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First Year Results of a Trial of 'Tahiti' Lime and Three Lime-hybrid Scions Grafted to Five Citrus Rootstocks Established at TREC

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A research-extension 1.65-acre lime and lime-hybrid scion and citrus greening rootstock planting was established in March 2017 at the Tropical Research and Education Center, Homestead, Florida. The purpose of the project was to evaluate and compare tree growth and performance and fruit quality of 'Tahiti' lime (TL) and three lime-like hybrid scions (C33, C14, and C11) grafted on Florida standard lime rootstock, *Citrus macrophylla* (CM) and four putatively tolerant citrus greening (huanglonbing, HLB) rootstocks (US-897, US-942, US-802, and SO+50-7). There were zero to 22-single-tree replications arranged in four blocks for the 20 scion × rootstock combinations, for a total of 239 trees. Simulating a standard cultural plan for 'Tahiti' lime citrus management in south Florida, the planting was managed intensively with frequent and high rates of fertilizer and an intense Asian citrus psyllid (ACP) management program. Surveys for pests were conducted biweekly and all trees were screened for citrus greening every three months. Six trees subsequently succumbed to damage caused by Hurricane Irma. At the end of year one, C11 scion and trees grafted to CM and US-802 rootstocks were tallest trees (mean, 1.1 m to 1.3 m) and C11/CM had the greatest mean stem diameter (46 mm). Mean incidence of rootstock sprouting was highest for trees grafted to US-802 and US-942 rootstocks compared to other rootstocks. C14 (seedless triploid lime hybrid) was the first scion–rootstock combination to flower and by March 2018 all C11 and TL scions had flowered regardless of rootstock. Thirteen insect pests were identified within the planting, including the ACP on six trees. Repeated HLB screening detected 23 trees positive for HLB by March 2018. To assist with determining the economic feasibility of growing any of the lime scion–rootstock combinations for commercial purposes, data on resources needed for grove establishment and maintenance were evaluated.

Since 2005, citrus greening also called huanglongbin (HLB) has been a very serious problem affecting citrus production in Florida (Halber and Manjunath, 2004). This disease, caused by the bacterium Candidatus *Liberibacter asiaticus* (Las), has been responsible for an estimated 26% and 42% reduction in orange acreage and production respectively in Florida (Singerman and Useche, 2016). Tens of thousands of acres have been rendered non-productive and/or abandoned. This phloem-limited bacterium generally causes severe tree decline (e.g., leaf drop, stem dieback), drastically reducing fruit production and quality, and eventually killing the tree.

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Prior to 2001, south Florida produced 50% of the limes consumed in the United States. One result of the citrus canker eradication program was the elimination of Florida's entire 'Tahiti' lime industry by the end of 2001 (Schubert et al., 2001; Singerman and Useche, 2016). Prior to citrus greening (Dewdney and Graham, 2016) the major concerns for lime production were wood pocket (a genetic disorder) and several graft transmissible viral diseases such as exocortis and xyloporosis (Cohen et al., 1961; Knorr and Childs, 1957; Tarnowski et al., 2009). An additional requirement for any successful lime rootstock in south Florida is tolerance to high pH, calcareous soil, low incidence of magnesium and iron deficiencies (called lime-induced chlorosis), and high fruit production (Campbell, 1991; Castle et al., 2004; Colburn et al., 1963).

'Tahiti' lime (*Citrus latifolia*; syn. *C. aurantifolia*) is susceptible to citrus greening, however a number of investigations rate 'Tahiti' lime as tolerant (little or no symptoms) to greening compared to sweet orange (*C. sinensis* 'Valencia'), grapefruit (*C. paradisi* 'Duncan'), some mandarins (e.g., *C. reticulata* cv

'Nules') and tangelos (*C. paradise* × *C. reticulate* cv 'Minneola') (Folimonova et al., 2009; da Graça, 1991; Lopes and Frare, 2008). Similarly, various lemon types (*C. limon*, *C. limonia*, *C. macrophylla*, and *C. jambhiri*) have been reported as tolerant to citrus greening (as reported by Altamirano et al., 1976 and Shokrollah et al., 2011; Koizumi et al., 1993). In the Philippines, greening infected lime, lemon and calamondin trees were reported vigorous and productive compared to other citrus species (Viñas and Honrade, 1974 reported in Altamirano et al., 1976; Batool et al., 2007; Folimonova et al., 2009; Gonzales and Vinas, 1982). A recent economic feasibility study reported that in south Florida, 'Tahiti' lime production could be economically viable even in the presence of citrus greening (Evans et al., 2014).

Citrus rootstocks have a profound impact on disease tolerance of citrus trees. Rootstocks and rootstock-interstock combinations vary in their tolerance to citrus greening and affect scion growth and fruit production (Albrecht and Bowman, 2011; Albrecht and Bowman, 2012; Albrecht et al., 2012; Bowman and McCollum, 2015; Shokrollah et al., 2011; Lopes and Frare, 2008; Cheema et al., 1982). Recently several trifoliate hybrids (e.g., US-897, US-802, and US-942) and one sour orange hybrid (SO+50-7) have been reported to have superior greening tolerance compared to numerous other hybrids and commonly used older rootstocks (Albrecht et al., 2012; Bowman et al., 2016a, 2016b). Two of these (US-897 and US-802) also performed well in two short-term 'Tahiti' lime rootstock trials under south Florida conditions (Castle et al., 2004).

