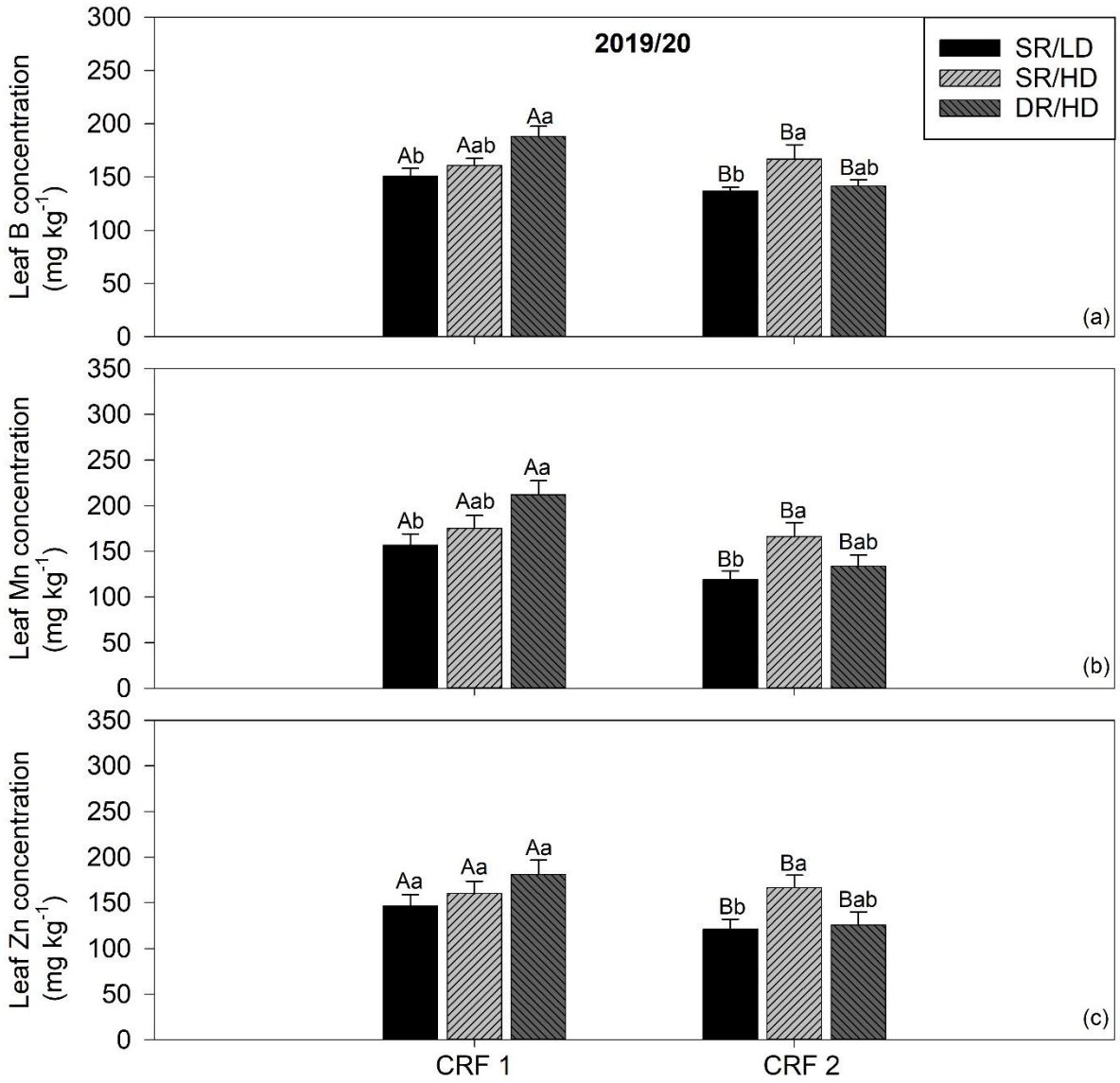

Figure 3-8. The concentration of B (a), Mn (b), and Zn (c) in the leaves under two controlled-release fertilizers (CRF) blends [CRF 1 (12N-1.31P-7.47K with 2.5× micronutrients and 2× Mg) and CRF 2 (16N-1.31P-16.6K)]; and three planting density (PD) [single row low-density (SR/LD), single row high-density (SR/HD), and double-row high-density staggered in diamond setting (DR/HD)]. Means ± standard errors followed by different letters (uppercase compares CRF and lowercase compare PD) are significantly different by Tukey's test at $P < 0.05$.

APPENDIX A
LEAF NUTRIENT INTERPRETATION

Table A-1. Guidelines for interpretation of orange tree leaf analysis based on 4 to 6-month-old spring flush leaves from non-fruiting twigs (Koo et al., 1984).

