CRDF Commercial Product Delivery

Project 1.a. Bactericide Strategies FY 2016-18

Project 15-049C. Biopesticides. Final Report Nov 1, 2018

Project 1 Title: Candidatus Liberibacter asiaticus PATHOGEN INTERVENTION

The goal of this project is to identify bactericides effective against Huanglongbing (HLB). Project managers will identify bactericides from various sources from products in the market to materials in early stages of development that are effective against HLB, and assist with formulation for effective delivery, provide regulatory guidance by engaging regulatory consultants and EPA and assist with commercialization if necessary. This is an ongoing project that will build on the development of an assay pipeline for screening bactericides and the in vitro screening of more than eight hundred compounds including material libraries from agriculture, biotech and pharmaceutical companies. Bactericides that have been identified by project managers, as potential short to long-term solutions will continue to be tested in assays and in field trials and steps will be taken to encourage commercialization of these materials to provide a solution to growers for HLB.

Subproject Title: 1a, 3. Bactericide Strategies: Bactericide Field Testing
Narrative of Progress against Goals:
Obj. 1 - Managing existing field trials including analyzing data, refining treatments and reporting progress to CPDC.

15-049C. Biopesticides. Situation Statement. This study was to provide a side-by-side comparison of five essential oil products as a preventative treatment and a therapy for HLB on young trees. The impact of treatments on psyllids, tree health, foliar nutrition, disease rating, and HLB status will be evaluated. The trial was large enough and replicated sufficiently to allow statistical evaluation. Since these products are materials that are “minimal risk” or classified as biopesticides by EPA, if they are shown to have a significant effect on bacterial titer, the products can be available to growers in a short time frame. Materials were applied to young trees that are both uninfected and infected by *Candidatus* Liberibacter asiaticus.

Objectives of the Project:

1) Determine ability of candidate materials to suppress CLas populations in trees (3-4 years old) already infected and showing symptoms of HLB
2) Determine ability of candidate materials to prevent or slow infection of new trees (3-4 years old) by ACP/CLas.

The biopesticide field trials, project 15-049C, were set-up in late February 2016 using 3-year-old Hamlin trees on US897 rootstock in the Ridge. Trees were planted in 2013 at two separate but similar sites at a spacing of 8 x 15 feet. The 6 treatments were an unsprayed

Control, **Ecotrol Plus** (Keyplex), a proprietary research oil product **(IPGN5)**, **Thymeguard** (Agro Research International, **OnGuard** EO and **Xplode** (AgXplore International).

Treatments, adjuvants, concentrations and rates.

Control (Untreated)	Rates of application
Adjuvants	
Ecotrol Plus	20 ml/tree 0.6762oz/tree * 363 trees/acre = 1.9gal/acre
KeyPlex 1400	20 ml/tree 0.6762oz/tree * 363 trees/acre = 1.9gal/acre
N-Sure	30 ml/tree 1.01oz/tree * 363 trees/acre = 2.9gal/acre
IPGN5 (Dean Gabriel)	0.8oz/tree x 363 trees/acre = 2.27 gal/acre
proprietary	0.0125 oz./tree x 363trees/acre = 7.71 ounces/acre
OnGuard EO (X-10)	1qt/100gal (32oz/100gal)
Sursil	6oz/100gal
Thymeguard	1qt/100gal (32oz/100gal)
Protyx	1pt/100gal (0.125% v/v) (16oz/100gal)
Xplode (0-3)	1qt/100gal. (32oz/100gal)
Sursil	6oz/100gal.

All products were applied as foliar sprays at the recommended rates and with the recommended adjuvants by the registrants. Applications of these products were applied every 60 days (6 applications per year) for two years (2016,2017) by a contracted crop consultant (CC, Pacific Ag Research). In 2017, the IPGN5 treatment was dropped.

Pre-treatment tree and grove evaluation included scion, rootstock, soil type, soil pH, and good horticultural practices including irrigation, fertility program, leaf nutrition, recommended young tree psyllid and other pest control including appropriate rates of neonicotinoids (by the cooperating grower), subsequent yield and fruit quality. Good horticultural care was continued for the duration of the trial. The field sites were chosen by the contracted crop consultant (CC) in conjunction with the CRDF field trial administrator. After two years of treatments, we now have valid information on the impact of these treatments on HLB status, tree health, disease rating, foliar nutrition, yield and fruit quality.

