CRDF Thermotherapy Field Day

Thursday, December 3, 2015

Wheeler's Lake Wales TT Dec 3, 15 Leaves collected for PCR 6/9/2015;

Treatment Temp (F) Treatment duration TT: 15 trees and 15 control trees

6/12/2015 122 - 128° 20 sec

Ct = cycle threshold		Jun 9,15	Sept_15	Diff	pre to post trt	
Trt	Tr No.	Pre Ct	Post_Ct	post-pre	HLB change	
Control	1	20	20	0	no chg	
Control	2	35	21	-14	more	?
Control	3	24	36	12	less	
Control	4	27	23	-4	more	
Control	5	20	24	4	less	
Control	6	20	21	1	less	
Control	7	40	22	-18	more	?
Control	8	37	39	2	less	
Control	9	24	22	-2	more	
Control	10	20	23	2	less	
Control	11	24	24	0	no chg	
Control	12	40	38	-2	more	
Control	13	20	22	2	less	
Control	14	21	22	1	less	
Control	15	21	22	1	less	
	Avg	**26**	**25**	**-1**	**more**	
HLB neg		4 neg	3 neg			

Controls Pre to Post Summary	
more HLB	5
less HLB	8
no change=	2

3-4 HLB neg

		Jun 9,15	Sept_15	Diff	pre-post
Trt	Tr No.	Pre Ct	Post_Ct	post-pre	HLB change
TTtrt	1	19	24	5	less
TTtrt	2	20	24	3	less
TTtrt	3	21	22	1	less
TTtrt	4	19	24	5	less
TTtrt	5	21	22	1	less
TTtrt	6	21	23	2	less
TTtrt	7	20	23	2	less
TTtrt	8	22	25	3	less
TTtrt	9	20	25	5	less
TTtrt	10	19	22	3	less
TTtrt	11	20	23	3	less
TTtrt	12	22	22	0	no chg
TTtrt	13	20	23	3	less
TTtrt	14	19	23	4	less
TTtrt	15	19	21	2	less
	Avg	**20***	**23 ns**	**3**	**less**
	* signif. at P<0.05	15 pos	14 pos		
		more HLB			

Treated Pre to Post Summary	
more HLB	0
less HLB	14
no change=	1

All HLB +

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Wheeler's Lake Wales TT TT: 15 trees and 15 control trees

		Tree height (cm)			DI			
Trt	Tr No.	**TrHt 6_9**	**TrHt 9_10**	**TrH 10_20**	**DI 6_9**	**DI 7_17**	**DI 9_10**	**DI 10_20**
Control	1	160	160	170	13	14	13	18
Control	2	200	190	210	15	11	14	19
Control	3	170	160	150	14	8	14	19
Control	4	160	160	170	14	15	16	18
Control	5	180	170	165	13	14	13	17
Control	6	160	170	180	14	16	13	19
Control	7	190	180	195	14	12	12	19
Control	8	190	210	240	14	11	12	18
Control	9	180	170	190	15	14	14	19
Control	10	190	170	200	13	10	12	19
Control	11	190	180	190	16	15	18	19
Control	12	210	230	255	14	12	14	18
Control	13	210	200	220	13	12	14	18
Control	14	170	180	190	14	13	13	14
Control	15	200	180	215	14	11	14	15
	Avg	**184**	**180**	**196**	**14**	**13**	**14**	**18**
		taller	taller	= height	lower DI	= DI	= DI	higher DI