Element	Unit	Deficient	Low	Optimum	High	Excess
N	%	< 2.2	2.2 – 2.4	2.5 – 2.7	2.8 – 3.0	> 3.0
P	%	<0.09	0.09 – 0.11	0.12 – 0.16	0.17 – 0.30	> 0.30
K	%	<0.7	0.7 – 1.1	1.2 – 1.7	1.8 – 2.4	> 2.4
Ca	%	<1.5	1.5 – 2.9	3.0 – 4.9	5.0 – 7.0	> 7.0
Mg	%	<0.2	0.20 – 0.29	0.30 – 0.49	0.50 – 0.70	> 0.70
B	mg/kg or ppm[y]	< 20	20 – 35	60 – 120	101 – 200	> 200
Cu	mg/kg or ppm	< 3	3 – 4	5 – 16	17 – 20	> 20
Fe	mg/kg or ppm	< 35	35 – 59	60 – 120	121 – 200	> 200
Mn	mg/kg or ppm	<18	18 – 24	25 – 100	101 – 300	> 300
Zn	mg/kg or ppm	< 18	18 – 24	25 – 100	101 – 300	> 300
Mo	mg/kg or ppm	< 0.05	0.06 – 0.09	0.10 – 2.0	2.0 – 5.0	> 5.0
Cl	%	---	---	< 0.2	0.20 – 0.70	> 0.70[z]
Na	%	---	---	---	0.15 – 0.25	> 0.25

[z]Leaf burn and defoliation can occur at Cl concentration > 1.0%.
[y]ppm=parts/million.

APPENDIX B
SOIL NUTRIENT INTERPRETATION

Table A-2. Soil test interpretations for other extraction methods compared with Mehlich 1 and 3 (Obreza and Morgan, 2008)

Extractant	Nutrient	Very Low	Low	Medium	High	Very High
		(Less than sufficient)			(Sufficient)	
Mehlich 1	P	< 10	10–15	16–30	31–60	> 60
Mehlich 3[y]	mg/kg	< 11	11–16	17–29	30–56	> 56
Ammonium acetate pH 4.8[x]	(ppm)[z]	≤ 11			> 11	
Bray P1[x]		≤ 40			> 40	
Bray P2[x]		≤ 65			> 65	
	Mg		Low	Medium	High	
Mehlich 1	mg/ kg		< 15	15–30	> 30	
Mehlich 3[w]	(ppm)		< 25	25–33	> 33	
Ammonium acetate pH 4.8[v]			< 14	14–26	> 26	
		Less than sufficient			Sufficient	
Ammonium acetate pH 7.0[x]		≤ 50			> 50	
	Ca	Less than sufficient			Sufficient	
Mehlich 1		≤ 250			> 250	
Mehlich 3[w]	mg/ kg	≤ 200			> 200	
Ammonium acetate pH 4.8[v]	(ppm)	≤ 250			> 270	
Ammonium acetate pH 7.0[x]		≤ 270			> 250	

[z]parts/million (ppm) x 2 = lbs/acre.
[y]Estimated from unpublished correlation data (T. A. Obreza, 2006).
[x]From Koo et al. (1984).
[w]Estimated from correlation data (Alva, 1993).
[5]Estimated from correlation data (Sartain, 1978).

LIST OF REFERENCES

Albrecht, U., Alferez, F., and Zekri, M. 2019. 2019–2020 Florida Citrus Production Guide: Rootstock and Scion Selection. HS1308. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Albrecht, U., and Bowman, K. D. 2011. Tolerance of the trifoliate citrus hybrid US-897 (*Citrus reticulata Blancox Poncirus trifoliata L. Raf.)* to Huanglongbing. HortScience, 46: 16-22.

Al-Obeed, R. S., Ahmed, M. A. A., Kassem, H. A., and Al-Saif, A. M. 2018. Improvement of “Kinnow” mandarin fruit productivity and quality by urea, boron and zinc foliar spray. J. Plant Nutr., 41: 609–618.

Alva, A. K. 1993. Comparison of Mehlich 3, Mehlich 1, ammonium bicarbonate-DTPA, 1.0 M ammonium acetate, and 0.2 M ammonium chloride for extraction of calcium, magnesium, phosphorus, and potassium for a wide range of soils. Commun. Soil Sci. Plan., 24: 603-612.

Alva, A. K., Mattos Jr, D., Paramasivam, S., Patil, B., Dou, H., and Sajwan, K. S. 2006. Potassium management for optimizing citrus production and quality. Int J. Fruit Sci., 6: 3-43.

Barker, A. V., and Pilbeam, D. J. 2015. Handbook of plant nutrition. CRC press

Beeson Jr, R. C., and Silva, D. 2017. Development of a procedure to maximize production of hardy rootstocks of citrus using stem cuttings. Am. J. Plant Sci., 8: 2837.

Boaretto, A. E., Boaretto, R. M., Muraoka, T., Nascimento Filho, V. F., Tiritan, C. S., and Mourão Filho, F. A. A. 2002. Foliar micronutrient application effects on citrus fruit yield, soil and leaf Zn concentrations and 65Zn mobilization within the plant. Acta Hortic.594: 203–209.

