



RFP – Field Trial of Potential Year-Three HLB Treatments for OTC Treated Trees

CRDF is seeking bids to evaluate experimental HLB treatments that will identify whether trees that have been treated with OTC injections for two years will maintain health, yield, and fruit quality through the third year without an additional OTC treatment and if the proposed experimental treatments contained herein will have an effect on tree performance and productivity.

Background and scope of work:

CRDF has identified an opportunity to study HLB therapies in trees which have received two OTC injections and produced two crops of fruit. Pursuant to the label of the OTC injection materials, confirmed by state regulators, trees which have been injected two years in a row must be left untreated in the following year, including foliar-applied OTC. It is assumed that trees with two years of injections will not backslide in the noninjected third year, but growers are doubtful, and there is limited independent data from which to make a judgment.

There is a Valencia block on the east coast which has served as a trial site for Dr. Ute Albrecht's research on OTC injections which could be used to help address these questions. The original purpose of the trial was to look at different rates and volumes of OTC injections. In this trial there are six treatments. Three of the treatments from the original trial received 1.1 grams of active ingredient per tree and 2 treatments received 0.55 grams of active ingredient per tree. The last treatment was an untreated control. Each treatment has eight replicated plots with eight trees per plot. Using the established treatment plots, an experiment can be designed to inject trees in the third year with either EDTA, Aluminum-Fosetyl, or OTC, and compare them to trees left untreated during this third year, which will serve as a control.

Trial Design:

For the new trial, the eight-tree plot would be split in half. Four of the eight trees in each replicated plot would be treated and the remaining four trees would be untreated. The untreated groups of four trees will provide insight into the longevity of the previously applied OTC treatments and serve as a benchmark of performance for the third-year treatments.

The eight plots of the untreated control treatment from Dr. Albrecht's experiment will be used to evaluate EDTA and Aluminum-Fosetyl injections as a stand-alone treatment in trees that have never been injected. The untreated control plots will be split in half with four trees receiving an injection of the experimental treatment and the other four trees in the plot remaining untreated. There are eight plots of untreated control trees. To evaluate both EDTA and Aluminum-Fosetyl in these established treatment areas, only four replicated plots per treatment are possible. All other treatments will have eight replicated plots.

There will be eight replicated plots for five of the six treatments. Each replicated plot will have four trees. In the untreated control group from Dr. Albrecht's trial, there will be four replicated plots for EDTA and four replicated plots for the Aluminum-Fosetyl compared to an equal number of untreated plots.

There will be a total of 96 plots in the trial. Each plot has four trees. The total number of trees for the trial is 384. There will be 192 trees that are untreated and 192 trees that will be treated with the experimental bactericides. The plots have already been established. See Appendix A for trial design.

Treatments

Untreated control

EDTA injection

Aluminum-Fosetyl injection

OTC injection for a third year

Deliverables

1. Yield – weight of fruit per tree
2. Weight of a 50-fruit subsample for each tree
3. Fruit Quality per plot (brix, acid, ratio, pound solids at CREC Pilot Plant)
4. Fruit Drop (percent drop per tree, plot, and treatment)
5. DI ratings (trial initiation and at harvest)
6. Trunk damage (rating of severity)

All data collected will be provided on both an individual tree and plot basis using Excel. Tree health measurements will be taken prior to the beginning of the study and just prior to harvesting the fruit from each tree. Individual tree health will be rated using the DI rating method as outlined in the August 2016 Citrus Industry Magazine (https://crec.ifas.ufl.edu/media/crecifasufledu/extension/extension-publications/2016/2016_August_hlb.pdf).

Fruit drop counts will begin 3 months prior to the anticipated harvest date. Fruit drop should be assessed every 2 weeks. Prior to the start of evaluating fruit drop, all fruit under the measurement trees will be removed but not counted. From that point, fruit drop will be measured once every 2 weeks. Dropped fruit shall be counted and removed from underneath each measurement tree. Fruit drop counts will continue until harvesting. For each fruit drop evaluation, the total number of fruits counted under each measurement tree will be totaled and that data will be recorded on an Excel spreadsheet. The total number of dropped fruit per measurement tree will be submitted to CRDF.

Total yield will be collected from each measurement tree. Fruit weight (Kg) per tree will be submitted on Excel data sheets to be sent to CRDF at harvest. For each tree, a **50-fruit subsample** will be counted and weighed (Kg). The weight of the 50-fruit subsample will be used to calculate fruit drop percentage for each tree in the trial.

At harvest, a 65-fruit sample will be collected from each treatment plot (n=96) and put into labeled mesh bags for **fruit quality** analysis. Harvest lead time must be anticipated to facilitate coordination of delivery to the Pilot Plant at UF/IFAS, CREC, Lake Alfred, through the CRDF Research Coordinator (Brandon Page).

