MITIGATION OF HUANGLONGBING EFFECTS ON GRAPEFRUIT TREES USING ENHANCED NUTRITIONAL PROGRAMS

By

DIEGO FERNANDO RAMIREZ ACOSTA

A DISSERTATION 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

2016

To my wife and family, who support me and gave me the inspiration to think bigger

ACKNOWLEDGEMENT

I sincerely thank Dr. Jose Chaparro for trusting my abilities and give me the opportunity to start this program, for being an excellent role model not only as a scientist but as person and leader. I express my gratitude to Dr. Brian Boman and Dr. Alan Wright for all their support, for offering me the guidance, the resources and all the support needed to solve the different situations and problems that came out in this project. I also thank Dr. Barret Gruber for his help in the structuring and support in the initial stage of this project. In addition, a want to thank Randy Burton, Jerry Britt, Judy Gersony, Felix Palencia, Oscar Paguada, Cassie Kirkland and Darren Cole, for their valuable help in the development of activities in laboratory and in the field.

I also thank all the members of the Indian River and Education Center, headed by the directors Dr. Pete Stoffella and Dr. Ronald Cave for facilitating the different logistic resources needed to complete this work, as well as for the human warmth during all my stay in the center. I am grateful for all my friends in Fort Pierce and Gainesville for their support and for all the good and bad days.

Finally I thank my dear wife and family for all their love, support, and company during this process, they are the biggest motivation to keep improving myself to become a better person and member of the society.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HLB	Huanglongbing
ENP	Enhanced Nutritional Program
DKP	Dipotassium polyphosphate
KN	Potassium nitrate
KP	Potassium phosphite
C	Calcium nitrate
M	Microelements
GPV	Gross Packed Value
CL	*Candidatus Liberibacter*
ACP	Asian Citrus Psyllid
AAP	Acquisition Access Period
IAP	Inoculation Access Period
SAR	System Acquired Resistance
P_0	Vertical gap fraction
GIMP	GNU Image Manipulation Program
GPV_t	Gross Packed Value per acre
GPV_c	Gross Packed Value per acre (Control)
Cts	Costs

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

MITIGATION OF HUANGLONGBING EFFECTS ON GRAPEFRUIT TREES USING ENHANCED NUTRITIONAL PROGRAMS
By

Diego Fernando Ramirez Acosta

December 2016

Chair: Jose Xavier Chaparro
Cochair: Brian Boman
Major: Horticultural Sciences

The supplemental application of different Enhanced Nutritional Programs (ENPs) was evaluated in two commercial red grapefruit groves with presence of Huanglongbing and receiving granular fertilization and pesticide applications. The ENPs were composed by the combination of five commercial water-soluble components (DKP, potassium nitrate (KN), potassium phosphite (PK), calcium nitrate (C), and microelements (M)). Five combinations were evaluated in experiment 1 and nine in experiment 2, spraying the products between three and four times per year during 2014 and 2016. The canopy growth and volume, concentration of macro and microelements in leaves, fruit production and Gross Packed value (GPV) were established and compared to control trees. Additionally, an economic analysis of implementation was included to determine the cost-benefit relation using ENPs. The results indicated no significant effect of ENPs on canopy growth and density during both seasons. However significant increases in the concentration of Mn, Zn and B in leaves were reported in ENPs receiving microelements. Despite no significant differences were observed in fruit production, treatments with applications of N, K and PK, increased their productivity between 30 and 36% more than control. Changes in fruit size were reported, where the

treatment DKP+KP+M increased significantly the production of large size fruit up to 42% compared to control. The GPV was not significantly different compared to control trees. However, treatments DKP+KP+M and KN+KP obtained average increases greater than 60% compared to control, obtaining positive returns after the deduction of the costs associated to the implementation of these two ENPs in two years.

.

CHAPTER 1
LITERATURE REVIEW

The Panorama of Florida Citrus Production

The production of citrus in Florida has an important position in the social and economic activities in the state. The citrus industry represents an estimated of $8.91 billion impact to the local economy and a contribution of $4.62 billion to the gross domestic product. The citrus industry participates in the creation of at least 75,800 jobs (Hodges et al, 2012a).

Since the arrival of citrus to Florida in 1493 with Christopher Columbus, citrus crops have experienced multiple threats and obstacles to become the most important fruit industry in the state. Difficulties have included environmental events such as freezes in central Florida that resulted in the loss of large citrus acreages, hurricanes like those experienced in 2004 and 2005 that reduced productivity by destroying trees and facilitating the spread of citrus canker. Social factors such as the change in land due to urbanization has impacted the citrus industry as well, reducing the total area dedicated to citrus production (Farnsworth et al, 2014a; Farnsworth et al, 2014b). After the appearance of Huanglongbing (HLB) in 2005, the citrus industry in Florida is experiencing one of the largest tree loss events in its history.

According to data collected by the National Agricultural Statistic Service of the United States Department of Agriculture (NASS, 2016), during the time period from 1995 to 2015, the citrus acreage in Florida peaked during 1996-1997 at 815,100 acres (330,000 ha), representing a production of 13,156 citrus tons. One year later (1997), the production of citrus in Florida reached an estimated of 13,583 boxes, which is the maximum value reported since 1915. Since that time, citrus production has been negatively impacted by the reduction of acreage and the effect of the factors previously mentioned.

Since 1995, citrus area has declined at a rate of 3.1% per year, showing for 2015 a total of 459.100 acres (185,791 ha). Citrus productivity has declined 3.8% per year since 2005 with a production of 5,043 thousand of tons (reduction of 8,113 thousand of tons with season 1995) (NASS, 2016).

The Grapefruit Production in Florida

The grapefruit (*Citrus x paradisi* macfad.) is a hybrid of pummelo (*Citrus grandis)* and sweet orange (*Citrus x sinensis* (L.) Osbeck) and has been cultivated in Florida since 1809, starting with seed that were brought from the Bahamas (Davies and Jackson, 2009)). The establishment of this fruit crop in Florida has been affected in the same way as other citrus cultivars in Florida where instability of economic factors linked to supply and demand have reduced profitability on occasions during the 50's and the 80's where the oversupply resulted in a reduction in prices and acreage. However, new plantings were made during 1960s with the realization that older plantings were aging and started to decline. These new plantings benefited from increased consumer interest due to grapefruit's nutritional and health value, which increased consumer demand and fruit prices making it more profitable for growers. Other events such as the hurricanes during 2004-2005 favored an increase in prices due to the reduction of the supply (Davies and Jackson, 2009).

The production of grapefruit and other citrus fruit is based in the use of vegetative propagation to reduce the juvenility of the tree. The rootstocks used are based on the tolerance to environmental pressures including pests and low temperatures, and the effect on the vigor, health, productivity of the tree and fruit quality (Mossler, 2014).

The use of irrigation is important in grapefruit production due to the lack of a uniformly-distributed precipitation especially during the bloom and fruit set. Indeed, almost 90% of the citrus acreage uses micro-sprinkler systems for under canopy irrigation, and in many cases this system is used to deliver soluble fertilizers to the trees as a part of the fertilization program.

As well as in other citrus varieties, the fertilization in grapefruit is based in the application of the macro and micronutrients that are needed for the plant to grow and develop correctly, maintaining an optimal productivity. There are different fertilization programs, depending on factors related to the grove that includes mainly the age of the trees, the type of soil and nutrient concentrations in it. For example, in mature grapefruit trees (+8 years) it is recommendable the application of 138-180 Kg/ha of N, and the applications of P and K depend upon N concentration, ranging from 0 kg/ha to the same rate of N, according to the concentration of these nutrients in the soil (Obreza and Morgan, 2008). The timing complement the nutrition process, focused in apply the fertilizer in the correct time of the production cycle, maximizing the efficiency in the nutrition process. For citrus production, the spring vegetative growth is very important since is the time during the fruiting wood is created for the next year crop. For that reason, the fertilize application usually start early between February and the flowering stage. The second fertilization point continuous from flowering until May, and the third basic fertilization program occurs between September and October, avoiding the rainy season in summer (Obreza and Morgan, 2008). Different strategies have been used to increase the nutrient efficiency in Florida citrus production. In order to improve plant growth and fruit production, the soils can be adjusted to a pH between 6 and 7 and slow release fertilizers can be used to maintain a constant supply of nutrients to the tree. Other option is the use of fertigation, that as it was aforementioned, uses soluble minerals that are delivered to the plant through the irrigation line, with high efficiency and fast availability to be uptake by the plant. Usually this strategy is used for N and K applications as well as some microelements. The association between the nutritional programs and fertilization is closed and determine factors such as the nutrient uptake efficiency, the yield and other factors related to the water use and pollution processes due to nutrient leaching (Alva et al, 2003).

The most important microelements included in the citrus nutrition program are Mn, Zn, Cu, Fe, and B. Under normal conditions, the application of microelements is recommended only under deficiency symptoms or when deficient levels are found in leaves samples (Obreza and Morgan, 2008). One recommended way to apply these nutrients and accelerate the uptake and response of the plant to this nutritional treatment is through the use of foliar fertilization. (Mossler, 2014; Obreza and Morgan, 2008).

Grapefruit production in Florida is focused primarily on the fresh fruit market, but fruit having cosmetic defects can be used for juice processing. Near 50% of the total fruit produced do not meet the cosmetic requirements and are transported to processor plants where the fruit is washed and squeezed and juice extracted. The fruit selected for the fresh market is washed, waxed and treated with fungicides to prevent post-harvest decay (Mossler, 2014). One characteristic particular in the grapefruit commercialization is that the fresh market fruit price is based on the size of the fruit, where small fruits or excessively large fruits are lower in value in comparison with medium and large fruit sizes.

Huanglongbing Disease

Huanglonbing (HLB) is the most important disease in citrus production around the world and its relevance is directly related to the severity of its effects on tree health and productivity. The disease is caused by Candidatus *Liberibacter* (CL), a gram-negative, fastidious, phloem-limited α-proteobacteria that cannot be cultured at present (Bové, 2006). Three species have been identified that are related with this disease: C. *L* asiaticus, C. *L africanus* and C. *L americanus*, names based upon the possible origin of the species. C. *L asiaticus* (Las) is the species with the largest negative impact on citrus production around the world, since it is present in high production countries such as China, United States and India. (Hilf and Lewis, 2016). Brazil has reported the presence of Las and C.L. *americanus,* and South Africa has reported C. L. *africanus* (Achor et al., 2010; Batool et al, 2007).

The symptoms of this disease include reduction of the canopy in trees affected, increased fruit drop, a decrease in fruit quality and a general reduction in the productivity and vigorousness of the tree (Albrecht and Bowman, 2008). However, these symptoms can appear several months or years after the infection date, hindering the visual detection of the disease (Bové, 2006; Farnsworth et al, 2014b; Gottwald, 2010; Gottwald et al, 2012; Teixeira et al, 2005;). The leaf symptoms associated with the disease are partially or complete asymmetric coloring mottle, secondary leaves with abnormal growth and yellowing, and green corky leaf veins with chlorotic interveinal tissue (Achor et al, 2010; Batool et al, 2007). The symptomatic fruit are reduced in size with malformations and poorly colored rind with reduction in the flavor quality, juice percentage and aborted seeds (Achor et al., 2010; Albrecht and Bowman, 2008; Gottwald, 2010). Not all of the symptoms occur in all trees infected and are not symptoms exclusive to HLB infection. Most of the symptoms could result from the presence and interaction of other environmental or nutritional conditions, and these interactions can mask or disfigure the symptoms expressed by HLB. Example of these interactions are nutritional deficiencies such as Zn, Mn, Fe, other diseases including *Phytophthora*, gummosis, and viruses (Bové, 2006). Considering these arguments as well as the necessity to detect the disease in the early stages, the use of modern technology generates reliable results in the identification of CL, including the use of DNA hybridization and PCR (Batool et al, 2007; Bové, 2006; da Graça et al, 2015).

Economic Impact

Huanglongbing negatively affects citrus production in three different ways, including reducing the lifespan of the tree, diminishing marketable fruit production, and increasing production costs due to the application of control methods to enhance the health of the tree, reduce psyllid populations and bacterial inoculum (Farnsworth et al, 2014b).

According to Hodges et al (2012b), HLB has reduced the production of citrus by 23%, resulting in a decrease in revenues of around 16% and the loss of more than 8,000 jobs. Other studies have reported inflation in the production costs closer to 40% (Irey et al, 2008). By 2012, the Florida citrus industry was comprised of approximately 222,000 ha and more than 60 million trees, of which approximately 18% were infected with HLB (Gottwald et al, 2012). In 2015, it is estimated that approximately 90% of the acres cultivated with citrus and 80% of the trees are affected with HLB in Florida, generating a yield loss of 40% (Singerman and Useche, 2015).

Origins of HLB

As mentioned by Da Graça et al. (2015), the description of the origins of the CL remains inconclusive and controversial. According to the description of the symptoms by farmers and scientists, the appearance of the disease dates back to the late 1800s in China, indicating more than 100 years of infection in the citrus production areas located in that country, following by spread to countries such as India, Pakistan and Iran (Bové, 2006; da Graça et al, 2015; Nwugo et al, 2013,).

In Africa, the disease was reported in the 1920s and was recognized as “greening” and “yellow spot”, while in the western hemisphere the disease was reported in 2004 and 2005 in São Paulo-Brazil and in Florida, United States, which are two of the most important regions in citrus production of the world (Batool et al, 2007; Bové, 2006). After these initial reports, the disease has continued its expansion. In the United States, the disease expanded to neighboring states including Louisiana, Georgia and South Carolina and with time reached other important citrus-producing states such as California, Arizona and Texas. (da Graça et al, 2015; Gottwald, 2010; Hilf and Lewis, 2016). In South America, the disease spread from Brazil to Argentina and Paraguay. Currently, CL has been detected in Central American and Caribbean countries such as Mexico, Belize, Jamaica and Cuba. (Achor et al, 2010; Bové, 2006; da Graça et al, 2015).Other important citrus producing countries such as Australia and Spain, are also at high risk due to the presence of CL or the presence of the vector (da Graça et al, 2015).

The Process of Transmission and Infection of HLB, and Plant Response

Candidatus Liberibacter asiaticus (CLas), which is the pathogen reported in Florida, can infect citrus trees via several avenues. The bacteria can be spread through the use of infected plant material for grafting, but another less common avenue is infection through parasitic organisms such as dodder (*Cuscuta pentagona*), which was demonstrated to allow transmission of the bacteria from citrus to periwinkle and tomato (Hilf and Lewis, 2016; Zhou et al, 2007). However, natural insect vectors include the Psyllid *Diaphorina citri* commonly known as Asian Citrus Psyllid (ACP) which is present in Asia and U.S., and *Trioza eritrae* in Africa (Bové, 2006; Halbert and Manjunath, 2004; Hilf and Lewis, 2016). The ACP was initially described in Taiwan in 1907 and its transmission potential was described in China between 1941 and 1955 by Lin Kung Hsiang (Bové, 2006). In the US, ACP was identified in Florida in 1998, Texas in 2001, and in California in 2008 (Grafton-Cardwell et al, 2013; Hilf and Lewis, 2016).

The symptoms of ACP feeding on leaves include curly and notched leaves, and when ACP populations are high, the new sprouts can be killed. The ACP has a developmental cycle of approximately 11 to 15 days, and the adults can live several months. The capacity of female psyllids to lay around 750 to 800 eggs in a lifetime allows for a rapid buildup of populations (Halbert et al, 2004).