Boaretto, A. E., Tiritan, C. S., and Muraoka, T. 1997. Effects of foliar applications of boron on citrus fruit and on foliage and soil boron concentration. In Boron in soils and plants. pp. 121-123. Springer, Dordrecht.

Boman, B. J. 1994. Evapotranspiration by young Florida flatwoods citrus trees. J. Irrig Drain. Eng., 120: 80-88.

Boman, B., and Obreza, T. 2015. Fertigation nutrient sources and application considerations for citrus. Circular 1410. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Boman, B., and Obreza, T. 2018. Water Table Measurement and Monitoring for Flatwoods Citrus. Circular 1409. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Bove, J. M. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol., 88: 7-37.

Bowman, K. D., McCollum, G., and Albrecht, U. 2016. Performance of 'Valencia'orange (*Citrus sinensis [L.] Osbeck*) on 17 rootstocks in a trial severely affected by Huanglongbing. Sci. Hortic., 201: 355-361.

Brlansky, R. H., and Rogers, M. E. 2007. Citrus Huanglongbing: Understanding the vector-pathogen interaction for disease management. Plant Health Progr., 10.

Castle W. S., Bowman K. D., Grosser J. W., Ferrarezi R. S., Futch S. H., Rogers S. 2019. Florida Citrus Rootstock Selection Guide, 4th Edition. SP248. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Castle, W. S. 1980. Citrus rootstocks for tree size control and higher density plantings in Florida. In Proceedings of the Fla. State Hort. Soc., 93:24-27.

Castle, W. S. 2010. A career perspective on citrus rootstocks, their development, and commercialization. HortScience, 45: 11-15.

Chaudhry, F. M., Sharif, M., Latif, A., and Qureshi, R. H. 1973. Zinc-copper antagonism in the nutrition of rice (*Oryza sativa*.). Plant Soil, 38: 573-580.

Chung, K. R., and Brlansky, R. H. 2005. Citrus diseases exotic to Florida: Huanglongbing (citrus greening). PP-210. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Court, C., Hodges, A., Stair, C., Rahmani, M. 2018. Economic Contributions of the Florida Citrus Industry in 2015-16. 10 Jan 2020. < https://fred.ifas.ufl.edu/DEStudio/html/EconomicImpactAnalysis/FullReport-EconomicContributionsFloridaCitrusIndustry2016-17-12-4-2018.pdf>.

Cutts, A. 2020. Official rules affecting the Florida citrus industry. State of Florida Department of Citrus, (February). 10 Feb 2020. < https://fdocgrower.app.box.com/s/xn65jdjfsf93p1952no8a2sknpiv17k5/file/187106877628>.

Dalal, R. P. S., Sangwan, A. K., Beniwal, B. S., and Sharma, S. 2013. Effect of planting density on canopy parameter, yield and water use efficiency of Kinnow mandarin. Indian J. Hortic., 70: 587-590.

Dordas, C. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agron. Sustain. Dev., 28: 33-46.

Dou, H., and Alva, A. K. 1998. Nitrogen uptake and growth of two citrus rootstock seedlings in a sandy soil receiving different controlled-release fertilizer sources. Biol. Fertil. Soils, 26:169-172.

Du, C. W., Zhou, J. M., and Shaviv, A. 2006. Release characteristics of nutrients from polymer-coated compound-controlled release fertilizers. J. Polym. Environ., 14: 223–230.

Etxeberria, E., Gonzalez, P., Achor, D., and Albrigo, G. 2009. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. Physiol. Mol. Plant. P., 74: 76-83.

Farnsworth, D., Grogan, K. A., van Bruggen, A. H., and Moss, C. B. 2014. The potential economic cost and response to greening in Florida citrus. Choices, 29: 1-6.

Ferrarezi, R. S., Qureshi, J. A., Wright, A. L., Ritenour, M. A., and Macan, N. P. 2019. Citrus production under screen as a strategy to protect grapefruit trees from Huanglongbing disease. Front. Plant Sci., 10: 1598.

Ferrarezi, R. S., Rodriguez, K., and Sharp, D. 2020. How historical trends in Florida all-citrus production correlate with devastating hurricane and freeze events. Weather, 75: 77-83.

Folimonova, S. Y., Robertson, C. J., Garnsey, S. M., Gowda, S., and Dawson, W. O. 2009. Examination of the responses of different genotypes of citrus to Huanglongbing (citrus greening) under different conditions. Phytopathology, 99: 1346-1354.

Fried, N., Hudson, M. E. 2019. Florida Citrus Statistics 2017-2018. Florida department of agricultural and consumer services. 10 Jan 2020. < https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Statistics/2017-18/fcs1718.pdf>.