		Tree height (cm)			DI			
Trt	Tr No.	**TrHt 6_9**	**TrHt 9_10**	**TrH 10_20**	**DI_6_9**	**DI_7_17**	**DI_9_10**	**DI_10_20**
TTtrt	1	150	160	165	13	12	12	13
TTtrt	2	140	140	150	16	10	12	12
TTtrt	3	160	160	160	16	12	16	18
TTtrt	4	140	140	140	22	14	16	19
TTtrt	5	130	140	140	21	12	15	15
TTtrt	6	190	200	220	20	12	14	16
TTtrt	7	180	170	175	18	11	13	15
TTtrt	8	150	160	180	19	14	15	15
TTtrt	9	190	180	205	18	16	18	15
TTtrt	10	170	170	180	16	12	16	18
TTtrt	11	170	170	200	22	16	16	19
TTtrt	12	180	180	220	18	15	15	18
TTtrt	13	150	170	190	20	11	16	18
TTtrt	14	160	160	180	16	10	14	15
TTtrt	15	140	140	170	18	15	16	16
	Avg	**160***	**162***	**178 ns**	**18***	**13 ns**	**15 ns**	**16***
* signif. at P<0.05		shorter	shorter	= height	higher DI	= DI	= DI	lower DI
		TrHt 6/9	TrHt 9/10	TrH 10/20	DI_6_9	DI_7_17	DI_9_10	DI_10_20

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Wheeler's Lake Wales TT TT: 15 trees and 15 control trees

Trt	Tr No.	FrDr 7_17	FrDr 9	FrDr 10_20	TA6_9	TA10_20	CV 6_9	CV 9_10	CV 10_20
		Fruit drop			Tr Cr Sec Area (cm		Can vol (m3)		
Control	1	4	3	1	24	28	4	4	5
Control	2	11	30	9	33	37	6	6	8
Control	3	3	13	2	29	33	5	5	4
Control	4	0	3	1	27	33	4	5	5
Control	5	0	6	5	33	37	4	5	6
Control	6	1	7	0	36	36	5	6	8
Control	7	0	3	2	48	50	8	8	9
Control	8	0	0	2	55	62	6	8	11
Control	9	1	9	2	39	42	5	5	6
Control	10	0	12	3	59	65	8	7	10
Control	11	0	3	3	28	32	5	6	6
Control	12	0	3	16	36	39	6	9	10
Control	13	0	3	17	43	48	6	7	10
Control	14	1	8	9	48	53	7	8	9
Control	15	2	3	26	48	52	8	7	9
	Avg	**2**	**7**	**7**	**39**	**43**	**6**	**6**	**8**
		ns	ns	ns	larger	larger	larger	larger	larger

Trt	Tr No.	FrDr 7_17	FrDr 9	FrDr 10_20	TA6_9	TA10_20	CV 6_9	CV 9_10	CV 10_20
		Fruit drop			Tr Cr Sec Area (cm		Can vol (m3)		
TTtrt	1	1	5	3	37	39	6	7	7
TTtrt	2	0	4	1	35	37	4	4	6
TTtrt	3	11	5	2	20	23	4	4	4
TTtrt	4	5	9	18	24	27	3	3	4
TTtrt	5	0	6	5	18	21	2	2	3
TTtrt	6	7	0	1	39	40	6	7	9
TTtrt	7	1	6	8	37	39	5	6	6
TTtrt	8	2	14	7	23	26	4	4	4
TTtrt	9	4	2	8	47	48	7	8	9
TTtrt	10	2	11	12	40	41	5	5	6
TTtrt	11	0	1	1	27	29	5	5	8
TTtrt	12	2	15	14	39	43	4	5	6
TTtrt	13	1	3	0	28	29	4	6	6
TTtrt	14	1	4	9	28	31	4	5	5
TTtrt	15	1	4	10	28	31	4	4	5
	Avg	**2 ns**	**5 ns**	**7 ns**	**31***	**33***	**4***	**5***	**6***
	* signif. at P<0.05	**No Leaf Drop**			smaller	smaller	smaller	smaller	smaller
		FrDr 7_17	FrDr 9	FrDr 10_20	TA 6_9	TA 10_20	CV 6_9	CV 9_10	CV 10_20

THE COST OF THERMOTHERAPY

Joseph Trotochaud & Reza Ehsani – UF/IFAS Citrus Research & Education Center

A – *EQUIPMENT COSTS*

Construction of thermotherapy machines using steam or hot water is relatively simple. If only a few acres need to be treated, it may be more economical to rent a thermotherapy machine. However, growers with several thousand acres may decide to build a machine themselves. The following factors must be considered when constructing a thermotherapy machine.