At the initiation of the trial, all the selected trees from one site tested PCR negative for HLB (designated the HLBNEG site) and all trees at the nearby second site tested positive for HLB (HLBPOS site). At each site, studies focused on a subset of 6 measurement trees in the middle of each of the 10 tree plots; 6 trees x 4 blocks = 24 trees x 6 treatments = 144 measurement trees at each site. The two end trees on each end of the 10 tree plots were avoided as buffer trees. The experimental design consisted of four replications of blocks of six trees (n=24 trees per treatment). The evaluations consisted of periodic measurements of polymerase chain reaction (PCR) to quantify bacterial titer in leaves, canopy volume, trunk cross-sectional area, disease severity (disease index, DI), leaf nutrient analyses fruit yield, fruit drop counts, canker

evaluations, juice quality characteristics and ACP counts. After two full years of treatments, data collection was completed and analyzed for treatment effects in 2018. We now have valid information on the impact of these treatments HLB status, disease rating, tree health, foliar nutrition, yield and fruit quality.

Tree measurements. Leaves were samples for PCR from all 144 trees per site. Three mature leaves from 4 branches for a total of 12 leaves (with petioles attached) from each tree were sampled by CRDF staff twice per year and promptly sent to the Southern Gardens Diagnostic Laboratory (http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf) for evaluation of cycle threshold (CT) and copy number (CN) per DNA. Tree canopy dimensions were measured twice per year and used to calculate canopy volume (CV in m^3) and trunk cross sectional area (TCSA in cm^2). Disease index (DI) was rated 4-5 times per year from 2016 to 2018. For leaf nutrient analysis, two mature leaves from around the 6 measurement trees per main plot, were sampled in summer (July-August) by CRDF staff, pooled into 1 sample (12 leaves total from each of the 24 plots per site; n=4 per treatment) and immediately forwarded to Waters Ag Lab for analysis. Fruit were harvested from the 6 measurement trees per main plot (n=4 per treatment) and weighed to get total yield after year 1 in Jan 2017 and after year two in Dec 2017. Total percentage fruit drop (%FD) from these trees was also estimated at these times. For juice quality analysis from each harvest, 50-fruit samples were pooled from various canopy positions from the 6 measurement trees (8-9 fruit from each tree; n=4 per treatment) and delivered to the CREC standard testing lab.

Tap Sampling for ACP: Monthly tap samples from 3 separate locations on each measurement tree were done by CRDF staff and the number of psyllids collected were totaled along with visual counts of adults and nymphs. After monthly pesticide sprays, appropriate rates of neonicotinoids and these biopesticide treatments, ACP counts were almost always zero so there were not enough data to statistically analyze for treatment effects. All other tree measurement and fruit data were analyzed using factorial ANOVAs and means were separated using Tukey's least significant difference. Tables summarizing these results follow.

Results. PCR CT and copy number (CN) values in Feb 2016 were used to select the two contrasting sites, HLBNEG and HLBPOS (Table 1, 2). At the initially HLBNEG site, there were no treatment differences in Aug 2016 but after one year of the biopesticide treatments, average CT values of the IPGN5 were highest and CT of the Ecotrol and Onguard (OnGrd) treatments had become HLB positive (CT<31; Table 1). By Dec 2017, however, Ecotrol trees had become HLB negative again (CT >31) and were significantly higher than the Control and Thymeguard (Thyme) treatments which were HLB positive. All treatments including the untreated control had lower CT values in Dec 2017 than initially in Feb 2016 so none of the treatments prevented infection. The Ecotrol treatment which had the lowest CT in Feb 2017, was able to delay infection better than the Control after 2 years.

HLBNEG	Table 1. PCR CT_Values and Copy Number_100NgDNA in 2016, 2017									
Trt	CT_Feb2016	CN_Feb2016	CT_Aug2016	CN_Aug2016	CT_Feb2017		CN_Feb2017		CT_Dec2017	
CON	39.68	0.13	33.92	741.99	33.41	B	867.03	AB	30.05	B
Ecotrol	39.16	24.17	31.53	12484.30	27.77	C	1366.48	AB	35.18	A
IPGN5	38.77	635.16	32.61	550.61	39.04	A	12.27	B		
OnGrd	39.74	0.07	33.36	11723.36	28.05	C	1877.14	A	31.89	AB
Thyme	37.57	4793.47	33.46	5703.22	31.24	BC	918.94	AB	30.89	B
Xplode	38.55	43.80	33.79	4311.80	33.45	B	726.39	AB	33.19	AB
AVG:	38.91	916.13	33.11	5919.21	32.16		961.38		32.24	

Mean values followed by different letters are significantly different at P< 0.05.

