

A Survey of the Commercial Application of Bactericides in the Florida Citrus Industry

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Overview

At the direction of the Citrus Research and Development Foundation's (CRDF) Commercial Product and Delivery committee, the CRDF staff began an extensive survey of the commercial use of bactericides in Florida citrus. Four variables were assessed, viz. fruit yield (kg/tree), fruit drop (%), visual disease index score (DI) and PCR analysis of HLB infection (i.e. cycle threshold count; CT), and. Data were collected over two production seasons (2016/2017 and 2017/2018).

Volunteer grower cooperators within the citrus industry were identified and informed of the proposed project details. CRDF staff approached growers in the three defined production regions of the state; Central Ridge, Indian River, and Southwest Florida. CRDF staff collected data from a total of 48 cooperator locations (Table 1). Participating sites represent multiple growers, in some cases multiple sites per grower, along with a variety of rootstocks, scions and a range of tree ages.

Survey Methods

Trial Layout

Two trial design layouts were implemented at the discretion of the cooperator location (single and double block layout; Figure 1). Choice of bactericide products, timing, dosage, number of applications, and tank mix protocol including choice of adjuvants, were left to the discretion of each cooperator. Thus, within the label guidelines, the bactericide "treatment" category was not standardized and could have been variable from location to location.

For the single block design, a selected number of rows (typically 4 rows) were identified to remain untreated with bactericides (Control). The remainder of the rows in the block received bactericide application. Twenty sentinel trees for monitoring and data collection were identified in the two middle rows of the four untreated rows. In addition, twenty sentinel trees were identified in two nearby rows which received bactericide applications (Treated). The treated rows with the twenty sentinel trees, were within ten rows of the four untreated rows (Figure 1). For the two block design, two adjacent blocks with same scion x rootstock combination and planting date were selected. One block received bactericide applications (Treated) and the other block remained untreated (Control; Figure 1). Twenty sentinel trees in both blocks were selected for monitoring and data collection. Within both trial designs, the sentinel trees were selected for uniformity by visual evaluation. The twenty trees in each category (treated or untreated) were set up in four groups of five trees. The location of the each grouping was determined by the number of viable trees available and the characteristics of the block. Efforts were made to place each group of treated trees within the same position in the row as the corresponding untreated group.

Data Collection

Yield was calculated by the total weight of fruit harvested from individual sentinel trees. Monthly fruit drop counts began in August for grapefruit and Hamlin trials, October for Midsweet trials, and December for Valencia trials. Regardless of scion, the fruit drop counts continued on a monthly basis until the trials were harvested. The weight of a fifty-fruit subsample was measured from each sentinel tree. The total yield per tree was compared to the weight of the fifty-fruit subsample to estimate the

number of fruit per tree. The total number of dropped fruit was calculated from the monthly fruit drop evaluation. Using the estimated number of fruit per tree and the total number of dropped fruit, the percentage of fruit dropped prior to harvest was calculated.

Evaluation of disease severity (Disease Index Score; DI) and PCR assay results (Cycle Threshold Value; CT) were collected throughout the two growing seasons. Within the growing season, most locations had multiple measurements, which were averaged for final analysis. Disease severity (DI) was assessed by a protocol involving subjective scoring of individual trees. The tree was assessed on each side with sides divided into four quadrants. Within individual canopy quadrant, a score was entered based on visual disease severity (0 to 5 scale). A score of 0 would indicate there are no visual HLB symptoms and a score of 5 would indicate symptoms throughout. Results were reported as the sum of the scores for the eight quadrants. The maximum score one side of a tree can receive is 20 and the maximum score the entire tree could receive is 40 (totally symptomatic).

Real time PCR analysis for HLB infection was conducted by a commercial laboratory (Southern Gardens Diagnostic Laboratory, Clewiston, FL). A positive reaction is detected by accumulation of a fluorescent signal. The CT value (cycle threshold) is defined as the number of cycles required to achieve a fluorescent signal, therefore, a lower CT value would indicate a greater level of infection. In the Florida citrus industry, a CT value < 32 is generally considered an infected tree, although any CT value < 40 indicates some level of infection.

Statistical Analysis

Groups of 5 trees (replicate plot) at each trial site were nested within treatments (control vs. treated); hence the experiment design was a completely randomized design. The analysis was performed on plot averages. For trial sites with two-year data, trial site, treatment, year and all interactions served as fixed effects. For sites for which there were only single-year data available (i.e. only Year 1 or Year 2) the model was simplified to include trial site, treatment, and the two-way interaction as fixed effects. To account for potential correlation of the residuals for the two-year data sets (repeated measures design), we modeled the residual variance using a compound symmetric model (CSH) with heterogeneous variances. For two time points, these models are equivalent to the unstructured variance (UN) and first order autoregressive (ARH(1)) models. Based on the AICC fit statistic, the CSH did not offer any improvement over the split-plot in time model, which assumes independence of residuals. Least squares means were calculated and compared using simple t-tests. No adjustments for multiple comparisons were made based on the arguments put forth by Saville (2018); this lack of adjustments in essence makes it easier to detect differences among treatments.