- ***Tree Sizing and Spacing***
  Bigger trees require more heat, a larger enclosure, and more water. If the spacing in a grove is close enough, or double resets are common, then it may be possible to treat two trees at a time.
- ***Heat Sources and Delivery***
  Steam heats the canopy faster than hot water and requires less water, but the equipment costs more.
- ***Water Demand, Capacity, and Quality***
  Clean, soft water is necessary for steam and hot water systems to prevent clogging or shortened life. Smaller steam systems can operate for half of a day with just 200 gallons of water. Hot water may need at least twice that amount.
- ***Vehicle***
  The vehicle must hold the weight of the equipment, water, and travel in sand. A self-propelled system is easier to maneuver in a grove than a pull-behind system. Self-propelled machines can be transported easily from one grove to another.
- ***Tree Enclosure***
  The tree enclosure should match the size of the trees being treated. An oversized enclosure will require more heat and time to raise the temperature while an undersized enclosure can be damaged by larger trees.
- ***Power Source***
  A power source is needed for oil burners on steam or hot water systems, fans and water pumps. Power can come from a small generator or from the vehicle directly if it is powerful enough. 3000 to 5000 watts is enough to power most thermotherapy systems.
- ***Heat Distribution***
  Since hot air rises, circulation must be added to prevent burning the tops of trees. For steam, this can be accomplished with the placement of fans at the ceiling of the enclosure, while for hot water the placement and angle of spray nozzles will need to be considered.
- ***Number and Placement of Thermometers***
  The temperature in the tree enclosure must be monitored to prevent over- or under-treatment. A single thermometer may not give a representative picture of the temperature distribution, so a series of 4 or more thermometers should be placed within the tree enclosure and monitored in real-time by the operator.
- ***Operator Vision and Equipment Placement***
  Most thermotherapy machines require two people to operate them, one driving the machine, and one person on the ground helping the driver line the machine up with the tree. Installing side-facing cameras or positioning equipment to create good sightlines for the driver will eliminate the need for a second operator.

The following tables lists the approximate cost of the improved thermotherapy system used by the University of Florida Citrus Research and Education Center.

FUNCTION	ITEM	CAPACITY	COST
Mobile Platform	Military Surplus M1078	2-1/2 Ton, 4 X 4, 8'wide	$ 15,000.00
Steam Generator	Sioux Steam-Flo® SF-20	690 lb/hr steam	$ 20,000.00
Water Storage	IBC Tote Tank	275 gallon	$ 285.00
Water Pump	Flowjet Sprayer Pump	3.8 gpm @ 45psi	$ 1,000.00
Water Filter	4-1/2" X 20" Sediment Filter	20 micron, 10 gal/min	$ 60.00
Water Softener	Kinetico Signature Series®	300 gal/recharge	$ 2,300.00
Electricity Generator	Military Surplus M802A	5 kW	$ 600.00
Tree Enclosure	Custom Hydraulic Frame with Cloth Cover	10'L x 8'W x 9'H	$ 15,000.00
Electronics	Cameras, Controls, Thermometers	Single Operator Required	$ 600.00
	TOTAL ESTIMATED:		**$ 54,845.00**

THE COST OF THERMOTHERAPY

Joseph Trotochaud & Reza Ehsani – UF/IFAS Citrus Research & Education Center

B – *OPERATING COSTS*

For thermotherapy machines using steam or hot water, labor and fuel are the primary costs for operation. Since steam and hot water generators typically operate continuously, the cost of thermotherapy then becomes dependent on how many trees can be done per hour. If only a few trees are treated per acre, costs will rise because more time is spent moving from tree to tree than actually applying thermotherapy. This means that blocks with higher rates of HLB infection will actually have a lower cost per tree for thermotherapy. Combining tree size and infection rate, the operational cost of thermotherapy can be estimated.