During the past three years, several producers have established small 'Tahiti' lime plantings (~60 acres) to test production techniques and economic viability. Trees in these plantings are on *C. reticulate* or *C. jambhiri* rootstocks which are non-greening tolerant genotypes and therefore plantings are under an intense ACP control program. Despite the intensive management, these trees are now showing symptoms (e.g., defoliation and dieback) of HLB (J.H. Crane, personal observation).

To address the local need for citrus greening tolerant citrus and for alternative commercial fruit crops for south Florida, a small scion × rootstock demonstration planting was established. Tree scions included 'Tahiti' lime, three new seedless triploid lime hybrid selections and five rootstocks including alemow (*C. macrophylla*) the traditional rootstock for acid fruit grown on the calcareous soils of southern Miami-Dade County. Herein we report on the first year of this investigation.

Materials and Methods

Planting and establishment. On 15, 16, and 30 Mar. 2017, a 0.6636 ha (1.65-acre) demonstration grove of 'Tahiti' lime and three seedless lime triploid hybrids grafted onto four citrus greening tolerant rootstocks plus an industry standard was established at the University of Florida/IFAS Tropical Research and Education Center (TREC), Homestead (Table 1). Trees were spaced 4.5 m (15 ft) in-row and 6.1 m (20 ft) between-rows. A high volume [6.36 mm (0.25-in) per hour] irrigation system for cold protection and a micro-sprinkler system were installed [Maxijet®, 320 one-piece fanjet, 39.75 lph (10.5 gph)]. The 20 scion–rootstock combinations were planted in a randomized complete-block design with four blocks and an uneven number of replications of each scion–rootstock combination in each block. Nearly all trees (97%) were tested for citrus greening prior to planting. An intense plant nutrition and irrigation management program was immediately implemented that included a seven-month slow release fertilizer (Harrells 70-day 16–6–12), a moderately quick release granular fertilizer (8–3–9), repeated foliar minor element applications (KeyPlex 350), and repeated soil drench applications of Sequestrene-138 chelated iron plus phosphorous acid (NutriPhite Magnum 2–40–16). Two ten-tree rows of 'Tahiti' lime trees severely infested with ACP and infected with HLB were located about 398 m (1307 ft) to the southwest of the planting. The latter served as a natural source of HLB inoculum.

Plant growth and development. Tree height (9 June and 3 Nov. 2017) and trunk dia. were recorded twice (16 June and 12 Dec. 2017). Dates of flowering were recorded on five occasions (31 Oct. and 10 Dec. 2017; 8 Jan., 29 Jan. and 19 Mar. 2018). Presence of rootstock sprouts was recorded nine times (5 and 12 Apr., 15 May, 9 June, 27 June, 14 July, 25 Oct. and 12 Dec. 2017 and 28 Mar. 2018). Rootstock sprouts were removed after each evaluation and all trees were out fitted with plastic trunk covers on 15–17 July 2017 in an effort to stop repeated rootstock sprouting. Leaves were sampled for leaf nutrient analysis on 18

Table 1. Scions and rootstocks in lime trial at University of Florida/IFAS TREC, Homestead.

	Scions	
Common name or selection ID	Abbrev.	Genetic background
'Tahiti' lime ('Persian' lime)	TL	*C.* × *latifolia*. Trihybrid intergeneric cross involving citron (*C. medica*), pummelo (*C. grandis*), and *C. micrantha*.[z]
DPI-435-0061	C11	Key lime × (Key lime + Valencia sweet orange somatic hybrid).
DPI-435-0027	C14	Lakeland limequat × tetraploid Femminello lemon
DPI-435-9-33	C33	Todo el Ano lemon × (Key lime + Valencia sweet orange somatic hybrid).
	Rootstocks	
Alemow	CM	*Citrus macrophylla* was the most widely used 'Tahiti' lime rootstock. Propagated from apomictic seed.
SO+50-7	SO+50-7	Tetraploid somatic hybrid – sour orange (*C. sinensis*) + *Poncirus trifoliata* (trifoliate orange 50-7).
US-802[y]	US-802	*C. grandis* (L.) 'Siamese' × *P. trifoliata* 'Gotha Road'
US-897[y]	US-897	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Flying Dragon'
US-942[y]	US-942	*C. reticulata* 'Sunki' × *P. trifoliata* 'Flying Dragon'

[z]Moore, G.A. 2001. Oranges and lemons: clues to the taxonomy of *Citrus* from molecular markers. Trends in Genetics 17:536–540 and Mabberley, D.J. 2004. Citrus (Rutaceae): A review of recent advances in etymology, systematics and medical applications. Blumea 49:481–498.

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

Oct. 2017 as per Orth and Campbell (1973). Nearly all trees were leaning west after Hurricane Irma (9–10 Sept. 2017). They were righted and staked within three days post-storm. Plant height, trunk diameter and leaf nutrient data were analyzed using a linear mixed model to account for blocking associated with row. Tukey's multiple comparison method was used for pairwise comparisons of means. A Fisher's exact test was used to analyze the flowering and rootstock sprouting data.

Pest and disease management. A comprehensive Asian citrus psyllid (*Diaphorina citri*; ACP) control program was implemented using systemic and contact insecticides. A weekly insect pest-monitoring program was implemented immediately after planting. Five trees were randomly sampled biweekly in each of the 20 rootstock–scion combinations. The average number of adult ACPs per 10 flushes (or less depending on the size and developmental stage of the tree) was determined in each experimental plot using the stem tap monitoring method (Hall et al., 2007). Management of ACP was in accordance with the 2016 Florida Citrus Pest Management Guide <http://edis.ifas.ufl.edu/in686> recommendations. Young trees produce multiple flushes throughout the year and are at greater risk of greening infection. Therefore soil-applied systemic insecticides (i.e. imidacloprid, thiamethoxam, sulfoxaflor and clothianidin) and contact insecticides were used to manage ACP. Citrus canker (*Xanthomonas axonopodis* pv. *citri*) was managed with periodic copper applications (Gottwald et al., 2002; Schubert et al., 2001; Dewdney and Graham, 2016). Standard weed control and mowing was implemented as needed.