Results

Fruit Yield and Fruit Drop – Two Year Data

There were 16 locations with yield data from both harvest seasons (Table 2). For these locations, there was a tendency ($P = 0.11$) for bactericide application to reduce fruit yield (63 vs. 60 kg/tree for Control and Treated trees, respectively; $SEM = 1.3$); however, there was a location x treatment x year interaction ($P = 0.004$; Table 2). Grapefruit 3/Year1 and Grapefruit 7/Year 2 (Indian River) experienced an average of 34% greater ($P \leq 0.061$) yield when treated with bactericides. In contrast, Hamlin 4/both years (Indian River) and Valencia 38/Year1 (Ridge) experienced an average of 18% less ($P \leq 0.082$) yield when treated with bactericides.

For fruit drop, there were 11 locations with fruit drop data from both harvest seasons (Table 3). For these locations, there was no effect ($P = 0.84$) of bactericide application on fruit drop (33 vs. 33 % for Control and Treated trees, respectively; $SEM = 1.0$); however, there was a location x treatment x year interaction ($P = 0.009$; Table 3). Grapefruit 1 and 3 (Year 1; Indian River) experienced an average of 28% less ($P \leq 0.024$) fruit drop when treated with bactericides. In contrast, in Year 2, Grapefruit 1 (Indian River) experienced a 23% greater ($P = 0.012$) fruit drop when treated with bactericides.

Yield - Single Year Data

There were 15 locations with yield data from Year 1 only (Table 4). For these locations, bactericide application resulted in reduced ($P = 0.035$) fruit yield (80 vs. 72 kg/tree for Control and Treated trees, respectively; $SEM = 2.4$); however, there was a location x treatment interaction ($P = 0.036$; Table 4). Grapefruit 5 (Indian River) and Valencia 22 (Southwest) experienced an average of 41% less ($P \leq 0.069$) yield when treated with bactericides. In contrast, Midsweet 2 (Southwest) experienced a 24% greater ($P = 0.050$) yield when treated with bactericides.

There were 12 locations with yield data from Year 2 only (data not shown). For these locations, bactericide application had no impact ($P = 0.761$) on fruit yield (60 vs. 61 kg/tree for Control and Treated trees, respectively; $SEM = 1.8$). There was no location x treatment interaction ($P = 0.153$).

Fruit Drop Single Year Data

There were 15 locations with fruit drop data from Year 1 only (Table 5). For these locations, bactericide application resulted in greater ($P = 0.021$) fruit drop (16 vs. 18 % for Control and Treated trees, respectively; $SEM = 0.7$); however, there was a location x treatment interaction ($P = 0.037$; Table 5). Hamlin 8 (Southwest), Midsweet 3 (Southwest), and Valencia 22 (Southwest) experienced an average of 40% greater ($P \leq 0.083$) fruit drop when treated with bactericides.

There were 17 locations with fruit drop data from Year 2 only (Table 6). For these locations, bactericide application had no impact ($P = 0.850$) on fruit drop (53 vs. 53 % for Control and Treated trees, respectively; $SEM = 0.7$); however, there was a location x treatment interaction ($P = 0.009$; Table 6). Valencia 29 (Ridge) and Valencia 32 (Southwest) experienced an average of 19% greater ($P \leq 0.051$) fruit drop when treated with bactericides. In contrast, Valencia 28 (Ridge) experienced a 17 % lesser ($P = 0.037$) fruit drop when treated with bactericides.

Tree Disease Index Score

Disease Index (DI) scoring data were collected from all locations in both years (Table 7). Overall, bactericide application reduced ($P = 0.009$) tree health score (22.0 vs. 22.2 for Control and Treated trees, respectively; $SEM = 0.06$); however, there was a location x treatment x year interaction ($P = 0.002$; Table 7). A total of 96 entries are represented in this dataset (48 locations x 2 harvest seasons). Within this interaction, DI was impacted by bactericide application at 20 locations (Table 7). Among these, 14 locations experienced an average of 8.4% worsening in tree DI score, while 6 locations experienced an average of 7.1% improvement in tree DI score as a result of bactericide application. Individual results on the remaining 76 non-impacted entries is provided in Appendix A.

PCR Assay; Cycle Threshold (CT Value)

There were 39 locations with CT Values from both harvest seasons. For these locations, there was no main effect ($P = 0.62$) of bactericide application on CT value (29.4 vs. 29.3 for Control and Treated trees, respectively; $SEM = 0.10$); however, there was a location x treatment x year interaction ($P = 0.07$).

This interaction revealed both advantages and disadvantages relative to bactericide application and subsequent PCR measure of tree infectivity (Table 8). In Year 1, 5 locations revealed an advantage of bactericide application, while 6 locations experienced a disadvantage. In Year 2, no locations experienced an advantage of bactericide application, while 2 locations experienced a disadvantage.

There were 9 locations with CT Values on a single year only with no impact ($P \geq 0.15$) of bactericide application on CT value (32.5 vs. 33.6 for Control and Treated trees, respectively, in Year 1, and 28.6 and 28.2 for Control and Treated trees, respectively, in Year 2 (SEM = 0.55 and 0.29).