Based on field trials applying thermotherapy to approximately 5000 trees, the following costs per tree have been observed using thermotherapy systems constructed by the University of Florida:

TREE SIZE	PERCENT INFECTED	TREES PER DAY	COST PER TREE
6-9’	40%	75	$4.00
6-9’	80%	100	$2.50
<6’	80%	120	$1.75
<6’	100%	150	$1.50

Most field trials to-date have been conducted in groves with trees 6-9’ tall and infection rates around 60%, resulting in the following cost per tree and cost per acre:

COST PER TREE

3.5 Gallons of Water
0.25 Gallon of Fuel
1 operator
3 minutes

$1.70

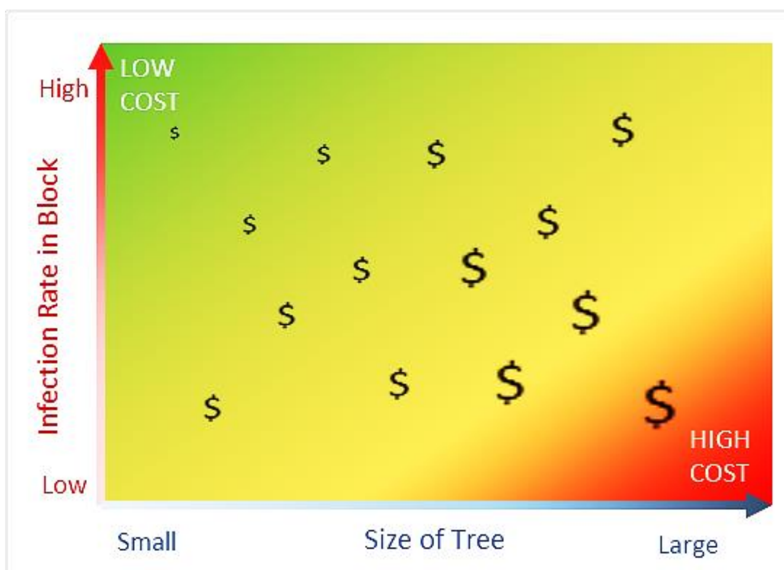
COST PER ACRE

10’ Spacing,
60% Infected

$125

In general, the cost per tree of thermotherapy will be lowest in groves with small trees and high rates of infection. Vice versa, the cost of thermotherapy will be highest in groves with large trees and low infection rates. The size of the tree influences cost more than the infection rate since larger trees require larger heat generators, vehicles, water tanks, pumps, and tree enclosures. Larger trees also take longer to reach treatment temperature because there is more tree to be heated.

Cost savings can be found by recycling spare equipment such as water tanks, pumps, electricity generators, and hydraulic components. The steam or hot water generators will be the most expensive single component but can be purchased off the shelf eliminating any need for fabrication.



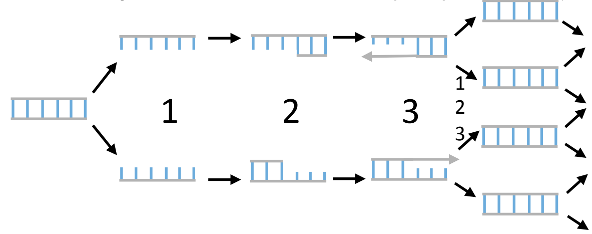
The tree enclosure will be the second most expensive component will be the tree enclosure, since this is a custom item. A large cost savings can be found if a vehicle does not need to be purchased or can be purchased second hand. Reducing to a single operator by using simple side-facing cameras can reduce operating costs by as much as a third.