By Aug 2016 at the initially HLBPOS site (Table 2), the Control had the highest CT value and Ecotrol had the lowest. These differences in CT disappeared by Feb 2017 but the CN of Ecotrol was lowest. By Dec 2017, only the Xplode treatment was still HLB positive (CT<31) but the other treatments (excluding IPGN5) had improved to become HLB negative with CTs >31. This result could have been remarkable but the untreated Control treatment also increased CT by Dec 2107 so this increase in CT could not be attributed to the treatments. Thus, CT values of the Ecotrol treatment were lower (with more HLB) than the untreated Control during the first year but all treatments had less HLB by Dec 2017 and none differed from the Control.

HLBPOS	Table 2. PCR CT_Values and Copy Number_100NgDNA in 2016, 2017									
Trt	CT_Feb16	CN_Feb16	CT_Aug16		CN_Aug16		CT_Feb17	CN_Feb17		CT_Dec17	
CON	26.25	6648.38	26.61	A	4261.09	B	25.66	4850.78	A	32.27	AB
Ecotrol	25.45	7481.05	24.00	B	15966.24	A	27.70	499.72	B	33.47	A
IPGN5	25.10	6968.49	25.38	AB	5171.81	B	24.50	3314.52	AB		
OnGrd	25.05	8438.01	26.11	AB	9715.66	AB	25.60	2757.31	AB	33.44	A
Thyme	24.35	7910.67	25.29	AB	5971.40	B	25.53	2580.94	AB	33.05	A
Xplode	24.84	10293.14	25.21	AB	9962.39	AB	24.56	2821.64	AB	29.02	B
AVG:	25.17	7956.62	25.43		8508.10		25.59	2804.15		32.25	

At the HLBNEG site prior to treatments in Feb 2016, OnGrd treated trees had larger canopy volumes (CV) than Thyme trees (Table 3). By Aug 2016, all trees had grown but the Control trees were larger than the Thyme trees. Control trees were largest and Ongrd and Thyme trees smallest in Feb 2017 and by June 2018, Control and Ecotrol trees were largest and Ongrd trees were smallest. Ongrd trees were smaller than Control trees after 2 years of treatments. The untreated Control trees consistently had the largest trunk cross sectional area (TCSA) and thyme trees had the smallest TCSA.

HLBNEG	Table 3. CanopyVolume (m^3) & TCSA (cm^2) in 2016, 2017.							
Trt	CV_Feb2016		CV_Aug2016		CV_Feb2017		CV_June2018	
CON	27.76	AB	36.47	A	42.8	A	39.73	A
Ecotrol	24.51	BC	31.89	AB	38.14	AB	40.3	A
IPGN5	27.07	AB	35.76	AB	37.7	AB		
OnGrd	29.37	A	34.67	AB	35.05	B	30.67	B
Thyme	22.61	C	29.87	B	34.83	B	38.32	AB
Xplode	26.84	AB	31.99	AB	38.47	AB	37.87	AB
AVG:	26.36		33.44		37.83		37.38	

HLBNEG	Trunk Cross Sectional Area (TCSA)							
Trt	TCSA_Feb2016		TCSA_Aug2016		TCSA_Feb2017		TCSA_June2018	
CON	28.33	A	32.49	A	35.64	A	41.99	A
Ecotrol	24.86	BC	29.93	AB	34.94	AB	37.07	AB
IPGN5	28.32	A	33.38	A	36.53	A		
OnGrd	27.58	AB	32.7	A	33.31	AB	38.3	AB
Thyme	23.24	C	27.86	B	31.1	B	35.8	B
Xplode	26.89	AB	31.67	AB	34.8	AB	38.49	AB
AVG:	26.54		31.34		34.39		38.33	

Canopy volumes of the HLBPOS trees were overall smaller (Table 4) than HLBNEG trees from the beginning and there were no treatment effects on CV of the HLBPOS trees. The TCSA of Control trees was initially largest and that of the Thyme trees smallest in Feb 2016 but these differences disappeared by Aug 2016.