Literature Cited

Saville, D.J. 2018. Chapter 5: Multiple Comparison Procedures: The Ins and Outs. In: B. Glaz and K. M. Yeater, editors, Applied Statistics in Agricultural, Biological, and Environmental Sciences. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Inc., Madison, WI. p. 85-106.

Table 1. Grower cooperator locations and dataset contribution.

Region	Trial Site Code	Planting date	Rootstock	Scion	Yield Season 1	Yield Season 2	Drop Season 1	Drop Season 2	DI Season 1	DI Season 2	CT Season 1	CT Season 2
River	Grapefruit 1	2013	Sour	RayRuby	X	X	X	X	X	X	X	X
River	Grapefruit 3	2013	Sour	RayRuby	X	X	X	X	X	X	X	X
River	Grapefruit 5	1989	Swingle	Flame	X		X		X	X	X	X
River	Grapefruit 7	1989	Swingle	Flame	X	X	X	X	X	X	X	X
River	Grapefruit 8	1989	Swingle	Flame					X	X	X	X
SW	Hamlin 3	2012	Carrizo	Hamlin	X		X		X	X	X	
River	Hamlin 4	1985	Sour	Hamlin	X	X	X	X	X	X	X	X
SW	Hamlin 5	2014	Swingle	Hamlin					X	X	X	X
SW	Hamlin 6	1991	F80	Hamlin	X		X		X	X	X	
SW	Hamlin 7	2011	Carrizo	Hamlin	X		X		X	X	X	
SW	Hamlin 8	2012	Swingle	Hamlin	X		X		X	X	X	X
Ridge	Hamlin 12	2007	Cleo	Hamlin		X		X	X	X	X	X
Ridge	Hamlin 13	2007	Swingle	Hamlin		X		X	X	X	X	X
Ridge	Hamlin 14	2007	Carrizo	Hamlin		X		X	X	X	X	X
Ridge	Hamlin 15	2008	Swingle	Hamlin		X		X	X	X	X	X
SW	Hamlin 16	2007	Kuharske	Hamlin					X	X	X	X
Ridge	Hamlin 17	2007	Cleo	Hamlin		X		X	X	X	X	X
SW	Midsweet 2	2009	Carrizo	Midsweet	X		X		X	X	X	
SW	Midsweet 3	2008	Swingle	Midsweet	X		X		X	X	X	X
SW	Midsweet 4	2007	Swg/Kuhr	Midsweet		X		X	X	X		X
SW	Valencia 3	2015	Carrizo	Valencia	X		X		X	X	X	
SW	Valencia 4	2002	Kuharske	Valencia	X	X	X	X	X	X	X	X
SW	Valencia 5	2002	Kuharske	Valencia	X	X	X	X	X	X	X	X
SW	Valencia 6	2008	Kuharske	Valencia	X	X	X	X	X	X	X	X
River	Valencia 7	2008	Swingle	Valencia	X		X		X	X	X	X
River	Valencia 8	2008	Swingle	Valencia	X		X		X	X	X	X
River	Valencia 9	2008	Swingle	Valencia	X		X		X	X	X	X
SW	Valencia 12	1992	Carrizo	Valencia	X		X		X	X	X	X

Region	Trial Site Code	Planting date	Rootstock	Scion	Yield Season 1	Yield Season 2	Drop Season 1	Drop Season 2	DI Season 1	DI Season 2	CT Season 1	CT Season 2
SW	Valencia 13	1992	Carrizo	Valencia	X		X		X	X	X	X
SW	Valencia 14	1992	Carrizo	Valencia	X		X		X	X	X	X
Ridge	Valencia 15	2000	Swingle	Valencia	X	X	X	X	X	X	X	X
Ridge	Valencia 16	2000	Swingle	Valencia	X	X	X	X	X	X	X	X
Ridge	Valencia 17	1997	Swingle	Valencia	X	X	X	X	X	X	X	X
SW	Valencia 21	1991	Carrizo	Valencia					X	X	X	
SW	Valencia 22	1992	Carrizo	Valencia	X		X		X	X	X	
SW	Valencia 23	2011	Swingle	Valencia	X	X	X	X	X	X	X	X
Ridge	Valencia 28	2007	Swingle	Valencia	X	X		X	X	X	X	X
Ridge	Valencia 29	2008	Kuharske	Valencia	X	X		X	X	X	X	X
Ridge	Valencia 30	2008	Kuharske	Valencia	X	X		X	X	X	X	X
Ridge	Valencia 31	2007	Kuharske	Valencia	X	X		X	X	X	X	X
Ridge	Valencia 32	10 + years	Kuharske	Valencia		X		X	X	X	X	X
Ridge	Valencia 33	2000	Kuhr/812	Valencia		X		X	X	X		X
Ridge	Valencia 34	15 + years	Kuharske	Valencia		X		X	X	X	X	X
Ridge	Valencia 35	15 + years	Kuharske	Valencia		X		X	X	X	X	X
Ridge	Valencia 37	2008	Kuharske	Valencia		X		X	X	X	X	X
Ridge	Valencia 38	20 + years	Kuharske	Valencia	X	X		X	X	X	X	X
Ridge	Valencia 39	15 + years	Kuharske	Valencia		X		X	X	X	X	X
Ridge	Valencia 40	2011	Swingle	Valencia				X	X	X	X	X

Table 2. Effect of bactericide application on citrus fruit yield in kg/tree (40 kg = 1 box) in locations with two years of yield data.

