Citrus Research and Development Foundation, Inc.

# RMC-18 Request for Project Funding

Revision 2 (5/2/2018)

**Application Schedule:**

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| April 25, 2018 | Call for pre-proposals announced |
| May 21, 2018 | Completed pre-proposals must be received by 5:00 pm EDT |
| June 14, 2018 | Invited list for full proposal submission posted to CRDF webpage |
| July 20, 2018 | Complete invited full proposals must be received by 5:00 pm EDT |
| Sept. 26, 2018 | Approved funded projects list posted to website |

**Purpose:**

The Mission of the Citrus Research and Development Foundation is to "Advance disease and production research and product development activities to insure the survival and competitiveness of Florida's citrus growers through innovation". The purpose of this CRDF request for proposals is to support the Foundation's mission by addressing key industry needs. Questions and research priorities were discussed during citrus grower input sessions, researcher brainstorming sessions, and from the recently completed National Academy of Sciences (NAS) study. These were developed into priorities for the two separate 2018 CRDF RFPs (RMC-18 & CPDC-18). The priorities identified are expected to lead proposals for hypothesis-driven, knowledge-building research projects, the results of which will lead to downstream solutions to HLB. These research priorities were selected and approved by the Research Management Committee (RMC) and the CRDF Board of Directors, which are composed of citrus industry members.

Pre-proposals and invited full proposals will be reviewed by the RMC, the Board of Directors, and a scientific advisory board. External, ad hoc expert reviewers will review invited full proposals. Decisions on pre-proposal and proposal approval will be made by the Board of Directors based on the recommendations of the RMC.

**RMC-18 Research Priorities:**

The CRDF Research Management Committee 2018 (RMC-18) call for funding applications focuses on priorities for hypothesis-driven, knowledge-building research projects, the results of which will lead to solutions to HLB downstream. Successful applicants to this program will have developed research projects addressing one or several of the research priorities in this section. These priorities should not be combined with priorities from the CPDC-18 RFP. Proposals addressing priorities from the CPDC-18 RFP must be submitted as independent pre-proposals.

1. **Effective bactericide use in Florida citrus groves**
   A. Examine the effect of bactericides on tree health including root health, yield and fruit quality, in different aged trees and different disease severity levels.
   B. Test or develop tools to accurately measure and track live bacterial titers in citrus trees.
   C. Examine the dynamics of bactericide introduction into the tree and systemic movement within the vascular system of trees.
   D. Develop novel, commercially viable application technologies to improve the uptake of bactericides.
2. **Asian citrus psyllid (*Diaphorina citri*)**
   A. Improve the understanding of ACP population pressure and repeated inoculation of mature citrus on disease development and decline.
   B. Examine the impacts of bactericides or other molecules on ACP survival and fitness and determine if bactericide or other molecule use impacts transmission of *C*Las.
3. **Horticultural practices for disease management**
   A. Investigate how root systems are impacted by HLB and how they can be treated to restore or prevent further tree decline and interactions with other root pathogens?
   B. Investigate the most efficient use of micro and macro nutrients on HLB diseased trees and possible correlations with *C*Las titer.
   C. Investigate chemicals or horticultural practices that can mitigate or exacerbate the symptoms of HLB including phloem collapse.
4. **Plant Improvement**
   A. Identify the genetic basis, of citrus host responses to HLB to identify targets for conventional or biotechnological approaches for the development of HLB resistant or tolerant citrus varieties.
      i. Seek new resistance R-genes in citrus or other species that counteract *C*Las effectors.
      ii. Describe the varietal target and experimental approach targeting HLB resistance. Indicate the percent effort, time and resources dedicated to each variety.
      iii. Evaluate germplasm developed specifically for HLB resistance through conventional or biotechnological techniques. Describe phenotypic and molecular characterization protocols for laboratory, greenhouse and field experiments; reference the appendix for phenotyping protocols.
   B. Develop tools for reliable, high-throughput characterization of citrus germplasm for HLB resistance or tolerance using current knowledge of HLB symptomology and the molecular characterization of citrus.
5. **Citrus black spot (*Phyllosticta citricarpa*) management**
   A. Develop strategies for citrus black spot management in Florida citrus groves, including strobilurin and non-strobilurin fungicide use and cultural practices.
   B. Develop management strategies to control the spread of citrus black spot throughout Florida, e.g. tarping and CHMAs.

## Pre-proposal Submission

The first step in the RMC-18 RFP is the submission of a pre-proposal. Pre-proposals forms are due on May 21, 2018 by 5:00pm Eastern time. Pre-proposals will be reviewed by the CRDF Research Management Committee and full proposal invitation recommendations will be considered and approved by the CRDF Board of Directors. The pre-proposal form requests applicant information and the narrative portion is composed of four sections.

Complete the top portion of the Pre-proposal form: check the correct box for either RMC-18 or CPDC-18 depending on which priority is being addressed, Project Title, all Investigator fields, Total Estimated Budget in US dollars, Duration, Current Date and Collaborators.

Projects may be funded for a period of one, two or three years.

**Section One: Non-Technical Abstract**

For this *non-technical* abstract, please provide the following information to summarize your project (250 word limit):

1. What specific priorities are being addressed (refer to priority number and letter)
2. What is the purpose of this proposed project, and how does it address a specific priority or priorities from this RFP?
3. Briefly describe the approaches or methods used to accomplish the project goals.
4. What is the practical application and timeline of this project and what will be the impact on the citrus industry?

**Section Two: Objectives, Timelines and Milestones**

For this section, please provide the following information (1000 word limit)

1. List the specific objectives of the project with the corresponding hypotheses to be tested.
2. Briefly describe the experimental approach taken in this project to meet each objective, including methods and technologies.
3. List the technical requirements and expertise needed to accomplish the project objectives.
4. For each objective, provide a timeline with specific milestones. How will progress and success of each objective and milestone be determined?
5. Do subsequent objectives rely on the success of a prior objective? If so, describe if/how subsequent objectives can be investigated if early objectives are not met?

**Section Three: Expertise**

Briefly describe each investigator's expertise/experience and how the available resources/facilities are suited to accomplish the project objectives. (100 word limit)

**Section Four: Expected Results**

For this section, provide the following information (100 word limit):

1. What are the anticipated deliverables of this project in terms of knowledge, recommendations and/or products?
2. How specifically will the results from this project be applied for the management of citrus, HLB or other diseases?

## Invited Full Proposal Submission

Full proposals are due on July 20, 5:00 pm EDT. Only complete, invited full proposals will be accepted for funding consideration.

**Content and Form of Application Submission:**

All documents described below are required.

There are three documents that comprise the Full Proposal package:

1. The multi-page Full Proposal Cover Page and Budget (Form CB-18)
2. Project Narrative Document (up to 15 pages) plus appendices
3. Subcontract Budget (Form SC-18) (complete if applicable, using a separate form for each proposed subcontract).

**Cover Page and CB-18 Instructions:**

Complete the fields on the first page of the Full Proposal Cover Page.

**Biohazard Regulation/DNA:** If you are working with regulated agents or materials, and have an approved IBC plan, check "Yes" to indicate you are in compliance with their guidelines. Otherwise, check "No".

**Today's Date:** Enter the date on which you complete and submit your proposal.

**Project Title:** Enter a clear and concise title for your project.

**Principal Investigator:** Enter a single point-of-contact for the Principal Investigator that will be heading the project. List Co-PIs with collaborators.

**Project #:** As shown on posted list of invited pre-proposals.

**Email:** Use a valid email address for your point-of-contact. Email will be the primary means of communication within the program.