HLBPOS	Table 4. Canopy Volume (m^3) & TCSA (cm^2) in 2016, 201			
Trt	CV_Feb2016	CV_Aug2016	CV_Feb2017	CV_June2018
CON	15.71	15.96	19.21	19.99
Ecotrol	15.31	16.18	17.19	18.26
IPGN5	13.41	14.87	17.25	
OnGrd	14.56	15.94	18.37	17.78
Thyme	13.84	15.6	16.19	17.48
Xplode	14.55	15.38	16.73	17.88
AVG:	14.56	15.66	17.49	18.28

HLBPOS	Trunk Cross Sectioal Area_cm^2				
Trt	TCSA_Feb16		TCSA_Aug16	TCSA_Feb17	TCSA_Jun18
CON	17.45	A	18.38	20.07	23.62
Ecotrol	17.09	AB	17.52	18.92	22.06
IPGN5	15.35	AB	16.14	17.75	
OnGrd	15.93	AB	17.01	18.99	22.24
Thyme	14.06	B	15.32	17.28	20.16
Xplode	15.68	AB	16.91	18.28	20.67
AVG	15.93		16.88	18.55	21.02

The average visual disease index (DI) rating of HLBNEG trees gradually increased from about 10 in Feb 2016 to about 23 in Feb 2018 (Table 5). Ecotrol trees had the lowest DI on several dates but there were no consistent treatment differences throughout as no treatment DI differed from the control.

HLBNEG	Table 5. Disease Index (DI) 2016-2018.						
Trt	DI_Feb16	DI_May16	DI_June16	DI_Aug16	DI_Nov16	DI_Feb17	
CON	10.13	10.25	11.29 A	15 AB	17.63	14.38	AB
Ecotrol	10.21	10.13	10.25 C	13.67 B	16.71	14.17	AB
IPGN5	10.5	10.58	11.13 AB	14.79 AB	17.46	14.63	A
OnGrd	10.75	10.63	11.17 AB	13.71 B	17.08	13.83	B
Thyme	10.33	10.46	10.63 BC	14 B	16.33	14.17	AB
Xplode	10.83	10.08	10.54 BC	15.58 A	17.13	14.17	AB
AVG:	10.46	10.36	10.84	14.46	17.06	14.23	
HLBNEG							
Trt	DI_June17	DI_Sep17	DI_Nov17	DI_Feb18	DI_May18		
CON	19.5 AB	18.08	22.67 A	23.38	19.79		
Ecotrol	18.29 B	17.83	20.79 B	23.04	18.96		
IPGN5	20.54 A	18.08	23.25 A	23.92	20.13		
OnGrd	19.21 AB	18.38	22.38 AB	22.67	19.33		
Thyme	18.71 B	18.38	23.29 A	23.5	19.83		
Xplode	20.38 A	18.04	22.63 AB	23.54	19.42		
AVG:	19.44	18.13	22.50	23.34	19.58		

The average disease index (DI) rating of HLBPOS trees also gradually increased from about 17 in Feb 2016 to about 21 in Feb 2018 (Table 6). Ecotrol trees had the highest DI on June 2017 but there were no consistent treatment differences throughout.

HLBPOS	Table 6. Disease Index (DI) 2016-2018.							
Trt	DI_Feb2016	DI_May2016	DI_June2016	DI_Aug2016	DI_Nov16	DI_Feb2017	DI_June2017	
CON	16.58	17.50	12.13	15.13	17.17	18.21	19.63	AB
Ecotrol	17.75	17.63	12.70	15.63	17.17	18.25	20.92	A
IPGN5	17.46	18.00	12.58	15.75	17.17	16.92	20.71	AB
OnGrd	17.54	17.46	12.67	15.63	17.54	17.63	19.13	B
Thyme	17.63	17.54	12.71	15.92	18.21	18.00	20.67	AB
Xplode	17.21	17.25	12.58	15.08	17.13	17.00	20.79	AB
AVG:	17.36	17.56	12.56	15.52	17.40	17.67	20.31	
HLBPOS								
Trt	DI_Sep2017	DI_Nov2017		DI_Feb2018	DI_May2018			
CON	17.25	18.29	A	22.33	20.54			
Ecotrol	16.83	18.38	A	21.33	19.29			
IPGN5	16.46	17.79	AB	20.96	20.46			
OnGrd	16.13	16.46	B	20.28	19.29			
Thyme	17.33	17.63	AB	21.92	20.79			
Xplode	17.42	18.71	A	21.54	20.63			
AVG:	16.90	17.88		21.39	20.17			

At both the HLBNEG and HLBPOS sites, there were no treatment effects on any of the major nutrients in July 2016, 6 months after the treatments began (Table 7). At both sites, leaf N, K, Mg and Fe concentrations were relatively low compared to standard optimum target values. Leaf B levels were high. Ecotrol treatment leaves had highest Mn and Zn concentrations.