For questions or more information, please contact Joseph Trotochaud (jtroto@ufl.edu) or Reza Ehsani (ehsani@ufl.edu).

What is PCR?

Polymerase chain reaction (PCR) is a technology used to make many copies of a specific piece of DNA. PCR is a heating and cooling process that pulls apart the double stranded DNA (1), adheres small pieces of DNA to "prime" the creation of new DNA strands (2), and then builds a new strand of DNA that is the same as the original (3). This cycle repeats many times, doubling the amount of DNA each cycle. This increases the amount of DNA in a sample to a detectible level.

Polymerase Chain Reaction (PCR)



PCR detects the presence of DNA of the bacterium that causes HLB, but it does not distinguish between live or dead cells. However, the type of PCR that is being funded by the CRDF is a quantitative PCR (qPCR) that does estimate the amount of DNA present based on a Copy threshold (C_t) value.

What is a copy threshold?

Quantitative PCR will cycle 40 times. This type of PCR uses a signal that tells the PCR machine how much DNA is in the sample during each of the cycles. The machine will go through several cycles before it can even detect the target DNA, the cycle in which the DNA is finally detected by the machine is the cycle threshold or C_t. This is when enough DNA has been made to hit the threshold for detection. For example, if only one copy is in the sample, the machine may take 38 cycles to make enough copies until there is enough DNA for the machine to detect; in this case the C_t is 38. If there are 100 copies, the machine may take only 20 cycles and the C_t would be 20.

Do PCR results tell us how much bacteria is in the sample?

PCR labs produce C_t values which cannot be directly translated into bacterial concentrations. The C_t values will tell you if your tree is HLB positive or negative and to what extent the tree is infected compared to all of the samples in that sample batch. Extra steps are necessary to estimate the number of bacterial cells in a sample; this would limit the number of samples that could be processed and does not provide important decision-making information to growers.

My sample has a C_t value of 32, why does the testing lab consider this HLB free?

A number of falsely positive or falsely negative samples are expected when screening large numbers of samples from many sources; because of this a threshold is set by the PCR lab. The Southern Gardens threshold, for example, is a C_t value of 32, this value was selected after screening large numbers of known positive and negative samples. PCR results should be combined with a visual assessment of the tree to get the most accurate knowledge of tree health status.

If my sample has a C_t value of 40 does that mean it is HLB free?

No test is perfect. A C_t value that is above the" PCR negative" threshold may not mean the tree is disease-free; sampling methods including the type of tissue selected for sampling and the number of samples per tree, and the time of year effect the ability to get accurate results. Again, a visual assessment of the tree, combined with PCR results, will give a more accurate read on the health of the tree. A document describing methods to evaluate tree health titled "Field trial tree evaluation methods", can be found on the CRDF website (citrusrdf.org).

What is the best tissue to submit to the PCR labs?

Leaf tissue that has shown symptoms of HLB such as blotchy mottle and yellow vein symptoms is the best tissue to select. New flush tissue should be avoided and only fully expanded leaves should be sampled. For more detailed instructions see this document: http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf.

What is the best time of year to sample?

During the summer when the weather is hot and the trees look their best is the time of year when HLB is the most difficult to detect by PCR. This may be because the tree is growing more rapidly than the bacteria is growing, the heat may suppress the bacteria, or the tree may be able to use defense mechanisms to suppress the bacterial growth. Southern Gardens recommends sampling from September through March for the most accurate results.

PCR Labs (these CRDF funded labs provide PCR analysis of plant and psyllid samples at no cost to growers):

SWFREC HLB Lab:
2685 State Road 29 North
Immokalee, Florida 34142
239-658-3431
www.imok.ufl.edu/programs/plant-path/extension-roberts/hlb-lab/

Southern Gardens Lab:
United States Sugar Corporation
Technical Operations
111 Ponce de Leon Ave.
Clewiston, FL 33440
863-902-2249
www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf