Influence of Oscillating Stress in HLB Infected Trees

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Abstract

Huanglongbing (HLB), otherwise known as citrus greening is considered the most devastating disease of citrus. Extensive efforts from scientists around the world are directed at saving the citrus industry. With a decline in production, effective methods are needed to screen for HLB resistance. Identification of cultivars that demonstrate HLB resistance will be the most sustainable long-term solution for citrus production where HLB is endemic. Some growers and researchers have observed that environmental stress appears to accelerate HLB progression, and this study was conducted to see if stress evaluation could quickly differentiate between susceptible and resistant trees. The objective was to assess the influence of drought and nutrient stresses on acceleration of HLB symptom development (HLB mottling, nutritional, growth, and Ct (crossing threshold) data), and whether such stresses aided in efficiently distinguishing between susceptible, resistant, and tolerant trees.

A study was conducted in a hoophouse at the USDA-ARS in Fort Pierce, FL using three test factors (genotype, fertilizer stress, and water stress). Trees in the study included Valencia/Kuharske (known HLB-susceptible), Carrizo (known HLB-resistant), and Temple (reportedly HLB-tolerant).

Treatments included a (1) non-inoculated control with full water and fertilizer and the following treatments applied to inoculated trees: (2) full water without fertilizer, (3) full water and fertilizer every three weeks (a full fertilizer and water treatment), (4) full water and fertilizer every twelve weeks (1/4 of the rate of the full treatment), (5) stress water (trees allowed to slightly wilt before watering) and fertilizer ever three weeks, and (6) stress water and fertilizer every twelve weeks (both water and nutrient stress).

By June 2013 treatments across all genotypes, application of either water-stress or nutrient-stress resulted in higher CLas (*Candidatus* Liberibacter asiaticus) titer as indicated by Ct (higher Ct is lower CLas titer), accelerating our ability to identify Carrizo as having HLB-resistance compared to the HLB-susceptible Valencia while these single stresses resulted in Ct similar to the full water full fertilizer treatment in Temple. Combined nutrient and water stress resulted in the greatest tree diameters among all CLas-inoculated treatments for each genotype, with the divergence in size occurring after the stresses were removed. These highly stressed trees may have been less generally conducive to CLas establishment and growth. This was best reflected by Ct especially in Valencia, which displayed CLas development in this treatment through August 2013 and then subsequent loss of the pathogen after stress application. Of all the genotypes, Carrizo was the most resistant, while Temple and Valencia were equally susceptible, and ability to distinguish these difference was accelerated by single stress treatments in Valencia but not Temple. Since single stress treated Temple were still in the lowest Ct (highest titer) group at 6-8 months after inoculation, and statistically different from Carrizo, it appears that application of moderate stress may accelerate screening for HLB-resistant citrus genotypes.

Introduction

Citrus has been a critical part of agricultural economies around the world. Preceding World War II, Florida grove owners increased production of oranges resulting in a harvest surpassing California for the first time (Michael Gannon 1996). Between 1942-1953 Florida farmers produced 80 million boxes of oranges and grapefruit, and a total yield of 100 million boxes total for all citrus genotypes through the 11-year period. Agriculture reached substantial gains during the war, improving Florida's economy (Michael Gannon 1996). Today, Florida is still the largest producer of citrus yield in the United States (Hodges & Spreen 2012). Huanglongbing (HLB), also known as citrus greening, is considered the most serious disease of citrus worldwide (Gottwald, 2007). Citrus greening is associated with the *Candidatus* Liberibacter asiaticus bacteria (Grafton-Cardwell et al., 2013). The psyllid (*Diaphorina citri)* is the vector of *Candidatus* Liberibacter asiaticus. Liberibacter is injected into the phloem of a citrus leaf via the feeding psyllid (Grafton-Cardwell et al., 2013). Since this bacteria is non-culturable, it creates limitations on microbiological research (Hung, et al. 2004). The bacteria causes leaf HLB mottling, nutritional deficiency, abnormal fruit growth, and bitter juice (Rogers et al 2014). Transmission from vector to host occurs in one to seven hours of feeding; timeline of inoculation to death of citrus trees can occur within three to five years (Rogers et al 2014). Sensitive assays that are based on polymerase chain reactions (PCR) are used to detect the bacteria to diagnose HLB and are used in research to assess effects of treatments on disease progression (Manjunath et al., 2007).

There are still many unknowns about HLB (Manjunath et al., 2007), as different citrus genotypes and various environmental conditions appear to influence rate and severity of disease development (Stover et al., 2016). This variability causes difficultly in the initial diagnosis of the disease as trees take considerable time (1-2 years) to express symptoms. Specific genotypes respond differently to environmental stresses (Folimonova et al., 2009). Identifying interactions between these environmental stresses and conditions, *Candidatus* Liberibacter asiaticus (CLas) strains, and citrus genotype could shorten the time it takes to identify HLB-resistant trees (Stover et al., 2016), and may also aid and speed up HLB research projects (Zhang et al., 2012).

Ct is the number of PCR cycles it takes to amplify a targeted gene to a level considered detectable, so a lower Ct means the original gene of interest is present at higher levels (Zhang et al., 2009). To quantify *Candidatus* Liberibacter asiaticus (CLas) PCR primers and probes (such as HLBasf, HLBr, and HLBp) are used (Zhang et al. (2014). Zhang et al. in 2012 developed a graft-based chemotheraphy method for screening to identify therapies that might revitalize Huanglongbing-affected citrus plants.. Another study by Ramadugu et al. in 2014 conducted research on the screening of citrus and its close relatives for tolerance to Huanglongbing as identification of tolerant or resistant trees is very important to develop sustainable citrus production. Other studies focus on the ability to screen for resistance to *Phytophthora* (Graham et al., 2007). Since *Phytophthora* is a fungal disease that affects the root system of citrus trees, it is important to quickly identify the disease and implement methods to aid the tree. In citrus, it is critical to identify such diseases as they present stress to an already HLB stressed tree. Optimizing screening methods for HLB will allow us to be more effective in managing the disease (Stover et al., 2009). Identification of these screening methods may help speed up the research process, identify resistance, and help us to understand how particular genotypes respond under environmental stress.

