Request for Proposals – 2: Neutral pH

Testing the Injection of Oxytetracycline (OTC) in a pH Neutral Solution

**Situation Statement:** CRDF is seeking bids to test an adjuvant developed by Dr. Santra which, when added to an injectable OTC formulation, creates a pH of 7.5, which is higher than registered OTC formulations. Dr. Santra claims that this new method for mixing OTC has shown promising efficacy in treated trees infected with huanglongbing (HLB), with low risk of trunk damage at the injection site.

An experiment is proposed to evaluate the efficacy of Dr. Santra’s adjuvant and OTC mixture compared to other treatments of injectable OTC administered under current label instructions. All treatments would be compared to a water-treated control. Data to be collected includes yield, fruit quality, fruit drop, DI ratings, trunk damage, OTC detection in leaves, and residue testing in whole fruit.

**Methodology:** A single trunk injection for each of the existing OTC products (Remedium TI and Rectify) shall occur according to label requirements for standard commercial treatments. The experimental treatments – those using the adjuvant developed by Dr. Santra - would be injected following his protocol for mixing and application. Water treated controls shall be injected with DI water. The active ingredient rate for the OTC treatments, as well as injection volume, shall be the same for all trees in the trial.

**Objectives of the Project:** The trial is intended to evaluate the relative effectiveness of the experimental OTC injection mixture. Data shall be collected on the following elements:

1. Yield per tree (total fruit weight)
2. Weight of a 50-fruit subsample for each tree
3. Fruit quality per plot (brix, acid, ratio, pound solids)
4. Fruit drop (percent drop per tree, plot, and treatment)
5. DI ratings (trial initiation and at harvest)
6. Trunk damage (rating of severity)
7. qPCR
8. OTC detection in leaves
9. Residue testing in whole fruit (USDA lab in North Carolina)

**Trial Design:** The trial will consist of trees between five years old and fifteen (5 to 15) years old. All trees used in this trial shall be the same age, rootstock, and scion. Tree health and appearance will be as uniform as possible. The trees shall be sufficiently healthy as to allow normal uptake of injected crop protection products.

Seven (7) treatments shall be administered. The trees shall be of similar visual health and must be the same rootstock, scion, and age. The seven (7) treatments shall be replicated eight (8) times with each plot containing five (5) trees. Evaluations shall focus on the entire 5-tree plot for each replicated plot. There will be a total of 280 trees used for this trial.

**Treatments:**
1. DI Water injected control
2. OTC injection with Remedium at label instructions (low pH)
3. OTC injection with Rectify at label instructions (low pH)
4. OTC injection with adjuvant (provided by Dr. Santra for the experimental treatments, which shall be mixed at the time of application with the OTC by the PI)
5. OTC injection with adjuvant and zinc (adjuvant and zinc to be provided by Dr. Santra for the experimental treatments, which shall be mixed together at the time of application by the PI)
6. Streptomycin and adjuvant (which are both provided by Dr. Santra, which shall be mixed at the time of application by the PI)
7. Streptomycin + OTC mixed with adjuvant (streptomycin and adjuvant to be provided by Dr. Santra for the experimental treatments, which shall be mixed at the time of application by the PI)

**Deliverables:** Data collected shall be provided on both an individual tree and plot basis using Excel. Tree health measurements shall be taken prior to the beginning of the study and just prior to harvesting the fruit from each tree. Individual tree health shall 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).

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

At harvest, a 65-fruit sample shall be collected from each treatment plot 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.

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

**Trunk Damage** shall be assessed by a simple number rating. The damage scale shall 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.

The researcher shall include field notes and digital pictures for trunk damage that is considered extreme and/or outside the boundaries of this rating system.
**qPCR** shall be evaluated at 3 specific times during the trial. The first sampling shall be at the initiation of the trial prior to any treatments being applied. The second sampling shall occur in the month of December. The final sampling shall be conducted a few days prior to harvesting.

Four (4) branches on each tree shall be flagged before any leaf sampling occurs. These four branches shall be the only place on the tree where leaves for qPCR analysis are collected. From each flagged branch, four leaves shall be collected (4 branches x 4 leaves = 16 leaves per tree) and placed into a sealed plastic bag and immediately placed into a cooler filled with ice. The leaves to be collected are to be the youngest fully expanded and mature leaves on the branch. All samples shall remain protected from light and placed in cold storage until delivery to the Southern Gardens Diagnostic Laboratory.

**OTC Detection:** Three days after injection, a sample consisting of twenty (20) leaves shall be collected from each tree in the trial. There shall be 280 samples. Leaf collection shall be a random sampling from the entire canopy of a tree. Once a sample is collected, it is to be stored in a cooler with dry ice and not exposed to light. The 280 leaf samples are to be promptly shipped to a laboratory chosen by CRDF for OTC detection. The laboratory results shall be submitted to CRDF on an Excel spreadsheet.

**Residue Testing** shall be conducted by the USDA National Science Laboratories in Gastonia, North Carolina. One collective fruit sample from each individual plot shall be submitted to the USDA laboratory. In total, there will be fifty-six (56) samples. Each sample shall consist of two fruits from each tree in a measurement plot, ten fruit total for the plot. For each measurement plot, the ten fruits shall be washed to remove external spray residues. Once washed and properly dried, the ten fruits shall be placed in a blender and processed into a smooth, homogeneous liquid. From the liquefied whole fruit sample, a volume of 100 ml shall be collected and placed into sealed container. The fifty-six (56) samples shall be properly packaged with dry ice and shipped to the USDA laboratory. The USDA laboratory analysis results shall be submitted to CRDF on an Excel spreadsheet.

**Proposal Submission:**

Proposals must be submitted to catp@citrusrdf.org, and received by 5:00 p.m. Eastern time on October 20, 2023.

The following documents shall comprise the Proposal package:

1. **Proposal Cover Page and Budget** - Form CB-22 -link to download form:
   
   
   (5 pages; pdf file).

2. **Budget Justification** (Word file)

3. **Subcontract Budget** - Form SC-22 -link to download form:


   (4 pages; pdf file. SC-22 forms do not count in the 7-page limit) - *complete if applicable*, using a separate form for each proposed subcontract. See detailed instructions later in this file.

All questions should be directed to catp@citrusrdf.org.
Questions/Answers Related to:

RFP 1: Phos acid and Copper
RFP 2: Neutral pH

Is it an issue if the trees have already been injected with OTC this year?

The trees in these trials should not have been treated with OTC previously.

Will all treatments be applied at the same time? The instructions say to follow manufacturer directions and, if PHI’s are different timings, could be an issue.

Yes. PHI will not be affected for the OTC treatments.
The fruit from the Strep trees will be destroyed, so PHI is not an issue.
PHI for Remedium and Rectify is 180 days according to the label; we must abide by this.

Are all 280 trees considered measurement trees? In past CRDF projects end trees were excluded from measurements.

Yes, all 280 trees in this trial are measurement trees. End trees for each plot are not excluded.

What is the timing of trunk damage ratings?

Two assessments will be sufficient:
• Assessment 1 will be 3 months after injection.
• Assessment 2 will be just prior to harvest.