A tri-monthly citrus greening (Candidatus *Liberibacter asiaticus*) screening was implemented immediately after planting and an additional screening was conducted post-Hurricane Irma. Three to five leaves were randomly selected, from different parts of the tree, and processed immediately for DNA extraction. Midribs were excised and placed into 2 mL conical screw-cap microcentrifuge tubes containing 5–10 2.3 mm diam. zirconia/silica beads (BioSpec Products, Bartlesville, OK). Due to the fibrous nature of citrus, tissue was lysed twice with a Bead Mill 24 homogenizer (Fisher Scientific, Pittsburgh, PA, USA). Genomic DNA was extracted using Thermo Scientific Gene Jet Genomic Purification Kit (Fisher Scientific), following protocol described in Gazis et al., 2018. Conventional PCR was carried out using the *Las* specific primer set targeting the 16S rDNA, OI1 and OI2c (Jagoueix et al., 1996). PCR reactions were assembled as follows: 12.5 μL GoTaq®G2 Hot Start Master Mix (Promega Corp., Madison, WI, USA), 1.25 μL 10 mM reverse primer, 1.25 μL 10 mM forward primer, 1 μL dimethyl sulfoxide (DMSO, Sigma-Aldrich, St Louis, MO, USA), 1 μL of genomic DNA and double-distilled water to complete a total volume of 25 μL. Thermocycler conditions were as described in Tatineni et al., 2008. Five positive controls (fresh citrus samples gathered from an infected planting) and two negative controls (water) were included in each experiment. Amplified PCR products were confirmed with gel electrophoresis and exemplar positive amplicons (5) were sent to MCLAB laboratories (www.mclab.com) for cleaning and sequencing. Sequencher TM 4.9 (Gene Codes Corp., Ann Arbor, MI, USA) was used to assess the quality of the chromatograms and assemble the strands into contigs. Amplicons were confirmed to represent *Las* based on results from the Basic Local Alignment Search Tool (BLAST), using the NCBI nucleotide database (www.ncbi.nlm.nih.gov/BLAST).

Development of crop enterprise budgets. Our approach to determining the economic viability of a lime enterprise began with the creation of a representative grove establishment budget that reflects the amount needed and current costs of all production inputs including labor, fertilizer, infrastructure, equipment, and water. The grove establishment budget was based on information obtained from the scientists, input suppliers and a grower collaborator. Primary field data collected at the research site included: i) treatment-level quantities of material inputs applied; ii) treatment level type, timing, and number of field operations performed; and iii) cost of infrastructure (e.g., irrigation systems). Supplementary secondary data required for estimating cropping-system costs included prices paid for inputs and materials, commodity prices received for crops.

Results and Discussion

Tree survival. Prior to the category 2, Hurricane Irma (10–11 Sept. 2017) all 239 trees were alive. Post-storm nearly all trees were leaning to the west. Within three days of the storm trees were carefully righted and staked; there was very limited limb breakage. However, over the next five months some trees showed signs of drought stress and it was determined that in most cases the trunk area just at and below the ground level was severely girdled. A copper paint solution was applied to the lower trunk and some canopy was removed in an effort to save these trees, however, six eventually died. These trees were excluded from the data analysis.

Plant growth and development. There was significant difference among the tree height of the scions and rootstocks during a six-month period (June to Nov.) (Table 2). However, there was a significant difference in trunk diameter among the scions × rootstock combinations (Table 3). Rootstocks grafted with the C11 scion were tallest (1.3 ± 0.2 m) but similar to TL scion trees (1.2 ± 0.2 m); TL scion trees were similar in height to C14 scion trees (1.1 ± 0.2 m) but slightly taller than C33 scion trees. Trees grafted with C33 were significantly smaller than all other trees. All scions grafted to CM (1.4 ± 0.2 m) and US-802 (1.1 ± 0.2 m) rootstocks were significantly taller than scions grafted to SO+50-7 rootstock, with US-897 and US-942 not significantly different from all other rootstocks. In contrast, rootstock diameter was significantly different among the scion × rootstock combinations (Table 3). The trunk diameters of the scion–rootstock combinations of C11/CM, C11/US-802, TL/US-802 and TL/CM were larger (415 mm–464 mm) than C14/SO+50-7, C14/US-942, C14/US-897, C11/US-897, and C33 grafted to US-897, US-942, and SO+50-7 (304 mm–364 mm). C11/CM trees had the largest trunk dia. (464 mm) and TL/US-942 the smallest diameter (303 mm).

Table 2. Change in tree height of four lime scions and five rootstocks after a 5-month period (June–Nov.).[z]

Selection	Tree height ± SD (m)
Scion	
C11	1.3 ± 0.2 a
TL	1.2 ± 0.2 ab
C14	1.1 ± 0.2 bc
C33	1.1 ± 0.2 c
Rootstock	
CM	1.4 ± 0.2 a
US-802	1.1 ± 0.2 a
US-897	1.1 ± 0.2 ab
US-942	1.1 ± 0.2 ab
SO+50-7	1.1 ± 0.2 b

Levels not connected by the same letter are significantly different.