García-Tejero, I., Jiménez-Bocanegra, J. A., Martínez, G., Romero, R., Durán-Zuazo, V. H., and Muriel-Fernández, J. L. 2010. Positive impact of regulated deficit irrigation on yield and fruit quality in a commercial citrus orchard [*Citrus sinensis* (L.) Osbeck, cv. salustiano]. Agric. Water Manag., 97: 614–622.

Gerendás, J., and Führs, H. 2013. The significance of magnesium for crop quality. Plant Soil, 368: 101-128.

Goldberg, S., Lesch, S. M., and Suarez, D. L. 2000. Predicting boron adsorption by soils using soil chemical parameters in the constant capacitance model. SOIL SCI. SOC. AM. J., 64: 1356-1363.

Goldman, C. R. 2010. Micronutrient elements (Co, Mo, Mn, Zn, Cu). Biogeochemistry of Inland Waters. Academic Press, San Diego, 378-382.

Gonzalez, P., Reyes-De-Corcuera, J., and Etxeberria, E. 2012. Characterization of leaf starch from HLB-affected and unaffected-girdled citrus trees. Physiol. Mol. Plant Pathol., 79: 71-78.

Gottwald, T. R., Graham, J. H., Irey, M. S., McCollum, T. G., and Wood, B. W. 2012. Inconsequential effect of nutritional treatments on Huanglongbing control, fruit quality, bacterial titer and disease progress. Crop Prot., 36: 73-82.

Graham, J. H., Johnson, E. G., Gottwald, T. R., and Irey, M. S. 2013. Presymptomatic fibrous root decline in citrus trees caused by Huanglongbing and potential interaction with Phytophthora spp. Plant Dis., 97: 1195-1199.

Gransee, A., and Führs, H. 2013. Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. Plant Soil, 368: 5-21.

Hailes, K. J., Aitken, R. L., and Menzies, N. W. 1997. Magnesium in tropical and subtropical soils from north-eastern Australia. II. Response by glasshouse-grown maize to applied magnesium. Soil Res., 35: 629-642.

Halbert, S. E., and Manjunath, K. L. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol.,87: 330-353.

Halbert, S. E., Manjunath, K., Roka, F. M., and Brodie, M. 2008. Huanglongbing (citrus greening) in Florida, 2008. Food and Fertilizer Technology Center. 10 Jan 2020. <http://www.fftc.agnet.org/library.php?func=viewandid=20110712080736andtype_id=4>.

Hall, D. G., Albrecht, U., and Bowman, K. D. 2016. Transmission rates of ‘Ca. Liberibacter asiaticus’ by Asian citrus psyllid are enhanced by the presence and developmental stage of citrus flush. J. Econ. Entomol., 109: 558-563.

Hall, D. G., Hentz, M. G., and Adair Jr, R. C. 2008. Population ecology and phenology of Diaphorina citri (Hemiptera: Psyllidae) in two Florida citrus groves. Environ. Entomol., 37: 914-924.

Hall, D. G., Richardson, M. L., Ammar, E. D., and Halbert, S. E. 2013. Asian citrus psyllid, Diaphorina citri, vector of citrus Huanglongbing disease. Entomol. Exp. Appl., 146: 207–223.

Huber, D. M., Graham, R. D. 1999 The role of nutrition in crop resistance and tolerance to disease, in: Rengel Z. (Ed.), Mineral nutrition of crops fundamental mechanisms and implications, Food Product Press, New York, pp. 205–226.

Huber, D. M., and Jones, J. B. 2013. The role of magnesium in plant disease. Plant Soil, 368: 73-85.

Hutton, R. J., and Loveys, B. R. 2011. A partial root zone drying irrigation strategy for citrus-Effects on water use efficiency and fruit characteristics. Agric. Water Manag. 98: 1485–1496.

Ibrahim, M., Ahmad, N., Anwar, S. A., and Majeed, T. 2007. Effect of micronutrients on citrus fruit yield growing on calcareous soils. Advances in plant and animal boron nutrition, 179-182.

Iglesias, D. J., Lliso, I., Tadeo, F. R., and Talon, M. 2002. Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. Physiol. Plant., 116: 563-572.

Ilyas, A., Ashraf, M. Y., Hussain, M., Ashraf, M., Ahmed, R., and Kamal, A. 2015. Effect of micronutrients (Zn, Cu and B) on photosynthetic and fruit yield attributes of citrus reticulata Blanco var. kinnow. Pakistan J. Bot., 47: 1241–1247.

Imtiaz, M., Alloway, B. J., Shah, K. H., Siddiqui, S. H., Memon, M. Y., Aslam, M., and Khan, P. 2003. Zinc nutrition of wheat: II: interaction of zinc with other trace elements. Asian J. Plant Sci., 2: 156-160.

Irfan, S. A., Razali, R., KuShaari, K., Mansor, N., Azeem, B., and Versypt, A. N. F. 2018. A review of mathematical modeling and simulation of controlled-release fertilizers. J. Control. Release, 271: 45-54.