The transmission process of HLB through ACP is composed of two steps, the Acquisition Access Period (AAP), and the Inoculation Access Period (IAP). The AAP is related to the time in which the nymphs and adults that are feeding on infected trees acquire the pathogen, and this time can last between 0.25 to 24 hours, where acquisition efficiencies varies between 13 and 90% in adults (Grafton-Cardwell et al, 2013). The IAP was 67% efficient in adults that originated from nymphs raised on HLB-positive trees. Although one insect can infect a tree, the transmission rate is variable and may depend on different factors such as the number of infected ACP, the distribution of HLB titer, the citrus variety, and the age of the tree (Grafton-Cardwell et al, 2013). Once the pathogen is transmitted and present in the phloem tissue, it obstructs the translocation of assimilates throughout the plant, resulting in accumulation of starch in the leaves, generating a failure in the structure of the leaf tissue and induces chlorosis. The limitation in the translocation of nutrients results in the starvation of the root system and a decline of the tree (Achor et al, 2010). A considerable period of latency between the infection and the appearance of symptoms is observed during this process, suggesting that trees are responding to the disease (Nwugo et al, 2013). The mechanisms used by the tree in this response are still unknown, in part from the inability to culture CL, and the activation of a programmed cell death, which occurs in the foliar tissue affected by HLB. Studies have reported comparisons of gene expression in healthy and infected tissue, demonstrating significant differences in gene expression between healthy and diseased tissues (Albrecht and Bowman, 2008; Bové, 2006; Nwugo et al, 2013; Zhao et al, 2013;).

Nwugo et al (2013) reported that susceptibility of grapefruit to HLB may be related to the inability to express the antioxidants that help to mitigate the effect of CLas. According to this study, the main response of grapefruit to HLB is the reduction in the concentration of nutrients, especially Fe, Zn and Cu, and diminishing the production of proteins related to metabolic activities such as photosynthesis, protein transport, and synthesis of molecules related to energy production. Other observations suggest that the tree increase the concentration of K to maintain the activity of proteins related with the production of starch, and increases the production of low effectiveness pathogen-response compounds that despite the low potential to control HLB, have been used in the development of early CLas detection techniques (Nwugo et al., 2013).

Methods to HLB Control

Since the disease cycle has multiple steps in the transmission and infection processes, the measures taken to ameliorate the damage caused by HLB have multiple targets including the reduction of HLB inoculum, the limitation of disease dispersion, and the mitigation of the negative effects in infected trees. One strategy is based on the production of clean tree material by nurseries using secure insect-proof screen houses, and the reduction of psyllid populations primarily using insecticides. Other strategies include the restriction of access of the psyllid to citrus groves including the establishment of high density groves under screen houses, thermal therapy to reduce the titer of the bacteria, and the application of biological control agents to reduce the population of the psyllid (Arevalo et al, 2009; Farnsworth et al, 2014a; Zhang et al, 2014).

Propagation of Clean Material and Development of Resistant or Tolerant Citrus Trees

The initial measure used to reduce the spread of the disease was the production of clean propagation material to avoid the spread of the pathogen in new groves (Bové, 2006). In Florida, it is now mandatory to use certified pathogen-free budwood and the propagation must be done in certified HLB-free and ACP-proof facilities (Brlansky et al, 2011). Another management measure applied, but later discarded, was the removal of symptomatic trees. It was soon realized that this strategy was not economically sustainable for the industry and that the infection might be present in asymptomatic trees (Farnsworth et al, 2014b). Without doubts, the best long-term strategy to control HLB is the development of resistant varieties with high commercial potential. Among commercial citrus germplasm, 'Temple' tangor demonstrates the highest levels of HLB tolerance (Stover et al, 2015). However, studies indicate that the citrus source with the highest levels of HLB resistance is *Citrus trifoliata* L. These results suggest that sources of genetic resistance are present in cultivated and wild citrus, showing potential for the development of resistant varieties (Stover et al, 2010; Stover et al, 2015).

Reduction of HLB Titer

Techniques to reduce the concentration of CL in citrus trees, including the use of chemical compounds such as antibiotics, have shown some positive results for HLB management. This group of compounds includes Ampicillin, Penicillin, Carbenicillin, and Sulfadimethoxin, which are considered the most effective in Las suppression (Zhang et al, 2014). The use of nano-emulsions to enhance the permeation of substances through leaf cuticles have shown increases in the effectiveness of these chemical compounds as well the application of other foliar substances, such as nutrients (Yang et al, 2015).

Another long term strategy used in the reduction of Las titer is thermotherapy. This method exposes infected trees to high temperatures (between 45° and 48°C) during at least 48 hours. Thermotherapy obtained significant reductions in the titer of the canopy in HLB-trees (Hoffman et al, 2013).

Reduction of ACP

This method is considered the easiest way to control HLB. It is based in the control of the ACP populations through the use of chemical and biological control. The effectiveness of the chemical control has been demonstrated in different publications, with the foliar and soil application of compounds that affect the life cycle of ACP

(Abdullah et al, 2009; Brlansky et al, 2011). The insecticides that have been reported as efficient in ACP reduction include Fenpropathrin, Imidacloprid, Spinetoram as foliar and Aldicarb in the soil, showing significant reductions of the number of adult ACPs in comparison with treatments without the chemical (Arevalo et al, 2009; Brlansky et al, 2011). The other option to control the populations of ACP is the use of *Tamarixia radiata* as a biological control agent of ACP. This exotic parasitoid has been released successfully in Florida to decrease ACP populations and spread, and the parasitism percentage ranged from between 39% and 56% (Qureshi et al, 2009). It is important to recognize that other generalist organisms such as spiders and insects that belong to the orders Diptera, Hymenoptera, Hemiptera are also predators of ACP (Michaud, 2002).

The Use of Enhanced Nutritional Programs

As aforementioned, HLB infection affects the translocation of nutrients affecting their concentration in leaves and roots, as well the productivity and fruit quality (Shen et al, 2013). Different authors have reported deficiencies of different macro and micronutrients including Ca, P, Mg, Fe, Mn, and Zn in HLB-infected trees as well as an increase in the concentrations of potassium in the leaves (Aubert, 1987, Brlansky et al, 2011, Zhao et al, 2013).

Masaoka et al, (2011) reported a decrease in the concentration of Fe and Zn in rough lemon trees infected by HLB by approximately 50% in comparison with healthy trees. A comparison of the microelement concentrations between healthy and HLB-infected flat lemon (*C.reticulata* Blanco cv. Depresa), indicated that all micronutrients except Cu were significantly reduced in infected trees. Pustika et al (2008) investigated the effect of HLB on mandarin nutrition and reported reductions in leaf nutrient concentrations of N, Mg, and Fe of approximately 12, 21 and 42% respectively, compared to healthy trees.

This alteration in the concentration of nutrients not only affects the overall tree productivity but influences the microbiota associated with the rhizosphere and endosphere of the plant (Gu et al, 2013). This microbial community plays a role in the type of response that trees can have against the diseases inducing or not systemic resistance (Musetti et al, 2007). Different studies have demonstrated the potential of certain mineral nutrients in the activation of specific defense mechanisms used against stress situations, so they play an important role in disease control (Razi et al, 2011). Based on that premise, the application of a supplemental source of nutrients that can be delivered directly to the foliar tissue has been proposed as a viable treatment to maintain the productivity and extend the life of citrus trees.

After the appearance of HLB, different alternatives have been developed to improve the nutrition in HLB-infected trees and ameliorate the effects in nutrition by this disease. The alternative includes the development of a Diagnosis and Recommendation Integrated System (DRIS) which gives a citrus nutrition decision support in nutrition based in the ratios of the nutrients, focused in a fertilization based in maintain the rations among elements (Schumann, 2016). In terms of sources and rates, most of them are based in the application of integrated programs that includes granular fertilization, companied by application of macro and micronutrients as foliar. These programs are known as Enhanced Nutritional Programs.

The Enhanced Nutritional Programs (ENPs) are based on the supplemental application of foliar fertilizers, companied by other elements such as fungicides, bactericides and Systemic Acquired Resistance (SAR) inductors, which help in the mitigation of HLB symptoms by supplying the nutrients depleted by the disease, and supporting the recovery of the health and productivity and HLB-infected trees (Stansly and Roka, 2013).

The use of this strategy was initiated by Maury Boyd, a citrus grove owner, who decided to use this cocktail of nutrients and SAR products since 2006, obtaining positive results in the maintenance of marketable fruit production of HLB infected trees (Spann et al, 2011). Since the base of the formulation was empirical, different studies have attempted to corroborate and understand the role of the compounds in HLB control. The program includes elements such as K and B, which are associated with cell wall development, decreasing the susceptibility to pathogens in the plant, as well as the synthesis of proteins, starch and cellulose (Spann and Schumann, 2009). Magnesium is known to be involved in carbohydrate metabolism (Jones and Huber 2007). Microelements such as Mn, Zn, and Mo were added since they were reported as deficient in citrus trees under HLB infection, and additionally, commercial products that include hydrogen dioxide, potassium phosphate, *Bacillus subtilis*, salicylic acid and potassium phosphite, are combined to form a fungicide/bactericide cocktail with the objective of microbial pathogenic population reduction and enhancement of the trees response against the infection.

The Boyd combination has been tested by several researchers, obtaining mostly positive results when it is accompanied by good psyllid control (Shen et al, 2013, Rouse, 2013; Stansly and Roka, 2013; Spann and Schumann, 2012). However, other combinations of macro and microelements different from Boyd's cocktail have also been evaluated obtaining positive results in the recovery of the infected trees. Rouse (2013), evaluated the application of different ENPs based on the addition of a source of N (KNO_3 or urea) and P (P_2O_5) as macroelements, and combined Ca and Mg with microelements, similar to the micronutrients included in Boyd's cocktail. The results indicated an increase in tree productivity between 18 and 53% in comparison to untreated trees. Pustikas (2008) reported a significant recovery of HLB positive mandarin trees after foliar application of macro and microelements (N, K, Ca, Mg, Fe, Mn, Zn, and Cu), showing a reduction between 36 and 40% in the disease severity based on the visual symptoms related with HLB.

It is important to recognize that the application of ENPs does not affect psyllid populations. Gottwald (2012) indicated no significant differences between control and ENPs applications in tree productivity and canopy recovery when ACP was not controlled. Different research indicated the importance of controlling ACP populations in groves under the application of ENPs to maximize the results, limiting the spread of the bacteria and its effect on plant recovery (Rouse et al, 2010; Spann and Schumann, 2012). One example of this recommendation is the study conducted by Stansly and Roka (2013) that reported the highest yields in treatments including pesticide and foliar nutritional applications in comparison with no treatment and pesticide and foliar sprays applied separately.

The Economic Effect of ENP Application

It is known that the application of nutritional programs has an effect on the costs related with the citrus maintenance. Stansly and Roka (2013) affirm that the estimated costs of the application of ENP in addition to psyllid control is about $2230/ha/year in sweet oranges ($690 insecticide + $1540 ENP), suggesting that only prices greater or equal to $2 dollars/lb. solids would provide positive revenues for growers. Economic analysis by Spreen and Baldwin (2014) indicated similar costs with ENPs showing increases between $500 up to $1500 per hectare. However, despite the increase in production costs, economic models that simulate the cost-benefit relation under HLB citrus production indicate that the application of strategies to control the spread of HLB and/or enhance the productivity in HLB-infected trees are more beneficial in the long term (Lopez and Durborow, 2015, Salifu et al, 2014).

Considering all the factors regarding the application of ENPs, further studies that allow the identification of the best components for a better cost-benefit relation based on their efficacy on tree recovery and productivity should be conducted to ensure positive revenues for the growers and the long-term survival of the citrus industry in Florida

CHAPTER 2
ENHANCED NUTRITIONAL PROGRAMS FOR INCREASING MINERAL CONCENTRATIONS AND TREE GROWTH OF HLB-AFFECTED GRAPEFRUIT TREES

Huanglongbing is recognized as the major threat to citrus production around the world (Batool et al, 2007; Bové, 2006), a its damage and infection involve the blocking of the phloem tissue, generating alterations in the translocation of nutrients within the tree, reducing the longevity and productivity of citrus trees (Achor et al, 2010). During the infection, the pathogen causes an initially localized affection, that with no control, the bacteria would colonize the tree using the vascular tissue as the dispersion path, reaching the canopy and the root system, inducing dieback and eventually death of the tree (Achor et al, 2010, Cimò et al, 2013).

It is known that N and K are the most important elements in citrus fertilization, where the productivity of citrus cultivars increase for N and K_2O application rates between 112 to 224 Kg per hectare (Koo, 1979). However, development of the citrus tree also depends on the availability and distribution of these minerals and photo assimilates within the tree. Normally, citrus does not accumulate high levels of starch in the leaves except when this accumulation of starch and other carbohydrates is related with new flush seasons or under Zn-deficiency conditions (Yelenosky and Guy, 1977). Starch is accumulated at high concentrations in the aerial parts of the tree as a consequence of infection by HLB, contrasting with the low or absence of starch in the root system (Etxeberria et al, 2009).

Some alterations in macroelements have been found in citrus trees affected by HLB. Razi et al (2011), collected information from 43 citrus groves in the presence of HLB in Pakistan, reporting that 72% of the HLB-positive trees presented deficiencies in

N, 85% in P, and 60% in K. Even though the deficiencies were not related directly with HLB, the disease can be a factor that contributes to a decrease the nutritional status of the tree and favors the appearance of secondary diseases, making the nutritional program in HLB-infected groves a critical point in the maintenance of the economic activity.

The concentration of micronutrients has been reported as influenced by HLB, where different authors have reported deficiencies in Fe, Mn and Zn, (Koen, 1978, Masaoka et al, 2011, Zhao et al, 2013). However, these deficiencies seem to be variable, depending of the location and cultivar, among other factors. Masaoka et al (2011) reported significant decreases in the concentrations of Zn and Fe in different cultivars under HLB infection (Rough lemon, flat lemon, and Tankan) in comparison with healthy trees. However, HLB infected plants of the cultivar Siem showed significant reduction only in Fe concentrations when planted in pots, showing differences with trees planted in the field, which reported significant reductions only in Zn even when adequate fertilization programs were used in both cases and similar temperature ranges. In the same study, the variety flat lemon reported significant reductions in the concentration of Mn.

The use of foliar sprays has been used in different crops to ameliorate nutrient deficiencies, mitigating iron deficiencies in alkaline soils (Álvarez-Fernández et al, 2004). In citrus, applications of low biuret urea has resulted in significant increases in yield (Lovatt, 1999). Taking this into account, to maintain adequate nutrition and productivity in HLB-infected trees, the use of ENP has generated favorable results in the recovery of the tree and the productivity where different studies using this strategy on citrus have reported levels of productivity similar to those observed before the appearance of the disease (Arevalo et al, 2009, Rouse, 2013, Stansly et al, 2014). Given the positive role of applications of foliar nutrients in the reduction of nutritional disorders in citrus trees, the objective of this study is to evaluate the effects of ENPs on nutrient concentration, and canopy growth and density in grapefruit trees infected with HLB. The hypothesis being tested infers that the use of ENPs increases the concentration of nutrients in the leaves and enhances the tree growth and canopy density in the trees.