Table 3. Change in tree trunk diameter of four lime scions and five rootstocks during a six-month period (June-Dec.).z

Scion–rootstock	Trunk dia. ± SD (mm)
C11/CM	46 ± 4 a
TL/US-802	45 ± 5 ab
C11/US-802	42 ± 6 abc
TL/CM	43 ± 4 abc
C14/CM	41 ± 5 abcd
C33/CM	44 ± 4 abcde
C14/US-802	38 ± 3 bcdef
C33/US-802	37 ± 4 cdefg
TL/SO+50-7	36 ± 4 defg
C11/US-942	32 ± 2 defg
C14/SO+50-7	33 ± 4 fg
C14/US-942	31 ± 4 fg
C11/SO+50-7	32 ± 4 fg
TL/US-897	31 ± 5 fg
C11/US-897	31 ± 4 fg
C33/US-897	33 ± 4 fg
C33/US-942	30 ± 4 efg
C33/SO+50-7	33 ± 4 fg
C14/US-897	31 ± 4 fg
TL/US-942	30 ± 3 g

Levels not connected by the same letter are significantly different.

Flowering. One C14/US-802 tree flowered during Dec. 2017 (Table 4). In general, most trees did not commence flowering until late Jan. 2018. Overall C11 and TL scions had more flowering than C14 and C33 scions. In general, scions grafted to CM, SO+50-7, US-802 and US-897 rootstocks had more flowering than scions grafted to US-942 rootstocks. By the end of Jan. most scion–rootstock combinations were flowering (Table 4). Only C33/CM, C33/US-802 and C33/US-942 had no trees flowering.

Rootstock sprouting. Rootstock sprouting was periodically a serious problem for all scions but continued at a higher percentage for trees grafted with TL and C11 even after plastic trunk covers were installed at the end of July (Table 5). From April to July, rootstock sprouting was more problematic for scions grafted to US-802, US-897, and US-942 rootstocks compared to CM and SO+50-7 rootstocks (Table 6). After trunk covers were installed, rootstock sprouting declined significantly for all scion–rootstock combinations except C11/US-802, C33/US-802, TL/US-802, C11/US-897, TL/US-897 and TL/US-942.

Leaf nutrient content. There was no significant difference among scions, rootstocks and scion × rootstock combinations for leaf phosphorus (P; 0.26%), potassium (K, 2.29%), and zinc (Zn, 26 ppm). There was no scion × rootstock interaction for calcium (Ca), iron (Fe), and manganese (Mn) but there were significant differences among scions and rootstocks for Ca and

Table 4. Effect of scion and rootstock on the percentage of trees with flowers.

Selection	31 Oct. 2017	10 Dec. 2017	8 Jan. 2018	29 Jan. 2018	9 Mar. 2018
Scion					
TL	0	6.76	10.81	28.38	22.97
Cl1	0	1.82	3.64	43.64	38.18
C14	1.82	7.27	9.09	21.82	10.91
C33	0	0	0	2.08	2.08
Rootstock					
CM	0	0	11.11	37.78	26.67
SO+50-7	0	4.41	7.35	25.00	16.18
US-802	2.38	4.76	4.76	16.67	23.81
US-897	0	2.38	4.76	26.19	16.67
US-942	0	11.43	2.86	17.14	14.29
Scion–rootstock					
C11/CM	0.0	0.0	0.0	6.7	50.0
C11/US-802	0.0	0.0	0.0	40.0	50.0
C11/US-897	0.0	0.0	9.1	36.4	36.4
C11/US-942	0.0	9.1	9.1	27.3	27.3
C11/SO+50-7	0.0	0.0	0.0	45.5	27.3
C14/CM	0.0	0.0	25.0	41.7	25.0
C14/US-802	8.3	8.3	8.3	8.3	16.7
C14/US-897	0.0	0.0	0.0	9.1	9.1
C14/US-942	0.0	25.0	0.0	12.5	0.0
C14/SO+50-7	0.0	8.3	8.3	33.3	0.0
C33/CM	0.0	0.0	0.0	0.0	0.0
C33/US-802	0.0	0.0	0.0	0.0	0.0
C33/US-897	0.0	0.0	0.0	10.0	0.0
C33/US-942	0.0	0.0	0.0	0.0	0.0
C33/SO+50-7	0.0	0.0	0.0	0.0	8.3
TL/CM	0.0	0.0	18.2	36.4	27.3
TL/US-802	0.0	10.0	10.0	20.0	30.0
TL/US-897	0.0	10.0	10.0	50.0	20.0
TL/US-942	0.0	10.0	0.0	20.0	20.0
TL/SO+50-7	0.0	6.1	12.1	24.2	21.2

Table 5. Effect of scion and rootstock on the percentage of trees with rootstock sprouts by scion and rootstock.[z]

	Scion				
Date	TL	C11	C14	C33	
5 Apr. 2017	50.0	50.1	41.8	35.4	
12 Apr. 2017	25.7	10.9	10.9	2.1	
15 May 2017	32.4	7.3	7.3	31.3	
9 June 2017	44.6	54.5	18.2	43.8	
27 June 2017	14.9	10.9	5.5	4.2	
14 July 2017	17.8	38.2	10.9	33.3	
25 Oct. 2017	6.7	12.7	3.6	8.3	
12 Dec. 2017	12.1	14.6	1.8	8.3	
28 Mar. 2018	13.5	9.1	0.0	0.0	
	Rootstock				
Date	CM	SO+50-7	US-802	US-897	US-942
5 Apr. 2017	13.3	41.2	64.3	50.0	65.7
12 Apr. 2017	4.4	20.6	19.1	4.8	17.1
15 May 2017	0.0	20.6	38.1	21.4	22.9
9 June 2017	11.1	52.9	50.0	33.3	51.4
27 June 2017	2.2	58.8	21.4	11.9	8.6
14 July 2017	15.6	19.1	23.8	31.7	37.1
25 Oct. 2017	6.7	1.5	9.5	19.0	5.7
12 Dec. 2017	0.0	2.9	28.6	7.1	14.3
28 Mar. 2018	0.0	5.9	11.9	4.8	11.4

[z]Trees were outfitted with plastic trunk covers during this time-period in an effort to eliminate rootstock trunk sprouting.