Isaac, R. A., and Johnson, W. C. 1985. Elemental analysis of plant tissue by plasma emission spectroscopy: collaborative study. J. Assoc. Off. Anal. Chem.;(United States), 68.

Johnson, E. G., Wu, J., Bright, D. B., and Graham, J. H. 2014. Association of ‘Candidatus Liberibacter asiaticus’ root infection, but not phloem plugging with root loss on Huanglongbing-affected trees prior to appearance of foliar symptoms. Plant Pathol., 63: 290-298.

Jones, C., and Jacobsen, J. 2005. Plant nutrition and soil fertility. Nutr. Manag. Modul., 2.

Kadyampakeni, D. M. 2019. Interaction of soil boron application with leaf B concentration, root length density, and canopy size of citrus affected by Huanglongbing. J. Plant Nutr., 43: 186–193.

Kadyampakeni, D. M., Morgan, K. T., Nkedi-Kizza, P., and Kasozi, G. N. 2015. Nutrient Management Options for Florida Citrus: A Review of NPK Application and Analytical Methods. J. Plant Nutr., 38: 568-583.

Kadyampakeni, D. M., Morgan, K. T., Schumann, A. W., and Nkedi-Kizza, P. 2014a. Effect of irrigation pattern and timing on root density of young citrus trees infected with Huanglongbing disease. HortTechnology, 24: 209-221.

Kadyampakeni, D. M., Morgan, K. T., Schumann, A. W., Nkedi-Kizza, P., and Obreza, T. A. 2014b. Water use in drip-and microsprinkler-irrigated citrus trees. Soil Sci. Soc. Am. J., 78: 1351-1361.

Kafkafi, U., Kant, S., 2005. Fertigation. Encycl. Entomol. 1–6.

Kobori, Y., Nakata, T., Ohto, Y., and Takasu, F. 2011. Dispersal of adult Asian citrus psyllid, Diaphorina citri Kuwayama (Homoptera: Psyllidae), the vector of citrus greening disease, in artificial release experiments. Appl. Entomol. Zool., 46: 27-30.

Koo, R. C. J., Anderson, C. A., Stewart, I., Tucker, D. P. H., Calvert, D. V., and Wutscher, H. K. 1984. Recommended fertilizers and nutritional sprays for citrus. Florida Agricultural Experiment Station Bulletin D, 536.

Labanauskas, C. 1963. Foliar sprays correct manganese deficiencies on desert grapefruit. Calif. Agric., 17: 14-15.

Ladaniya, M. S., Marathe, R. A., Das, A. K., Rao, C. N., Huchche, A. D., Shirgure, P. S., and Murkute, A. A. 2020. High density planting studies in acid lime (*Citrus aurantifolia* Swingle). Sci. Hortic., 261: 108935.

Levy, Y., Bielorai, H., and Shalhevet, J. 1978. Long-term effects of different irrigation regimes on grapefruit tree development and yield. J. Am. Soc. Hortic. Sci., 103: 680-683.

Li, C. P., Qi, Y. P., Zhang, J., Yang, L. T., Wang, D. H., Ye, X., … Chen, L. S. 2017. Magnesium-deficiency-induced alterations of gas exchange, major metabolites and key enzymes differ among roots, and lower and upper leaves of Citrus sinensis seedlings. Tree Physiol., 37: 1564–1581.

Li, J., Li, L., Pang, Z., Kolbasov, V. G., Ehsani, R., Carter, E. W., and Wang, N. 2019. Developing Citrus Huanglongbing (HLB) Management Strategies Based on the Severity of Symptoms in HLB-Endemic Citrus-Producing Regions. Phytopathol., 109: 582-592.

Li, S. L., Li, Z. G., and He, Z. L. 2014. Effect of Enhanced Zinc Nutrition on Mitigation of Huanglongbing (HLB)-affected Citrus. J. Citrus Pathol.,1.

Lin, K. H. 1956. Yellow shoot of citrus. Symptomatology. Investigations in the cause of Huanglongbing. Natural transmission and spread. General conclusions. Acta Phytopathol. Sin, 2: 1-42.

Lovatt, C. J. 1999. Timing citrus and avocado foliar nutrient applications to increase fruit set and size. HortTechnology, 9: 607–612.

Lovatt, C. J. 2013. Properly timing foliar-applied fertilizers increases efficacy: A review and update on timing foliar nutrient applications to citrus and avocado. HortTechnology, 23: 536-541.

Malhotra, S. K. 2016. Water soluble fertilizers in horticultural crops–An appraisal. Indian J. Agric. Science, 86: 1245-56.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. Academic press.

McCauley, A., Jones, C., and Jacobsen, J. 2009. Soil pH and organic matter. Nutr. Manag. Modul., 8: 1-12.

Mead, F.W., Fasulo, T.R. 2017 Asian Citrus Psyllid, *Diaphorina citri Kuwayama* (Insecta: Hemiptera: Psyllidae). EENY-033. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plan., 15: 1409-1416.