HLBNEG	Table 7. Leaf nutrient concentrations in Jul 2016. Optimun ranges are included for reference											
Leaf Opt	2.5 – 2.7	.12 – .16	1.2 – 1.7	3.0 – 4.9	.30 – .49	60 – 12	25 – 100		25 – 100		5 – 16	36 – 100
Trt	%_N	%_P	%_K	%_Ca	%_Mg	ppm_Fe	ppm_Mn		ppm_Zn		ppm_Cu	ppm_B
CON	2.43	0.13	0.92	4.05	0.24	45	96	AB	56	AB	20	143
Ecotrol	2.35	0.15	1.09	3.71	0.28	55	131	A	7	A	17	147
IPGN5	2.47	0.15	1.16	3.67	0.26	46	69	B	35	B	16	141
OnGrd	2.46	0.15	1.11	3.62	0.24	47	71	AB	38	AB	15	134
Thyme	2.33	0.15	1	3.69	0.29	41	77	AB	40	AB	19	141
Xplode	2.36	0.13	1	3.78	0.26	49	97	AB	51	AB	26	149
AVG:	2.40	0.14	1.05	3.75	0.26	47.2	90.2		37.8		18.8	142.5
HLBPOS												
Trt	%_N	%_P	%_K	%_Ca	%_Mg	ppm_Fe	ppm_Mn		ppm_Zn		ppm_Cu	ppm_B
CON	2.24	0.14	1.1	3.74	0.3	62	68	B	45	B	28	125
Ecotrol	2.29	0.15	1.16	3.27	0.29	61	94	A	61	A	22	109
IPGN5	2.19	0.13	0.972	3.36	0.29	44	51	B	36	B	21	105
OnGrd	2.21	0.15	1.22	3.4	0.3	49	57	B	38	B	23	109
Thyme	2.09	0.15	1.17	3.25	0.3	45	59	B	40	B	23	104
Xplode	2.23	0.15	1.08	3.63	0.32	87	61	B	33	B	26	118
AVG:	2.21	0.15	1.12	3.44	0.30	58.0	65.0		42.2		23.8	111.7

n = 4 from 6 trees pooled in 4 reps.

By Aug 2017, leaf N, K, Mg and Fe were within optimum ranges at the HLBNEG site (Table 8). Since leaf K, Mn and B were all within optimum ranges, the small treatment differences are probably not important. Since all leaf nutrient values at the HLBPOS site are within optimum ranges (except Fe), the small treatment effects on leaf P, Mg, Mn and B are probably of little consequence.

HLBNEG	Table 8. Leaf nutrient concentrations in Aug 2017. Optimun ranges are included for reference.															
Leaf Opt:	**2.5 – 2.7**	**.12 – .16**		**1.2 – 1.7**		**3.0 – 4.9**	**.30 – .49**		**60 – 120**	**S**	**25 – 100**		**25 – 100**	**5 – 16**	**36 – 100**	
Trt	%_N	%_P		%_K		%_Ca	%_Mg		ppm_Fe	%_S	ppm_Mn		ppm_Zn	ppm_Cu	ppm_B	
CON	2.71	0.21		1.6	AB	3.76	0.31		65	0.31	25	C	21	12	84	AB
Ecotrol	2.77	0.19		1.42	AB	3.43	0.34		68	0.32	34	A	20	12	89	AB
OnGrd	2.56	0.2		1.58	AB	3.77	0.36		57	0.32	33	AB	20	11	93	B
Thyme	2.84	0.19		1.38	B	3.5	0.34		61	0.33	27	BC	19	15	83	A
Xplode	2.54	0.21		1.63	A	3.59	0.34		59	0.33	27	BC	20	13	86	AB
AVG:	2.68	0.20		1.52		3.61	0.34		62.0	0.32	29.2		20.0	12.6	87.0	
HLBPOS																
Trt	%_N	%_P		%_K		%_Ca	%_Mg		ppm_Fe	%_S	ppm_Mn		ppm_Zn	ppm_Cu	ppm_B	
CON	2.63	0.21	A	1.59		3.12	0.37	AB	58	0.3	26	B	26	15	65	A
Ecotrol	2.58	0.2	AB	1.47		2.96	0.37	AB	59	0.29	46	AB	45	11	57	AB
OnGrd	2.64	0.22	A	1.68		3.01	0.41	A	59	0.31	35	B	33	12	63	AB
Thyme	2.76	0.18	B	1.39		3.12	0.32	B	69	0.28	76	A	70	8	57	B
Xplode	2.67	0.21	A	1.58		3.03	0.39	A	51	0.3	27	B	30	12	61	AB
AVG:	2.66	0.20		1.54		3.05	0.37		59.2	0.30	42.0		40.8	11.6	60.6	