**Phone:** Include your phone number, along with country and city codes.

**Organization:** Enter the name of the organization through which you are working on this project.

**Co-PIs and Collaborators:** Enter the names and affiliation of your Co-PIs and primary collaborators. Secondary collaborators may be included here if there is sufficient space. A Co-PI is anyone who has a separate budget under the project, or otherwise has a significant role in completing the proposed work. A collaborator may provide advice or materials, or receive materials for analysis. Collaborators cannot have a budget in the project, and have no contractual obligation.

Please attach a table (**appendix i**) that lists the Co-PI's and Collaborators, and each of their roles by Objective; this table is not counted in the 15-page limit.

**Address:** Enter your physical mailing address. This will be a secondary means of contact.

**Project Duration (years):** Enter the duration of your entire project in years (not greater than 3 years).

**Total Funds Requested (total project):** This field is populated automatically from page 2 of Form CB-18. Verify the accuracy of the total funding for your project factoring all years included in Project Duration in $US. **CRDF does not allow inclusion of indirect costs in project budgets.**

**Year 1 Funding Request:** This field populates automatically from page 3 of Form CB-18. Verify the accuracy of the Total Direct amount from Page 3 of 5 representing funding you are requesting for the first year of the project in $US.

**Abstract:** Summarize your project in up to a 300-word abstract. Include sufficient detail that will allow reviewers to have a clear idea of your project design and anticipated benefits. Be sure to state project objectives in the abstract.

**Instructions for Project Budget Forms**

Budget forms are included within Form CB-18 (pages 2-5), and should be completed and saved at the same time as the Full Proposal Cover (page 1) is completed. Refer to the forms menu on the Funding/Proposal page to download the budget form. Budget forms do not count towards the 15-page limit of the body of the proposal.

Stipends for graduate student involvement in the project should be included as part-time OPS, not staff. In addition, tuition waiver for graduate students is not a personnel expense but, if requested, should be included under "**Other Direct**" costs.

Complete a budget form for each year of the project as designated on Pages 3 to 5. These amounts automatically populate to Page 2, the composite budget form for the entire project period. If applicable, the Subcontract total (line 21) for each year of the project from all Form SC-18's must be manually inserted into pages 3 to 5 of the Project Budget Forms under Subcontracts (line 17 on CB-18). Verify that all amounts are correct, including Subcontractor totals.

When completing entries for personnel to be employed under this project, consider shifting the salary request to the 2nd quarter of the first year of the project if an employee is a new hire. This will more realistically reflect actual employee cost in the first year of the project. Reasonable cost of living increases can be included in personnel entries in the budget and should be described in the budget justification.

Requests for travel expense should consider costs of travel related to conduct the research, as well as reasonable expenses related to presenting project results to peers and stakeholders. Details of proposed travel should be provided in the budget narrative. Do not include purchase of gasoline or vehicle maintenance as travel costs. Instead, include them as Supplies/Materials expense.

Do not leave any fields blank. Be sure to complete all three yearly budget forms, adding zeroes in any years of the form if needed. This will allow the forms to properly function in calculating sums and transferring values to the composite forms.

**Budget Justification (narrative):** This is a Word file, saved as a PDF and combined with Form CB-18. Provide a brief statement that details the following expense categories:

**Personnel:** Their role and specific responsibilities in the project. Please be certain to rely on your current institutional fringe rates when calculating personnel costs. Except for PI and Co-PI's, list specific position titles instead of employee names unless the individual is critical to the project. Replacement of personnel listed in this section must be pre-approved by CRDF.

**Supplies/Materials:** Provide general details of types of supplies and materials that will be utilized for the project and are included in the budget request. This description should demonstrate the need for these expenses to complete the objectives of the proposed research.

**Travel:** Provide details for in-state and out-of-state travel and purpose of the travel for each trip. Sufficient detail should be provided to assess the value to the project and to CRDF. Include the number

of trips, etc. Do not include fuel or maintenance expenses in travel, but move to the **Supplies/Materials** category.

**Subcontracts:** Summarize the participation of other institutions or entities who will participate in the project and their required budget. (A separate Form SC-18 must be completed and submitted for each entity. Each subcontractor should also complete a budget justification.) (See 10.a.)

**Other Direct:** Provide justification of all costs shown.

**Equipment:** Provide details for equipment with cost in excess of $500. Define the value of the equipment to completion of the project objectives. A specific quote should accompany this request.

**Project Narrative:**

This section is subject to a 15-page length limit. Submit this document as a PDF file.

1. **Introduction:**
    - **a.** What is the purpose of this proposed project and how does it address a specific priority or priorities from this RFP?
    - **b.** Summarize the body of knowledge in the literature as it relates specifically to this project.
    - **c.** Describe and cite ongoing or recently completed significant research related to the proposed project including the work of co-PIs and collaborators.
    - **d.** Describe preliminary work pertinent to the proposed work. Attach important preliminary data as **appendix ii**; appendices will not count towards the 15-page limit.
2. **Objectives:**
    - **a.** List specific objectives to be accomplished, including the overall goal of the project and relevance of the project to the RMC-18 program priorities.
    - **b.** Do subsequent objectives rely on the success of a prior objective? If so, how will this affect the project and how will this be managed?
3. **Project Deliverables:**
    - **a.** What knowledge, information, recommendations, products or tools will result from this research?
    - **b.** How and when will the results of this research be communicated to growers?
4. **Experimental Approach:** Provide a description of the project experimental design.
    - **a.** Plant improvement projects refer to the appendix of this document for greenhouse and field trial requirements.
    - **b.** Non-plant improvement projects that include field trials are required to clearly state:
        - **i.** Experimental design: Plot size, layout, treatments, blocks, replications and experimental and sampling unit.
        - **ii.** Site description including the number of sites and criteria for site(s)
        - **iii.** Application methodology (equipment, coverage, etc.) and timing
        - **iv.** Data collection: What will be measured, how will it be measured and how often data will be collected.
        - **v.** Permitting or crop destruction requirements.
5. **Project Timeline:** Timeline of the project with objectives and specific project accomplishments as milestones including subcontractor milestones.

6. **Cost/Benefit of Proposed Intervention (if applicable)**

7. **Regulatory & Commercialization Considerations**

   This section is required for all projects developing research concepts, products or tools that are regulated by any state or Federal agency and/or have commercialization needs. Applicants must provide evidence that state and Federal regulations have been considered and a plan has been developed to improve the likelihood of successful regulatory compliance. The pathway to commercialization of products and tools must also be described to give evidence for the feasibility of commercialization. Please provide the following as **appendix iii**; this document will not count towards the 15-page limit.

   **a.** Describe the regulatory requirements of the concept, product or tool during the research and development stage and for implementation.
   **b.** Describe how this proposal accounts for regulatory considerations including:
      i. Acquisition of necessary permits for material movement and trial work
      ii. SOPs for all pertinent agencies to ensure compliance.
   **c.** Identify institutional experts, relevant state and federal agencies or regulatory consultants that have been engaged to discuss the regulatory aspect of this research concept.
   **d.** Describe what will be required to achieve commercialization of project deliverables.
   **e.** Identify collaborators or consultants that have been engaged with demonstrated expertise in product development and commercialization of this research concept.
   **f.** Describe intellectual property status including any tools used to develop the product that may affect commercialization.
   **g.** Describe plans and processes to communicate technology status in reports, presentations and publications without compromising the ability to secure the appropriate patent protection.

Questions regarding regulatory issues may be addressed to aescares@freshfromflorida.com. Commercialization questions should be addressed to institutional offices of technology transfer.

8. **Summary of previously CRDF-funded work** – If the proposed project is a follow-up to a previous CRDF-funded project, please provide the following in a 2-page summary as **appendix iv**, which will **not** count towards the 15-page limit: Include only work accomplished by you in a previously funded CRDF project.

   **a.** Title and CRDF number of previous project
   **b.** Objectives of previous project
   **c.** Significant results of previous project, including publications.

   Please note that PIs with any current CRDF funding must be up to date on required project reporting including quarterly and annual reports, at time of full proposal review, to be considered for 2018 project funding.

9. **Bibliography of Literature Cited in the Project Narrative**

10. **Additional budget forms and narrative:** The project budget forms are attached to the cover page, so will precede the project narrative. However, the following additional budget information is requested here; these forms are not included in the 15-page limit

    **a. Supplemental: Subcontract Budget (Form SC-18):** If you have co-PI (consultants, professional services or institutions) who will receive funds from your project, complete a separate Form SC-18 for each subcontractor from another entity (the budget of cooperators

from your own institution should be included in the main budget). Please include these forms for each year of subcontract and a composite budget form for the entire subcontract period as provided for on Form SC-18. A budget justification (narrative) for the subcontract budget also should be prepared and attached. The total for each year of a subcontract budget form must be entered manually into the subcontract field of the project budget form for each corresponding year. If more than one subcontractor, verify that all are included in the Subcontract Total for each year. The Subcontract total on page 2 of Form CB-18 should calculate automatically. Refer to the application forms menu to download the Subcontract Budget forms. Download Subcontractor Budget Form. **NOTE: While many .pdf forms can usually be combined into one form, each Form SC-18 must be saved as a separate file to avoid fields being overwritten.**

**b. Current and Pending Support**: This excel spreadsheet follows project budget information and is used by reviewers to provide context (and funding detail) of ongoing research by the PI as well as other pending proposals from the team. The purpose of this form is to provide an overview of funding available to the PIs, and to communicate current and planned research that may be related to that proposed to CRDF. The form should be completed with information for each of the PIs that are contributing to the proposal. Download Current and Pending spreadsheet Download Personnel Allocations / Current and Pending Support Spreadsheet

**c. Personnel Allocation Form:** Like the current and pending form, this spreadsheet information is useful to project reviewers and to CRDF to assess level of effort and expertise to meet objectives. Refer to the application form menu to Personnel Allocation Spreadsheet**.**

**11. Appendices:**

**i.** Table of roles of the Co-PIs and Collaborators

**ii.** Preliminary Data (see 1d)

**iii.** Regulatory and Commercialization Considerations (see 7)

**iv.** Summary of CRDF Funded Projects (see 8)

**v.** Brief resumes with only relevant publications are required for all key personnel. It is important to know who will be doing the work, and what their capabilities are. Full CVs are not required. Collaborators should be included in key personnel.

**vi.** Institutional Authorization form. This is typically the routing form used by institutions to document review and approval of project proposals prior to their submission. Where available, a copy of the completed institutional authorization form (e.g. UF, IFAS DSR-1) should be included here. All USDA-ARS applicants must submit an approved ARIS form. If your institution does not use such a form, a generic authorization form is provided for your use in the column at the upper left of this page.

**vii.** Letter of authorization from each institution for which a Subcontractor Budget form has been completed. This statement allows CRDF to know that subcontractors have agreed, if the project is approved, to complete the objectives with the budget details as provided in the proposal.

**viii.** Provide a statement of commitment from each Co-PI and Collaborator.

**ix.** Other Relevant Information

## Proposal Layout Details

Proposals should be written in English and submitted as email attachments in Adobe PDF format. Page margins should be one inch on all sides. Use Times New Roman or a similar typeface set to 12-point and single line spacing. Documents exceeding word or page limits will not be considered.

Page numbers should be placed on the lower right hand corner with the Principal Investigator name and the page number (e.g., Robins-2).

Each file submitted as a component of your full proposal should be named with the Principal Investigator name and CRDF-assigned project number provided in your email and form identifier (e.g., Robins_18-0##_proposal, Robins_18-0##_CB18, Robins_18-0##_SC18). When naming files to be included in your Full Proposal submission avoid using special characters (! @ # $ % &) in the filename. Invited Full Proposal packages should be emailed to catp@citrusrdf.org.

Questions may be emailed to catp@citrusrdf.org

# Appendix

**Plant improvement germplasm evaluation guidelines**

## Section 1. Guidelines for proposals seeking funding to collect data on existing germplasm evaluation trials

CRDF recognizes that there are existing preliminary greenhouse and field trials as well as advanced stage field trials that may require funding for data collection. Although, these trials may not fit the criteria outlined for new trials, they may be considered for funding for data collection. Programs seeking funding should follow these guidelines when preparing a proposal to seek funding to evaluate existing trials.

### Preliminary greenhouse and field trials

These trials should be embedded in core breeding proposal objectives as they occur earlier in the breeding cycle. Principal investigators seeking funding for data collection for these projects should describe the objectives, population development, targeted traits of interest, evaluation materials and methods, statistical design and analysis of ongoing experiments. PI's should submit available summary preliminary data for each cycle of evaluation on the trials.

New greenhouse experiments and preliminary (early stage) field trials should adhere to the minimum guidelines below which were adopted from a document developed by the team of plant improvement researchers from Arizona, California, Florida and Texas, nominated at the National Citrus Breeding Collaboration meeting on Feb 27$^{th}$ in Dever, Colorado.

### Greenhouse experiments

*Experimental design:*

- Experiments must use control (non-inoculated) plants of the same variety and age as the inoculated or infected plants.
- Experiments must maintain controls and infected plants under the same conditions. Plants should be randomized.
- It is suggested to use at least 10-12 plants per treatment and variety to conduct disease/health evaluations. For additional genomics, metabolomics, or other analyses, a small but representative subsample of plants can be used.
- Plants to be used in the experiments should be as homogeneous as possible
- Use a minimum of 10 psyllids and 2 tissue pieces per plant for psyllid transmission and graft transmission of *C*Las, respectively. Number of psyllids and tissue pieces may be adjusted based on the size of experimental plants. Bud stick grafting is also a suitable method for inoculation. Provide information on percentage of infection of psyllid colony and titer levels of plant variety used for inoculation. Use standardized PCR methods and provide Ct-values and DNA concentration of plants from which inoculation material was obtained.

- Experiments must define the source of *C*Las used and reference to any previous publications or work with the same source.
- Researchers must do one of the following:
    - Use a *C*Las strain that is maintained in the exotic pathogens collection of citrus at USDA-ARS, Beltsville, MD.
    - Use a strain that has been sequenced or extensively genotyped.
    - Maintain the strain that they use.
    - Maintain the strain that they use as -80 frozen tissue for future sequencing or other analysis.

*Data collection:*

- One or more measures of plant size must be recorded at beginning and end of the experiment:
    - Stem diameter (preferred). For non-grafted trees measure at 10 cm above soil level. Height above graft union for measuring stem diameter of grafted trees is preferred at 5 cm but can vary depending on factors such as site of graft inoculation. Must be defined and consistent within an experiment.
    - Plant height or, if plants were pruned, the sum of the length of all branches after regrowth. Indicate times and frequency of pruning.
    - Biomass at the end of experiment. May be separated into shoot, leaf, and root portions.
- Greenhouse conditions and management practices should be recorded in as much detail as possible, including pot size, potting medium, nutritional program, temperature ranges, light levels, and any trimming or training of plants.
- Conduct foliar disease symptom ratings at different time intervals throughout the experiment using 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.
- Document type of foliar symptoms (chlorosis, blotch mottle, reduced leaf size, vein corking, etc.).
- Collect at least 3 leaves (number may vary based on plant size) at different time intervals for *C*Las detection (may have this coincide with disease ratings). Choose mature leaves randomly from different areas throughout the canopy to account for variation. Pool tissue for analysis. Use petioles/midribs for *C*Las detection.
- If *C*Las testing of roots is to be part of the experiment, fibrous roots (≤ 2mm) should be used.
- Use the "Li primers" (Li et al., 2006) for real-time PCR detection of *C*Las. New guidelines on primers may follow.
- Report DNA concentration, Ct-values, and percent of infected plants. If the Ct values are used for classification of which trees are infected and which are not infected by *C*Las, the cutoff should be clearly indicated on reports.
- Duration of experiment and time intervals for disease ratings will vary depending on the age of plants used. Duration of experiments should be a minimum of 6 months following inoculation, but preferably 12 months. Time intervals for PCR detection may vary based on resources and main purpose of the experiment.