Mengel, K., and Kirkby, E. A. 1980. Potassium in crop production. In Advances in agronomy (Vol. 33, pp. 59-110). Academic Press.

Moreira, A. S., Stuchi, E. S., Silva, P. R. B., Bassanezi, R. B., Girardi, E. A., and Laranjeira, F. F. 2019. Could tree density play a role in managing Citrus Huanglongbing epidemics? Trop. Plant Pathol., 44: 268–274.

Morgan, J. B., and Connolly, E. L. 2013. Plant-soil interactions: nutrient uptake. Nature Education Knowledge, 4: 2.

Morgan, K. T., Cushman, K. E., and Sato, S. 2009b. Release mechanisms for slow-and controlled-release fertilizers and strategies for their use in vegetable production. HortTechnology, 19: 10-12.

Morgan, K. T., Kadyampakeni, D. M., Zekri, M., Schumann, A. W., Vashisth, T., and Obreza, T. A. 2018. 2019–2020 Florida Citrus Production Guide: Nutrition Management for Citrus Trees. CMG13. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Morgan, K. T., Rouse, R. E., and Ebel, R. C. 2016. Foliar applications of essential nutrients on growth and yield of 'valencia' sweet orange infected with Huanglongbing. HortScience, 51: 1482–1493.

Morgan, K. T., Wheaton, T. A., Castle, W. S., and Parsons, L. R. 2009a. Response of young and maturing citrus trees grown on a sandy soil to irrigation scheduling, nitrogen fertilizer rate, and nitrogen application method. HortScience, 44: 145-150.

Nasir, M., Khan, A. S., Basra, S. A., and Malik, A. U. 2016. Foliar application of moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow'mandarin. Sci. Hortic., 210: 227-235.

Nava, D. E., Torres, M. L. G., Rodrigues, M. D. L., Bento, J. M. S., and Parra, J. R. P. 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. J. Appl. Entomol., 131: 709-715.

Nwugo, C. C., Lin, H., Duan, Y., and Civerolo, E. L. 2013. The effect of '*Candidatus Liberibacter asiaticus'* infection on the proteomic profiles and nutritional status of pre-symptomatic and symptomatic grapefruit (*Citrus paradisi*) plants. BMC Plant Biol., 13: 59.

Obreza, T. A. and Morgan T. K. 2008. Nutrition of Florida Citrus Trees 2nd edition. SL 253. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Obreza, T. A. 2003. Importance of potassium in a Florida citrus nutrition program. Better Crop., 87: 60–63.

Obreza, T. A., and Rouse, R. E. 1993. Fertilizer Effects on Early Growth and Yield of Hamlin Orange Trees. HortScience, 28: 111-114.

Obreza, T. A., and Sartain, J. B. 2010. Improving nitrogen and phosphorus fertilizer use efficiency for Florida's horticultural crops. HortTechnology, 20: 23-33.

Obreza, T. A., Rouse, R. E., and Sherrod, J. B. 1999. Economics of controlled-release fertilizer use on young citrus trees. J. Prod. Agric., 12: 69–73.

Obreza, T. A., Rouse, R., and Hanlon, E. A. 2006. Advancements with Controlled-Release Fertilizers for Florida Citrus Production: 1996-2006. SL-243. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Philpot, B. 2008. Field Guide to Soil Moisture Sensor Use in Florida, prepared for the St. Johns River Water Management District (SJRWMD) by the Program for Resource Efficient Communities (PREC) at the University of Florida (UF), Gainesville. 10 Jan 2020. <https://floridawaterstar.com/pdfs/SMS_field_guide.pdf>.

Pustika, A. B., Subandiyah, S., Holford, P., Beattie, G. A. C., Iwanami, T., and Masaoka, Y. 2008. Interactions between plant nutrition and symptom expression in mandarin trees infected with the disease Huanglongbing. Australas. Plant Dis. Notes, 3: 112.

Ranade-Malvi, U. 2011. Interaction of micronutrients with major nutrients with special reference to potassium. Karnataka J. Agric. Sci, 24: 106–109.

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., and Prado, F. E. 2009. Soluble sugars-metabolism, sensing and abiotic stress a complex network in the life of plants. Plant Signal. Behav., 4: 388–393.

Rouse, R. E., Ozores-Hampton, M., Roka, F. M., and Roberts, P. 2017. Rehabilitation of Huanglongbing-affected citrus trees using severe pruning and enhanced Foliar nutritional treatments. HortScience, 52: 972–978.

Sartain, J. B. 1978. Adaptability of the double-acid extractant to Florida soils. Proc.- Soil Crop Sci. Soc. Fl.

Satranjoe, J. 2014. Citrus Greening Forces Florida Growers to Trust a Controversial Savior. 10 Jan 2020. <http://news.caloosahatchee.org/docs/Huffington_Post_130830.pdf>.