Mn and rootstocks for Fe (Table 7). Scions TL and C33 had significantly higher Ca leaf content than C14 scions with Ca content of C11 scions similar to all scions. Scions C11 and TL had significantly greater leaf Mn content than C14 scions. Trees on rootstock US-897 had significantly more leaf Ca than trees on US-942 rootstock whereas scions grafted to CM, SO+50-7 and US-802 rootstocks had similar leaf Ca content. Trees grafted to CM, US-897, and US947 rootstocks had significantly greater leaf Mn content that trees grafted to SO+50-7 rootstock. Trees grafted to US-802 rootstock had intermediate leaf Mn content. Leaf N, Mg, S. Cu and B leaf content varied significantly among scion × rootstock combinations (Table 8). Leaf N content was highest in C14 scions on US-802, US-897 US-942, and TL/SO+50-7 rootstocks compared to C11/SO+50-7. Magnesium leaf content was highest in C14/US-897 and TL/SO+50-7 compared to C11/CM, C110US-942, C14/CM, C14/SO+50-7, TL/US-897 and TL-942. Sulfur leaf content was highest in C11/CM and lowest in C14/SO+50-7. Copper leaf content was similar among most scion × rootstock combinations with C33/SO+50-7 the highest and C14/US-942 the lowest. C33/US-897 had the highest B leaf content compared to most C14/CM, C14/SO+50-7, TL/CM, TL/SO+50-7 and TL/US-942.

Insect detections. ACP monitoring and management started at planting. The monitoring program involves weekly visual inspections to ~70% of the trees including all rootstock–scion combinations for presence of ACP and other pests. The first ACP detection was on 10 May 2017 and since then ACPs have been detected infesting 60 trees. Scion/Rootstock combinations did make a difference on the number of psyllid finds through the year. The highest number of finds on any scion–rootstock combination was 11 on TL/CM trees (Table 9). There were no psyllids found on three combinations (C33/SO+50-7; C33/US-942; C-14-CM); two of these last three had the same scion (C33). Most of the psyllids found were still at the egg stage (56%), followed by shoots with nymphs (15%) and nymphs + eggs (15%), only adults (10%), adults + nymphs (2%) and only one shoot with all of the stages (2%). Of the trees found infested with psyllids, most were found to be infested only one time (83%), six trees had two infestations (10%), three had three (5%) and only one tree was observed to have psyllids five times (2%). Four of those five times was continuous over a six-week period. More psyllids were found on the north and south edges of the grove, however they were fairly evenly distributed between east and west. In general, no significant pest problems have been observed and the pest management program

Table 6. Effect of scion × rootstock combination on the percentage of trees with rootstock sprouts.

	Date								
Scion–rootstock	4-5-17	4-12-17	5-15-17	6-9-17	6-27-17	7-14-17	10-25-17[z]	12-12-17[z]	3-28-18[z]
C11/CM	8.3	8.3	0.0	8.3	0	25.0	16.7	0.0	0.0
C14/CM	8.3	0.0	0.0	8.3	0.0	0.0	0.0	0.0	0.0
C33/CM	10.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0
TL/CM	27.3	9.1	0.0	27.3	9.1	18.2	9.1	0.0	0.0
C11/US-802	80.0	20.0	20.0	50.0	20.0	20.0	1.1	40.0	40.0
C14/US-802	58.3	16.7	8.3	25.0	16.7	16.7	0.0	8.3	0.0
C33/US-802	40.0	0.0	70.0	70.0	10.0	30.0	30.0	40.0	0.0
TL/US-802	80.0	40.0	60.0	60.0	40.0	30.0	10.0	30.0	10.0
C11/US-897	63.6	0.0	0.0	54.6	27.3	36.4	36.4	18.2	9.1
C14/US-897	27.3	9.1	9.1	27.3	0.0	18.2	0.0	0.0	0.0
C33/US-897	50.0	0.0	30.0	20.0	0.0	50.0	10.0	0.0	0.0
TL/US-897	60.0	10.0	50.0	30.0	20.0	22.2	30.0	10.0	10.0
C11/US-942	72.3	18.2	18.2	9.1	9.1	81.8	9.1	18.1	0.0
C14/US-942	62.5	37.5	0.0	12.5	0.0	12.5	12.5	0.0	0.0
C33/US-942	33.6	0.0	33.6	33.6	0.0	16.7	0.0	0.0	0.0
TL/US-942	80.0	10.0	40.0	50.0	20.0	20.0	0.0	30.0	40.0
C11/SO+50-7	36.4	9.1	0.0	72.7	0.0	27.3	0.0	0.0	0.0
C14/SO+50-7	58.3	0.0	16.7	16.7	8.3	8.3	8.3	0.0	0.0
C33/SO+50-7	41.7	8.3	25.0	83.3	8.3	41.7	0.0	0.0	0.0
TL/SO+50-7	36.4	36.4	27.3	48.5	6.1	12.1	0.0	6.1	12.1

[z]Trees were outfitted with plastic trunk covers during this time-period in an effort to eliminate rootstock trunk sprouting.