Trunk Damage shall be assessed by a simple number rating. The damage scale will be as follows:

1. No damage to the trunk aside from the drill hole.
2. Bark splitting extending no more than 2 inches from the drill hole.
3. Bark splitting extending no more than 4 inches from the drill hole.
4. Bark splitting extending no more than 6 inches from the drill hole.
5. Bark splitting extending more than 6 inches from the drill hole.

Proposal Submission:

Proposals must be submitted to catp@citrusrdf.org, and received by 5:00 p.m. Eastern time on Tuesday, April 9, 2024.

The following documents shall comprise the Proposal package:

1. **Proposal Cover Page and Budget** - Form CB-24 -*link to download form:*
<https://citrusrdf.org/wp-content/uploads/2024/03/CB-24-Cover-Page-and-Budget.pdf>
(5 pages; pdf file).
2. **Budget Justification** (Word file)
3. **Subcontract Budget** (*complete if applicable*) - Form SC-24 -*link to download form:*
<https://citrusrdf.org/wp-content/uploads/2024/03/SC-24-Subcontract-budget.pdf>
(4 pages; pdf file. SC-24 forms do not count in the 7-page limit). Complete a separate form for each proposed subcontract.

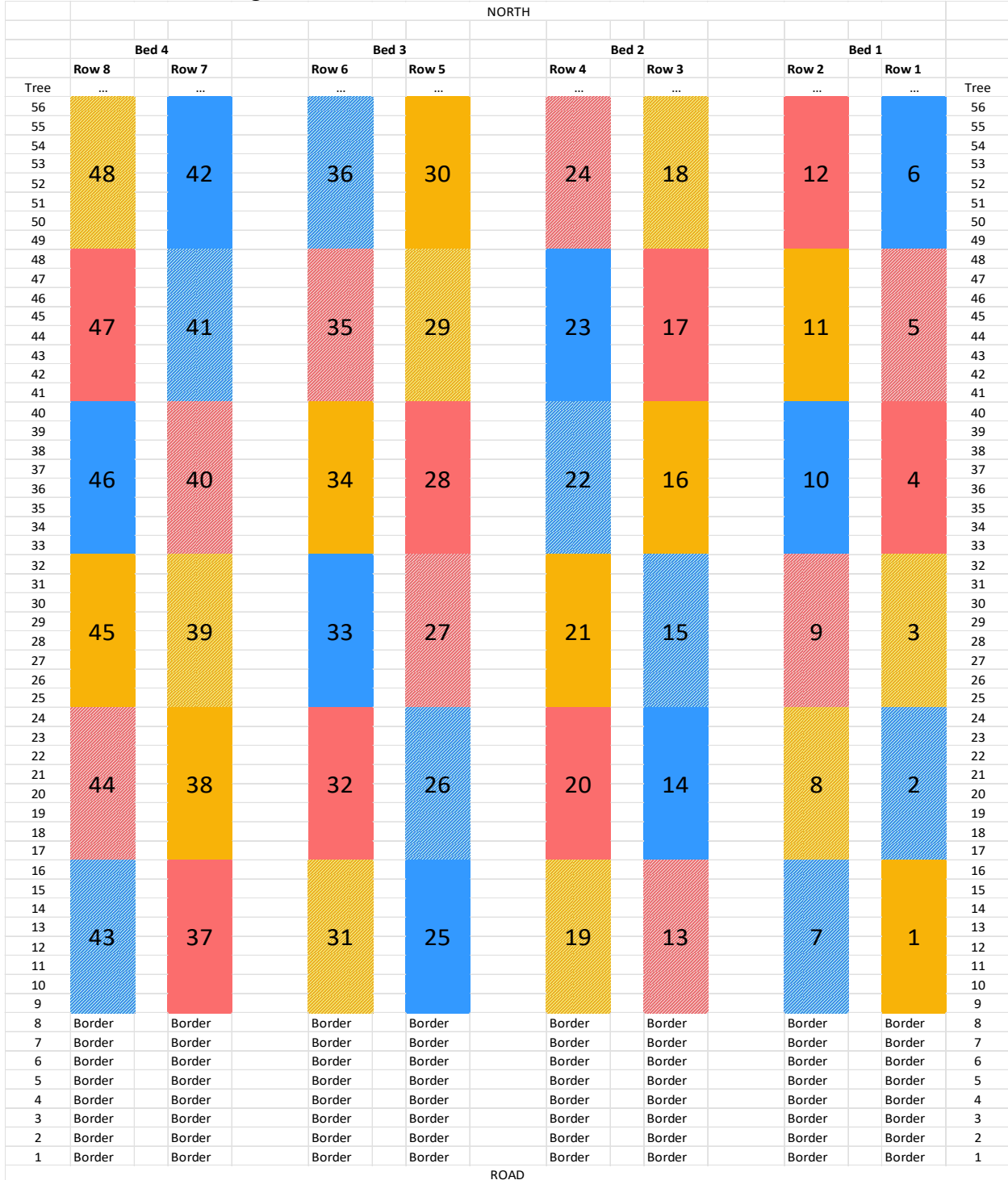
Detailed instructions can be found at http://citrusrdf.org/wp-content/uploads/2024/03/Instructions-to-complete-submit-preproposals-and-full-proposals_March-2024.pdf

All questions should be emailed to catp@citrusrdf.org.