Schofield, R. K., and Taylor, A. W. 1955. The Measurement of Soil pH 1. Soil Sci. Soc. Am. J.19: 164-167.

Schumann, A. W., Fares, A., Alva, A. K., and Paramasivam, S. 2003. Response of 'Hamlin' orange to fertilizer source, annual rate and irrigated area. Proc. Fla. State Hort. Soc. 116:256-260. 2003.

Schumann, A., Hostler, K., and Waldo, L. 2012. Advanced citrus production systems — grove designs for higher efficiencies. Citrus Ind., (September), 6–9.

Shen, W., Cevallos-Cevallos, J. M., Nunes da Rocha, U., Arevalo, H. A., Stansly, P. A., Roberts, P. D., and van Bruggen, A. H. C. 2013. Relation between plant nutrition, hormones, insecticide applications, bacterial endophytes, and *Candidatus Liberibacter* Ct values in citrus trees infected with Huanglongbing. Eur. J. Plant Pathol., 137: 727–742.

Shin, K., and van Bruggen, A. H. C. 2018. Bradyrhizobium isolated from Huanglongbing (HLB) affected citrus trees reacts positively with primers for *Candidatus Liberibacter asiaticus*. Eur. J. Plant Pathol., 151: 291–306.

Shober, A. L., Wiese C., Denny G. C., and Mylavarapu R. 2018. Soil pH and the Home Landscape or Garden. SL 256. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Singerman, A., and Burani-Arouca, M. 2017. Evolution of Citrus Disease Management Programs and Their Economic Implications: The Case of Florida's Citrus Industry. FE915. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Singerman, A., Burani-Arouca, M., and Futch, S. H. 2018. The profitability of new citrus plantings in Florida in the era of Huanglongbing. HortScience, 53: 1655–1663.

Singerman, A., Lence, S. H., and Useche, P. 2017. Is area-wide pest management useful? The case of citrus greening. Appl. Econ. Perspect P., 39: 609-634.

Spreen, T. H., and Zansler, M. L. 2016. Economic analysis of incentives to plant citrus trees in Florida. HortTechnology, 26: 720–726.

Spreen, T. H., Baldwin, J. P., and Futch, S. H. 2014. An economic assessment of the impact of Huanglongbing on citrus tree plantings in Florida. HortScience, 49: 1052-1055.

Spyke, P., Sherrod, J., and Grosser, J. 2017. Controlled-Release Fertilizer Boosts Health of HLB Trees. Citrus Ind., (April), Part 1.

Stansly, P. A., Arevalo, H. A., Qureshi, J. A., Jones, M. M., Hendricks, K., Roberts, P. D., and Roka, F. M. 2014. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by Huanglongbing. Pest Manag. Sci., 70: 415-426.

Stover E, Castle WS, Spyke P. 2008. The citrus grove of the future and its implications for Huanglongbing management. Proc. Florida State Hortic. Soc., 121:155–159

Stover, E., Inch, S., Richardson, M. L., and Hall, D. G. 2016. Conventional citrus of some scion/rootstock combinations show field tolerance under high Huanglongbing disease pressure. HortScience, 51: 127-132.

Sumner, M. E., Farina, P. M. W., and Hurst, V. J. 1978. Magnesium fixation—a possible cause of negative yield responses to lime applications. Commun. Soil Sci. Plan., 9: 995-1007.

Tang, N., Li, Y., and Chen, L. S. 2012. Magnesium deficiency–induced impairment of photosynthesis in leaves of fruiting Citrus reticulata trees accompanied by up-regulation of antioxidant metabolism to avoid photo-oxidative damage. J. Plant Nutr., 175: 784-793.

Tansey, J. A., Vanaclocha, P., Monzo, C., Jones, M., and Stansly, P. A. 2017. Costs and benefits of insecticide and foliar nutrient applications to Huanglongbing-infected citrus trees. Pest Manag. Sci., 73: 904–916.

Trejo-Pech, C. J., Spreen, T. H., and Zansler, M. L. 2018. Is growing oranges in Florida a good investment? Am. J. Agr. Econ., 100: 625-639.

Truog, E. 1947. Soil reaction influence on availability of plant nutrients. Soil Sci. Soc. Am. J., 1: 305-308.

Tsai, J. H. 2008. Asian Citrus Psyllid *Diaphorina citri Kuwayama* (Hemiptera: Psyllidae). Encycl. Entomol. Springer, Dordrecht, 306-308.

USDA. 2019a. Citrus Fruits 2019 Summary. ISSN, 1948, 9048. 10 Jan 2020 .<https://www.nass.usda.gov/Publications/Todays_Reports/reports/cfrt0819.pdf>.

USDA. 2020. Natural Resources Conservation Service. Web soil survey. 1 Jan 2020. < https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx>

USDA. 2012. United States Standards for Grades of Grapefruit Juice. 10 Jan 2020. < https://www.ams.usda.gov/sites/default/files/media/Canned_Grapefruit_Juice_Standard%5B1%5D.pdf>.