Several reports and numerous grove observations suggest that stress to *Candidatus* Liberibacter asiaticus infected trees increases progression of symptom development (Stover et al., 2016). In natural conditions, citrus groves experience different stresses at the same time which may include flooding, drought, nutrient deficiency, high irradiance, high temperature, and high atmospheric evaporative demand (Syversten, 2014). For example, water stress markedly affects tree physiology in several ways. Stress can trigger fruit drop within a citrus tree by carbohydrate deficiency, ABA rise, and ethylene release (Iglesias et al., 2007). Drought stress is a key component associated with increased nematode populations in the soil, which causes a rapid decline in root health (Luc et al., 2005). Stresses either alone or in combination can either delay development or damage trees, but, not all stress is deleterious. A study done by Southwick & Davenport (1986) demonstrated water stress resulted in induced early flowering, and this is sometimes used to achieve off-season or earlier cropping.

There is minimal literature information available on how citrus trees infected with HLB directly respond to stress versus non-stressed environments. Further complicating these effects, different scion/rootstocks may respond differently to individual environmental stresses (Mafra et al., 2012). For example, trifoliate genotypes are less tolerant of high soil bicarbonates concentrations and high pH, but when grown as whole trees (e.g. such as when grown to produce seed for new genotypes) generally have low levels of HLB symptom expression (Espeleta et al. 1999). Monofoliate genotypes are more tolerant of the bicarbonates and high pH, but entire trees of such genotypes generally show a higher level of symptom expression of citrus greening or HLB.

There is general agreement that HLB-resistant or tolerant citrus trees will provide a long-term sustainable solution to HLB (Espeleta et al. 1999). The study reported here is part of a large project with the overall objective to develop consistent, routine, and efficient methods for assessing the susceptibility of citrus genotypes. This will advance identification of resistant materials, could assist other research programs studying HLB, and may enhance comparison of results between studies (Stover et al., 2016). The specific goal of this study was to assess the influence of drought and nutrient stresses on development of HLB symptoms (HLB mottling, nutritional, growth, and Ct (crossing threshold) data), and effects of such stresses on efficiently distinguishing between susceptible, resistant, and tolerant trees to provide faster screening methods for HLB.

Methodology

Graft Inoculations

This project was led by the USDA/ARS (Ed Stover and Robert Shatters) in collaboration with the University of Florida (Gloria Moore and Jude Grosser), and was funded by the USDA Animal and Plant Health Inspection Service (APHIS). Three sets of trees (Valencia/Kuharske, Carrizo, and Temple/Sour orange) were ordered from Harris Citrus Nursery of Lithia, FL. Plants were in 4” x 13.5” citripots set in soilless mix. The study included 10 graft-inoculated plants of each genotype (30 total) with each of five different treatments, plus 5 non-inoculated control trees of each genotype for a total 180 trees. Plants were kept in a hoophouse at USDA-ARS in Fort Pierce, FL.

Treatments

There were a total of six treatments (stress versus non-stress). All trees except the controls were bud-inoculated with the HLB-pathogen in December 2012. Each tree was inoculated with two buds that tested positive for the bacteria CLas. Each set of trees was labeled with a tag and color coordinated to provide the instructions for treatment. Stress treatments were initiated March 2013 and continued until January of 2014, after which all trees were maintained with normal water and fertilizer

Treatment	Description	Amount
1 Control	Non-inoculated control with normal water and fertilizer every three weeks.	Full watering (50oz) and fertilization (6 grams N per year/pot (supplied using Peter’s Professional 20-10-20; 0.6 gm Fe from Brandt-Microkey, mixed chelated micronutrients) every 3 weeks
2	Full water and no fertilizer.	Full watering (50oz) and no fertilizing.
3	Full water and fertilizer every three weeks.	Full watering (50 oz) and fertilization (6 g N per year/pot; 0.6 g Fe) every 3 weeks.
4	Full water and fertilizer every twelve weeks. (1/4 of the rate of the full treatment)	Full watering (50 oz) and ¼ fertilization (1.5 g N per year/pot; 0.15 g Fe) every 12 weeks.
5	Stress water and full fertilizer every three weeks.	Stress watering (let wilt before watering) every other month and fertilization (6 g N per year/pot; 0.6 g Fe) every 3 weeks.
6	Stress water and reduced fertilizer every 12 weeks.	Stress watering (let wilt before watering) every other month and ¼ fertilization (1.5 g N per year/pot; 0.15 g Fe) and fertilization every 12 weeks.

Assessment

Starting March 2013, bimonthly measurements taken of each tree included: diameter, HLB symptom assessment, and leaf collection for PCR analysis. The average of the two diameters (north/south and east/west) was calculated for analysis. HLB symptom leaf assessments were slight nutritional, HLB leaf mottle, and HLB severe mottle. Since there is variation in the CLas titer throughout the tree, we collected three leaves per tree. Leaves were selected with the most HLB-like symptoms (Stover & McCollum, 2011).

Determination of CLas titer

The Shatters laboratory at the USDA in Fort Pierce, FL performed DNA extraction and polymerase chain reaction (PCR). All leaf samples were stored in a freezer at -20°C until processed. Each sample was extracted using the bottom 2 cm of the leaf petiole into the mid-rib tissue of each of the three leaves. Qiaqen kits were used for the processing and PCR was run to determine the level of CLas (*Candidatus* Liberibacter asiaticus) titer in each leaf collection. Tables and figures indicate the months in which the data was recorded to depict both progression and seasonality of disease symptoms, pathogen development, and growth. During the first stage of the trial trees were monitored on a bi-monthly basis for symptoms and titer, then were later moved to quarterly assessments. The first diameter measurement, assessment of symptom expression, and leaf harvest were collected in March of 2013 and the final collection was in November of 2014.