Trial	Location	Rootstock	Scion	Year	Control	Treated	SE	P ≤
Grapefruit_01	Indian River	RayRuby	Sour Orange	1	39	39	4.5	0.983
Grapefruit_01	Indian River	RayRuby	Sour Orange	2	35	25	4.5	0.141
Grapefruit_03	Indian River	RayRuby	Sour Orange	1	30	45	4.5	0.023
Grapefruit_03	Indian River	RayRuby	Sour Orange	2	26	22	4.5	0.581
Grapefruit_07	Indian River	Grapefruit	Sour Orange	1	119	119	6.4	0.979
Grapefruit_07	Indian River	Grapefruit	Sour Orange	2	96	113	6.4	0.061
Hamlin_04	Indian River	Hamlin	Sour Orange	1	143	118	6.4	0.007
Hamlin_04	Indian River	Hamlin	Sour Orange	2	106	91	6.4	0.082
Valencia_04	Southwest	Valencia	Kuharske	1	26	28	6.4	0.887
Valencia_04	SouthWest	Valencia	Kuharske	2	26	23	6.4	0.688
Valencia_05	SouthWest	Valencia	Kuharske	1	41	41	6.4	0.918
Valencia_05	SouthWest	Valencia	Kuharske	2	43	41	6.4	0.830
Valencia_06	SouthWest	Valencia	Kuharske	1	58	53	6.4	0.564
Valencia_06	SouthWest	Valencia	Kuharske	2	41	46	6.4	0.587
Valencia_15	Ridge	Valencia	Swingle	1	80	78	6.4	0.904
Valencia_15	Ridge	Valencia	Swingle	2	82	71	6.4	0.224
Valencia_16	Ridge	Valencia	Swingle	1	83	86	6.4	0.723
Valencia_16	Ridge	Valencia	Swingle	2	60	69	6.4	0.322
Valencia_17	Ridge	Valencia	Swingle	1	48	54	3.2	0.186
Valencia_17	Ridge	Valencia	Swingle	2	115	111	3.2	0.300
Valencia_23	Southwest	Valencia	Swingle	1	25	26	6.4	0.894
Valencia_23	Southwest	Valencia	Swingle	2	14	13	6.4	0.880
Valencia_28	Ridge	Valencia	Swingle	1	76	68	5.7	0.303
Valencia_28	Ridge	Valencia	Swingle	2	62	73	5.7	0.160

Trial	Location	Rootstock	Scion	Year	Control	Treated	SE	P ≤
Valencia_29	Ridge	Valencia	Kuharske	1	56	58	6.4	0.831
Valencia_29	Ridge	Valencia	Kuharske	2	47	43	6.4	0.591
Valencia_30	Ridge	Valencia	Kuharske	1	36	33	6.4	0.728
Valencia_30	Ridge	Valencia	Kuharske	2	35	22	6.4	0.147
Valencia_31	SouthWest	Valencia	Kuharske	1	62	56	6.4	0.493
Valencia_31	SouthWest	Valencia	Kuharske	2	36	42	6.4	0.469
Valencia_38	Ridge	Valencia	Kuharske	1	185	145	6.4	0.0001
Valencia_38	Ridge	Valencia	Kuharske	2	90	80	6.4	0.267

Table 3. Effect of bactericide application on fruit dropped prior to harvest (% of total) in locations with two years of fruit drop data.

Trial	Location	Rootstock	Scion	Year	Control	Treated	SE	P ≤
Grapefruit_01	Indian River	RayRuby	Sour Orange	1	50.8	40.4	3.18	0.024
Grapefruit_01	Indian River	RayRuby	Sour Orange	2	52.3	63.8	3.09	0.012
Grapefruit_03	Indian River	RayRuby	Sour Orange	1	55.5	37.5	3.11	< 0.0001
Grapefruit_03	Indian River	RayRuby	Sour Orange	2	64.7	64.3	3.07	0.933
Grapefruit_07	Indian River	Grapefruit	Sour Orange	1	5.3	5.9	1.83	0.834
Grapefruit_07	Indian River	Grapefruit	Sour Orange	2	25.0	19.7	3.40	0.302
Hamlin_04	Indian River	Hamlin	Sour Orange	1	12.5	18.9	3.33	0.140
Hamlin_04	Indian River	Hamlin	Sour Orange	2	32.8	40.0	4.45	0.244
Valencia_04	SouthWest	Valencia	Kuharske	1	44.9	43.9	4.54	0.877
Valencia_04	SouthWest	Valencia	Kuharske	2	60.3	60.8	4.44	0.938
Valencia_05	SouthWest	Valencia	Kuharske	1	24.1	29.3	4.04	0.347
Valencia_05	SouthWest	Valencia	Kuharske	2	49.0	47.4	4.57	0.807
Valencia_06	SouthWest	Valencia	Kuharske	1	15.5	14.8	2.95	0.873
Valencia_06	SouthWest	Valencia	Kuharske	2	51.6	41.7	4.50	0.127
Valencia_15	Ridge	Valencia	Swingle	1	10.9	13.0	2.76	0.578
Valencia_15	Ridge	Valencia	Swingle	2	20.2	27.8	3.96	0.154
Valencia_16	Ridge	Valencia	Swingle	1	16.9	11.2	2.56	0.162
Valencia_16	Ridge	Valencia	Swingle	2	35.0	28.8	4.01	0.292
Valencia_17	Ridge	Valencia	Swingle	1	33.1	32.7	2.10	0.900
Valencia_17	Ridge	Valencia	Swingle	2	22.4	24.2	1.87	0.468
Valencia_23	Southwest	Valencia	Swingle	1	40.9	43.6	4.54	0.681
Valencia_23	Southwest	Valencia	Swingle	2	73.6	74.2	3.84	0.911

Table 4. Effect of bactericide application in year 1 (2016-17) only on citrus fruit yield in kg/tree (40 kg = 1 box).

Trial	Location	Rootstock	Scion	Control	Treated	SE	P ≤
Grapefruit_05	Indian River	Swingle	Grapefruit	136	80	9.18	< 0.0001
Hamlin_03	Southwest	Carrizo	Hamlin	41	37	9.18	0.750
Hamlin_06	Southwest	F_80	Hamlin	160	139	9.18	0.113
Hamlin_07	Southwest	Carrizo	Hamlin	31	29	9.18	0.872
Hamlin_08	Southwest	Swingle	Hamlin	44	29	9.18	0.279
Midsweet_02	Southwest	Carrizo	Midsweet	109	135	9.18	0.050
Midsweet_03	SouthWest	Swingle	Midsweet	98	94	9.18	0.729
Valencia_03	SouthWest	Carrizo	Valencia	3	0	9.18	0.834
Valencia_07	Indian River	Swingle	Valencia	73	71	9.18	0.883
Valencia_08	Indian River	Swingle	Valencia	70	73	9.18	0.803
Valencia_09	Indian River	Swingle	Valencia	77	80	9.18	0.815
Valencia_12	Southwest	Carrizo	Valencia	89	93	9.18	0.716
Valencia_13	Southwest	Carrizo	Valencia	104	100	12.99	0.773
Valencia_14	Southwest	Carrizo	Valencia	100	89	9.18	0.417
Valencia_22	Southwest	Carrizo	Valencia	60	36	9.18	0.069

Table 5. Effect of bactericide application in year 1 only (2016-17) on fruit dropped prior to harvest (% of total)

Trial	Location	Rootstock	Scion	Control	Treated	SE	P ≤
Grapefruit_05	Indian River	Swingle	Grapefruit	5.7	10.0	2.14	0.106
Hamlin_03	Southwest	Carrizo	Hamlin	18.4	19.3	2.89	0.827
Hamlin_06	Southwest	F_80	Hamlin	6.0	6.8	1.75	0.756
Hamlin_07	Southwest	Carrizo	Hamlin	34.9	35.9	3.55	0.843
Hamlin_08	Southwest	Swingle	Hamlin	25.4	48.5	3.71	0.000
Midsweet_02	Southwest	Carrizo	Midsweet	13.4	12.6	2.40	0.824
Midsweet_03	SouthWest	Swingle	Midsweet	8.1	14.1	2.52	0.063
Valencia_03	SouthWest	Carrizo	Valencia	12.5	10.8	3.14	0.671
Valencia_07	Indian River	Swingle	Valencia	19.1	19.6	2.90	0.913
Valencia_08	Indian River	Swingle	Valencia	21.2	17.9	2.80	0.423
Valencia_09	Indian River	Swingle	Valencia	19.2	14.7	2.57	0.242
Valencia_12	Southwest	Carrizo	Valencia	22.3	22.4	3.06	0.995
Valencia_13	Southwest	Carrizo	Valencia	14.5	18.9	4.05	0.344
Valencia_14	Southwest	Carrizo	Valencia	14.0	17.3	2.76	0.371
Valencia_22	Southwest	Carrizo	Valencia	22.4	30.5	3.40	0.083

Table 6. Effect of bactericide application in year 2 only (2017-18) on fruit dropped prior to harvest (% of total)