Appendix A

TRT	Device	Compound	Dose (a.i.) per tree	Reps	Trees/rep	Total
T1	None	None	0 mg	8	8	64
T2	Chemjet	Arbor-OTC	550	8	8	64
T3	Chemjet	Arbor-OTC	1100	8	8	64
T4	TJ Bio	ReMedium TI	550	8	8	64
T5	TJ Bio	ReMedium TI	1100	8	8	64
T6	Chemjet	ReMedium TI	1100	8	8	64

Dr. Albrecht's trial design.



New treatments randomized across the old treatments.

	Bed 4		Bed 3		Bed 2		Bed 1		
	Row 8	Row 7	Row 6	Row 5	Row 4	Row 3	Row 2	Row 1	
Tree	Tree
56	AL Con	OTC Con	CON	EDTA	EDTA Con	AL Con	AL Con	AL Con	56
55									55
54	AL Con	OTC Con	CON	EDTA	EDTA Con	AL Con	AL Con	AL Con	54
53									53
52	AL	OTC	AL	EDTA Con	EDTA Con	AL	AL	AL	52
51									51
50	AL	OTC	AL	EDTA Con	EDTA Con	AL	AL	AL	50
49									49
48	AL	EDTA	EDTA Con	OTC Con	OTC Con	EDTA	EDTA Con	OTC Con	48
47									47
46	AL	EDTA	EDTA Con	OTC Con	OTC Con	EDTA	EDTA Con	OTC Con	46
45									45
44	AL Con	CON	EDTA	OTC	OTC	EDTA Con	EDTA	OTC Con	44
43									43
42	AL Con	CON	EDTA	OTC	OTC	EDTA Con	EDTA	OTC Con	42
41									41
40	EDTA Con	AL Con	AL Con	AL	EDTA	AL	EDTA Con	EDTA	40
39									39
38	EDTA Con	AL Con	AL Con	AL	EDTA	AL	EDTA Con	EDTA	38
37									37
36	EDTA	AL	AL	AL Con	CON	AL Con	EDTA	EDTA Con	36
35									35
34	EDTA	AL	AL	AL Con	CON	AL Con	EDTA	EDTA Con	34
33									33
32	EDTA	EDTA Con	AL Con	AL Con	EDTA	AL	AL Con	EDTA Con	32
31									31
30	EDTA	EDTA Con	AL Con	AL Con	EDTA	AL	AL Con	EDTA Con	30
29									29
28	EDTA Con	EDTA	AL	AL	EDTA Con	CON	AL	EDTA	28
27									27
26	EDTA Con	EDTA	AL	AL	EDTA Con	CON	AL	EDTA	26
25									25
24	OTC Con	AL	EDTA Con	CON	AL	OTC Con	OTC Con	CON	24
23									23
22	OTC Con	AL	EDTA Con	CON	AL	OTC Con	OTC Con	CON	22
21									21
20	OTC Con	AL Con	EDTA	EDTA	AL Con	OTC	OTC	AL	20
19									19
18	OTC Con	AL Con	EDTA	EDTA	AL Con	OTC	OTC	AL	18
17									17
16	AL	EDTA	OTC Con	EDTA Con	AL Con	EDTA Con	CON	AL	16
15									15
14	AL	EDTA	OTC Con	EDTA Con	AL Con	EDTA Con	CON	AL	14
13									13
12	CON	EDAT Con	OTC	EDTA	AL	EDTA	EDTA	AL Con	12
11									11
10	CON	EDAT Con	OTC	EDTA	AL	EDTA	EDTA	AL Con	10
9									9
8	Border	Border	Border	Border	Border	Border	Border	Border	8
7	Border	Border	Border	Border	Border	Border	Border	Border	7
6	Border	Border	Border	Border	Border	Border	Border	Border	6
5	Border	Border	Border	Border	Border	Border	Border	Border	5
4	Border	Border	Border	Border	Border	Border	Border	Border	4
3	Border	Border	Border	Border	Border	Border	Border	Border	3
2	Border	Border	Border	Border	Border	Border	Border	Border	2
1	Border	Border	Border	Border	Border	Border	Border	Border	1

ROAD