Statistical Analysis

Statistical analysis was conducted using ANOVA for parametric tree diameter data and using the non-parametric Kruskal-Wallis test for all other data, all using SAS programing. At each time point, comparisons were made between main effects (genotype and stress treatments), as well as the interaction between genotype and stress. Samples were considered statistically significant having a p value < or = to 0.05. The GLM procedure was run to determine parametric results. The GLIMMIX procedure was conducted on the non-parametric data. All figures are labeled with the Duncan grouping of mean significance to compare the differences between each treatment. For each comparison at each time point, means with the same letter are not significantly different.

Results and Discussion

Interactions between genotypes and stress vs non-stress treatments: Growth

As expected, un-inoculated control trees had consistently the largest stem diameter in comparison with all other treatments across all genotypes (fig 1a-1c). In the age of HLB, root health can be negatively affected, consequently reducing plant growth and vigor (Johnson & Graham, 2015). With the addition of stress to an already stressed tree, trees can encounter reduced vigor, leaf loss, and premature fruit drop (Graham et al., 2014). The treatment of full water and full fertilizer treatment resulted in the greatest growth among inoculated trees of all three genotypes until the stress application was complete in January of 2014 (fig. 1). Thereafter, stress water and stress fertilizer treatments showed an increase in diameter growth (fig. 1). Temple trees treated with full water and no fertilizer application resulted in the smallest diameter, while application of stress water and stress fertilizer had the largest diameter of the treatments (fig 1b). Similar results were seen for Valencia diameter growth, full water and full fertilizer treatments had the highest diameters until completion of the stress treatment in January of 2014 (fig 1). Interestingly, following the end of stress treatments, the combination of water and fertilizer stress surprisingly produced the second largest diameter in all three genotypes with growth rates similar to the un-inoculated controls following conclusion of stress treatments on January 2014 (Fig. 1).

A study by Beattie et al. in 2009 showed that fertilizer application reduced severity of HLB disease symptoms. Foliar fertilizer may improve color and foliage abundance in HLB trees due to the presence of micronutrients (Gottwald et al., 2012). However, we propose that the treatment combining stress water and stress fertilizer application produced so much stress that it compromised establishment of the CLas bacteria and as a result, trees grew relatively well after stress was terminated (fig. 1a-1c). Treatments with one parameter stress (full water and stress fertilizer) showed the lowest growth rate in both Valencia and Carrizo (fig. 1a and 1c). In Temple there were no differences in growth between treatments applied to inoculated trees at most timepoints (fig. 1b)

A study by Hanlon and Syversten (2008) demonstrated that water stress could affect the root to shoot ratio by root loss. Root loss could consequently reduce stem diameter growth as observed in our study (fig. 2). Comparison of diameter in genotype main effect showed that Carrizo genotypes have an overall larger diameter than Temple or Valencia (fig. 2). Temple consistently had the smallest diameter of the three genotypes. Interestingly, effect of treatment on stem diameter was most evident after the stress application was completed in January of 2014 (fig. 1a-1-c). Nutrient uptake by plants is limited under stressed conditions due to decreased transpiration rates, impaired active transport, and membrane permeability (Pessarakli, 1999). Therefore, after completion of the stress applications, trees were better able to utilize their energy for growth.

Looking at the main effect of treatment across all three genotypes, un-inoculated trees had the largest diameter (mm) of all six treatments followed by the combination of both water and fertilizer stress treatment (fig. 3). Water and fertilizer stress treatment stem diameter was lower than the control but similar to the full water/full fertilizer treatment, which was unexpected. When analyzing the treatments over the course of the experiment, all treatments show a consistently tight growth pattern during the application of stress (fig. 3). Upon completion of the stress exposure in January of 2014, thereafter, treatments stay linear and no significant growth changes are indicated (fig. 1a-1c and fig.3).

Interactions between genotypes and stress vs non-stress treatments: Foliar symptoms

Studies have shown CLas identification in PCR was often associated with leaf nutrient deficiency symptoms (Hajivand et al., 2011). Figures 4a-4c compare the nutritional deficiencies of Valencia, Temple, and Carrizo. A study by Graham et al. in 2014 documented that trees that were exposed to stress experienced increased HLB-symptomatic expression (fig 4a, 4b, and 4c). Full water and no fertilizer treatment showed the highest level of nutritional deficiency symptoms, while full water and fertilizer every three weeks (normal full fertilization for greenhouse trees) had the lowest symptomatic expression across all three genotypes in main effect and interactions between genotype and stress, which is seldom statistically different from water stress and full fertilizer treatment (fig. 4a, 4b. 4c and fig. 6). This shows the expected significance of fertilizer application in nutritional deficiency symptoms (fig 4b). All treatments showed a constant deficiency pattern through stress exposure and thereafter (fig. 4a, 4b. 4c and fig. 6). In Carrizo, full water and no fertilizer showed a peak in nutritional deficiency in October of 2013, while the remaining treatments show an slight increase in deficiency in October 2013, then stayed relatively consistent for the remainder of the trial (fig 4c).

Across all genotypes, Carrizo sometimes showed a lower level of nutritional deficiency compared to Temple and Valencia (fig. 5). At the final time point, Temple and Carrizo are statistically different, however, Valencia was not significantly different from either of the other two genotypes (fig. 5). When comparing nutritional deficiencies for the main effect of genotype, Temple had an average of around 30 percent deficiency, compared to Carrizo with an average of 10 percent deficiency (fig 5). This is consistent with many reports that Carrizo is a more resistant genotype (Albrecht and Bowman, 2011).

When comparing the main effects of treatment on nutritional deficiencies, overall full water and no fertilizer application show to have the highest amount of nutritional deficiency in all treatments (fig. 6). Un-inoculated trees and stress water and full fertilizer express little to no deficiency symptoms (fig. 6). After completion of stress exposure in January of 2014, all 6 treatments show a decrease in nutritional deficiency symptoms (fig. 6).