Materials and Methods

Experimental Setting

Experiment 1

The first experiment was located in a commercial grove located in Saint Lucie County, Fl. The grove consisted of ‘Flame’ red grapefruit (*Citrus paradisi*) on ‘Swingle’ citrumelo (X. *Citroncirus* spp.) rootstock approximately 27 years old. Trees were set at 5.48m in-row by 7.62m between row spacing on 15.24m double row beds (240 tree/ha). Analysis of HLB titer by real time PCR and psyllid traps were used in the grove to confirm the presence of HLB and ACP. Prior analysis showed an infection rate of 100% of trees in the grove. The soil series in the selected block was described as a Pineda sand containing a surface sand layer of 96.5 cm overlaying 106.7 cm of loamy sand. The trees were treated with supplemental water-soluble nutrients using micro-sprinklers and received insecticide applications according to the UF-IFAS guidelines for ACP management. Additionally, all trees received “grower standard” ground applications of dry, water-soluble granular fertilizer (179 kg N/ha) applied at three specific times (pre-bloom (February-March), post-bloom (April-May), and late summer (September)], according to UF-IFAS guidelines (Obreza and Morgan, 2008). An additional application was made during December 2014.

Supplemental foliar treatments consisted of water-soluble fertilizers that vary with regard to levels of macro and micronutrients. Six different treatments were established (Table 2-1**)** including a control without foliar application. Each treatment had five replications composed of eight to ten trees set in a randomized block design. The amount of each foliar fertilizer was based on the recommended rates: Dipotassium poly phosphate (DKP) companied by low biuret urea (4N-20P-22K) applying 19L/ha, potassium phosphite (0N-30P-27) applied at 2.4 L/ha, and a micronutrient mix containing B (1.2%), Mg (3.8%) Mo (0.01%), Mn (0.5%), Zn (0.5%), Fe (0.2%), S (0.1%) at a rate of 1 19 kg/ha (see Table A-1 for more details). The fertilizer components were dissolved in water and applied by spraying. The trees under each treatment received approximately 8 L of the solution. A total of 10 applications of the ENP were sprayed between March 2014 and Sep 2016 (Mar/14, Jun/14, Sep/14, Dec/14, Feb/15, May/15, Oct/15, Jan/16, Apr/16, and Sep/16).

Experiment 2

The second experiment was conducted at a commercial grapefruit grove in Saint Lucie County as well but planted with ‘Ruby Red’ red grapefruit (*Citrus paradisi*) on Sour Orange (Citrus x *aurantium*) rootstock of approximately 6 years age. In this grove, the presence of ACP and HLB was confirmed by psyllid monitoring and real time PCR analysis on leaves samples. The soil in the selected block is a Riviera sand containing a sand layer 56 cm inches deed above a 56 to 106.7 cm sandy loam horizon. Trees were spaced at 5.6 m in-rows by 7.6 m between row spacing of 15.2 m double-row beds (242 trees/ha). The trees received water-soluble nutrients using micro-sprinklers, granular fertilizers (179 kg N/ha) and insecticide applications according to the UF-IFAS guidelines for ACP management (Obreza et al, 2008).

The ENPs evaluated are the combination of five different fertilizers which were applied according to recommended rates: two commercial products were used as a major source of macroelements: one was DKP, and the other was the combination of KNO_3 (14N-46K) and urea (18N), applying 8kg/ha and 19L/ha respectively. Calcium nitrate (15.5N-19Ca) was included in some treatments and applied directly to the soil at 47 L/ha, and potassium phosphite as well as the microelement component were supplied with the same commercial mixture than the used in experiment 1. Combinations of these components were used to generate nine treatments and a control, with each treatment having five replicates composed of at least 4 trees set in a randomized block design (Table 2-2). The fertilizers were dissolved in water and applied as foliar sprays. The trees received approximately 8.7 L of the solution (2,105 L/ha). A total of seven applications were sprayed between March 2014 and Aug 2015 (Mar/14, Jun/14, Sep/14, Dec/14, Feb/15, May/15, Sep/15, Jan/16, Apr/16, and Sep/16).

Variables Measured

Mineral concentration in leaf tissue and soil

The impact of the fertilization treatments was determined by performing nutrient analysis of the foliage eight times in experiment 1 and seven times in experiment 2 during 2014 and 2016. Approximately 20 randomly selected leaves were used to estimate the concentrations of N, P, K, Ca, Mg, Fe, Cu, Mn, B, and Zn. The leaves were washed to remove contaminants and dried to a constant weight at 105 °F prior to analysis. The methods used for nutrient evaluation are shown in Table 2-3. Soil samples were collected during July 2015 to establish the nutrient concentrations and pH. The sampling method followed the protocol described by Obreza and Morgan (2011), collecting one soil core close to the microsprinkler irrigated area of 20 cm deep per tree. This process was repeated for two to four trees in the same replication and these samples were mixed to create one composite sample. The pH of the soil as well as the concentrations of P, K, Mg, Ca, S, B, Zn, Mn, Fe and Cu were determined using the methods listed in Table A-2.

Tree volume growth rate

Canopy volume was evaluated seven times between 2014 and 2016 using two to four trees per replicate. The measures included the canopy height (CH), and width taken in north/south (N-Sw), and east/west (E-Ww) orientation. These measures were used to determine the average radius (*r*) of the tree:

$$tree\ radius = \left(\frac{A}{2}\right)^3$$

Where A is the average of the three measures obtained (CH, N-Sw and E-Ww). Subsequently, the canopy volume (*V)* was estimated according to the formula:

$$V = \frac{4}{3}\ \pi r^2$$

Once the tree volume was established, the rate of change was calculated through the time in terms of percentage of change between the initial time and the next evaluation.

Leaf area index

The Leaf Area Index measures were evaluated for the seasons 2015 and 2016. For 2015 pictures from below of the canopy were taken using a GoPro® at 0.5 m from the trunk base facing north. The pictures taken were processed using GNU Image Manipulation Program (GIMP). Through this software, the vertical gap fraction (P_0) was established for each image, calculating the proportion of background pixels to the total pixels within the frame of the photo. The LAI was calculated using the formula *LAI= -2 ln (P0)* as proposed by Liu and Pattey (2010).

For 2016, the values were obtained through the use of a ceptometer ACCUPAR LP-80 (Decagon Devices). The device was located below the canopy, facing north. The evaluations were made with high sun intensity (>1000 μmol m^2/s). In both evaluations, 2 to 4 trees from each replication were used to evaluate this variable.

Statistical Analysis

All the variables evaluated were analyzed using an Analysis of Variance (ANOVA) to determine significant differences between treatments. When the differences between treatments were significant ($p < 0.05$), a Tukey HSD test was used to compare the means. The software R was used to carry out the analysis (R, 2014).

Results

Leaf Nutritional Content

In Experiment 1, the concentrations of N, P, and K in the foliar tissue were maintained stable throughout the time of the experiment (Table 2-4), where no significant differences were detected between treatments. For N, the average concentration ranged between 1.83 and 2.74%. The concentrations of P ranged between 0.10 and 0.15% and between 0.50 and 1.39% for K.   Other major nutrient such as Ca and Mg exhibited low variation through time and this variation was not significantly different between ENP treatments. The Ca concentration ranged between 3.32 and 5.74% and Mg between 0.25 and 0.37%, and these levels are considered to be within the normal range.

On the other hand, the micronutrient concentrations varied significantly during the experimental period as well as between the ENP treatments. Only the concentrations of Fe and Cu were not significantly different among treatments with concentrations between 32.9 and 67.2 mg/kg for Fe, whilst for Cu, the concentrations oscillated between 89 and 382 mg/kg, being highly influenced by the application of Cu-containing fungicides used for canker control sprays.

The concentration of the microelements Mn and Zn were highly affected by the application of microelements in the ENP formulations, where the treatments DKP+M and DKP+KP+M obtained the highest concentrations in comparison with other treatments. After one year of application, the Mn concentrations were 362 mg/kg and 252 mg/kg for DKP+M and DKP+KP+M, respectively (Figure 2-1). These significantly higher values represented increases of between 276 and 440% more than the concentration in control trees or treatments without microelements. For Zn, the results were similar, where treatments DKP+M and DKP+KP+M showed significant increases with respect to the control. After one year, the concentration of Zn increased significantly in the aforementioned treatments ranging between 258 and 402% more Zn compared to the control (Figure 2-2). During the second year, the concentrations of Mn and Zn for DKP+M and DKP+KP+M were higher in comparison to other treatments, except for Zn where no significant differences were detected in the last evaluation (Aug 2016) with data oscillating between 37 and 57mg/kg.

The leaf tissue concentrations of B followed a similar pattern to those of Mn and Zn, showing significant increases in treatments receiving foliar-applied microelements (Figure 2-3). The treatments including microelements (DKP+KP+M, DKP+M and KP+M) showed increases ranging between 453% and 711% more B in the first year with respect to other treatments. These differences were significant during the experiment, however, a reduction in these differences was observed in the last evaluation where only KP+M produced significantly higher B than the control.

Within the evaluation dates, (August 2014, August 2015 and July 2016 can be used to compare the concentrations obtained with the deficient, optimal, and excessive ranges proposed by Obreza and Morgan (2011) (Table 2-5). The results obtained with this comparison did not detect deficient levels in any of the elements evaluated during the three sample dates. However, the concentrations of Cu were excessive in all treatments, and high concentrations of B, Mn and Zn were observed for treatments with foliar applications of micros.

Similar to the results obtained for experiment 1, in experiment 2 the macronutrient concentrations were not affected by application of ENPs in comparison to control, since no significant differences were reported among treatments (Table 2-4). In this trial, the concentrations of N-P-K presented were similar to experiment 1, with N concentrations between 2.10 and 2.71%, P concentrations between 0.10 and 0.17% and K concentrations of 0.78 to 1.53%. The concentrations of Ca and Mg were similar to those obtained in experiment 1 with ranges between 3.73 and 4.33% for Ca and 0.17 to 0.22% for Mg. The concentrations of microelements Mn, Zn and B were significantly different among treatments, showing a trend for increased levels in the leaves of trees with the application of ENPs containing micronutrients. These significant increases were detected three months after the first application. For Mn, the treatments KN+K+M and KN+K+M+Ca showed the highest concentration of 182mg/kg in the first year of application, showing increases of 115% with respect to the control (Figure 2-4). After 2.5 years, the differences were maintained with the treatment KN+M having the highest concentration of 103mg/kg. In this evaluation, the use of micronutrients represented increases of between 32 and 176% more Mn than in treatments not receiving this element.

After one year, treatments receiving microelements showed increases from 55% to 500% in Zn and B concentrations with respect to treatments not receiving microelement application (Figure 2-5). Microelement application increased leaf B concentrations 127 and 621% compared to treatments not receiving microelement application (Figure 2-6). However, during the last evaluation, the concentrations of these elements were reduced, with ranges between 27 and 60 mg/kg Zn and 101 and 150 mg/kg B.

Similar to the results obtained in experiment 1, the concentration of elements evaluated showed concentrations above the deficient level during the evaluations made in Aug 2014 and July 2015. However, excessive concentrations of Cu were detected for all treatments evaluated and as well as in experiment 1, and treatments receiving microelements applications, increased the concentration of B, Mn and Zn to high levels.

Soil Nutrient Content

The results of soil analysis made during the season 2015 indicated similar values in most of the parameters evaluated in both experiments (Table 2-5). Since both experimental locations are geographically close, similar soil nutrient characteristics were expected. The soil pH had an average value of 7.56 in experiment 1 and 7.39 in experiment 2. The ENP treatments did not significantly affect the concentration of N, K, Ca, Mg, S, Fe, B, and Cu concentrations in the soil. However in Experiment 1, ENP receiving microelements showed significant increases in the concentration of Zn and Mn. Compared to control. In these treatments, the Mn concentration was in average 67% greater than the concentration in control trees, whilst the concentration of Zn was in average 30% greater in trees with application of micronutrients compared to control. The analysis of the soil for the Experiment 2 did not show significant differences in the concentration of macro and microelements between treatments. When the results are compared to the concentrations recommended by Obreza and Morgan (2008), the concentrations of Ca, Mg, P and Cu were considered to be high in all treatments evaluated.

Tree volume growth rate

The application of ENPs did not affect significantly the canopy volume in any of the evaluations made during 2014 and 2016 for both experiments. In experiment 1 the average of the initial volume of the trees was estimated to be 24.7 m^3, which increased during the first year to 29.5 m^3 (Figure 2-7). During 2015, and after the hedging and topping activity, the initial volume was 29.4 m^3, but during the year the canopy volume decreased, reaching a value of 27.7 m^3 registered in June. During 2016 the canopy volume averaged of 34.5 m^3, indicating a recovery in the canopy. In experiment 2 the initial volume was established in 32.3 m^3 and the last evaluation for 2014 showed an increase in the average volume to 38.5 m^3. After the annual pruning, the average volume for 2015 was slightly reduced to 35.6m^3, but unlike the experiment 1, the tree volume at experiment 2 was stable up to the last evaluation in March 2015. During the evaluation of July 2016, the average tree volume increased to 55.27m^3.

Leaf Area Index

The observations of Leaf Area Index in grapefruit trees during 2015 and 2016, did not reported significant differences under the use of ENPs in any of the experiments. For Experiment 1 the LAI presented an average of 5.75 for 2015 and 3.53 for 2016. (Figure 2-9). Experiment 2 presented average of 3.1 and 4.9 for 2015 and 2016 respectively (Figure 2-10).

Discussion

Leaf and Soil Nutrient Analysis

The deficiencies in nutrients are presumed to be in part to the low mobilization of some microelements, added to the inability to translocate the elements due to obstructions of the sieve elements caused by HLB (Achor et al, 2010). Although no microelement deficiencies were detected, the results indicated significant increases in the concentration of Mn, Zn and B in ENPs receiving microelements. These results agree with the results obtained by Tian et al (2014) that claimed decreases in the concentration of microelements, especially Zn in HLB infected grapefruit leaves. These elements that are reported as deficient under HLB-infection, play important roles in metabolism processes including respiration, chlorophyll production, mobilization of carbohydrates, and activation of enzymes (Zekri and Obreza, 2008). So it was expected that increases in the concentration of these microelements would induce a positive effect in the health of the tree that would be reflected in fruit production. The foliar concentrations were based upon Mn, Zn and B treatments reported by Obreza and Morgan (2011) as high, however no visual symptoms of excess were detected in leaves, suggesting that the concentrations greater than 100mg/kg for Mn, Zn and B in leaves can be tolerated per grapefruit trees affected by HLB.

Regarding the lack of significant differences in the concentration of macroelements especially N and K among treatments in both experiments, suggests that these nutrients were rapidly translocated through the plant. N and K are highly mobile in the plant, so it is expected that increases in these elements should be reflected in fruit production as has been reported in various studies (Boman and Hebb, 1998, Boman, 2001, Boman, 2002).

In experiment 2 where two sources of nitrogen were used, treatments using KNO_3 and microelements showed larger increases in B, Mn and Zn when compared to treatments using DKP and microelements. As mentioned in Table 2-4, the urea used in KNO_3 treatments contains microelements including Mn, Zn and B, which would explain at some point the differences between KN and DKP treatments, however treatments without microelements such as DKP+KP and KN+KP did not show significant differences in the concentration of any mineral suggesting that the use of KNO_3 could maximize the absorption of micros by the leaves.

The results of soil test reflected similarities in the concentrations of nutrients in the soil for all treatments, where in general, the application of ENPs had a low influence. Only in experiment 1, the concentrations of B, Mn, and Zn increase significantly in ENPs receiving microelements compared to the rest of the treatments. A possible cause for this increases would be the runoff material that fell from the canopy to the ground, since the trees in experiment 1 were older, with lower canopy density and volume.

Contrasting with this canopy structure, the trees in experiment 2 had a denser canopy that reached the ground level, presumably reducing the runoff of the ENP solution.