n = 4 from 6 trees pooled in 4 reps.

Total fruit yield from these 3 to 4-year-old trees was quite low (about .3 to .5 boxes; 40 kg = 1 box) at both sites in both Jan 2017 and Dec 2017 harvests and there were no treatment effects on yield (Table 9, 10). Overall percentage fruit drop was quite high, 41-71% and again, there were no treatment effects. There was a little more visible canker in the IPGN5 treatment at the HLBNEG site in 2017 but very little canker at the HLBPOS site and no treatment effects.

HLBNEG Table 9. Fruit Yield (kg), Percentage Fruit Drop (FD) & Canker rating in Jan & Dec 2017.							
Trt	Yield_Jan2017	%FD_Jan2017		Yield_Dec2017	%FD_Dec2017	Canker_% 2017	
CON	17.85	54.9	AB	14.67	69.96	4.92	AB
Ecotrol	18.58	46	BC	16.37	66.05	4.24	AB
IPGN5	14.82	59.86	A			6.56	A
OnGrd	20.76	45.74	BC	14.03	70.95	6.28	AB
Thyme	22.03	41.09	C	12.84	70.55	3.44	B
Xplode	16.92	54.06	ABC	13.17	71.22	6.22	AB

n = 4 from 6 trees pooled in 4 reps.

HLBPOS	Table 10. Fruit Yield (kg), Percentage Fruit Drop (FD) and Canker rating in 2017.				
Trt	Yield_Jan2017	%FD_Jan2017	Yield_Dec2017	%FD_Dec2017	Canker_percent
CON	14.92	50.24	14.19	59.34	0
Ecotrol	8.74	68.68	9.16	65.7	0
IPGN5	11.97	58.73			0
OnGrd	12.85	57.87	11.38	66.65	0
Thyme	10.42	62.09	9.93	62.42	0.23
Xplode	10.88	58.93	10.68	62.04	0.1
AVG:	11.63	59.42	11.07	63.23	0.06

n = 4 from 6 trees pooled in 4 reps.

After 11 months of treatments (Jan 2017), there were no treatment effects on any of the measured juice quality characteristics at either site (Table 11). Fruit from the HLBPOS site tended to have higher % juice, Brix and pound solids per box than the HLBNEG site.

HLBNEG	Table 11. Fruit quality characteristics, Jan 2017.						
Trt	pc_juice	LbsJuicePerBox	Acid	TotalBrix	Ratio	LbsSolidsPerBox	JuiceColor
CON	0.55	49.28	0.55	8.68	15.71	4.28	34.88
Ecotrol	0.53	48.03	0.57	8.55	15.19	4.1	35.33
IPGN5	0.5	44.87	0.58	8.78	15.31	3.94	35.08
OnGrd	0.49	44.52	0.55	8.49	15.4	3.78	35.08
Thyme	0.54	48.75	0.54	8.79	16.47	4.29	35.35
Xplode	0.53	47.5	0.54	8.79	16.36	4.18	34.83
Avg:	0.52	47.16	0.56	8.68	15.74	4.10	35.09
HLBPOS	Fruit quality characteristics, Jan 2017.						
Trt	pc_juice	LbsJuicePerBox	Acid	TotalBrix	Ratio	LbsSolidsPerBox	JuiceColor
CON	0.57	50.89	0.6	10.68	17.83	5.43	35.03
Ecotrol	0.56	50.59	0.61	10.23	16.86	5.17	35.03
IPGN5	0.56	50.62	0.6	10.43	17.41	5.28	35.1
OnGrd	0.55	49.68	0.58	9.79	16.98	4.86	35.05
Thyme	0.58	52.55	0.56	9.99	17.78	5.25	35.3
Xplode	0.57	50.98	0.61	10.49	17.29	5.35	35.18
Avg:	0.57	50.89	0.59	10.27	17.36	5.22	35.12
6 Trts x 4 reps (6 trees pooled per rep)							

Untreated Control fruit from the second harvest (Dec 2017) at the HLBPOS site, tended to have the highest brix, ratio and lb solids per box compared to the Thyme treatment which had the lower lb solids per box than the Control. (Table 12).

