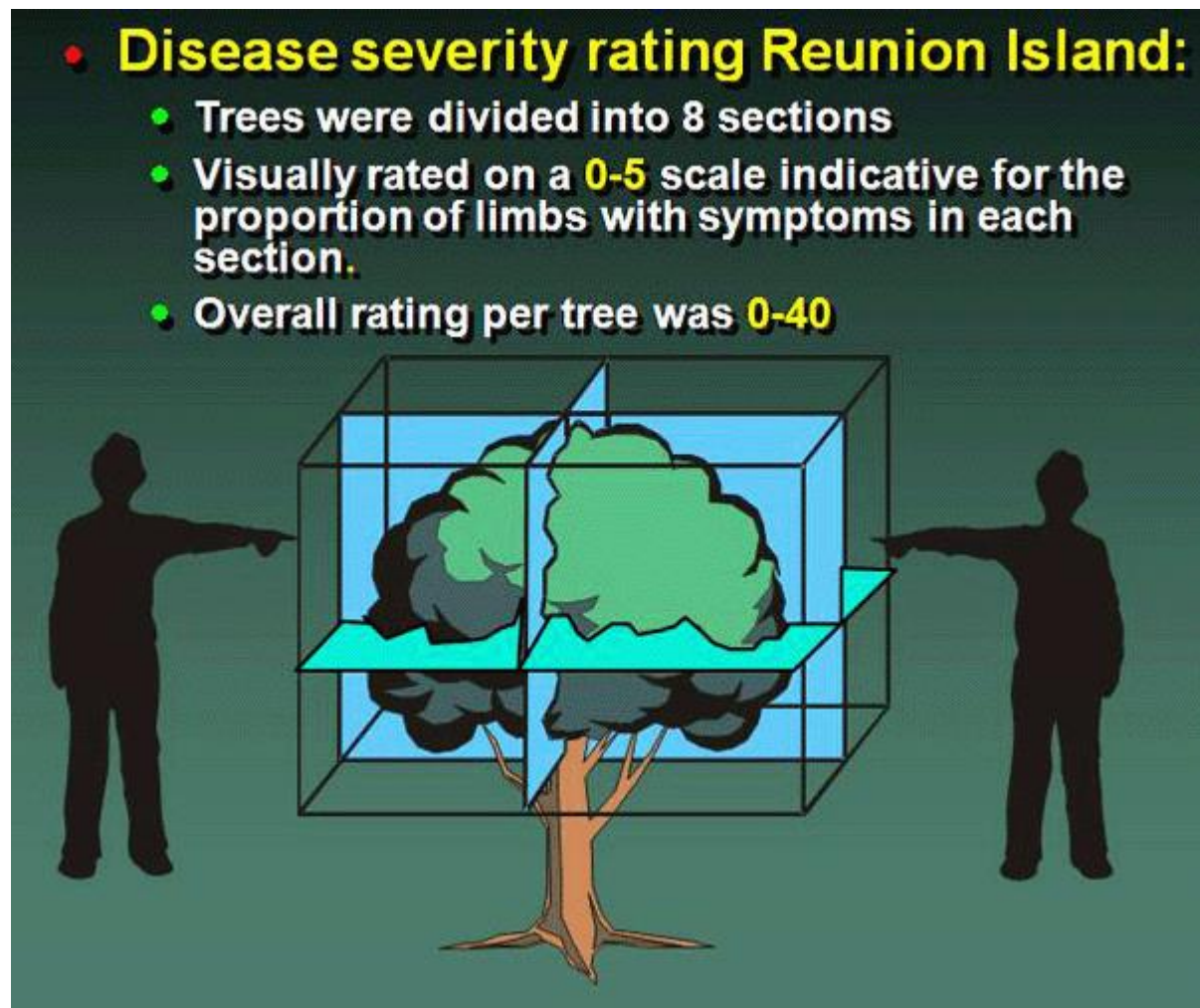# Field Trial Tree Evaluation Methods

This field evaluation protocol has been developed by CRDF project managers to evaluate CRDF field trials. This evaluation should provide valid information on the impact of various treatments on tree health, as well as the impact of treatments on bacterial infection and HLB disease. Methods are intended to be straightforward and easily adaptable to different locations and treatments.

The most basic field trial evaluation could include a visual disease index (DI, see below) rating before and after treatment and twice a year thereafter. DI ratings could be done on 12 treated trees and 12 control trees in about 1 hour. This rating plus yield data will provide sufficient information to compare the treated trees with the untreated control trees. It is important to include untreated control trees or standard practices or treatments in a field trial to serve as a basis for comparison of results. Additional potential tree evaluations follow.

Gottwald, T. R., Aubert, B., and Xue-Yuan, Z. 1989. Preliminary analysis of citrus greening (Huanglungbin) epidemics in the People's Republic of China and French Reunion Island. Phytopathology 79:687-693.

Tree canopy decline index (DI) score: Each canopy hemisphere will be subdivided into four equal quadrants by two imaginary perpendicular planes (vertical and horizontal at mid canopy height) passing through the axis of the tree trunk. The resulting eight sections (4 on each side of the canopy) are scored individually on a 0-5 scale indicative of the proportion of limbs expressing HLB disease symptoms within each section (0 = no limbs, 5 = all limbs). The summation of the eight scores for each tree will result in a severity rating of 0-40 for each tree on each survey date. For example, trees with a DI score of 32, have an average DI of 4 x 8 sections = 80% declined with symptoms.

A set of more complete evaluations which are used in CRDF field trials, includes a number of other measures. Some details are provided below; questions should be directed to the CRDF Project Managers.

Pre-treatment tree evaluation: It is important to collect measures of tree health and disease status prior to treatments to establish a baseline for comparisons of treatments.

Pre-treatment tree and grove documentation should include tree age, tree spacing/density, scion, rootstock, soil type, soil pH, general moisture status, and cultural practices including irrigation scheduling, water quality (salinity, bicarbonates), fertility programs, previous leaf nutrition, pest/psyllid control, yield records and fruit quality. Good horticultural care should continue uniformly in the entire trial area. At each site, there should be at least 12 replicate trees of each treatment plus 12 similar untreated control trees. 3-4 replicates of these 24 tree sets are desirable. Tree evaluations should focus on the middle 10 trees (excluding end buffer trees) of each of the treated and untreated trees for a total of 20 measurement trees in each replicate per site. Each of the 20 trees in each group should be assigned a unique treatment and replicate number. Treatment and control trees could be in rows of trees or in blocks with buffer trees on the borders.

Photographs of each of the 20 measurement trees at a standard distance, direction and size, should be taken just prior to the treatment for comparison with later photos.

Disease Index (DI) is a visible estimator of HLB disease developed by Gottwald et al., 1989, and detailed above. The DI numerical scale of 0-40 will be evaluated prior to treatment on each of the 20 measurement trees using 8 canopy sectors (4 on each side; maximum DI = 40) in trees greater than 3 years old. Smaller trees that are 1-2 yr-old, can be evaluated using only 4 sectors, 2 on each side (maximum DI =20).

Leaves for PCR. Please contact the US Sugar Diagnostic Lab to make arrangements prior to sampling leaves for PCR. The lab requires the completion of a Disclaimer, Indemnification and Hold Harmless Agreement prior to any samples being sent to the lab. All sampled leaves should be accompanied by the site information and their sample form from http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf .
Six-8 mature leaves (with petioles attached) should be sampled from around the canopy before treatments from the 20 measurement trees. If visible blotchy mottle symptoms are present, sampled leaves should be mature symptomatic leaves and placed into a sealable (e.g. zip lock) plastic bag, labeled with the tree unique ID code (as above) for each measurement tree and GPS coordinates if available. Sealed sample bags should kept cool and out of the sunlight (eg., in ice chests) and immediately transported to the US Sugar Diagnostic Lab.

Trunk diameter. Two perpendicular trunk diameters (D) can be measured with calipers and averaged to get D on each of the 20 measurement trees before treatment and annually thereafter. Diameters can be measured at exactly 8 to 12 inches above the ground (or above the graft union) depending on tree size. It is important to be consistent so you can return to the same spot on the trunk next year. You can also use a tape measure to measure trunk circumference (C) as trunk diameter can come from C/π; $C=\pi D$. The trunk cross sectional area (CSA) can be calculated geometrically from the trunk radius (r=D/2) using the formula πr^2 (where $\pi = 3.14$).

Canopy volume. Canopy volumes (in cu ft or cu m) can be calculated using a geometric prolate spheroid formula: $[(4 / 3)(\pi)(\text{tree height}/2)(\text{average canopy radius})^2]$. π=3.14, D = average diameter, r = radius, and ht. = height. Canopy dimensions can be sighted using a pre-measured
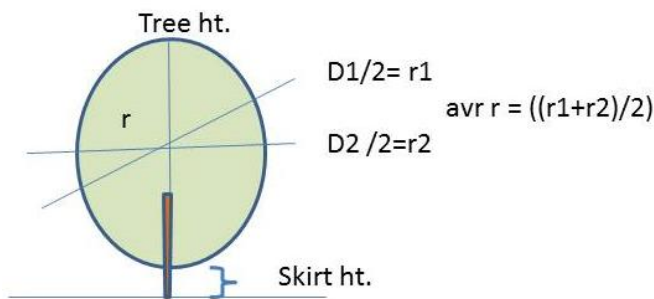
marked pole. Tree height is the distance from the ground surface to canopy top ignoring any escaped branches. If there is no skirt height, then subtract about 10 % from the canopy volume for the flat bottom. Estimated volume corrections can be made for raised skirt heights. An average canopy radius can be calculated from ½ of the average diameter width.

Canopy density can be qualitatively estimated by visually classifying overall tree canopy density into 3 classes: **Healthy** = thick green canopy, few visible woody branches, good crop load. **Moderate** = some yellow leaves, some leaf loss, woody branches visible, a few fruit dropped. **Declined** = some die back, visible leaf loss, obvious fruit drop and an open declined canopy.

Fruit drop. All fruit on the ground of the 20 trees can be raked out prior to treatment and fruit drop counts can be made approximately every 2 weeks after treatment. Percentage fruit drop can be calculated by dividing the number of total fruit dropped by the (total fruit dropped + fruit harvested).

<u>**Post-treatment tree evaluations:**</u>

Post-treatment tree evaluations can begin 1 week after treatments to evaluate any incidental short-term tree injury from treatments.

Photographs of the 20 measurement trees at a standard distance, direction and size, will be taken 1 week after treatment, 3 months later and annually thereafter.

Disease Index. Depending on tree condition, DI can be evaluated again from 6 to 12 months after treatment on each of the 20 trees using, 4 canopy sectors per side, in trees greater than 3 years old and on 2 sectors on each side of the canopy for trees 1-2 years-old as described above.

Leaves for PCR test. 6-8 mature leaves on the 20 trees will be sampled as above 1 week after treatment, and either 3 months later or annually thereafter (winter season preferable) and submitted to the US Sugar Diagnostic Lab as above after prior arrangements have been made.

Trunk diameter. Two perpendicular trunk diameters or a trunk circumference can be measured on each of the 20 trees annually in December. Diameters will be measured at exactly the same height as described above.

Canopy volume. Canopy volumes can be evaluated twice per year using the prolate spheroid formula from measurements of average canopy radius, tree height and skirt height as in the tree canopy volume illustration above.

Canopy density can be rated twice per year and rated as **Healthy, Moderate** or **Declined** as defined above.

Fruit drop. All fruit on the ground of the 20 trees will be raked out prior to treatment and fruit drop counts will be made approximately 1 week after heart treatment every 2 weeks thereafter until harvest.

Leaves for nutrient analysis. 12 mature, 6 month old spring flush leaves from each of the 20 measurement trees (10 treated and 10 untreated controls) will be sampled during July-August for nutrition analyses and submitted to labs for routine analysis of major and minor elements.

Fruit. At the first harvest after treatment, 50-fruit samples will be sampled from each of the 20 trees and put into labeled net bags for fruit quality analyses and will be delivered to a testing lab after prior arrangements have been made. Total yield and remaining fruit counts will be collected from the 20 measurement trees. Weight of the 50-fruit quality samples will be added to calculate total yield per measurement tree. Fruit counts will be used to calculate percentage fruit drop (total fruit drop / (total fruit drop + remaining on-tree fruit) for each of the 20 trees.

Data interpretation and analysis: Comparisons can be made between the 10 treated trees and 10 control trees. Tree photographs, DI ratings, trunk diameter, fruit drop and PCR data can be used as covariates in data analyses and collectively illustrate the effects of treatments.

Please contact Dr. Jim Syvertsen, jmsn@ufl.edu with any questions or comments.