

Attachment
Data Collection Protocol (taken from Denver Protocol)
RFP for services highlighted in yellow with blue optional)

Data to be collected from a field trial may vary by trial objectives, conditions, and resources available, but generally the following metrics should be used in CRAFT program data collection for the factors indicated:

- Tree size
 1. Measure at one or more time intervals before the completion of the trial.
 2. Trunk diameter for scion and rootstock
 - Measure at 5 cm above and below graft union.
 - Be consistent and return to the same spot on the trunk every year.
 - Measure in two perpendicular directions and use average.
 - Alternately, trunk circumference can be measured, and trunk diameter calculated using the formula $[\text{circumference}/\pi]$.
 - Report trunk cross-sectional area (TCSA) using the formula $[\pi \times (\text{diameter}/2)^2]$.
 3. Tree height to top of canopy
 - Do not include height of vigorous shoots that extend significantly past the top of the canopy.
 4. Canopy diameter
 - Parallel and perpendicular to the row.
 - If hedging and/or topping are done to the block, this needs to be clearly noted, and may significantly change the value of subsequent canopy size measurements.
 - Once tree height and diameter are measured, calculate canopy area and/or volume.
 - Measure canopy diameter parallel and perpendicular to row.
 - Calculate standard canopy volume according to the formula: $[(\text{diameter parallel to row} \times \text{diameter perpendicular to row}) \times \text{height}]/4$, modified from Wutscher and Hill (1995).
- Determine leaf macro and micronutrient concentrations annually during July-August from 12 mature, 4 to 6-month-old spring flush leaves from each or a subset of trees depending on experimental design.
- Report percentage of dead trees periodically or at the end of a trial period.
 5. Dead trees should be excluded from further ratings and analyses, or if included, this should be noted.
 6. Inferred or hypothesized cause of tree death may be noted. In many cases, trees that die in the first year are not the result of CLAs effects and may be excluded from HLB-associated assessments.

- If a trial is located in an HLB-endemic environment, conduct foliar disease ratings
 7. Use a scale from 1 to 5, with 1 = no foliar disease symptoms, 2 = foliar symptoms on less than 25% of leaves, 3 = 25-50% of leaves with symptoms, 4 = 50-75% of leaves with symptoms, 5 = more than 75% of leaves with symptoms.
 8. Calculate disease index as described below based on tree size and age:
 - For very small trees, rate the entire canopy as one unit. The maximum score per tree will be 5.
 - For medium trees, divide canopy into two sectors and apply ratings to each sector. The maximum score per tree will be 10.
 - For larger trees, divide canopy into 4 sectors and apply ratings to each sector. The maximum score per tree will be 20.
 - If trees are very large, divide into 8 sectors for a maximum score of 40.
 - To standardize ratings across trees sizes, divide the total score by the number of sectors used, so that all tree ratings are expressed on a 1-5 scale.
- Conduct canopy thickness and color ratings using a scale from 1-5 as described below.
 9. Apply ratings to one, two, four, or eight sectors of the canopy depending on tree size, with a maximum score of 5 for smallest trees and 40 for large trees.
 - To standardize ratings across trees sizes, divide the total score by the number of sectors used, so that all tree ratings are expressed on a 1-5 scale.
 - Dead trees are not to be scored for canopy thickness or canopy color, and so will not affect average values in analyses.
 10. Canopy thickness 1 = very thin canopy, 2 = thin canopy, 3 = medium canopy, 4 = thick canopy, 5 = very thick canopy.
 - It is recommended to illustrate differences between ratings photographically.
 11. Canopy color 1 = very yellow unhealthy canopy, 2 = yellow unhealthy canopy, 3 = moderately healthy canopy, 4 = healthy green canopy, 5 = very healthy dark green canopy.
 - It is recommended to illustrate differences between ratings photographically.
- Document foliar diseases not associated with HLB if commercially relevant (e.g., canker) particularly when evaluating different scion varieties.
 12. Foliar disease and health ratings should be conducted at the same time of year. In Florida and Texas, fall is recommended for scoring disease symptoms, as that is the time they will usually be most pronounced (once temperatures are dropping).
 - Additional ratings during spring and/or summer can provide important information and are recommended, particularly when evaluating new scion varieties.

- Tree appearance may be documented photographically using a measuring pole as reference.
- PCR evaluation of trees for CLAs:
 13. Collect mature leaves from most recent flush and use petiole/midribs for CLAs detection.
 - Depending on tree size, collect one or more leaves randomly from each of the four cardinal directions.
 14. Collect fibrous roots ($\leq 2\text{mm}$) for CLAs detection.
 - Depending on tree size, collect fibrous roots from a minimum of two different cardinal directions, avoiding zones of overlap between adjacent trees.
 15. Conduct leaf and root sample collections annually or at the end of the evaluation period (such as the end of four years of harvest).
 - May coincide with disease and health ratings.
 - Use the “Li primers” (Li et al., 2006) for real-time PCR detection of CLAs. New guidelines may follow.
- Once trees reach maturity, collect fruit yield and fruit quality data each season.
 16. Conduct yield and fruit quality assessment at dates that are standard harvest times for that cultivar, or harvest times that are proposed for new cultivars. Report date of assessment.
 17. Yield - assess directly by weighing fruits per replicate or indirectly by counting number of fruits per tree.
 - Report as fruit weight per experimental unit.
 - Alternatively, yield can be measured as boxes of fruit per tree.
 18. Fruit weight – determine from random subsample of fruits from each tree, or group, depending on what is practical.
 19. Fruit size - determine from subsample of fruits from each tree, or group, depending on what is practical for the situation.
 - Measure the horizontal or vertical diameter (as appropriate) of the subsample of fruit collected for determination of fruit weight.
 20. Fruit quality – depending on the type of fruit and trial purpose, determine percent juice, brix, acid, brix/acid ratio, external color, and juice color from subsample of fruits according to standard laboratory methods.
 - Sampling time will vary based on scion variety maturity and other factors. Select time that is most appropriate for the scion variety under evaluation.
 21. If appropriate, assess percentage of visually abnormal putatively greening-affected fruit per tree.
 22. If appropriate, assess fruit drop pre-harvest.
 - Report as percent drop from fruit number data.