Table 7. Mean leaf calcium, manganese, and iron content of four scion and five rootstock combinations seven months after planting.

Selection	Calcium (% ± SD[z])	Manganese (ppm ± SD)	Iron (ppm ± SD)
Scion			
C11	3.35 ± 0.97 ab	34 ± 5 a	--
C14	2.70 ± 0.49 b	25 ± 8 c	--
C33	3.54 ± 1.04 a	26 ± 7 bc	--
TL	3.76 ± 1.15 a	31 ± 6 ab	--
Rootstock			
CM	3.11 ± 0.81 ab	31 ± 6 a	191 ± 30
SO+50-7	3.34 ± 1.11 ab	24 ± 7 b	192 ± 24
US-802	3.51 ± 0.75 ab	28 ± 9 ab	157 ± 43
US-897	3.89 ± 1.22 a	31 ± 5 a	166 ± 23
US-942	2.83 ± 0.87 b	31 ± 8 a	180 ± 42

[z]Levels not connected by the same letter are significantly different.

has been effective. Citrus leaf miner is the most common pest followed by root weevils, bagworms, and other Lepidopterans, but all of these have been maintained at low levels and do not threaten tree health. There were some beneficial arthropods found such as spiders, ladybugs, hymenopteran parasitoids, lacewings and Zelus assassin bugs.

Citrus greening status. All 233 surviving trees, representing the different scions × rootstock combinations, were screened for citrus greening. Pathogen screening was conducted on five occasions, including a pre-trial screening before saplings were set on the ground (Mar. 2017, June 2017, Sept. 2017, Dec. 2017 and Mar. 2018). All trees were found negative for the pathogen in the first four screenings; however, the fifth screening detected 23 HLB positive samples (Table 10). The first positive HLB trees were detected in the first two western rows and last six eastern rows however within rows there appeared to be no trend based on north-south location nor on scion–rootstock combination (Table 11). However, there were 8, 11, 4, and 0 HLB positive trees on C11, C14, TL and C33 rootstocks, respectively and there were 6, 4, 6, 6, and 1HLB positive trees with CM, SO+50-7, US8032, US-897, and US-942 scions, respectively. Overall, C14/US-802 had the most HLB positive trees with three HLB positive C11/CM and C14/CM trees (Table 11). Two C11/SO+50-7, C11/US-897, C14/US-897 and TL/US-897 trees were HLB positive and on C11/US-802, C14/SO+50-7, C14/US-942, TL/SO+50-7 and TL/US-802 trees were positive. No C11/US-942, C33-any rootstock, TL/CM, and TL/US-942 were HLB positive. From the 60 ACP samples collected from the weekly pest survey (42 eggs, 20 nymphs, and 4 adults), only six were found positive for the pathogen.

Economic analysis. To assess the costs and returns associated with the lime scion × rootstock combinations, a preliminary crop enterprise budget was developed; information collected for year 1 is shown in Table 12. Assumptions included the grower already owned the land or that the land could be rented for $1236/ha ($500/acre) (prevailing land rental rate in Miami-Dade County). In terms of machinery and equipment investments, the acquisition of a low volume irrigation system was included in the budget. Purchase of other capital equipment items were not considered in the proposed budget, it was assumed that the grower already owned the equipment or could contract a grove management services provider for activities such as pest control applications. The establishment costs for the first year amounted to about $25,229/ha ($10,210/acre) (Table 12). Of this amount, land preparation and planting material totaled $12,326/ha ($4,988/A) (48.85% of total cost for year 1). Trees were spaced at 4.5 m (15 ft) × 6.1 m (20 ft); a plant density of 358 trees/ha (145 trees/acre). The cost of orchard inputs such as pest control chemicals and fertilizers, labor costs for grove operations, and irrigation water and electricity charges amount to $7,349/ha ($2,974/A) (29.13% of total cost for year 1). Total variable cost for year 1 is about $21,696/ha ($8,780/A) (85.99% of the total cost for year 1) while fixed costs, which include depreciation for the irrigation system, mainline and pump, and other fixed costs were estimated at $3534/ha ($1,430/A) (14.01% of the total cost for year 1). It is anticipated that the grove will require another 3–5 years before the trees are considered fully mature.

Table 8. Mean leaf nitrogen, magnesium, sulfur, sodium, copper, and boron content of 20 scion × rootstock combinations seven months after planting.