Trial	Location	Rootstock	Scion	Control	Treated	SE	P ≤
Hamlin_12	Ridge	Cleo	Hamlin	58.2	51.6	2.96	0.114
Hamlin_13	Ridge	Swingle	Hamlin	52.4	48.1	2.96	0.304
Hamlin_14	Ridge	Carrizo	Hamlin	62.3	56.8	2.93	0.184
Hamlin_15	Ridge	Swingle	Hamlin	73.5	67.6	2.77	0.124
Hamlin_17	Ridge	Cleo	Hamlin	66.4	68.0	2.77	0.672
Midsweet_04	Southwest	Swingle_&_Kuharske	Midsweet	58.6	57.9	3.38	0.875
Valencia_28	Ridge	Swingle	Valencia	48.0	40.1	2.60	0.037
Valencia_29	Ridge	Kuharske	Valencia	40.9	55.4	2.95	0.001
Valencia_30	Ridge	Kuharske	Valencia	53.8	59.0	2.91	0.213
Valencia_31	SouthWest	Kuharske	Valencia	23.6	23.8	2.51	0.958
Valencia_32	SouthWest	Kuharske	Valencia	58.8	66.8	2.79	0.051
Valencia_33	Ridge	Kuharske_&_US812	Valencia	63.5	61.3	2.88	0.584
Valencia_34	Ridge	Kuharske	Valencia	59.9	65.0	2.82	0.213
Valencia_35	Ridge	Kuharske	Valencia	48.2	53.4	2.96	0.216
Valencia_37	Ridge	Kuharske	Valencia	39.0	40.5	2.91	0.725
Valencia_38	Ridge	Kuharske	Valencia	49.3	50.5	2.96	0.787
Valencia_39	Ridge	Kuharske	Valencia	46.3	40.9	2.91	0.196

Table 7. Effect of bactericide application on disease index score ((DI; 20 treatment-impacted locations only)

Trial	Location	Scion	Rootstock	Year	Control	Treated	SEM	P ≤
Grapefruit_03	Indian River	RayRuby	Sour Orange	1	13.1	12.2	0.41	0.096
Grapefruit_08	Indian River	Grapefruit	Sour Orange	2	25.8	27.2	0.58	0.092
Hamlin_04	Indian River	Hamlin	Sour Orange	1	21.7	24.4	0.58	0.001
Hamlin_06	Southwest	Hamlin	F_80	2	19.0	21.7	0.58	0.001
Hamlin_16	Southwest	Hamlin	Kuharske	1	27.9	30.2	0.58	0.006
Hamlin_16	Southwest	Hamlin	Kuharske	2	29.6	31.0	0.58	0.084
Midsweet_03	SouthWest	Midsweet	Swingle	2	19.7	21.3	0.58	0.049
Valencia_05	SouthWest	Valencia	Kuharske	1	20.2	21.6	0.58	0.089
Valencia_05	SouthWest	Valencia	Kuharske	2	18.8	20.9	0.58	0.011
Valencia_12	Southwest	Valencia	Carrizo	2	26.7	25.1	0.58	0.057
Valencia_13	Southwest	Valencia	Carrizo	2	24.6	26.5	0.58	0.018
Valencia_14	Southwest	Valencia	Carrizo	1	22.4	20.9	0.58	0.063
Valencia_14	Southwest	Valencia	Carrizo	2	25.4	23.9	0.58	0.067
Valencia_21	Southwest	Valencia	Carrizo	2	23.7	25.8	0.58	0.011
Valencia_23	Southwest	Valencia	Swingle	2	21.6	20.1	0.58	0.060
Valencia_30	Ridge	Valencia	Kuharske	1	25.3	27.0	0.58	0.039
Valencia_32	SouthWest	Valencia	Kuharske	2	23.6	25.4	0.58	0.025
Valencia_35	Ridge	Valencia	Kuharske	2	24.9	26.9	0.58	0.015
Valencia_37	Ridge	Valencia	Kuharske	2	24.2	22.5	0.58	0.049
Valencia_38	Ridge	Valencia	Kuharske	2	23.0	24.4	0.58	0.074

Table 8. Effect of bactericide application on PCR detection of HLB infection in (CT Value; in 13 treatment-impacted locations only).

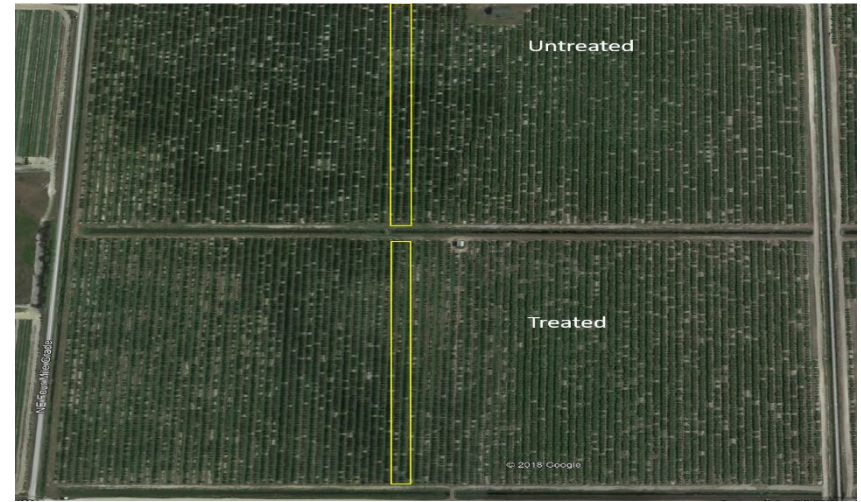
Trial	Location	Scion	Rootstock	Year	Control	Treated	SEM	P ≤
Grapefruit_01	Indian River	RayRuby	Sour Orange	1	38.7	36.1	0.615	0.003
Hamlin_05	Southwest	Hamlin	Swingle	1	36.3	34.2	0.870	0.093
Hamlin_08	Southwest	Hamlin	Swingle	1	33.8	30.1	0.870	0.003
Hamlin_13	Ridge	Hamlin	Swingle	1	26.4	29.6	0.870	0.011
Hamlin_17	Ridge	Hamlin	Cleo	1	30.1	27.9	0.870	0.075
Valencia_07	Indian River	Valencia	Swingle	2	31.7	28.3	0.870	0.006
Valencia_13	Southwest	Valencia	Carrizo	1	25.4	27.8	0.870	0.052
Valencia_23	Southwest	Valencia	Swingle	1	34.5	36.9	0.870	0.058
Valencia_28	Ridge	Valencia	Swingle	2	30.9	27.7	0.778	0.004
Valencia_31	SouthWest	Valencia	Kuharske	1	25.4	27.8	0.870	0.060
Valencia_34	Ridge	Valencia	Kuharske	1	30.2	26.7	1.227	0.020
Valencia_38	Ridge	Valencia	Kuharske	1	36.9	33.6	0.870	0.008
Valencia_40	Ridge	Valencia	Swingle	1	28.9	34.3	0.870	< 0.001

Figure 1. Treatment layout options



Single Block Design

The yellow box indicates the 4 untreated rows (Control). The blue box indicates the 2 rows (treated and untreated) that were evaluated. The red bars within the evaluation rows represent the 4 groups of 5 sentinel trees for treated and untreated.



Double Block Design

The entire block is designated to be treated or untreated. The yellow boxes indicate the rows that contain the 4 groups of 5 sentinel trees.

Appendix A: Effect of bactericide application on disease index score (non-impacted locations only).

Trial	Location	Scion	Rootstock	Year	Control	Treated	SEM	P ≤
Grapefruit 01	Indian River	RayRuby	Sour Orange	1	13.1	13.4	0.41	0.694
Grapefruit 01	Indian River	RayRuby	Sour Orange	2	21.6	21.3	0.41	0.596
Grapefruit 03	Indian River	RayRuby	Sour Orange	2	22.3	21.9	0.41	0.510
Grapefruit 05	Indian River	Grapefruit	Swingle	1	19.4	18.7	0.58	0.376
Grapefruit 05	Indian River	Grapefruit	Swingle	2	26.5	25.7	0.58	0.309
Grapefruit 07	Indian River	Grapefruit	Sour Orange	1	18.2	18.0	0.58	0.815
Grapefruit 07	Indian River	Grapefruit	Sour Orange	2	26.2	25.2	0.58	0.231
Grapefruit 08	Indian River	Grapefruit	Sour Orange	1	18.3	18.4	0.58	0.938
Hamlin 03	Southwest	Hamlin	Carrizo	1	16.6	17.3	0.58	0.430
Hamlin 03	Southwest	Hamlin	Carrizo	2	15.1	15.1	0.58	1.000
Hamlin 04	Indian River	Hamlin	Sour Orange	2	25.3	26.5	0.58	0.137
Hamlin 05	Southwest	Hamlin	Swingle	1	15.1	15.5	0.58	0.606
Hamlin 05	Southwest	Hamlin	Swingle	2	20.2	20.5	0.58	0.757
Hamlin 06	Southwest	Hamlin	F 80	1	16.1	15.8	0.58	0.746
Hamlin 07	Southwest	Hamlin	Carrizo	1	16.5	16.0	0.58	0.574
Hamlin 07	Southwest	Hamlin	Carrizo	2	23.5	23.0	0.58	0.592
Hamlin 08	Southwest	Hamlin	Swingle	1	16.6	17.9	0.58	0.122
Hamlin 08	Southwest	Hamlin	Swingle	2	21.9	22.2	0.58	0.792
Hamlin 12	Ridge	Hamlin	Cleo	1	23.9	22.8	0.58	0.182
Hamlin 12	Ridge	Hamlin	Cleo	2	24.6	24.6	0.58	0.855
Hamlin 13	Ridge	Hamlin	Swingle	1	27.8	28.0	0.58	0.855
Hamlin 13	Ridge	Hamlin	Swingle	2	26.9	27.8	0.58	0.261
Hamlin 14	Ridge	Hamlin	Carrizo	1	26.2	25.8	0.58	0.627
Hamlin 14	Ridge	Hamlin	Carrizo	2	25.6	25.1	0.58	0.544