Another variable analyzed in this study was the pH. A study conducted by Shen et al (2013) showed significant reduction in the pH in the soil after the application of ENPs combined with pesticides. Although the soils used in that study were also sandy soils, in the present study no differences were observed between ENP treatments and control. The pH of the solution applied in this study oscillated between 6.3 and 6.8, very similar to the pH observed in the water used to mix the ENP components.

Tree Growth Rate and Leaf Area Index

Symptom development in HLB infected trees is related with a degeneration of the vegetative tissue due to the massive blocking generated by the presence of *C. Liberibacter* in the sieve elements of the phloem. This blocking limits the transport of the nutrients from the leaves to the roots and eventually induces the dieback of the tree, limiting the availability of nutrients absorbed by the roots to the rest of the plant (Achor et al, 2010, Folimonova and Achor, 2010). Under these conditions, canopy growth is highly affected by the disease and is expected that the use of ENPs generates a positive effect in the recovery of the canopy of HLB-infected trees. However in this study, no effect of ENP on canopy growth and density in terms of LAI were detected. The addition of macroelements such as N and K did not affect the volume of the tree in ENP treatments compared to control. As was mentioned earlier in this discussion, the macroelements would play significant effect in productivity, as is reported in Koo (1979) were results indicated that the use of different rates of N and K did not affect the tree size, but had a positive effect on fruit production. Other causes that can limit the canopy growth is the disease severity, which is influenced by different factors, such as the age of the tree, number of infections, time of infection, among others (Gottwald, 2010).

Field observations in both fields, indicated that dead branches presumably are product not only by HLB infection but the presence of many other pathogens (citrus canker, phytophthora, etc.), which can affect the canopy growth in different ways and at the same time, the damage can be affected as well by other factors not considered in this study such as the temperature, precipitation, etc. Despite management practices such as topping and hedging favor the growth of new branches and reduce the HLB inoculum present in infected limbs, many infected branches still infected and this infection could restrict the growth, making difficult observe the impact of the ENP treatments in terms of differences in tree volume and LAI. A study from Rouse (2013) reported that the use of supplemental application of foliar sprays companied by severe pruning, increased significantly the canopy volume, leaf size and fruit production, in comparison with unpruned HLB-affected citrus trees.

Despite the similarities in the LAI values among the treatments, the trend was different in both experiments. For experiment 1 the LAI results suggest a decrease in the LAI in time but for the trees in experiment 2 showed increases, suggesting differences between the groves. However, since the methodologies for LAI in 2015 and 2016 were different, no conclusions can be made in this comparison. Other factor important to determine the canopy size and recovery is the tolerance of the rootstock and scion to HLB, however Folimonova (2009),reported that both rootstocks used, Swingle in experiment 1 and Sour Orange in experiment 2 are considered moderately tolerant. Similarly, the scions, Flame used in experiment 1 and Ruby red in experiment

2 are considered sensitive to HLB infection (Folimonova et al, 2009, Stover et al, 2015), so the differences in the canopy growth and LAI trend in both experiments are not related with different tolerances to HLB.

Table 2-1. Treatments and fertilizers evaluated in experiment 1.

Treatments	(DKP)	(KP)	(M)[1,2]
Control			
DKP+PK+M	✓	✓	✓
PK+M		✓	✓
DKP+PK	✓	✓	
DKP+M	✓		✓
DKP	✓		

The treatments are composed of the addition of the different components. DKP (dipotassium mono and diphosphate), KP (potassium phosphite) and M (Microelements)
[1] During 2014-2015, the composition of micronutrients included B (1.2%), Mg (3.75%), Mo (0.07%), Mn (12.75%), and Zn (4.75%).
[2] For 2016, the composition of micronutrients included B (0.3%), Mo (0.3%), Mn (16.30%), and Zn (15.10%).

Table 2-2. Treatments and fertilizers evaluated in experiment 2.

Treatments	(DKP)	(KN)[3]	(KP)	(M)[1, 2,]	(Ca)
Control					
Ca					✓
KN + K		✓	✓		
DKP + K	✓		✓		
KN + M		✓		✓	
DKP + M	✓			✓	
KN+K+M		✓	✓	✓	
DKP+K+M	✓		✓	✓	
KN+K+M+Ca		✓	✓	✓	✓
K + M			✓	✓	

The treatments are composed of the addition of the different components. DKP (dipotassium mono and diphosphate), KN (potassium nitrate and urea), KP (potassium phosphite), M (Microelements), and Ca (calcium nitrate).
[1] During 2014-2015, the composition of micronutrients included B (1.2%), Mg (3.75%), Mo (0.07%), Mn (12.75%), and Zn (4.75%).
[2] For 2016, the composition of micronutrients included B (0.3%), Mo (0.3%), Mn (16.30%), and Zn (15.10%).
[3] The product used to supply the urea contained B (0.1%), Mg (0.75%), Mo (0.005%), Mn (0.5%), Zn (0.5%), Fe (0.2%) , and S (0.8%).

Table 2-3. Average concentrations of macro and microelements in leaves of grapefruit trees for the seasons 2014-2016

Sampling Date	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn	B
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Experiment 1										
Mar 2014	2.15	0.15	0.50	5.75	0.38	49	361	92	36	99
Jun 2014	2.51	0.12	0.65	4.32	0.30	46	382	68[2]	29[2]	84
Aug 2014	2.74	0.10	0.57	3.85	0.28	39	214	57[2]	34[2]	96[2]
Jan 2015	2.52	0.13	0.90	5.01	0.34	50	238	75[2]	40[2]	161[2]
Feb 2015	2.81	0.15	0.94	4.61	0.32	67	255	162[2]	70[2]	113[2]
May 2015	2.87	0.14	1.40	3.32	0.31	33	89	38	18	128[2]
Aug 2015[1]	1.83	0.10	0.78	3.32	0.25	45	411	136[2]	67[2]	153[2]
Jul 2016[1]	2.64	0.11	0.92	4.08	0.33	61	174	82	47	141[2]
Experiment 2										
Mar 2014	2.10	0.17	1.12	4.16	0.18	82	279	150	64	122
Jun 2014	2.36	0.10	0.92	4.14	0.18	55	491	125[2]	64[2]	113
Aug 2014	2.59	0.11	0.78	3.75	0.17	36	170	104[2]	37[2]	104[2]
Jan 2015	2.51	0.13	1.05	4.33	0.17	44	289	89[2]	37[2]	94[2]
Feb 2015	2.71	0.16	1.43	3.93	0.18	60	312	163[2]	72[2]	85[2]
May 2015	2.65	0.13	1.53	3.73	0.21	40	264	136[2]	89[2]	160[2]
Jul 2016[1]	2.47	0.12	1.31	4.23	0.22	56	89	57	35	204

[1] Results in this dates can be compared with standards proposed by Obreza and Morgan (2008).
[2] Significant differences were found in the element concentration among treatments ($p<0.05$)

Table 2-4. 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; Obreza and Morgan, 2008).

Element	Unit	Deficient	Low	Optimum	High	Excess
N	%	< 2.20	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	%	< 0.20	0.20 - 0.29	0.30 - 0.49	0.50 - 0.70	> 0.70
Na	%	-	-	-	0.15 - 0.25	> 0.25
Mn	mg/kg	< 18	18 - 24	25 - 100	101 - 300	> 300
Zn	mg/kg	< 18	18 - 24	25 - 100	101 - 300	> 300
Cu	mg/kg	< 3	3 - 4	5 - 16	17 - 20	> 20
Fe	mg/kg	< 35	35 - 59	60 - 120	121 - 200	> 200
B	mg/kg	< 20	20 - 35	36 - 100	101 - 200	> 200

Table 2-5. Concentrations of macro and micronutrients (lb/acre) in soil from 2015.

Treatment	pH	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
Experiment 1											
Control	7.74	160	67	166	2,852	3.0	1.0 ab[1]	38.6 b	12.0 b	41.8	89.8
DKP+KP+M	7.36	168	73	147	2,429	5.8	0.9 abc	45.1 ab	16.2 ab	44.4	78.7
KP+M	7.66	187	65	163	2,957	3.8	1.1 a	54.6 a	21.6 a	45.0	88.3
DKP+KP	7.48	148	64	146	2,331	3.4	0.7 bc	31.7 b	9.6 c	40.6	82.6
DKP+M	7.58	209	87	173	3,056	6.8	1.1 a	52.1 a	21.2 a	49.6	88.8
DKP	7.54	160	65	139	2,400	4.0	0.7 c	32.8 b	9.6 c	44.4	74.1
Experiment 2											
Control	7.20	166	44	178	3,309	3.2	0.9	61.7	18.0	20.8	71.7
KP+M	7.50	223	50	188	4,359	15.4	1.4	87.0	25.0	21.0	77.7
Ca	7.48	222	50	199	4,174	7.6	1.2	77.4	22.0	22.6	91.0
KN+KP+M+Ca	7.36	196	40	180	3,578	7.4	1.1	76.8	20.6	18.6	71.7
KN+KP+M	7.28	199	51	191	3,687	5.4	1.1	85.4	28.6	19.6	85.4
DKP+KP+M	7.32	205	57	195	3,670	6.6	1.2	81.4	22.4	20.4	74.4
KN+KP	7.46	206	45	168	3,794	5.2	0.9	72.8	17.0	22.4	82.2
DKP+KP	7.58	225	59	189	4,399	6.6	1.3	70.0	15.6	16.0	71.2
KN+M	7.38	195	55	193	3,453	3.4	1.2	82.67	26.80	20.60	73.31
DKP+M	7.32	244	48	190	3,875	18.40	1.13	85.96	32.80	27.60	87.77

[1] Means with the same letter in the same column are not significant different according to the Tukey test (p>0.05).

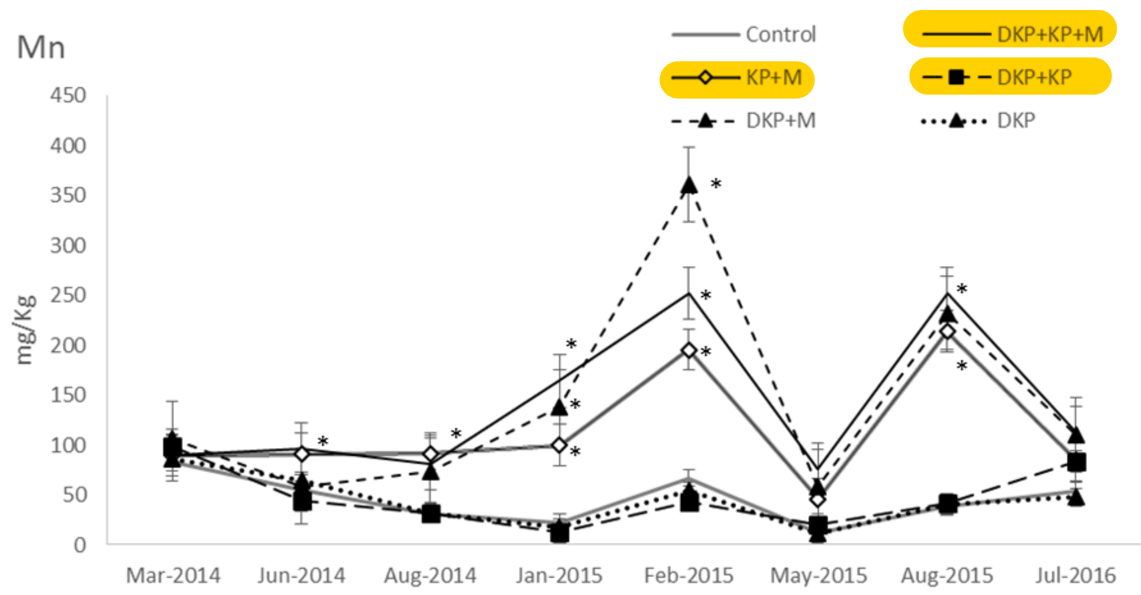

Figure 2-1. Temporal variation in the foliar concentration of Mn in trees treated with different combinations of ENPs in experiment 1. The components are KP (potassium phosphite), DKP (dipotassium polyphosphate + urea) and M (micronutrients). Error bars represent the standard error of the mean. *Treatments showing significant changes in Mn compared to control.

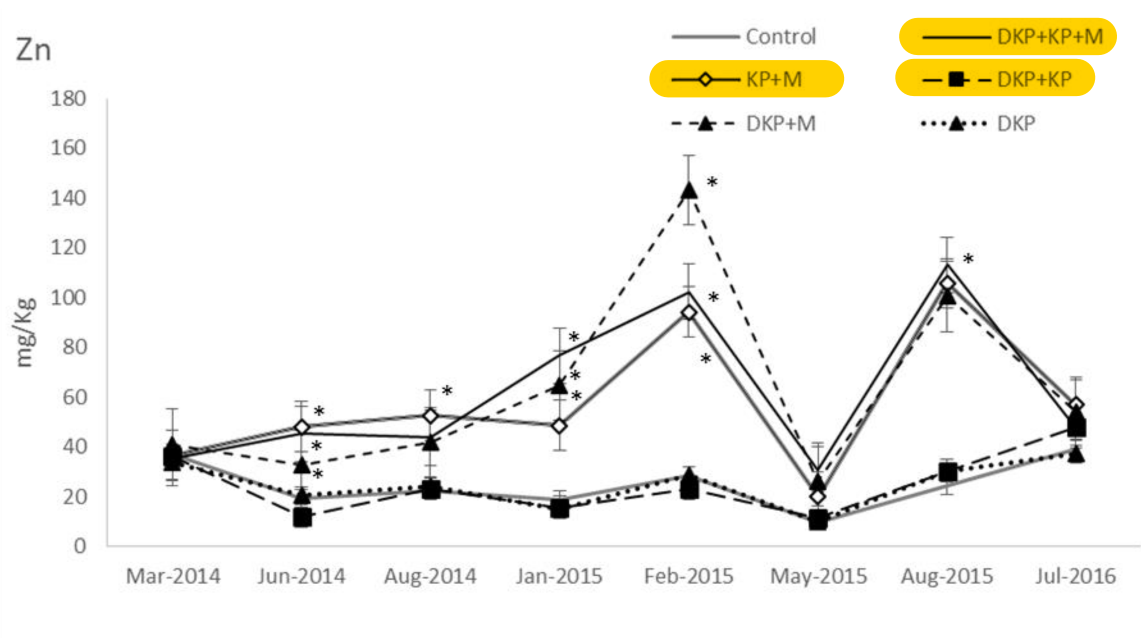

Figure 2-2. Temporal variation in the foliar concentration of Zn in trees treated with different combinations of ENPs in experiment 1. The components are KP (potassium phosphite), DKP (dipotassium polyphosphate + urea) and M (micronutrients). Error bars represent the standard error of the mean. *Treatments showing significant changes in Zn compared to control.