HLBNEG	Table 12. Fruit quality characteristics, Dec 2017											
Trt	pc_juice		LbsJuicePerBox		Acid	TotalBrix		Ratio		LbsSolidsPerBox		JuiceColor
CON	0.58	AB	52.23	AB	0.59	9.39		16.05		4.91		34.15
Ecotrol	0.6	A	53.63	A	0.59	9.79		16.61		5.25		34.65
OnGrd	0.57	B	51.69	B	0.57	9.29		16.39		4.8		34.2
Thyme	0.58	AB	52.34	AB	0.58	9.79		16.82		5.12		34.58
Xplode	0.58	AB	52.2	AB	0.56	9.65		17.15		5.04		34.43
Avg:	0.58		52.42		0.58	9.58		16.60		5.02		34.40
HLBPOS	Fruit quality characteristics, Dec 2017											
Trt	pc_juice		LbsJuicePerBox		Acid	TotalBrix		Ratio		LbsSolidsPerBox		JuiceColor
CON	0.58		52.23		0.58	10.07	A	17.3	A	5.26	A	34.63
Ecotrol	0.56		50.76		0.59	9.95	AB	16.92	AB	5.05	AB	34.58
OnGrd	0.57		51.59		0.58	9.79	AB	16.95	AB	5.05	AB	34.73
Thyme	0.56		50		0.61	9.36	B	15.43	B	4.68	B	34.35
Xplode	0.57		51.33		0.6	10.08	A	16.96	AB	5.17	A	34.33
Avg:	0.57		51.18		0.59	9.85		16.71		5.04		34.52
5 Trts x 4 reps (6 trees / rep)												

Conclusions. Since ACP counts were almost always zero, there was essentially no psyllid pressure throughout the two-year study at either site. At the HLBNEG site Table 1), all Biopesticide treatments including the untreated control had lower CT values in Dec 2017 than initially in Feb 2016 so none of the treatments prevented infection but the Ecotrol treatment was able to delay infection better than the Control after 2 years. At the HLBPOS site (Table 2), CT values of the Ecotrol treatment were lower (more HLB) than the untreated Control during the first year but all treatments had less HLB by Dec 2017 and no treatment differed from the Control.

At the HLBNEG site, Ongrd trees had smaller canopy volumes than untreated Control trees and Thyme trees had smaller TCSA than Control trees after 2 years of treatments (Table 3). Canopy volumes and TCSA of the HLBPOS trees were overall smaller than HLBNEG trees from the beginning and there were no treatment effects on tree size or trunk girth of the HLBPOS trees after 2 years (Table 4).

HLBNEG trees gradually increased visual disease index rating (DI) but there were no consistent treatment differences throughout as no treatment DI differed from the control (Table 5). HLBPOS trees initially had higher DIs than HLBNEG trees but again, there were no consistent treatment differences and both sites were similar in appearance after 2 years (Table 6).

At both the HLBNEG and HLBPOS sites, there were no treatment effects on any of the major nutrients in July 2016, 6 months after the treatments began (Table 7). Leaf N was initially low at the HLBPOS site but since all leaf nutrient values at the HLBPOS site were within optimum

ranges (except Fe) by the second year, the small treatment effects on leaf P, Mg, Mn and B were probably of little consequence (Table 8).

Total fruit yield in these 3 to 4-year-old trees was quite low (.3 to .5 boxes) at both sites in both years and there were no treatment effects on yield (Table 9, 10). Overall percentage fruit drop was quite high, 41-71% and again, there were no treatment effects.

There were no treatment effects on any of the measured juice quality characteristics at either site (Table 11). Fruit from the HLBPOS site tended to have higher % juice, Brix and lb solids per box than the HLBNEG site. Untreated Control fruit from the second harvest (Dec 2017) at the HLBPOS site, tended to have the highest brix, ratio and lb solids per box compared to the Thyme treatment which had the lower lb solids per box than the control. (Table 12).

Overall, there was little evidence to support the idea that these Biopesticide treatments suppress CLas populations or improve tree health in these well-managed 3 to 4-year old trees with good pesticide management of psyllids.