Trial	Location	Scion	Rootstock	Year	Control	Treated	SEM	P ≤
Hamlin 15	Ridge	Hamlin	Swingle	1	24.7	25.7	0.58	0.249
Hamlin 15	Ridge	Hamlin	Swingle	2	27.5	27.8	0.58	0.785
Hamlin 17	Ridge	Hamlin	Cleo	1	25.5	26.2	0.58	0.362
Hamlin 17	Ridge	Hamlin	Cleo	2	27.8	27.0	0.58	0.253
Midsweet 02	SouthWest	Midsweet	Carrizo	1	21.4	20.8	0.58	0.517
Midsweet 02	SouthWest	Midsweet	Carrizo	2	19.6	19.6	0.58	0.952
Midsweet 03	SouthWest	Midsweet	Swingle	1	18.2	19.4	0.58	0.145
Midsweet 04	Southwest	Midsweet	Swingle & Kuharske	1	30.2	30.3	0.58	0.808
Midsweet 04	Southwest	Midsweet	Swingle & Kuharske	2	31.2	30.2	0.58	0.274
Valencia 03	SouthWest	Valencia	Carrizo	1	1.0	0.8	0.58	0.879
Valencia 03	SouthWest	Valencia	Carrizo	2	3.3	4.0	0.58	0.362
Valencia 04	SouthWest	Valencia	Kuharske	1	20.6	20.5	0.58	0.968
Valencia 04	SouthWest	Valencia	Kuharske	2	20.7	21.2	0.58	0.571
Valencia 06	SouthWest	Valencia	Kuharske	1	20.3	21.5	0.58	0.140
Valencia 06	SouthWest	Valencia	Kuharske	2	20.6	19.2	0.58	0.106
Valencia 07	Indian River	Valencia	Swingle	1	17.6	18.1	0.58	0.517
Valencia 07	Indian River	Valencia	Swingle	2	19.0	19.8	0.58	0.384
Valencia 08	Indian River	Valencia	Swingle	1	17.9	18.3	0.58	0.656
Valencia 08	Indian River	Valencia	Swingle	2	19.1	20.1	0.58	0.225
Valencia 09	Indian River	Valencia	Swingle	1	18.5	18.6	0.58	0.968
Valencia 09	Indian River	Valencia	Swingle	2	20.3	19.2	0.58	0.195
Valencia 12	Southwest	Valencia	Carrizo	1	20.9	21.4	0.58	0.494
Valencia 13	Southwest	Valencia	Carrizo	1	21.7	21.2	0.58	0.544
Valencia 15	Ridge	Valencia	Swingle	1	19.1	19.2	0.58	0.919

Trial	Location	Scion	Rootstock	Year	Control	Treated	SEM	P ≤
Valencia 15	Ridge	Valencia	Swingle	2	20.4	19.8	0.58	0.494
Valencia 16	Ridge	Valencia	Swingle	1	20.2	20.3	0.58	0.855
Valencia 16	Ridge	Valencia	Swingle	2	20.2	20.5	0.58	0.761
Valencia 17	Ridge	Valencia	Swingle	1	17.4	17.4	0.29	0.968
Valencia 17	Ridge	Valencia	Swingle	2	20.4	20.2	0.30	0.501
Valencia 21	Southwest	Valencia	Carrizo	1	21.2	22.2	0.58	0.217
Valencia 22	Southwest	Valencia	Carrizo	1	20.4	21.5	0.58	0.182
Valencia 22	Southwest	Valencia	Carrizo	2	24.8	24.8	0.58	0.976
Valencia 23	Southwest	Valencia	Swingle	1	21.0	21.8	0.58	0.362
Valencia 28	Ridge	Valencia	Swingle	1	25.8	26.2	0.52	0.625
Valencia 28	Ridge	Valencia	Swingle	2	17.0	17.4	0.52	0.606
Valencia 29	Ridge	Valencia	Kuharske	1	25.1	24.8	0.58	0.716
Valencia 29	Ridge	Valencia	Kuharske	2	26.1	26.6	0.58	0.448
Valencia 30	Ridge	Valencia	Kuharske	2	27.3	28.0	0.58	0.379
Valencia 31	SouthWest	Valencia	Kuharske	1	25.5	25.8	0.58	0.671
Valencia 31	SouthWest	Valencia	Kuharske	2	25.1	25.0	0.58	0.952
Valencia 32	SouthWest	Valencia	Kuharske	1	26.1	25.9	0.58	0.903
Valencia 33	Ridge	Valencia	Kuharske & US812	1	25.8	25.7	0.58	0.903
Valencia 33	Ridge	Valencia	Kuharske & US812	2	25.8	24.7	0.58	0.192
Valencia 34	Ridge	Valencia	Kuharske	1	28.7	28.7	0.58	0.952
Valencia 34	Ridge	Valencia	Kuharske	2	27.8	28.1	0.58	0.761
Valencia 35	Ridge	Valencia	Kuharske	1	26.3	26.1	0.58	0.761
Valencia 37	Ridge	Valencia	Kuharske	1	23.5	23.7	0.58	0.903
Valencia 38	Ridge	Valencia	Kuharske	1	16.9	16.6	0.58	0.808

Trial	Location	Scion	Rootstock	Year	Control	Treated	SEM	P ≤
Valencia 39	Ridge	Valencia	Kuharske	1	27.3	27.6	0.58	0.716
Valencia 39	Ridge	Valencia	Kuharske	2	28.4	27.4	0.58	0.249
Valencia 40	Ridge	Valencia	Swingle	1	21.0	21.0	0.073	0.952
Valencia 40	Ridge	Valencia	Swingle	2	17.9	18.9	0.073	0.236