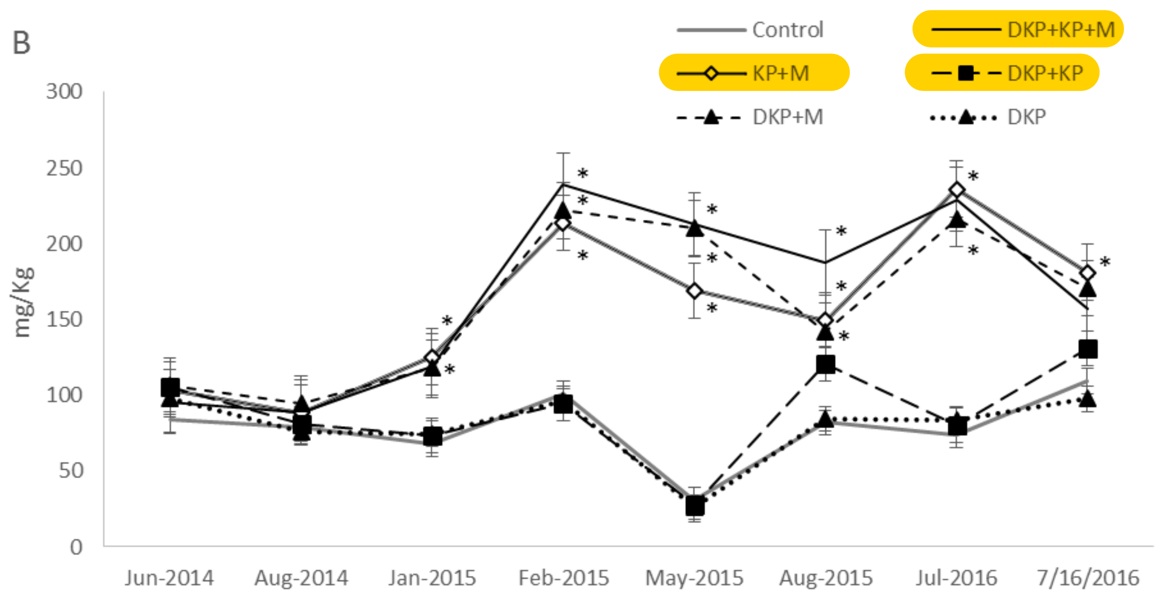

Figure 2-3. Temporal variation in the foliar concentration of B in trees treated with different combinations of ENPs in experiment 1. The components are KP (potassium phosphite), DKP (dipotassium polyphosphate + urea) and M (micronutrients). Error bars represent the standard error of the mean. *Treatments showing significant changes in B compared to control.

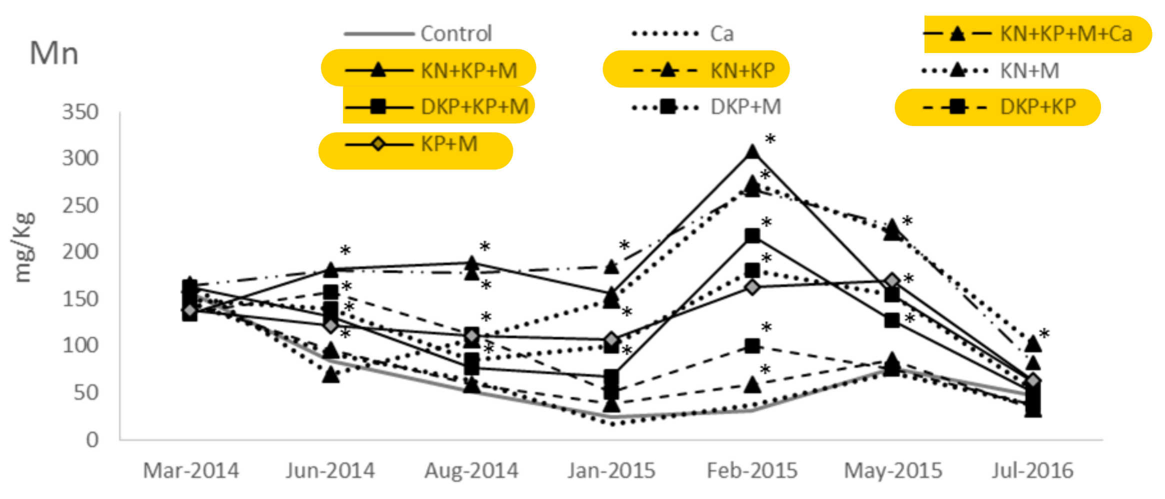

Figure 2-4. Temporal variation in the foliar concentration of Mn in trees treated with different combinations of ENPs in experiment 2. The components are KP (Potassium phosphite), DKP (Dipotassium polyphosphate + urea), KN (Potassium nitrate), Ca (Calcium nitrate) and M (Micronutrients). Error bars represent the standard error of the mean. *Treatments showing significant changes in Mn compared to control.

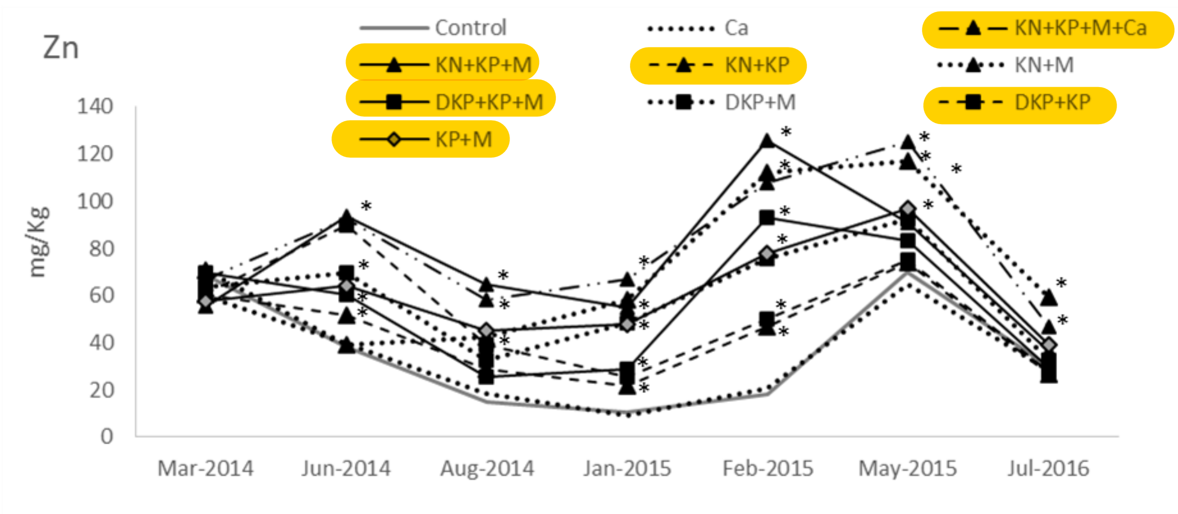

Figure 2-5. Temporal variation in the foliar concentration of Zn in trees treated with different combinations of ENPs in experiment 2. The components are KP (Potassium phosphite), DKP (Dipotassium polyphosphate + urea), KN (Potassium nitrate), Ca (Calcium nitrate) and M (Micronutrients). Error bars represent the standard error of the mean. *Treatments showing significant changes in Zn compared to control.

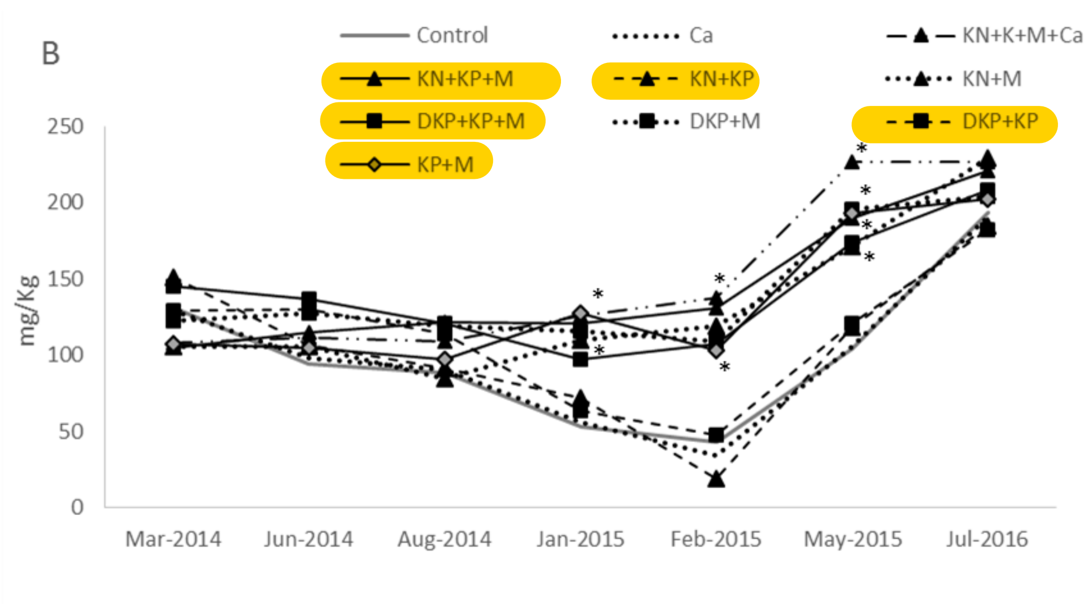

Figure 2 6. Temporal variation in the foliar concentration of B in trees treated with different combinations of ENPs in experiment 2. The components are KP (Potassium phosphite), DKP (Dipotassium polyphosphate + urea), KN (Potassium nitrate), Ca (Calcium nitrate) and M (Micronutrients). Error bars represent the standard error of the mean. *Treatments showing significant changes in B compared to control.

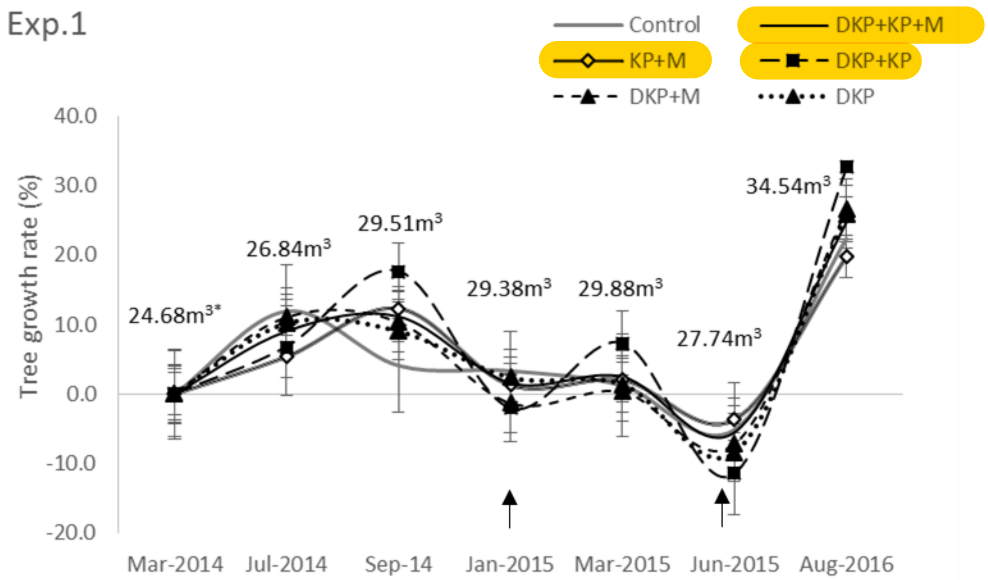

Figure 2-7. Variation in the growth rate along experiment 1. The rate growth was obtained calculating the difference in percentage between the initial canopy volume and the final volume. The values above the means, represent the average canopy volume obtained for the date. No significant differences were found among treatments (p>0.05). Arrows represent management practices related to reduction in the volume of trees (pruning).

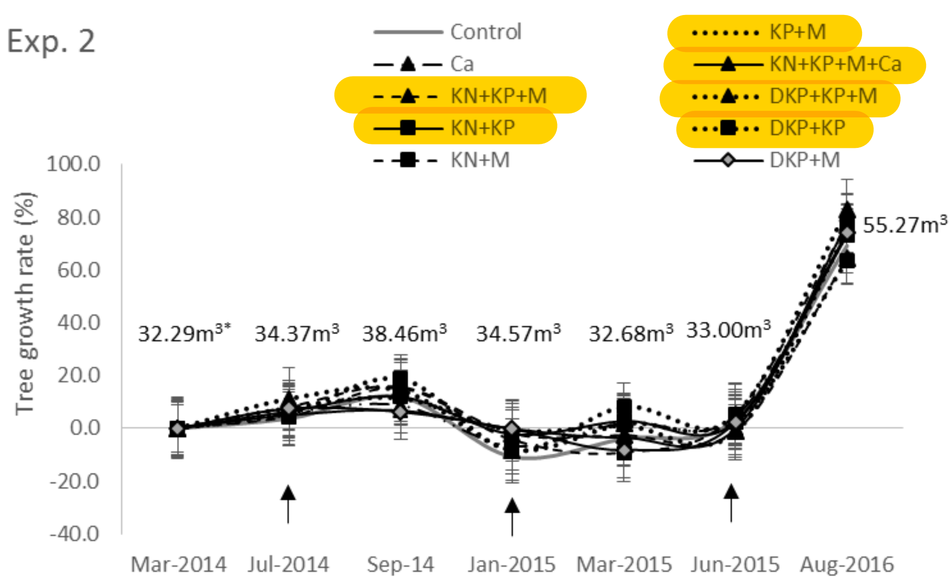

Figure 2-8. Variation in the growth rate along experiment 2. The rate growth was obtained calculating the difference in percentage between the initial canopy volume and the final volume. The values above the means, represent the average canopy volume obtained for the date. In any evaluation, significant differences were found among treatments (p>0.05). Arrows represent management practices related to reduction in the volume of trees (pruning).

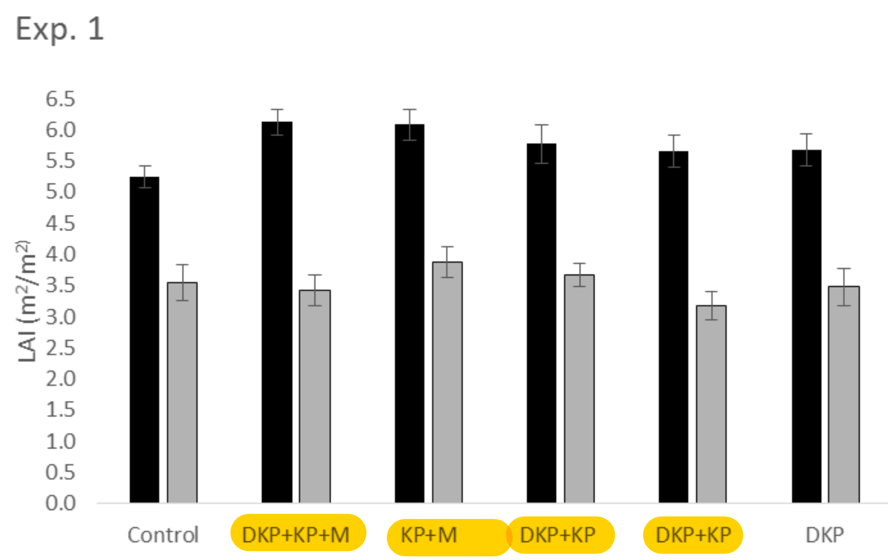

Figure 2-9. Average of leaf area index (LAI) for experiment 1 for the seasons 2015 and 2016. The error bars represent the standard error of the mean.