Figures 7a-7c shows the symptomatic HLB mottling percentages among the treatments in each genotype. Mottling is the most consistent visual diagnostic symptom for HLB identification (Gomez, 2009). The percentage was always low, less than 8% (fig. 7a-7c). Valencia trees showed a variation in mottling percentages throughout the treatments (fig. 7a). Stress water and stress fertilizer and full water and stress fertilizer treatments caused spikes in mottling in January of 2014, then reduced to less than one percent mottle (fig. 7a), which is consistent with field observations of greatest HLB mottling in the fall and winter. Temple trees showed similar results with combined stress water and stress fertilizer treatment trees having the highest HLB mottling, while water and stress fertilizer treatment trees with the lowest percentage of mottle (fig. 7b). Carrizo trees never showed significant HLB mottling and there was no difference between treatments as indicated by the mean separations (fig. 7c). As expected, in figures 7a-7c, un-inoculated trees had no HLB mottling.

When analyzing the main effect of genotypes Carrizo was least affected by HLB mottling (fig. 8), but differences were seldom statistically significant. Over the entire trial, Carrizo had less than 0.5 percent HLB mottling (fig. 8). Each of the three genotypes exhibited a decrease in HLB mottling after completion of stress exposure in January of 2014 (fig. 8). Trifoliate genotypes are commonly reported to have lower HLB symptom severity (Stover et al., 2010).

Across all three genotypes, full water and stress fertilizer had the highest percentage of HLB mottling of all treatments (fig. 9). When comparing all treatments, un-inoculated trees had the least amount of symptoms only second to stress water and stress fertilizer (fig. 9). Treatments at the final assessment in March of 2015 were not statistically different. Full water and stress fertilizer had the highest amount of HLB mottling in all the treatment.

Interactions between genotypes and stress vs non-stress treatments: Ct as indicator of CLas

Figure 10a shows the response of Valencia to the stress treatments as indicated by Ct values. Stress water and stress fertilizer was consistently in the grouping with the highest Ct values in Valencia (along with un-inoculated controls and full water with no fertilizer), indicating the lowest bacterial titers, and was significantly higher than most other treatments from Dec 2013. Two or more stress factors can limit physiological responses in citrus (Garcia-Sanchez and Syversten, 2013). Full water and fertilizer stress or stress water and full fertilizer had the lowest Ct at most timepoints in Valencia and Temple, which suggests that combined water and fertilizer stress may compromise the ability of the HLB-pathogen to develop inside the host. The comparison of treatment affect on Ct in Temple shows that un-inoculated trees have the highest Ct throughout the trial, indicating the lowest level of CLas, which was never significantly different from full water and no fertilizer (fig. 10b). However, the full water and full fertilizer treatment in Temple was always in the highest pathogen titer group, unlike in Valencia where the single stress treatments resulted in substantially faster disease development.

In Carrizo Ct analysis never showed statistically significant differences between treatments (fig. 10c). Overall Carrizo had the highest Ct values of the three genotypes, consistent with several reports of HLB-resistance in trees of this genotype. Valencia and Temple were seldom statistical different from each other in Ct.

Determination of susceptibility, resistance, or tolerance among genotypes

Citrus genotypes differ in their susceptibility, resistance, or tolerance to Huanglongbing. Trifoliate trees generally show HLB resistance (Albrecht and Bowman, 2011). Temple is fairly tolerant to HLB, and Valencia is highly susceptible (Stover and McCollum, 2011). Results of our study were consistent with Carrizo showing the highest Ct values (values at 40 are considered un-detectable) (fig. 11). Temple and Valencia had the lowest Ct values, indicating higher levels of CLas which were never statistically different from each other. Carrizo is statistically different (p value < or = to 0.05), from Valencia and Temple at most timepoints.

Ct values were analyzed to determine effect of treatments on development of the HLB pathogen (fig. 12). Stress water/stress fertilizer treatment had a higher Ct value compared to all treatments (figure 12). However, stress water/stress fertilizer was not statistically different from treatments un-inoculated, full water/full fertilizer, or full water/no fertilizer. One stress parameter (full water and stress fertilizer) had the lowest Ct (crossing threshold) indicating a high amount of bacteria. Ct value that is below some threshold(39), based on experimental error in PCR conducted, is considered to be HLB positive. For our work, any Ct <39 indicates detectable CLas.

These figures overall demonstrate that Carrizo was the most resistant to HLB, displaying the fewest symptoms, highest CTs, and lowest HLB mottle. Carrizo has been noted to show less severe symptoms of HLB, and lower Ct values (Stover et al., 2016).

Conclusions

Genotypes have different tolerances and susceptibility to environmental stresses (Folimonova et al., 2009). Analyzing interactions of genotype versus treatment effects on HLB development may allow acceleration of screening methods to identify resistance (Stover et al., 2009).

In a citrus industry that is overwhelmed with the rising cost of production, loss of yields, and deterioration of profits, it is critical to identify screening methods to help identify potential resistant cultivars. Understanding interactions between environmental stresses and genotypes may also allow us to develop a better understanding of each genotype's response to environmental stresses that influence HLB development.

Stress to *Candidatus* Liberibacter asiaticus infected trees increased progression of symptom development in Valencia. Trees seemed to increase in health after completion of treatment application in January of 2014, demonstrating that exposure to stress created unfavorable conditions for each genotype. Overall, we saw a reduction in CLas titer (higher Ct) and an increase in tree health with the combined stress water and stress fertilizer treatment. This is consistent with the two-parameter stress (stress water and stress fertilizer) decreasing ability of the bacterium to infect or grow inside the phloem. This is an interesting result and indicates such severe stress compromises our ability to screen for resistance. It must be noted that this treatment likely has no practical value in commercial citrus production since such high levels of stress would compromise growth, although it raises the interesting possibility that a transient stress may substantially reduce levels of the HLB pathogen. Based on the results from this study, one parameter stress showed signs of disease acceleration, and resulted in more rapid ability to distinguish the relative resistance of Carrizo compared to Valencia and was similar to non-stressed Temple . In particular, the treatment of full water and no fertilizer and full water and stress fertilizer both showed the most significant acceleration of disease progression. Based on the data in this experiment, Carrizo can be identified as a more resistant genotype then Valencia and Temple based on stem diameter data of all genotypes (fig. 2), percentage of nutritional deficiency in genotypes (fig. 5), percentage of mottling among genotypes (fig. 8), and Ct values among genotypes (fig. 11). Of the two monofoliates, Valencia exhibited less symptomatic expression compared to Temple. Carrizo displayed the lowest symptomatic expression, highest Ct values, and largest stem diameters out of all genotypes. Temple and Valencia had the most symptoms, lowest Ct, and smallest diameter showing they were more susceptible to HLB. This data supports the theory that Carrizo is a more resistant genotype compared to Valencia or Temple (Stover et al., 2016).