USDA. 2018. Florida citrus statistics 2017-2018. National Agricultural Statistics Service. 20 Jan. 2020. <https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Statistics/2017-18/fcs1718.pdf>.

USDA. 2019b. Citrus October forecast. 2016–2019. USDA, NASS, Florida Field Office. 20 Jan. 2020. <https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Forecast/2019-20/cit1019.pdf>.

Vashisth, T. 2017. Managing the Health and Productivity of HLB-Affected Groves. HS1288. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Vashisth, T., and Grosser, J. 2018. Comparison of Controlled Release Fertilizer (CRF) for Newly Planted Sweet Orange Trees under Huanglongbing Prevalent Conditions. J. Hortic, 5: 2376-0354.

Vincent C., Vashisth T., Zekri M., and Albrecht U. 2019. 2019–2020 Florida Citrus Production Guide: Grove Planning and Establishment. HS1302. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Wardowski, W., Whigham, J., Grierson W., and Soule J. 1995. Quality Tests for Florida Citrus. 20 Jan 2020. <http://irrec.ifas.ufl.edu/postharvest/pdfs/Quality_Tests_for_FL_Citrus-SP_99.pdf>.

Wheaton, T. A., Castle, W. S., Whitney, J. D., and Tucker, D. P. H. 1991. Performance of Citrus Scion Cultivars and Rootstock in a High-density Planting. HortScience, 26: 837–840.

Whitney, J. D., and Wheaton, T. A. 1984. Tree spacing affects citrus fruit distribution and yield12. In proc. Fla. State Hort. Soc 97:44-47.

Wright GC .2000. Irrigating citrus trees. Cooperative extension. University of Arizona. College of Agriculture. AZ1151. 5p.15 Jan 2020. <https://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az1151.pdf>.

Xia, Y., Ouyang, G., Sequeira, R. A., Takeuchi, Y., Baez, I., and Chen, J. 2011. A review of Huanglongbing (citrus greening) management in citrus using nutritional approaches in China. Plant Health Prog., 12: 24.

Yang, G. H., Yang, L. T., Jiang, H. X., Li, Y., Wang, P., and Chen, L. S. 2012. Physiological impacts of magnesium-deficiency in Citrus seedlings: photosynthesis, antioxidant system and carbohydrates. Trees, 26: 1237-1250.

Zaman, Q. U., and Schumann, A. W. 2005. Performance of an ultrasonic tree volume measurement system in commercial citrus groves. Precis. Agric., 6: 467–480.

Zambon, F. T., Kadyampakeni, D. M., and Grosser, J. W. 2019. Ground Application of Overdoses of Manganese Have a Therapeutic Effect on Sweet Orange Trees Infected with *Candidatus Liberibacter asiaticus*. HortScience, 54: 1077–1086.

Zekri, M. 2000. Evaluation of orange trees budded on several rootstocks and planted at high density on flatwoods soil. Proc. Florida State Hortic. Soc., 113: 119-123.

Zekri, M. and Obreza, T. 2003. Micronutrient Deficiencies in Citrus: Iron, Zinc, and Manganese. SL 204. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Zekri, M. and Obreza, T. 2013. Magnesium (Mg) for Citrus Trees. SL380. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Zekri, M., and Koo, R. C. J. 1992. Use of controlled-release fertilizers for young citrus trees. Sci. Hortic., 49: 233–241.

Zhang, M. Q., Guo, Y., Powell, C. A., Doud, M. S., Yang, C. Y., Zhou, H., and Duan, Y. P. 2016. Zinc treatment increases the titre of "*Candidatus Liberibacter asiaticus*" in Huanglongbing-affected citrus plants while affecting the bacterial microbiomes. J. Appl. Microbiol., 120: 1616–1628.

Zhu, D., Juan, W., Liao, S., and Liu, W. (2007). Relationship between plant availability of boron and the physico-chemical properties of boron in soils. In Advances in plant and animal boron nutrition (pp. 345-354). Springer, Dordrecht.

BIOGRAPHICAL SKETCH

Dinesh Phuyal was born in 1992, in Kathmandu, Nepal. He received his bachelor's degree from the Institute of Agriculture and Animal Science (IAAS) at Tribhuvan University, Nepal in Dec 2016. In 2012, he was awarded the Tribhuvan University Merit Admission Scholarship for securing a high score in the admission exam. During his undergraduate degree, he got an opportunity to learn interdisciplinary science of agriculture on projects related to various agronomic and horticultural crop management practices. Dinesh graduated from Tribhuvan University with Distinction and received scholarships during every semester due to his excellent academic performance. After receiving his degree, Dinesh worked as an Agriculture Instructor in Galkot Higher Secondary School, Nepal where he taught courses entomology and vegetable production. He desires to understand new techniques and strategies to produce crops in the face of changing environmental conditions. Dinesh has a dream to become a faculty member at a research education institution in his native Nepal.