Scion–rootstock	Nitrogen (% ± SD)	Magnesium (% ± SD)	Sulfur (% ± SD)	Copper (ppm ± SD)	Boron (ppm ± SD)
C11/CM	2.48 ± 0.35 ab[z]	0.17 ± 0.03 ef	0.53 ± 0.06 a	25 ± 5 cd	15 ± 6 bc
C11/SO+50-7	1.47 ± 0.34 b	0.26 ± 0.05 abcde	0.42 ± 0.04 abc	24 ± 2 cd	33 ± 17 abc
C11/US-802	1.74 ± 0.23 ab	0.22 ± 0.03 abcdef	0.44 ± 0.05 abc	27 ± 5 cd	26 ± 17 abc
C11/US-897	2.16 ± 0.41 ab	0.23 ± 0.07 abcdef	0.40 ± 0.01 abc	25 ± 3 abcd	29 ± 21 abc
C11/US-942	1.85 ± 0.31 ab	0.17 ± 0.03 ef	0.37 ± 0.10 abc	25 ± 3 cd	13 ± 3 bc
C14/CM	1.85 ± 0.45 ab	0.14 ± 0.02 f	0.29 ± 0.03 bc	24 ± 4 abcd	9 ± 3 c
C14/SO+50-7	2.39 ± 0.44 ab	0.18 ± 0.03 cdef	0.36 ± 0.03 c	22 ± 3 cd	10 ± 6 c
C14/US-802	2.78 ± 0.46 a	0.28 ± 0.04 abcde	0.35 ± 0.10 abc	23 ± 1 cd	14 ± 7 bc
C14/US-897	2.76 ± 0.25 a	0.31 ± 0.03 a	0.42 ± 0.05 abc	21 ± 2 cd	26 ± 16 abc
C14/US-942	2.81 ± 0.14 a	0.29 ± 0.02 abcdef	0.40 ± 0.03 abc	19 ± 3 d	52 ± 20 ab
C33/CM	2.49 ± 0.56 ab	0.30 ± 0.01 ab	0.44 ± 0.06 abc	34 ± 6 bc	24 ± 8 abc
C33/SO+50-7	2.34 ± 0.36 ab	0.29 ± 0.03 abcd	0.23 ± 0.06 abc	51 ± 7 a	26 ± 21 abc
C33/US-802	2.07 ± 0.34 ab	0.26 ± 0.02 abcde	0.29 ± 0.06 bc	33 ± 12 bcd	22 ± 10 abc
C33/US-897	2.50 ± 0.36 ab	0.26 ± 0.03 abcde	0.33 ± 0.05 abc	32 ± 9 bcd	57 ± 14 a
C33/US-942	2.35 ± 0.13 ab	0.21 ± 0.03 abcdef	0.30 ± 0.06 bc	42 ± 5 ab	26 ± 16 abc
TL/CM	2.34 ± 0.55 ab	0.26 ± 0.02 abcde	0.33 ± 0.05 abc	26 ± 5 cd	10 ± 2 c
TL/SO+50-7	2.86 ± 0.25 a	0.31 ± 0.02 a	0.47 ± 0.09 ab	22 ± 3 cd	9 ± 4 c
TL/US-802	1.73 ± 0.51 ab	0.23 ± 0.08 abcdef	0.37 ± 0.17 abc	20 ± 3 cd	29 ± 19 abc
TL/US-897	1.87 ± 0.40 ab	0.19 ± 0.03 bcdef	0.44 ± 0.04 abc	24 ± 2 cd	18 ± 8 abc
TL/US-942	2.41 ± 0.29 ab	0.18 ± 0.00 def	0.50 ± 0.07 ab	28 ± 3 bcd	8 ± 3 c

[z]Means having different letters are significantly different.

Table 9. Number of ACP finds by scion–rootstock combination, March 2018.

Scion × rootstock combination	Number ACP finds
C11/CM	2
C11/SO+50-7	1
C11/US-802	6
C11/US-897	2
C11/US-942	4
C14/CM	5
C14/SO+50-7	1
C14/US-802	1
C14/US-897	2
C33/CM	2
C33/US-802	4
C33/US-897	2
TL/CM	11
TL/SO+50-7	4
TL/US-802	6
TL/US-897	4
TL/US-942	3

Table 10. Number of HLB positive trees by rootstock, scion and scion–rootstock combination, Mar. 2018.

Selection	HLB positive (no.)
Rootstock	
C11	8
C14	11
TL	4
C33	0
Scion	
CM	6
SO+50-7	4
US-802	6
US-897	6
US-942	1
Scion × rootstock combination	
C11/CM	3
C11/SO+50-7	2
C11/US-802	1
C11/US-897	2
C11/US-942	0
C14/CM	3
C14/SO+50-7	1
C14/US-802	4
C14/US-897	2
C14/US-942	1
C33/CM	0
C33/SO+50-7	0
C33/US-802	0
C33/US-897	0
C33/US-942	0
TL/CM	0
TL/SO+50-7	1
TL/US-802	1
TL/US-897	2
TL/US-942	0

Table 11. Tree scion × rootstock combinations and grove locations positive for HLB in Mar. 2018 screening.[z]

Row no.	Tree no.	Scion × rootstock
1	11	TL/US-897
1	12	C11/SO+50-7
2	1	C11/US-897
2	2	C14/US-897
2	3	C14/SO+50-7
2	5	C14/CM
2	7	C14/US-897
10	3	C11/CM
10	16	C14/US-802
11	2	C11/US-802
11	3	TL/US-802
11	6	C14/US-942
11	11	C14/US-802
11	13	C11/CM
11	14	C11/SO+50-7
12	14	TL/SO+50-7
12	16	C14/US-802
13	11	C14/US-802
14	2	C11/US-897
14	5	C11/CM
14	6	C14/CM
14	14	C14/CM
15	15	TL/US-897

[z]Row 1 east; row 15 west.

Discussion

In general, the four scion and five rootstock combination trees established well and recovered from Hurricane Irma. The death of six of 239 trees may be attributed to storm damage. Preliminarily, rootstocks grafted to C11 and TL scions and CM and US-897 rootstocks were the tallest trees eight months after planting (Table 2). However, with the detection of citrus greening and its inevitable spread, this may change. In general, trunk diameters varied greatly among scions x rootstock combinations with trees grafted to scions C11 and TL grafted on CM and US-802 rootstocks having the largest trunk diameters nine months after planting (Table 3).