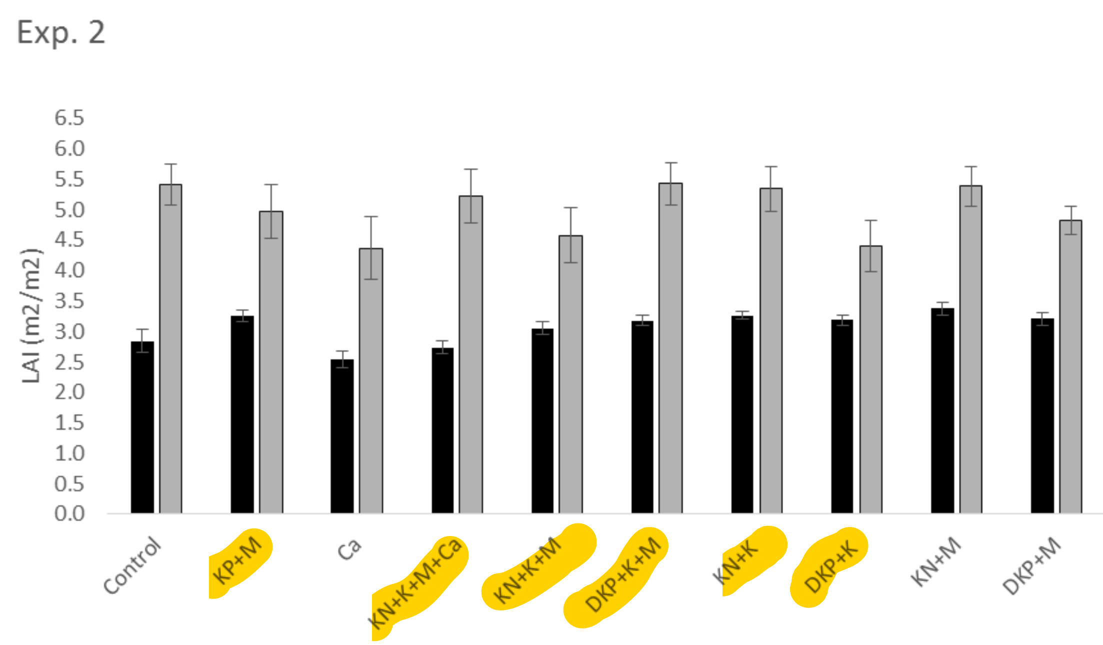

Figure 2-10. Average of leaf area index (LAI) in experiment 1 for the seasons 2015 and 2016. The error bars represent the standard error of the mean.

CHAPTER 3
IMPACT OF SUPPLEMENTAL FOLIAR FERTILIZATION PROGRAMS ON THE PRODUCTIVITY AND PROFITABILITY OF HLB-INFECTED GRAPEFRUIT TREES

Huanglongbing (HLB) disease is considered the most aggressive disease of citrus trees. In Florida. this disease is caused by the phloem-limited bacteria *Candidatus Liberibacter asiaticus* which is transmitted by the vector Diaphorina citri, commonly known as Asian citrus psyllid (ACP) (Bové, 2006). The consequences of this disease include massive fruit drop, alterations in fruit appearance, size and in general, reduction in the productivity and vigorousness of the tree (Albrecht and Bowman, 2008).

Eleven years have passed since discovery of HLB in Florida, and citrus acreage and production has declined 32% and 61%, respectively, since that time. According to Singerman and Useche (2015), about 90% of the acres cultivated with citrus and 80% of the trees are affected with this disease in Florida, generating a yield loss of about 40%. Since its detection in 2005, efforts have been focused on the control of the vector through spraying to reduce the spread of the disease, the development of new citrus varieties tolerant or resistant to the disease, and new strategies to improve the health and productivity of infected trees.

From a nutritional approach, HLB has been associated with nutritional imbalances, mostly deficiencies in macro and microelements including P, Mg, Mn, Fe, and Zn (Arevalo et al, 2009; Masaoka et al, 2011). One of the methods used to improve tree health is correcting these nutritional imbalances through the use of Enhanced Nutritional Programs (ENP). This strategy is based on the supplemental application of foliar fertilizers, usually accompanied by substances that help to ameliorate the infection such as bactericides, fungicides and system acquired resistance inducers (Stansly and

Roka, 2013). The ENPs include nutrients such as potassium, nitrogen, and other microelements, which are related to the synthesis of proteins, cell growth and protection and improvement of productivity and fruit size (Boman, 2001, Spann and Schumann, 2009). Studies evaluating the use of these ENPs have reported promising results in tree recovery and productivity when accompanied by strict control of ACP. Rouse (2013) reported a significant increase in the yield of 'Valencia' orange using the ENPs compared to control treatments. Stansly et al. (2014) also reported significant yield increases in 'Valencia' trees using a combination of pesticides and applications of ENPs, reaching a yield greater than 30% compared to the control. However, not all studies have shown positive results using ENPs. Gotwald et al (2012) did not report positive results in yield and tree health using ENPs in HLB-infected trees in a two-year study. These results suggest that the use of ENP have limitations, and additional studies are needed to understand the factors that affect the productivity of infected trees.

Additionally, costs related to ENP implementation are also limiting factors in the creation of a standard formulation of ENP for citrus trees. Different economic studies have described the costs associated to ENP application. Stansly et al (2014) calculated the ENP costs to be $700/ha per year ($283/acre per year), indicating that the costs of ENPs combined with pesticides application registered the highest yields. In another study, Stansly and Roka (2013) concluded that ENP costs in Valencia trees were $1600 per hectare, reporting positive returns per acre only when fruit prices reached values greater than $3.86/kg. If the program includes the ENP plus insecticides, the prices must be equal or greater than $4.41/kg. Spreen et al. (2014) simulated the future of orange production under the increase of costs related to the application of supplemental nutritional programs, and concluded that under no reduction of these extra costs, low revenues will be expected for growers and a reduction in the number of new plantings in Florida. The objective of this study was to evaluate the economic returns of different ENPs after two years of ENP implementation. The hypothesis proposed indicates that the application of ENPs increases the productivity and therefore economic returns to citrus producers.

Materials and Methods

Two mature commercial grapefruit groves were used to evaluate different ENPs combining compounds that supply macro and microelements. Both experiments were located in Saint Lucie County, Florida and were composed of two different rootstock-scion combinations as well as tree ages.

In Experiment 1 the trees consisted of 'Flame' red grapefruit (*Citrus* x *paradisi*) on 'Swingle' citrumelo (X. *Citroncirus* spp.) rootstock approximately 27 years old. Experiment 2 consisted of 'Ruby Red' red grapefruit (*Citrus x paradisi*) on Sour Orange (Citrus x *aurantium*) rootstock approximately 6 years old. For both experiments, the trees received applications of granular fertilizer, water soluble nutrients, and insecticide applications according to the UF-IFAS guidelines for ACP management (For more details see Chapter 2).

The treatments evaluated in experiment 1 are based on the combination of three different compounds: Di-potassium polyphosphate (DKP) accompanied by urea (4%), Potassium phosphite (KP), and a combination of microelements (M). Five different combinations of these fertilizers were evaluated in addition to a control without ENP application. These components were applied according to product instructions four times in 2014 and three times in 2015. Each treatment was replicated five times and each replication was composed of eight to ten trees (Table 1). Experiment 2 used the nutritional compounds included in experiment 1, in addition to the macroelements potassium nitrate and calcium nitrate, the latter being the only one applied to the soil. This experiment consisted of nine treatments and one control. Each treatment had five replications composed of six to ten trees (see chapter two for more details)

Fruit Drop and Yield

Four trees per replication (20 trees per treatment) were selected to evaluate the fruit yield under normal commercial harvest conditions. Fruit were collected, counted, and measured for size using a portable optical fruit sizing machine. We were able to collect data for experiment 1 during the 2014 season. The Gross Packed Value (GPV) was calculated according to Boman (1995) to establish the relationship between yield and fruit size. The size categories were established in accordance with the each commercial size (small (48 and 56), medium (36 and 40) and large (23, 27, and 32)), and the average Free on Board (FOB) price was obtained from the Market News Bulletin (Florida Citrus Mutual) published on the first day following the harvest. The unmarketable fruit percentage was established by calculating the number of fruit within a category size equal or less than 64, which due to the small size is not considered suitable for the fresh market.

Economic Analysis

An economic analysis was established using the yield data, calculating the costs per application of each ENP treatment in terms of boxes per hectare. Initially, the costs of the materials per hectare used in the ENPs were calculated as well the costs related to the 7 applications of the ENPs during 2014 and 2015 (Table 3-1). The costs per application were established from sale representatives of each component used in the experiment as well as the application cost. In order to know how many boxes per hectare represent the extra costs of using ENPs each year, the average control GPV($/tree) was used and divided per the average number of boxes per tree in control. The product ($/box) was used to compare the production among treatments. Since the costs of the conventional nutritional program as well as the management practices were not considered in this analysis, the control GPV per hectare was taken as the return baseline. This value and the ENP costs represented in boxes were subtracted from all treatments to observe whether the ENP treatments produced greater returns than control. Finally, the value accumulated during 2014 and 2015 was established for each ENP treatment and compared to the control.

Statistical Analysis

All the variables evaluated were analyzed using Analysis of Variance (ANOVA) to determine significant differences between treatments. When the differences between treatments were significant using ANOVA ($p < 0.05$), a Tukey HSD test was used to compare the means. The software R was used to carry out the analysis.

Results

Productivity, fruit size, and GPV

Experiment 1

Few differences were observed between treatments during 2014. The average fruit harvested oscillated between 346 and 383 units per tree, where no significant differences were observed (Table 3-2). The total fruit drop ranged between 1.1 and 1.9%, and the unmarketable fruit percentage ranged between 16% and 18%. For the marketable fruit, between 23.6% and 31.4% were small fruit, where control treatment produced the largest amount of small fruit compared to ENP treatments. The production of medium fruit oscillated between 37.1% and 43.7%, and for large size, between 10.2% and 19.5%. The DKP+KP treatment produced the highest percentage of medium and large fruit, increasing by 11% the production of medium size fruit and 91% the large size compared to the control. Despite the trends in changes in fruit size percentages due to ENPs, no significant differences were detected among the treatments. The number of boxes per tree were not significantly different among treatments either. In this case, the control treatment had the lowest yield with an average of 4.0 boxes per tree, whilst the maximum value occurred for the treatment DKP+KP with 4.8 boxes, showing an increase of 20% more boxes compared to control. The GPV maintained the same trend observed in the yield and in the percentage of large and medium fruit produced. The highest Gross Packed Value was observed for the treatment DKP+KP at $103/tree, an increase of 37% compared to the control ($75/tree). The treatment DKP+KP+M obtained the second highest GPV at $94/tree, and the remaining treatments had values of $85 and $86/tree.

Experiment 2

Similar to the results obtained in experiment 1, no significant differences were observed for the variables evaluated for season 2014 in experiment 2 (Table 3-3). However, the number of fruit varied widely, oscillating between 194 and 314 units per tree. The KN+KP+M treatment presented an average of 194 units per tree, which was the lowest yield compared to other treatments and the control (288 units/tree). The treatment KN+M presented the highest productivity, as the number of boxes per tree responded proportionally to the number of fruit, with a range between 1.8 and 3.4 boxes per tree. The KN+KP+M and KN+M treatments had the lowest and highest yields/ tree, respectively. The percentage of unmarketable fruit ranged between 10.0% and 15.8%, and the percentage fruit drop oscillated between 14.9% and 25.2%, with the control having the lowest percentage of dropped fruit.

Lower variation in fruit size percentages was observed when compared to fruit number. The range of the percentage of small and medium size fruit was 14.5% to 22.8% for small fruit and 26.1% to 35.3% for medium fruit respectively, where the control treatment had the highest percentage in medium size. For large size, the averages ranged between 11.9% and 23.5%, where DKP+M and KP+M treatments had the lowest and highest percentages, respectively. The GPV oscillated between $37 and $75, but the treatment with the highest value was KN+M and the lowest value was KN+KP+M.

The 2nd year of the ENP program in the 2015 season demonstrated greater differences between ENP treatments and the control (Table 3-4). The number of fruit ranged between 282 and 384 units per tree, and the lowest amount of fruit produced occurred for was the control. Although no significant differences were observed between treatments due to high variability, the DKP+KP+M and KN+M treatments tended to show the highest yields, with averages of 381 and 384 fruit per tree, respectively. These values represented an increase of around 35% over the control.

The percentage of unmarketable fruit varied widely among treatments with a range between 17.0% and 21.7%. The DKP+M treatment had the largest percentage of unmarketable fruit, showing a significant increase in comparison with the treatment KP+M, which showed the lowest unmarketable average. The control treatment showed a medium value of unmarketable fruit with 19.9%. Fruit drop percentages ranged from

11.8% to 20.6%, where the control had the highest value and the treatment DKP+KP the lowest, representing a reduction of 42%. The impact of low fruit set, fruit drop, and percent of unmarketable fruit affected the number of boxes per tree, with averages ranging from 3.0 to 4.6 boxes per tree. Once more, the control treatment showed the lowest values, while the DKP+KP+M and KN+KP treatments resulted in increases of between 50% and 53% more boxes per tree, respectively, than the control.

No significant differences were also observed in most of the fruit size analysis. Production of small size fruit ranged between 11.3% and 15.3%, where treatments DKP+M and KN+KP had the highest average and the lowest proportion of small fruit, respectively. Medium fruit size production ranged between 29.3% and 33.7%, where the control and the treatment with application of calcium to the soil, produced the lowest value. The KN+M and DKP+KP treatments increased the percentage of medium fruit between 12% and 15% relative to the control. The production of large fruit varied widely, ranging between 17.4% and 25.0%. The treatment with the highest percentage of large fruit was DKP+KP+M, showing a significant increase (42%) relative to the control.

The variation in the production of small, medium and large fruit affected the GPV calculated for each treatment, resulting in low values for the control treatment with $77 per tree and high values for the treatments DKP+KP+M and KN+KP with $127 and $125 per tree, respectively. However, despite these large differences in GPVs, the analysis of variance did not detect significant differences ($p = 0.08$).

Economic Analysis

Experiment 1.

The calculation of the average price per boxes produced by the control was estimated in $19/box in the season 2014. Taking this into account, the application of

ENPs generated additional costs per acre, oscillating between 31 (KP+M) and 64 boxes per hectare (DKP+KP+M) (Table 3-5). However, these increases in production costs were overcome by increases in productivity, where the results after the deduction of costs were positive, and additional return ranging between 7% and 31% greater than control were observed. The treatment DKP+KP obtained the largest productivity per acre obtaining 297 boxes more per hectare compared to the control.

Experiment 2

During the season 2014, the price per box produced by the control was estimated in $21/box. The results obtained (Table 3-4) indicate that most of the treatments presented reduction in the net production after the costs and control production were subtracted. These reductions oscillated between -5% and -50%, where the treatment KN+KP+M obtained the greatest reduction compared to control. The treatments KN+KP, KN+M and KP+M were the treatments that showed positive values after costs deduction, showing positive increases of 1%, 5%, and 10%, respectively. For the 2015 season, the price per box was established in $26/box and the costs of ENP application were reduced due to the elimination of an ENP application during the year, resulting in values between 18 and 35 boxes per hectare. Although the harvestable yield increased for all treatments (including the control), the value after the deduction of ENP costs were positive for all treatments and exceeded the gross income obtained by the control. These increases in ENPs oscillated between 14% and 61% where treatments DKP+KP+M and KN+KP showed the highest values of 61% and 59% more gross revenues than the control, respectively.

Most ENP treatments evaluated produced positive results for the two seasons (2014-2015), with increases of between 0.5% and 32% more net production than the control. The treatments KN+KP and DKP+KP+M produced the highest and second largest increases, respectively. After two seasons the treatments KN+KP+M+Ca, KN+KP+M and DKP+M still having a negative productivity compared to control.

Discussion

Although no significant differences in productivity were detected among treatments, multiple variables appeared to influence the final GPV obtained for each treatment. In 2015, six treatments increased the value by more than 40% compared to the control, and the treatments DKP+KP+M and KN+KP increased their value 60% more than the control. These results suggest that the application of ENPs improved the response in multiple aspects as it was mentioned in Chapter 2, increasing the concentration of nutrients, inducing canopy growth and density, and increasing the fruit number and size as has been reported in other studies (Stansly et al., 2014, Spann and Schumann, 2012, Rouse, 2013).