Understanding interactions among genotypes and how HLB development responds to stress helps to understand the physiological reactions occurring inside trees during host pathogen interactions. This study provided data to help determine resistance and tolerance in citrus genotypes, understand the influence of drought and nutrient stress on HLB mottle and nutritional deficiency, and presented information on the interactions of stress versus non-stress environment.

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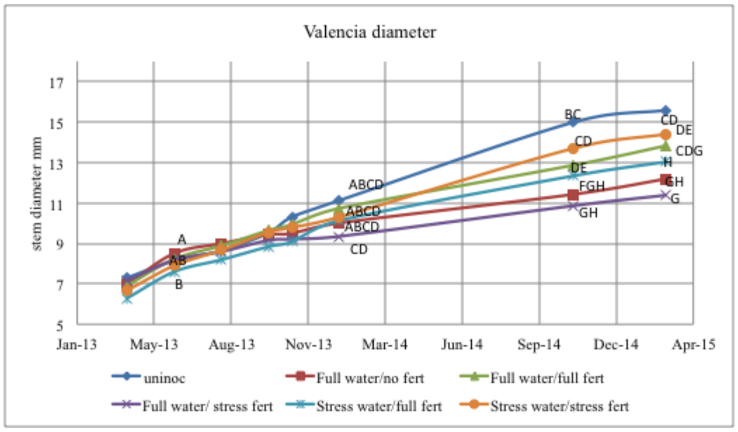
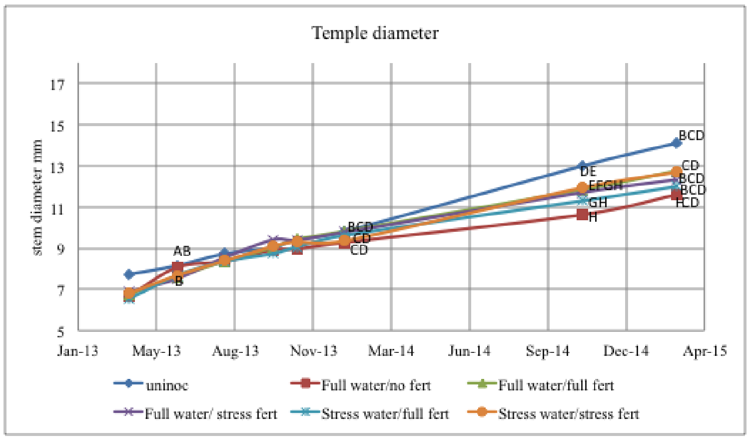




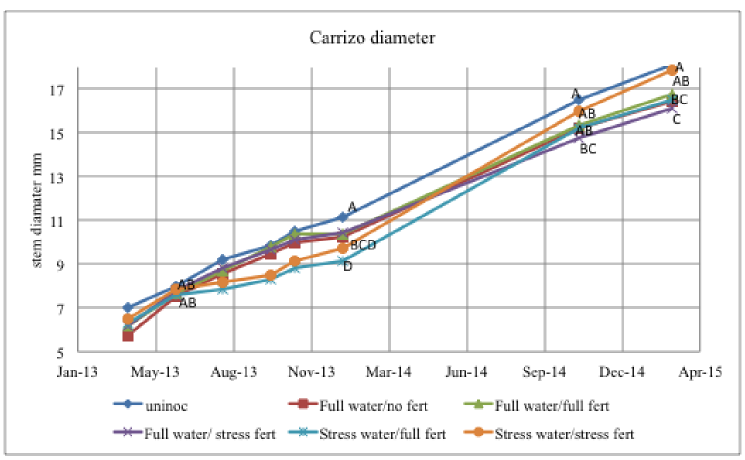


Figure 1. Growth in stem diameter (mm) in Valencia (Fig. 1a), Temple (Fig.1b), and Carrizo (Fig. 1c) as influenced by stress treatments. Beginning Jan. 2014 stress treatments were concluded and all trees received standard irrigation and nutrition. At each time point, means are not significantly different when followed by the same letter.

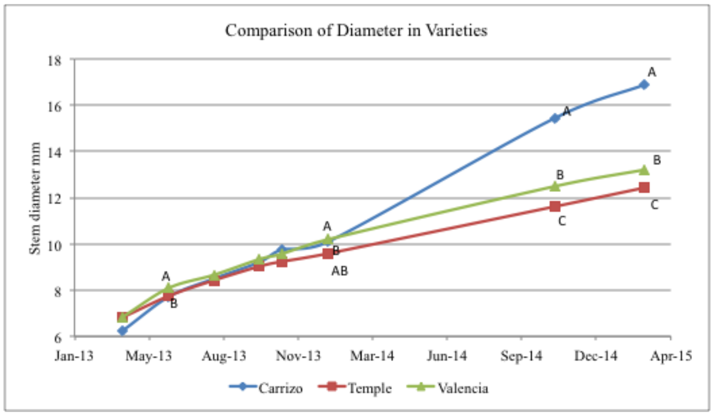


Figure 2 demonstrates growth as stem diameter, averaged across all treatments for Carrizo, Temple, and Valencia. Beginning Jan. 2014 stress treatments were concluded and all trees received standard irrigation and nutrition. At each timepoint, means are not significantly different when followed by the same letter.