In general, flowering of many scion–rootstock combinations commenced by the end of Jan., 2018. Exceptions were all rootstocks grafted to C-33 scions (Table 4). In general, rootstock sprouting was a significant problem for all rootstocks except CM (Tables 5 and 6). Scion × rootstock combination had no significant effect on leaf Ca, Mn, and Fe content with C11 and TL scions having the highest Ca and Mn leaf content and trees grafted to SO+50-7 the lowest (Table 5). There were significant scion × rootstock effects on the leaf content of N, Mg, S, Cu and B (Table 6). In general, leaf N (1.73-2.81%) and P (0.26%) content were within the range reported previously (Campbell and Orth, 1968). Leaf zinc concentrations (26 ppm) were at the low end of the range reported for oranges (Koo et al., 1984).

The experimental plot has been under constant ACP pressure. ACP findings seem to be associated more with the location in the grove more than with the scion–rootstock combination. ACP infestations were primarily found on the north and south edges of the grove. However, the chemical control strategy effectively disrupted the ACP cycle. Trees found infested with ACP during

Table 12. Cost and returns per acre of establishing a 1-acre lime planting in Miami-Dade County, FL.

Variable costs ($/acre):	Year 1[z]
Establishment	
Land preparation[y]	2,240.00
Planting material (trees, labor and materials)	2,747.75
Orchard activities	
Chemicals[x]	627.13
Fertilizer[w]	219.11
Labor[v]	1,711.90
Irrigation water & electric charge	415.80
Maintenance and repairs	
Maintenance & repair	300.00
Fuel & lube	100.00
Other variable costs	
Crop Insurance	
Interest (5% of variable costs)	418.08
Total Variable Costs	8,779.77
Fixed Costs ($/acre):	
Depreciation	
Irrigation system	150.00
Machinery, equipment, & building	100.00
Mainline & pump	45.00
Interest (5%)	
Irrigation system	140.00
Machinery, equipment, & building	0.00
Mainline & pump	45.00
Other fixed costs	
Land rental	500.00
Miscellaneous supplies	100.00
Land & property taxes	50.00
Liability insurance	100.00
Management cost	200.00
Total Fixed Costs	1,430.00
TOTAL COSTS	10,209.77
ESTIMATED NET RETURNS	(10,209.77)
Accumulated Establishment Costs	10,209.77

[z]The full production year is representative of all the remaining years the orchard is in full production (Year 8 to Year 20).

yLand preparation includes clearing previous orchard.

xIncludes the cost of materials only.

wIncludes the cost of materials only.

vIncludes the labor cost for chemicals application, fertilizers, application, mowing and weed removal, and pruning.

one monitoring event usually were not infested during the following monitoring event suggesting that the trees do not have established ACP populations but are subject to constant ACP reintroductions. In general, no other significant pest problems have been observed. Citrus leaf miner is the most common pest followed by aphids, bagworms and other Lepidopterans, but all of these are maintained at low levels and do not threaten tree health.

All trees were free of HLB for about 12 months after planting (Table 10). However, by March 2018 only trees grafted to C33 scions and scion–rootstock combinations TL/CM, TL/US-942 and C11/US-942 were HLB-free. Due to the proximity of the experimental plot to a source of the vector and the pathogen (see Materials and Methods), we expected the experimental plots to become infected with the disease fairly quickly. Infection was only confirmed in the 5th screening conducted in March 2018, one year after the trees were planted. However, the vector (at different life stages) had been found in the experimental plot since 10 May 2017. The lack of overlap between HLB positive trees and the presence of vector evidence could be explained by the disease epidemiology. Studies have shown that acquisition of the pathogen by ACP adults (from host to psyllid) can take from 15 min to 5 hrs., but transmission (from psyllid to host) requires a latent period of up to 35 days, depending on the developmental stage (Ammar E-D et al., 2016). In addition, systematic colonization of the host can take 30 or more days (Hilf and Luo, 2018) and therefore the pathogen may not be evenly distributed inside the host during the first stages of infection. Subsequently, we do not expect a perfect overlap between surveys (pest and pathogen) in the first year of the experiment and those which will be taken at the end of the project.

Even though, it is too early to confidently evaluate the difference in infection timing (a component of the tolerance evaluation) of the different rootstock × scion combinations, our results from the March 2018 screening do unveil some patterns. For instance, combinations with a C14 scion had the highest number of positive samples for the HLB (Table 11). On the other hand, screening results for the different scions were more evenly distributed with six samples being the highest number of positives for CM, US-802, and US-897 rootstocks. The scion × rootstock combination C14/US-802 had the highest number of positive samples (4). We predict that all the experimental trees will be infected with HLB at some point during the next sampling year but not all combinations will manifest the same degree of symptoms. We are expecting to find a rootstock x scion combination that can maintain an acceptable yield and quality under citrus greening pressure.

Some clarifications about the assumptions made for the crop budget enterprise are important; the proposed budget does not take into consideration all the expenses associated to start a fresh lime orchard from scratch. Rather, fresh lime production is being considered as an alternative crop for existing fruit growers in Miami-Dade County; therefore, the budget does not account for land, machinery and equipment acquisition. It is assumed that growers own the machinery and equipment needed or they may contract a grove management services provider for activities such as pest control applications. In terms of equipment, the proposed budget only accounts for the purchase and installation of an irrigation system. Establishment costs for the first year amounted to about $25,229/ha ($10,210/acre) (Table 12). Total variable cost for year 1 included land preparation and planting material, and orchard inputs; variable costs were $21,5780/ha ($8,780/acre) (85.99% of the total cost for year 1, while fixed costs, which include depreciation and other fixed costs were estimated at $3,515/ha ($1,430/acre) (14.01% of the total cost for year 1). Given that the grove is on the first year of the establishment, the trees are not bearing fruit; therefore, there were not revenues from the sale of fruit.

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