The lack of significant differences between ENPs and the control treatment in HLB citrus trees has been reported in other studies. Stansly et al (2014), studied the effect of the applications of ENPs and insecticides in ‘Valencia’ orange, obtaining significant differences in fruit number after only the third year of foliar application, showing a greater number of fruit in trees treated with ENP plus insecticides. Gottwald et al, (2012) also reported the lack of significant differences in ‘Valencia’ oranges during two years evaluating ENP treatments compared to control trees. Another factor that would have an influence on the results is the large variability in the infection level among trees in a grove. For the observations made in experiment 2, multiple diseases were visually identified during the first months of 2014, including citrus canker, melanose and root rot (suggesting the presence of *Phytophthora sp*.). These pathogens would impact the effects of the ENPs during both season and in part could explain the high variability in the results observed. However for 2015 season, the visual symptoms of these pathogens decreased substantially in the grove due to improvements in pest and disease management. On the other hand, Bassanezi et al (2011) studied the yield loss caused by HLB in sweet orange (*Citrus x sinensis* Osbeck) grafted on Rangpur lime (*Citrus x limonia* Osbeck), and reported high variability in yield and number of fruits in symptomless as well as in symptomatic trees with the same level of infection. Similarly, the results had similar relative fruit drop values among treatments, showing no effect of ENPs on the reduction of fruit drop.

These results contrast with those of Roberts et al. (2014) which showed that the application of ENPs reduced fruit drop caused by HLB infection. However, it is important to highlight that the mentioned study started in 2008, and only in 2011 and 2012 was the fruit drop significantly reduced in comparison to the control. The fruit size, which in grapefruit has an important effect on price, was affected by the application of some components included in the ENPs. In experiment 1, the application of DKP (dipotassium phosphate) and KP (potassium phosphite) increased the production of large fruit after one year of application. In experiment 2, applications of the same products obtained similar results, and for 2015, the treatment DKP+KP+M caused significant increases with respect to the control. All these observations suggest that the use of applications of N and K as foliar nutrients increase the production of large fruit. These results support the observations of Boman and Hebb (1998) that increases in grapefruit diameter occur following foliar application of KNO_3, mono potassium phosphate (MKP), and DKP. The treatments DKP+KP+M, KN+M and KN+KP increased fruit number in the 2015 season, suggesting that N and K have a positive effect on fruit production. Boman (1995) reported that increased N is associated with large fruit size and thicker rinds in citrus. Boman (2001) studied the foliar application of KNO_3 and MPK in Valencia orange and observed an increase in fruit production between 24-29%, generating 28% more boxes per tree. These applications increased gross income by 28% more than the control. For tangerines, the application of KNO_3 generated increases of 30% in fruit size, and an average $5 more gross returns per tree compared to the control (Boman, 2002). Additionally, foliar applications of low biuret urea during pre and post bloom in citrus have reportedly increased yields (Lovatt, 1999).

The combined effect of increased fruit number and larger size had a critical effect on GPV in grapefruit. Additionally, it is important to recognize that all treatments that stimulated fruit production or increased fruit size included the component KP (potassium phosphite). This component includes phosphorus as phosphite, and phosphite has been reported to have fungicidal activity, especially on oomycetes including *Pythium*, *Phytophthora*, and *Peronospora* (Silva et al., 2011). Phosphite was evaluated by Atwood and Brlansky (2011) to see if it could prevent the transmission of HLB in grafting budwood, however no effect was observed. This compound could potentially also play a role in limiting secondary diseases after HLB infection, improving tree health and recovery.

Generally, treatments that increased fruit number and size presented the greatest GPV, suggesting that ENPs focused on improving these characteristics can increase the marketable yield even under HLB-infection. The costs associated with the applications ranged between 29 up to 58 boxes per hectare, where the treatments with the highest net productivity during the experiment (DKP+KP+M and KN+KP) costed 47 and 58 boxes per hectare per year, respectively. In monetary terms, these values were less than the costs indicated by Rouse et al (2012) who calculated that application of Boyd's cocktail to be $600. After two years of application, positive net productivity was reported by most of the ENP treatments after the cost related to the application of ENPs. This analysis suggest that foliar applications of nutrients were generating a positive effect on tree productivity and fruit quality, even when no significant differences were observed. In particular, the treatments DKP+KP in experiment 1, and DKP+KP+M and KN+KP in experiment 2, had better response in fruit production and fruit size, positively affecting the GPV. In experiment 2, after two years, DKP+KP+M and KN+KP treatments generated net productivity greater than 380 boxes per hectare compared to productivity under no ENP application, suggesting that the use of these ENPs improve the factors that are highly related to an increase in economic revenues for growers, becoming a good strategy to maintain the viability of the grapefruit industry, despite the enormous negative impact of HLB in Florida.

Table 3-1. Costs of the components used in ENP treatments and their application costs.

Component	Costs		Rate per hectare		Total cost per hectare.
	L	kg	L	kg	
DKP	$3.3	-	19	-	$62.50
Urea (18%N)	$1.76	-	19	-	$33.50
KP	$6.33	-	1	-	$6.01
$CaNO_3$	-	$5.37	-	2.26	$12.21
Microelements	-	$3.19	-	1.1	$3.32
KNO_3	-	$0.53	-	18.0	$9.45
Application costs					$49.00

Table 3-2. Fruit production, unmarketable fruit, drop, fruit size, boxes produced, and Gross Packing Value (GPV) of the treatments for experiment 1 (2014). The treatments were generated by the addition of different components KP (Potassium phosphite), KN (Potassium nitrate), DKP (Dipotassium polyphosphate), M (Micronutrients), and Ca (Calcium nitrate).

Treatment	Fruit (units)			Fruit Size (units)				
	Total	Unmarketable[a]	Drop[b]	Small	Medium	Large	Boxes/tree	GPV
Control	346	60	6	109	136	35	4.0	$75
DKP+KP+M	364	62	4	97	135	67	4.5	$94
KP+M	383	67	6	118	147	46	4.5	$86
DKP+KP	381	45	5	90	166	74	4.8	$103
DKP+M	378	68	7	110	145	48	4.4	$85
DKP	364	58	6	104	147	48	4.3	$85
p-value	0.726	0.205	0.359	0.15	0.4	0.07	0.247	0.161

[a] Unmarketable for fresh market
[b] The data was collected two months before harvest.

Table 3-3. Fruit production, unmarketable fruit, drop percentage, fruit size, boxes produced and Gross Packing Value (GPV) of the treatments for experiment 2 (2014). The treatments were generated by the addition of different components K (Potassium phosphite), KN (Potassium nitrate), DKP (Dipotassium polyphosphate), M (Micronutrients), and Ca (Calcium nitrate).

Treatment	Fruit (units)			Fruit Size (units)				
	Total	Unmarketable[a]	Drop[b]	Small	Medium	Large	Boxes/ tree	PGV
Control	288	33	43	62	102	49	3.2	$66
KP+M	278	28	61	43	81	65	3.1	$73
Ca	283	45	47	65	88	38	2.9	$58
KN+KP+M	194	24	54	34	58	24	1.8	$37
KN+KP+M+Ca	256	35	44	57	87	34	2.6	$53
DKP+KP+M	289	30	72	45	82	60	2.9	$65
KN+KP	303	37	61	55	95	55	3.3	$72
DKP+KP	251	28	63	36	66	58	2.7	$64
KN+M	314	38	53	60	103	60	3.4	$75
DKP+M	255	37	58	53	77	30	2.5	$49
p -value	0.32	0.302	0.185	0.051	0.398	0.264	0.405	0.437

[a] Unmarketable for fresh market
[b] The data was collected two months before harvest

Table 3-4. Fruit production, fruit unmarketable, drop percentage, fruit size, boxes production and Gross Packing Value (GPV) of the treatments evaluated for experiment 2 (2015). The treatments are composed by the addition of different components K (Potassium phosphite), KN (Potassium nitrate), DKP (Dipotassium polyphosphate), M (Micronutrients), and Ca (Calcium nitrate).

Treatment	Fruit (units)				Fruit Size (units)				Boxes/ tree	PGV
	Total	Unmarketable[a]		Drop[b]	Small	Medium	Large			
Control	282	56	abc	58	36	83	49	b	3.0	76.7
KP+M	339	58	bc	50	42	107	82	ab	4.0	109.6
Ca	326	69	abc	59	46	96	56	b	3.6	89.3
KN+KP+M	335	64	abc	56	42	106	68	ab	3.7	98.7
KN+KP+M+Ca	352	61	c	69	45	108	69	ab	3.8	101.2
DKP+KP+M	384	71	abc	51	46	121	96	a	4.6	127.2
KN+KP	371	73	abc	56	42	112	89	ab	4.5	124.8
DKP+KP	367	80	ab	43	51	121	73	ab	4.3	112.9
KN+M	381	71	abc	58	57	128	66	b	4.2	108.2
DKP+M	350	79	a	44	54	112	61	b	4.0	99.5
p-value	0.462	0.002		0.052	0.08	0.316	0.001		0.13	0.08

[a] Unmarketable for fresh market

[b] The data was collected two months before harvest.

Means within a column followed by the same letter (a-b) are not significantly different at $p<0.05$, according to Tukey HSD test.

Table 3-5. Gross returns, in boxes per hectare, obtained in treatments evaluated in experiment 2. The calculations were done taking as baseline the average GPV and boxes/tree in control. $21/box (2014) and $26/box (2015).

Treatment	2014			2015			
	ENP_c	$Prod_t$	Net Prod.	ENP_c	$Prod_t$	Net Prod.	Net Prod. Accumulated 2014-2015
Experiment 2							
Control		759			725		
KP+M	49	889	82	29	1035	281	363
Ca	29	701	-87	18	844	101	14
KN+KP+M+Ca	54	601	-212	33	957	199	-13
KN+KP+M	49	424	-383	29	932	178	-205
DKP+KP+M	58	760	-57	35	1202	442	385
KN+KP	47	812	6	28	1179	426	432
DKP+KP	56	775	-40	34	1067	309	268
KN+M	46	880	75	28	1022	270	345
DKP+M	55	594	-219	33	940	183	-37

ENP_c= ENP cost per year, $Prod_t$= Production per treatment, $Prod_{ct}$=Control production, Net Prod.= $Prod_t$ – (ENP_c + $Prod_{ct}$).

Table 3-6. Gross returns, in boxes per hectare, obtained in treatments evaluated in experiment 1 (2014). The calculations were done taking as baseline the average GPV and boxes/tree in control. $19/box.

Treatment	ENP_c	$Prod_t$	Net Prod.
Control	19	959	
DKP+KP+M	64	1197	175
KP+M	31	1105	116
DKP+KP	62	1317	297
DKP+M	61	1086	66
DKP	59	1085	68

ENP_c= ENP cost per year, $Prod_t$= Production per treatment, $Prod_{ct}$=Control production, Net Prod.= $Prod_t$ – (ENP_c + $Prod_{ct}$).

CHAPTER 4
CONCLUSIONS

The general objective of this investigation was to evaluate the effect of different ENPs on growth and fruit yield of HLB- infected citrus trees. This objective was focused on the monitoring of canopy volume, leaf nutrient concentrations, productivity and economic viability of each ENP using two commercial grapefruit groves with a confirmed presence of HLB. After two years of evaluation, the results indicated important increases in the number of fruit per tree, fruit size, and increases in the gross incomes using most of the ENPs evaluated, in comparison to no ENP control. However, despite these positives results for most variables evaluated, significant differences were not observed due to high variability in the field. These lack of significant differences result from heterogeneity in the health of the trees (even under the same management) and different levels of infection and age of trees within the respective two groves. These differences generated large variation in the productivity of trees under each treatment. Also, it is important to point out that other researchers evaluating the use in the use of ENPs in citrus reported significant differences in different productivity variables only after the third year of evaluation. This relative long-time response can obey to the metabolism in mature citrus trees, where typically express changes in fertilization at least one year after the program was applied. Additionally all the trees received the same granular and fertigation program, where the foliar applications represented a small percentage compared to the granular and fertigation schedule.

The observation of the performance of the different treatments evaluated suggest not all the components are effecting in the same way the citrus trees. Most treatments containing KP produced positive results in large fruit production, and the four most productive ENPs in terms of number of fruit and GPV contained a source of macroelements (KN or DKP) and KP. On the other hand, No observable tree response was detected using ground applications of calcium nitrate. In none of the variables evaluated, this component produced noticeable changes or differences compared to control. On the other hand, the applications of micronutrients increase significantly the concentration of Mn, B and Zn in the leaves, but the effect of these increases was not reflected in more productivity or canopy growth during the experiment. However, since these elements are related to chlorophyll production, mobilization of carbohydrates and activation of enzymes, in a long term these nutrients can help in the recovery of the tree and increasing the yield, as well as the reduction of fruit drop.

The results obtained indicates that the foliar applications of macro and microelements in the foliar tissue can ameliorate the effects by HLB in citrus trees. Into the components evaluated the most important were the KP associated to a macronutrient source (DKP or KN), which favor in most of the cases the productivity in the trees. In terms of LAI and canopy growth, the ENPs produced minimal detectable effects. The canopy growth responds mainly to the irrigation program and not to nutritional applications. However, other studies using ENPs for orange trees, detected significant increases in canopy volume when the programs were accompanied by selective pruning to decrease the pathogen inoculum and promote the production of new leaves. The use of this complementary activity would be expected to generate a significant effect of ENPs in HLB-grapefruit trees.

Since the commercial grapefruit production in Florida is primarily focused on the fresh market, where the price per box is related to the size of the fruit. The recommended ENPs should be focused in high production of large size fruit that generates higher gross incomes to the producer. Taking this into account, the most prominent treatments in terms of fruit production were DKP+KP+M. This treatment reported outstanding results in variables such as boxes per tree, GPV, and large fruit production in both experiments. Additionally, DKP+KP+M produced significantly more large fruit than control, and one of the lowest amounts of unmarketable and dropped fruit, becoming the treatment with the best GPV after two seasons. These results indicate that the application of this ENP is not only economically viable, but that its application can increase the profitability of grapefruit production.

In order to obtain more precise data about the effect of ENPs and promote the observation of statistical differences between ENPs, especially for DKP+KP+M compared to the control, it is recommended to extend the evaluation at least one more year and utilize more field or grove replication. Further studies regarding differences between DKP and KNO_3 should be addressed to understand the associations between these compounds with microelements and potassium phosphite.

APPENDIX
ADDITIONAL TABLES

Table A-1. Composition of the fertilizer products used in experiments.

Component	N	P	K	Ca	B	Mg	Mo	Mn	Zn	Fe	S	Rate /ha	Observations
	Composition (%)												
KP	0	30	27	-	-	-	-	-	-	-	-	1L	Potassium phosphite.
DKP	4	20	22	-	-	-	-	-	-	-	-	19L	Low Biuret urea and potassium poly phosphate.
Urea (KN component)	18	-	-	-	0.1	1	0	0.5	0.5	0.2	1	19L	Low Biuret urea.
$CaNO_3$	16	-	-	19	-	-	-	-	-	-	-	19L	
Potassium Nitrate	14	-	46	-	-	-	-	-	-	-	-	8kg	
Microelements 1	-	-	-	-	1.2	4	0.1	13	4.2	-	-	19kg	Product used during 2014-2015 seasons.
Microelements 2	-	-	-	-	0.3	-	0.3	16	15	-	-	1kg	Product used during 2016 season.

Table A-2. Methods used in the analysis of leaves and soil samples.

	Analysis	Method	Sample size
Leaf tissue	Total N	LECO- Nitrogen Gas analyzer	0.5 g
	P, K, Ca, Mg, S, B, Zn, Mn, Fe, and Cu	ICAP - Open vessel wet digestion Digi Block 3000	5.0 g
Soil Analysis	M1 soil	Double Acid extraction/ ICAP for elements except sulfur	5 g
	pH	1:1 soil water read with a Hydrogen probe	25 g

LIST OF REFERENCES

Abdullah, T., H. Shokrollah, K. Sijam and S. Abdullah. 2009. Control of Huanglongbing (HLB) disease with reference to its occurrence in Malaysia. Afri. J. of Biotechnol. 8:17.

Achor, D., E. Etxeberria, N. Wang, S. Folimonova, K. Chung and L. Albrigo. 2010. Sequence of anatomical symptom observations in citrus affected with huanglongbing disease. Plant Pathol. J 9 (12): 56-40.

Albrecht, U. and K.D. Bowman. 2008. Gene expression in Citrus sinensis (L.) Osbeck following infection with the bacterial pathogen Candidatus Liberibacter asiaticus causing Huanglongbing in Florida. Plant. Sci. 175: 291-306.

Alva, A.K, S. Paramasivam, W.D. Graham, and T.A. Wheaton. 2003. Best nitrogen and irrigation management practices for citrus production in sandy soils. Water Air Soil Pollut.143:139–154.

Álvarez-Fernández, A., P. García-Laviña, C. Fidalgo, J. Abadía and A. Abadía. 2004. Foliar fertilization to control iron chlorosis in pear (Pyrus communis L.) trees. Plant and Soil 263: 5-15.

Arevalo, H.A., P.A. Stansly and R.E. Rouse. 2009. Preliminary effects of insecticidal control of Asian citrus psyllid and combinations of nutrients and systemic acquired resistance elicitors on incidence of greening disease in citrus. Resist. Pest Mgt. Nwsl. 18: 14-17.

Atwood, R., R.H. Brlansky. 2009. A Trial to Determine if Salicylic Acid and/or K-phite on Young Citrus Trees Grafted with HLB-infected Budwood Could Prevent Transmission of the Bacteria. Proc. Fla. St. Hort. Soc. 122:39.

Aubert, B. 1987. Epidemiological aspects of the greening (Huanglongbing) disease in Asia. Workshop on Citrus Greening Huanglongbing Disease, Fuzhou-China, (Review and Abstract): 5.

Bassanezi, R.B., L.H. Montesino, M.C.G. Gasparoto, A. Bergamin Filho and L. Amorim. 2011. Yield loss caused by huanglongbing in different sweet orange cultivars in São Paulo, Brazil. Eur. J. of plant pathol. 130: 577-586.

Batool, A., Y. Iftikhar, S.M. Mughal, M.M. Khan, M.J. Jaskani, M. Abbas, et al. 2007. Citrus Greening Disease–A major cause of citrus decline in the world–A Review. Hort. Sci. (Prague) 34: 159-166.

Boman, B.J. 1995. Effects of fertigation and potash source on grapefruit size and yield. Dahlia Greidinger International Symposium on Fertigation, Technion, Haifa.

Boman, B.J. 2001. Foliar nutrient sprays influence yield and size of 'Valencia'orange. Proc. Fla. St. Hort. Soc. 114:83-88.

Boman, B.J. 2002. KNO3 foliar application to ‘Sunburst’tangerine. Proc. Fla St. Hort. Soc. 115:6-9.

Boman, B.J. and J.W. Hebb. 1998. Post bloom and summer foliar K effects on grapefruit size. Proc Fla. St Hort. Soc. 111:128-134.

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

Brlansky, R.H., M.M. Dewdney and M.E. Rogers. 2011. Florida Citrus Pest Management Guide: Huanglongbing (Citrus Greening) (PP 225). Gainesville: University of Florida Institute of Food and Agricultural Sciences. Retrieved July 25, 2016, from www.imok.ufl.edu/hlb/database/pdf/00002825.pdf.

Cimò, G., R.L. Bianco, P. Gonzalez, W. Bandaranayake, E. Etxeberria and J.P. Syvertsen. 2013. Carbohydrate and nutritional responses to stem girdling and drought stress with respect to understanding symptoms of Huanglongbing in citrus. Hort. Sci. 48: 920-928.

da Graça, J.V., G.W. Douhan, S.E. Halbert, M.L. Keremane, R.F. Lee, G. Vidalakis, et al. 2015. Huanglongbing: An overview of a complex pathosystem ravaging the world's citrus. J. of integr. Plant biol.

Davies, F.S. and L.K. Jackson. 2009. Citrus Growing in Florida. 5th ed. University Press of Florida.

Farnsworth, D., K. Grogan, A. van Bruggen and C. Moss. 2014. The potential economic cost and response to greening in Florida citrus. Choices 29:3.

Folimonova, S.Y. and D.S. Achor. 2010. Early Events of Citrus Greening (Huanglongbing) Disease Development at the Ultrastructural Level. Phytopath. 100: 949-958.

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

Gottwald, T.R. 2010. Epidemiological Understanding of Citrus Huanglongbing. Annu. Rev. of phytopathol. 48:119-139. Ann. Rev. of Phytopath.

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

Grafton-Cardwell, E.E., L.L. Stelinski and P.A. Stansly. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Ann. Rev. of Entomol. 58: 413-432.

Gu, G., J.M. Cevallos-Cevallos, G.E. Vallad and A.H.C. van Bruggen. 2013. Organically managed soils reduce internal colonization of tomato plants by Salmonella enterica serovar Typhimurium. Phytopath. 103: 381-388.

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

Hilf, M.E. and R.S. Lewis. 2016. Transmission and Propagation of ‘Candidatus Liberibacter asiaticus’ by Grafting with Individual Citrus Leaves. Phytopath. 106: 452-458.

Hodges, A., D. Mulkey, R. Muraro and T. Spreen. 2012. County Property Values and Tax Impacts of Florida's Citrus Industry. (FE 437). Gainesville: University of Florida Institute of Food and Agricultural Sciences. Retrieved Aug 25, 2016, from http://edis.ifas.ufl.edu/fe437.

Hoffman, M.T., M.S. Doud, L. Williams, M.-Q. Zhang, F. Ding, E. Stover, et al. 2013. Heat treatment eliminates' Candidatus Liberibacter asiaticus' from infected citrus trees under controlled conditions. Phytopath. 103: 15-22.

2008. Economic impact of managing Huanglongbing in groves at Southern Gardens Citrus. I Taller Internacional sobre Huanglongbing de los cítricos (Candidatus Liberibacterspp) y el psílido asiático de los cítricos (Diaphorina citri), Hermosillo, Sonora Mexico. 2008.

Koen, T.J. and W. Langenegger. 1978. Effect of greening virus on the macroelement content of citrus leaves. Farm. In S. Africa 392:1.

Koo, R.C.J. 1979. The Influence of N, K, and irrigation on tree size and fruit production of 'Valencia Orange'. In Proc. Fla. St. Hort. Soc. 92:10-13.

Liu, J. and E. Pattey. 2010. Retrieval of leaf area index from top-of-canopy digital photography over agricultural crops. Agri. and For. Meteorol. 150: 1485-1490.

Lopez, J.A. and S.L. Durborow. 2015. Huanglongbing and the California Citrus Industry: A Cost Comparison of Do Nothing vs. Do Something Management Practices. Texas J. of Agri. and Nat. Resources 27: 51-68.

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

Masaoka, Y., A. Pustika, S. Subandiyah, A. Okada, E. Hanundin, B. Purwanto, et al. 2011. Lower concentrations of microelements in leaves of citrus infected with 'Candidatus Liberibacter asiaticus'. Japan Agri. Res. Qrtly. 45: 269-275.

Michaud, J.P. 2002. Biological control of Asian citrus psyllid, Diaphorina citri (Hemiptera: Psyllidae) in Florida: a preliminary report. Entomol. News 113: 216-216.

Mossler, M.A. 2014. Florida Crop/Pest Management Profiles: Citrus (Oranges/Grapefruit) (CIR 1241). Gainesville: University of Florida Institute of Food and Agricultural Sciences. Retrieved July 29, 2016, from https://edis.ifas.ufl.edu/pdffiles/PI/PI03600.pdf.

Musetti, R., R. Polizzotto, S. Grisan, M. Martini, S. Borselli, L. Carraro, et al. 2007. Effects induced by fungal endophytes in Catharanthus roseus tissues infected by phytoplasmas. Bul. of Insect. 60: 293.

NASS, N.A.S.S.N. 2016. Florida Citrus Statistics 2014-2015. In: U. S. D. o. Agriculture, editor retrieved on October 10 2016 from https://www.nass.usda.gov/ Statistics _by_State/Florida/Publications/Citrus/Citrus_Statistics/2014-15/fcs1415.pdf.

Nwugo, C.C., H. Lin, Y. Duan and E.L. Civerolo. 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. and K. Morgan. 2011. Nutrition of Florida Citrus Trees. 2 ed. University of Florida - IFAS, Gainesville Florida.

Obreza, T. and T.K. Morgan. 2008. Nutrition of Florida Citrus Trees, Second Edition. . University of Florida, Gainesville, Florida

Qureshi, J.A., M.E. Rogers, D.G. Hall and P.A. Stansly. 2009. Incidence of invasive Diaphorina citri (Hemiptera: Psyllidae) and its introduced parasitoid Tamarixia radiata (Hymenoptera: Eulophidae) in Florida citrus. J. of Econom. Entomol. 102: 247-256.

R, C.T. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing Viena, Austria. http://www.R-project.org/.

Razi, M.F., I.A. Khan and M.J. Jaskani. 2011. Citrus plant nutritional profile in relation to Huanglongbing prevalence in Pakistan. Pakistan J. of Agri. Sci. 48: 299-304.

Roberts, P.D., R.E. Rouse, S.S. Teems, R.E. Sytsma and Z. Shobert. 2014. The effect of nutritional spray programs applied to mitigate symptoms of Huanglongbing on fruit drop caused by HLB and citrus canker on ‘Hamlin’ orange trees. J. of Citrus Pathol. 1.

Rouse, B. 2013. Rehabilitation of HLB Infected Citrus Trees using Severe Pruning and Nutritional Sprays. Proc. Fla. State. Hort. Soc. p. 51-54.

Rouse, B., Irey, M., Gast, T., Boyd, M. and Willis, T., 2014, December. Fruit Production in a Southwest Florida Citrus Grove Using the Boyd Nutrient/SAR Foliar Spray. In Proc. Fla. State Hort. Soc. Vol. 125: 61-64.

Rouse, B., P. Roberts, I. Irey, M. Boyd and T. Willis. 2010. Monitoring Trees Infected with Huanglongbing in a Commercial Grove Receiving Nutritional/SAR Foliar Sprays in Southwest Florida. Proc. Fla. State. Hort. Soc.118-120.

Salifu, A.W., Grogan, K.A., Spreen, T.H. and Roka, F.M., 2012, February. Economic analysis of strategies to combat HLB in Florida citrus. In South. Agri. Econ. Assn. Ann. Mtg. 4-7.

Schumann, A. 2016. Getting started with the Diagnosis and Recommendation Integrated System (DRIS) for citrus nutrition decision support. UF/IFAS CREC in Citrus Nutrition Day, October 2016 Lake Alfred Florida.

Shen, W., J.M. Cevallos-Cevallos, U.N. Da Rocha, H.A. Arevalo, P.A. Stansly, P.D. Roberts, et al. 2013. Relation between plant nutrition, hormones, insecticide applications, bacterial endophytes, and Candidatus Liberibacter Ct values in citrus trees infected with Huanglongbing. Europ. J. of plant pathol. 137: 727-742.

Silva, O.C., H.A.A. Santos, M. Dalla Pria and L.L. May-De Mio. 2011. Potassium phosphite for control of downy mildew of soybean. Crop Protection. 30: 598-604.

Singerman, A. and P. Useche. 2015. Impact of citrus greening on citrus operations in Florida. UF IFAS, Gainesville.

Spann, T. and A. Schumann. 2009. The role of plant nutrients in disease development with emphasis on citrus and Huanglongbing. Proc. Fla. State. Hort. Soc. p. 169-171.

Spann, T. and A. Schumann. 2012. Using good horticultural practices to maintain yield of HLB-affected groves. Citrus Ind. 93:6-11

Spann, T.M., A.W. Schumann, B. Rouse and B. Ebel. 2011. Foliar nutrition for HLB. Citrus Ind. 92: 6-10.

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

Stansly, P. and F. Roka. 2013. Economies of HLB vector control and foliar nutrition. Citrus Ind. p. 6-9.

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

2010. Breeding citrus for HLB resistance at the USDA/ARS US Horticultural Research Laboratory, Ft. Pierce, Florida. 2010.

Stover, E., G.T. McCollum, R. Driggers, R. Lee, R. Shatters Jr, Y. Duan, et al. 2015. Resistance and tolerance to Huanglongbing in citrus. Acta Hortic. 1065: 899-903.

Teixeira, D.d.C., C. Saillard, S. Eveillard, J.L. Danet, P.I.d. Costa, A.J. Ayres, et al. 2005. ‘Candidatus Liberibacter americanus’, associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. Int. J. of System. and Evolut. Microbiol. 55: 1857-1862.

Tian, S., L. Lu, J.M. Labavitch, S.M. Webb, X. Yang, P.H. Brown, et al. 2014. Spatial imaging of Zn and other elements in Huanglongbing-affected grapefruit by synchrotron-based micro X-ray fluorescence investigation. J. of exper. Bot. 450.

Yang, C., C.A. Powell, Y. Duan, R. Shatters and M. Zhang. 2015. Antimicrobial nanoemulsion formulation with improved penetration of foliar spray through citrus leaf cuticles to control citrus huanglongbing. PloS one 10 7. Retrieved from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0133826

Yelenosky, G. and C.L. Guy. 1977. Carbohydrate accumulation in leaves and stems of'Valencia'orange at progressively colder temperatures. Bot. Gaz: 13-17.

Zekri, M. and T. Obreza. 2008. Plant Nutrients for Citrus Trees. IFAS publication SL200. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Zhang, M., Y. Guo, C.A. Powell, M.S. Doud, C. Yang and Y. Duan. 2014. Effective antibiotics against ‘Candidatus Liberibacter asiaticus’ in HLB-affected citrus plants identified via the graft-based evaluation. PloS one 9: e111032.

Zhao, H., R. Sun, U. Albrecht, C. Padmanabhan, A. Wang, M.D. Coffey, et al. 2013. Small RNA profiling reveals phosphorus deficiency as a contributing factor in symptom expression for citrus huanglongbing disease. Mol. plant 6: 301-310.

Zhou, L.J., D.W. Gabriel, Y.P. Duan, S.E. Halbert and W.N. Dixon. 2007. First report of dodder transmission of huanglongbing from naturally infected Murraya paniculata to citrus. Plant Dis. 91: 227-227.

BIOGRAPHICAL SKETCH

Diego Fernando Ramirez was born in Bogota, Colombia. He received a Bachelor of Science degree in Biology from the Nueva Granada Military University in 2009 focusing his research in the study of commercial aquaponics systems. After his graduation, Diego worked as research assistant for two years in the Hydrobiology lab at the same university, conducting research on water quality in water bodies affected by anthropogenic activities. In 2013, Diego started working at the Indian River Research and Education Center in Fort Pierce helping in the maintenance of citrus and peaches crops. His interest in crops nutrition and programs to enhance productivity using sustainable management practices prompted him to start his Master studies on citrus nutrition against HLB.