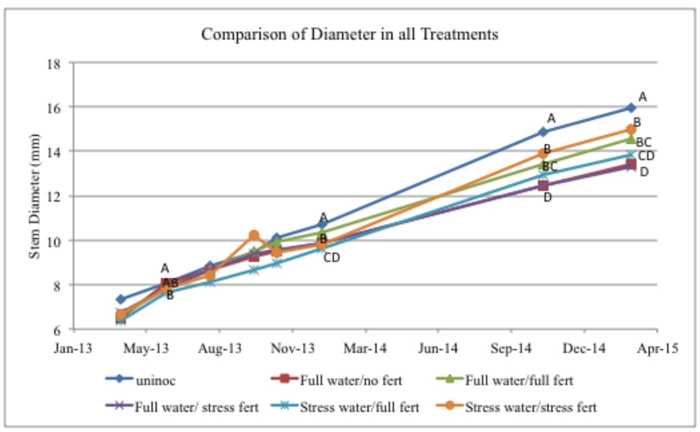
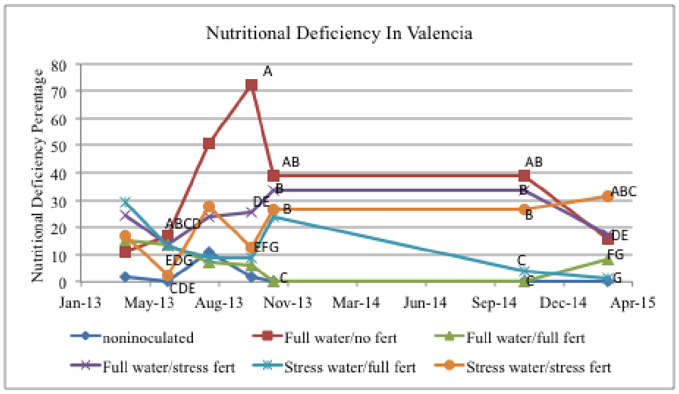
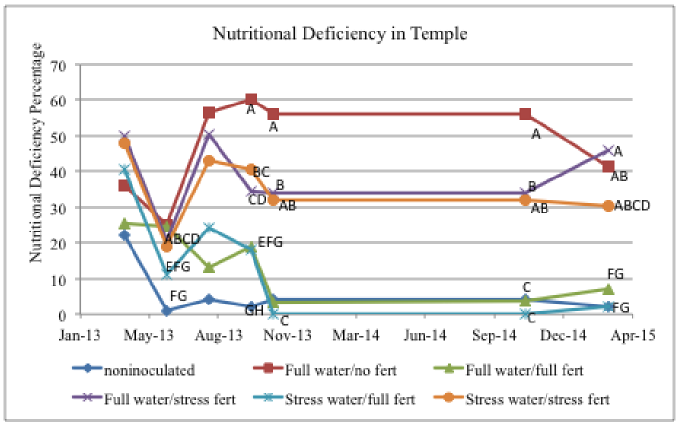


Figure 3 demonstrates growth as stem diameter, averaged across all genotypes for each treatment. Beginning Jan. 2014 stress treatments were concluded and all trees received standard irrigation and nutrition. At each time point, means are not significantly different when followed by the same letter.





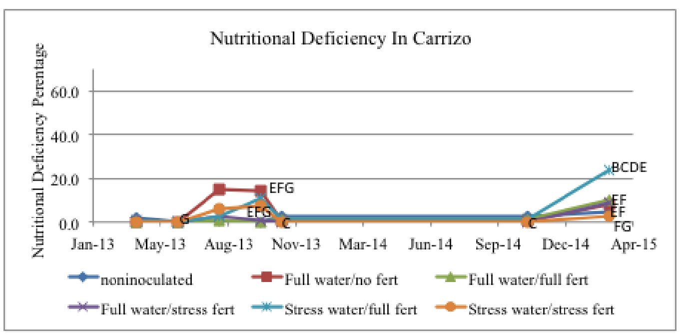


Figure 4a-4c compares nutritional deficiencies in Valencia (fig 4a), Temple (fig 4b), and Carrizo (fig. 4c) between Treatments 2-6 and the Non-inoculated control. Beginning Jan. 2014 all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

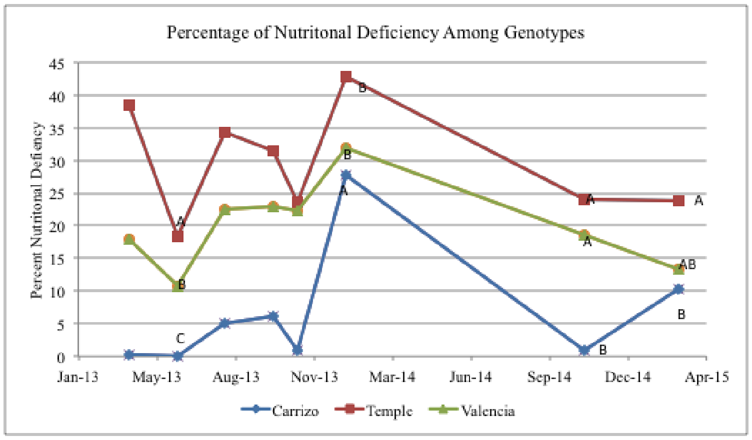


Figure 5 compares nutritional deficiency between Genotypes (Carrizo, Temple, and Valencia. Beginning Jan. 2014 all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

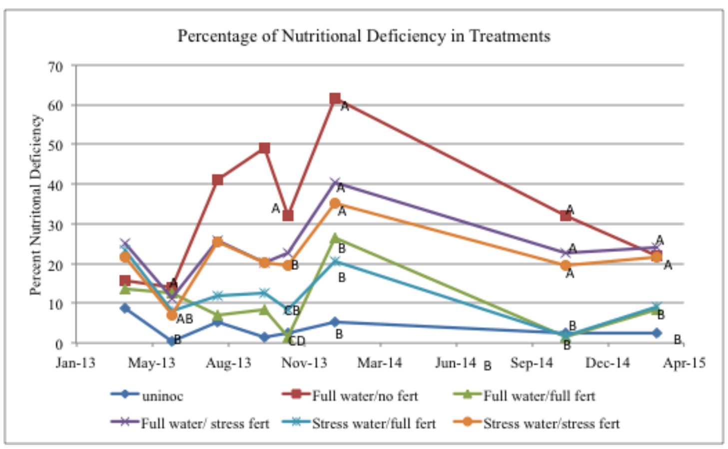


Figure 6 compares nutritional deficiency between Treatments and the Non-inoculated control. Beginning Jan. 2014 stress treatments were concluded and all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

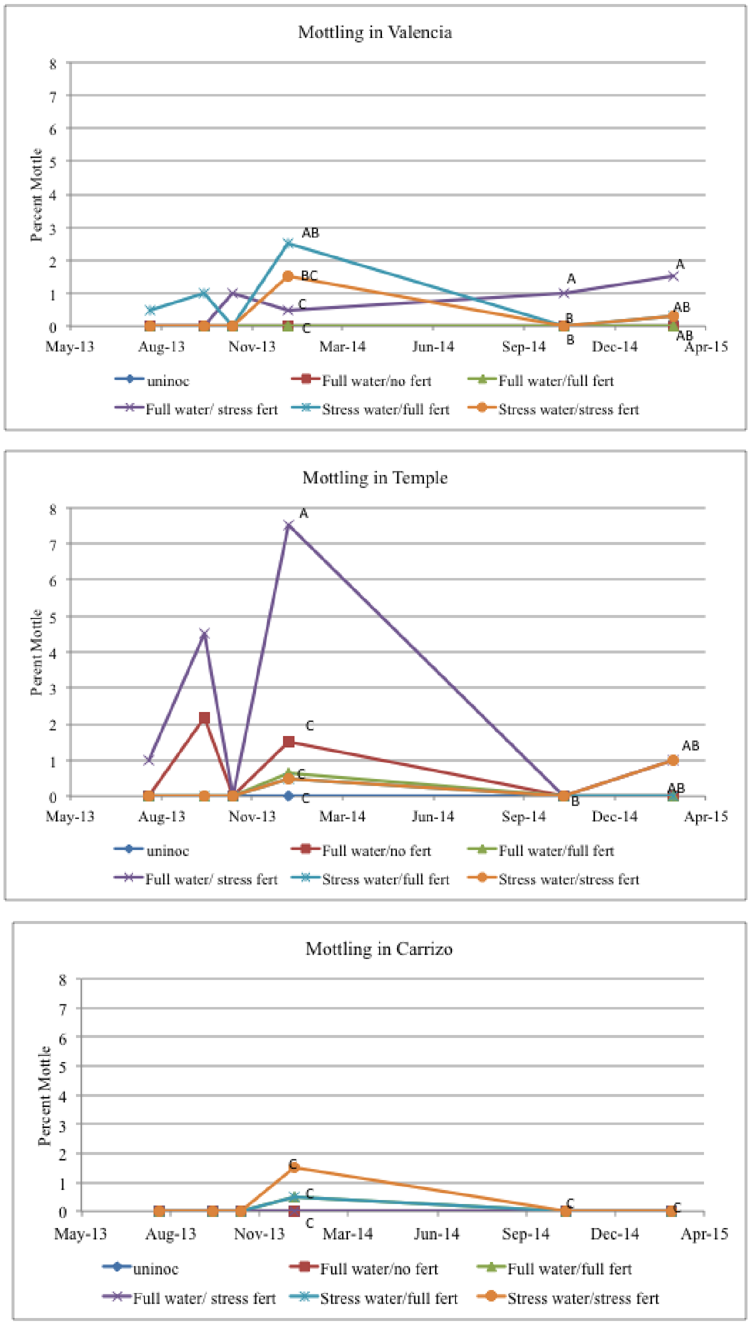


Figure 7. HLB mottling in Valencia (fig. 7a), Temple (fig. 7b), and Carrizo (fig. 7c). Beginning Jan. 2014 all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

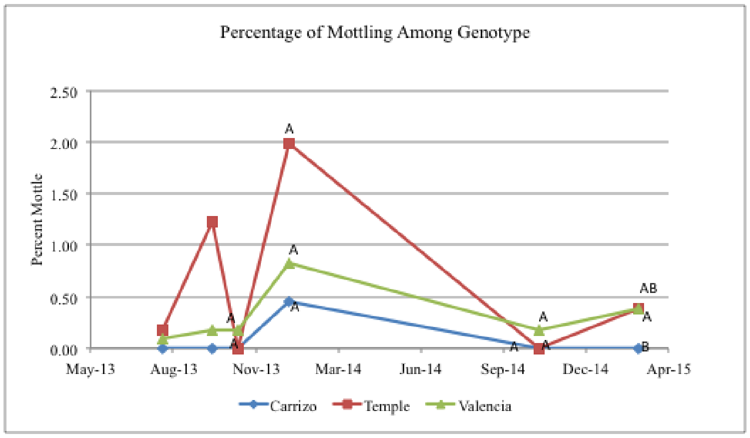


Figure 8 compares HLB mottling between genotypes. Beginning Jan. 2014 stress treatments were concluded and all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

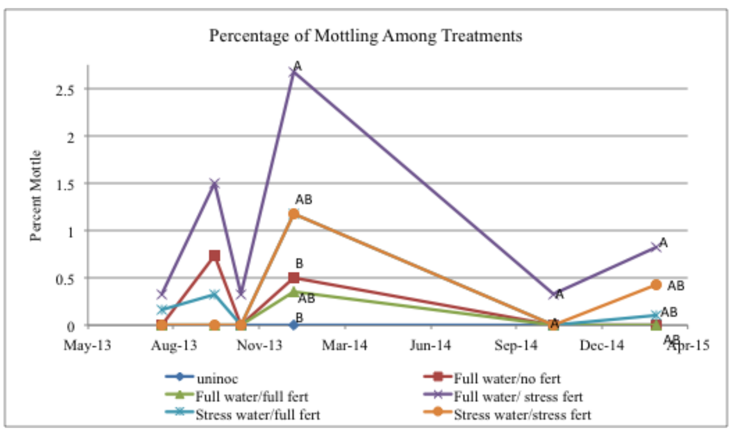


Figure 9 demonstrates the difference in HLB mottling among the various treatments. Beginning Jan. 2014 all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

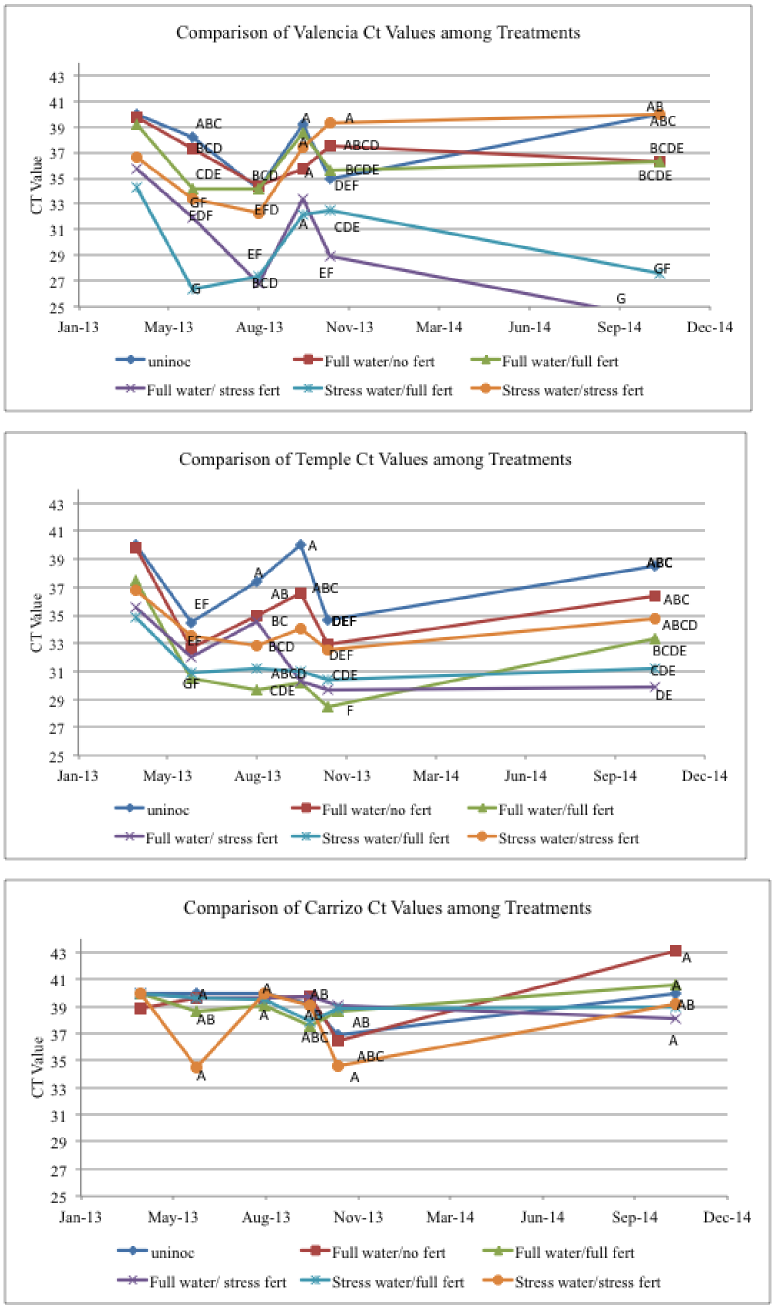


Figure10. Interaction of Ct between treatment and Valencia (fig. 10a), Temple (fig. 10b), and Carrizo (fig. 10c). Beginning Jan. 2014 all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

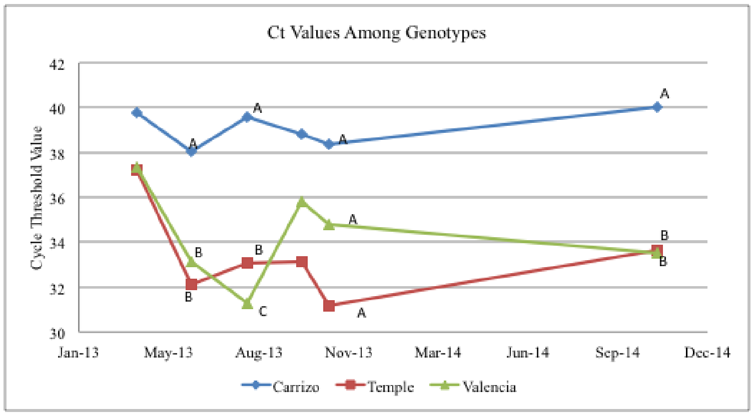


Figure 11 compare the Ct values Carrizo, Temple, and Valencia in three genotypes. Beginning Jan. 2014 stress treatments were concluded and all trees received standard irrigation and nutrition. Values over 40 are considered to be non-detectable. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.

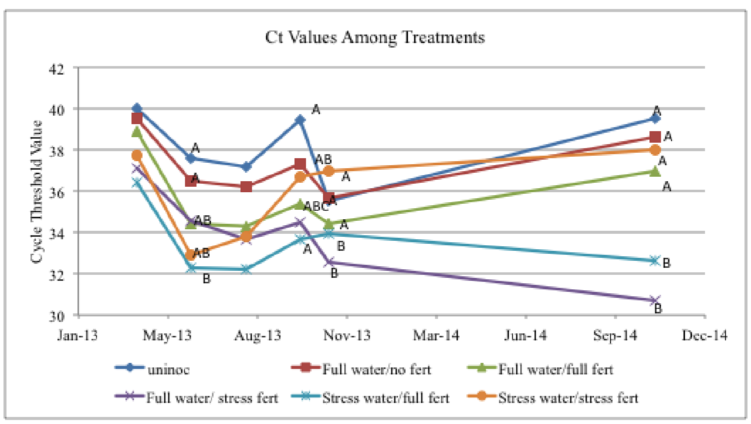


Figure 12 demonstrates the Ct values among the 6 treatments. Beginning Jan. 2014 all trees received standard irrigation and nutrition. Data displays Duncan grouping of means to determine the order of each class variable. Means with the same lettering are not significantly different.