**Early-stage field trials**

*Experimental design*

- Experimental design should be completely randomized or randomized blocked. Design will depend on trial objectives, number of rows available, row length, and tree spacing. Should be balanced across treatments/genotypes as far as numbers of individuals and reps in the test.
- As much as possible, all trees to be compared in a particular trial should come from the same nursery and be planted at the same time.
- Variability of soil and irrigation conditions should be taken into account in the experimental design (blocking).
- A minimum of 6 replicates should be used. Depending on the trial objectives, replicates can be 3 trees, or more; a minimum of 3 trees is preferred. The relative balance of number of replications and number of trees per replication may vary according to the particular situation.
- Plant border trees at end of rows, and in adjacent rows on each side, when possible.

*Data collection*

Data to be collected from a field trial may vary by trial objectives, conditions, and resources available. The following metrics should be used:

- Tree size. Measure at one or more time intervals before the completion of the trial.
    - 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. Alternatively, trunk circumference can be measured, and trunk diameter calculated using the formula [circumference/π]. Report trunk cross-sectional area (TCSA) using the formula [π x (diameter/2)$^2$].
    - Tree height to top of canopy (do not include height of vigorous shoots that extend significantly past the top of the canopy).
    - 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: [(diameter parallel to row x diameter perpendicular to row) x 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. Dead trees should be excluded from further ratings and analyses, or if included, this should be noted. 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 *C*Las effects and may be excluded from HLB-associated assessments.
- If a trial is located in an HLB-endemic environment, conduct foliar disease ratings using 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. 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. 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.
  - *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.
  - *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.
- 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 *C*Las:
  - 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.
  - Collect fibrous roots (≤ 2mm) for *C*Las detection. Depending on tree size, collect fibrous roots from a minimum of two different cardinal directions, avoiding zones of overlap between adjacent trees.
  - 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 *C*Las. New guidelines may follow.

- Once trees reach maturity, collect fruit yield and fruit quality data each season. 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.
    - 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.
    - Fruit weight – determine from random subsample of fruits from each tree, or group, depending on what is practical.
    - 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.
    - 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.
    - If appropriate, assess percentage of visually abnormal putatively greening-affected fruit per tree.
    - If appropriate, assess fruit drop pre-harvest. Report as percent drop from fruit number data.

**Existing Pre-commercial or large-scale field trials**

PI's seeking funding to collect data on existing field trials should describe the field trials using the following guidelines.

1. What are the objectives of the trial?
   Describe the criteria used to select each nominated candidate rootstock or scion variety. Include a brief description of pedigree, population development, and experimental design and phenotyping methods used to characterize populations at each screening stage. Submit summary data from preliminary trials on each candidate rootstock or scion.
2. Provide information on ownership of the genotypes and technology associated with the candidates selected including but not limited to transfer agreements or constructs containing proprietary technology in the case of engineered genotypes.
3. Provide a timeline for the experiment describing establishment date. Provide all planting dates in cases where some genotypes were planted later. What was the source of plant material for the field trial?
4. Provide information on the site characteristics, field layout and a field map of the trial. The field map should provide sufficient detail to identify blocks, treatments (rootstock or scion) and site characteristics (ponds, ditches, slope, windbreaks, etc.).
5. Describe the field trial in detail including the number of candidate genotypes, commercial standards, number of sites, experimental unit (number of trees), observational unit (number of trees), replications and statistical design.
6. What are the horticultural practices used to maintain the trial? Please identify all

treatments applied to the trial. If there are unbalanced, treatments explain how they are managed for statistical variation.
7. Describe in detail the traits of interest and data collection and analysis methods.
8. Submit summary data collected in the pre-commercial trial.
9. Identify candidates that have been submitted for disease indexing.

The scientific advisory board or ad-hoc reviewers, CRDF Committees and Board will review the information provided above to identify trials or portions of trials that merit funding for data collection will review the information provided above. As with new pre-commercial trials, CRDF will engage the citrus industry for comment on the relevance of the trials to industry needs to inform decisions on funding.

**Section 2. Proposal guidelines for new Pre-commercial citrus field trials:**

Proposals developed to solicit funding to conduct new large-scale pre-commercial variety field trials must describe the following.

**Description of genotypes nominated for pre-commercial citrus field trials**

1. Describe the criteria used to select each nominated candidate rootstock or scion variety including:
    a. A brief description of parental pedigree, population development, experimental design and phenotyping methods used to characterize populations at each screening stage.
    b. Provide information on ownership of the genotypes and technology associated with the candidates selected including but not limited to transfer agreements or constructs containing proprietary technology in the case of engineered genotypes.
    c. Submit summary data on each candidate rootstock or scion showing performance for all the traits of interest measured in preliminary trials. PI's should include a coded name for the candidates in a separate column. For example US2018R. To protect intellectual property these codes will be used when presenting information in public meetings where pedigrees and other confidential information **will not be disclosed.**
    d. Indicate whether candidates have been submitted for disease indexing.
    e. What is the source of plant material for the field trial?
    f. Synchronized planting of the pre-commercial field trial is vital for direct comparisons. How much disease free plant material is available for the field trial?
    g. Information provided in a – e above will be reviewed by a scientific panel and CRDF Board and Committees.

**Final candidate list determination**

The pre-commercial variety field trials serve as a powerful source of data for the citrus industry, and therefore it is important to engage the industry at large for input on the strength of the candidates and data summaries provided by the breeding programs. CRDF committees and Board will invite the principal investigators, collaborators, and industry stakeholders to evaluate the list of candidates and the associated summary data. Data presented at this public meeting will not provide confidential information such as pedigrees. Summary data of coded lines will be shared publically along with ranking

from the scientific panel, CRDF committees and Board, breeding program ranking and finally industry stakeholder ranking. At this meeting, the final list of candidates will be selected and approved for the pre-commercial field trial. Principal investigators MUST submit any/all revisions to the approved list of candidates for CRDF committees and Board consideration and approval before implementation.

**General Pre-commercial field trial design requirements**

The field trial design minimum requirements:

1. Number of sites: Dependent on the state geography and the targeted market. In Florida, at least 3 locations in the Central Ridge, Southeast, Southwest regions for most varieties.
2. Strategic site selection: To evaluate genetic, genetic x environmental and environmental effects on candidate variety performance.
3. At each site, the trial should be mapped to reduce variation and confounding factors.
    a. Account for topography, soil type changes, unbalanced windbreaks, drainage, ditches, ponds, etc.
    b. Multi-location trials should be set up given 3a so that genotypes can be compared for adaptation and regional performance.
4. Statistical design and analysis.
    a. A randomized complete block design (RCBD) or Latin square design is preferred. If more factor levels are necessary, the statistical design must be clearly described for review and approval.
    b. The trial should contain necessary standard (check) rootstocks or scions appropriate to the region for comparison to candidate varieties.
    c. Number of replications: Minimum of 5 replications.
    d. Experimental unit (plot) to which a treatment is applied: Minimum 8 trees. Treatments defined as the candidate varieties evaluated against industry standards.
    e. The observational unit or sampling unit (e.g., trees, branches, leaves) from which data is collected must be clearly defined in trials where the experimental unit has more than the 8 minimum trees required in b, above.
    f. The same observation trees will be measured in each data collection cycle. Due to the high variability of individual trees, a minimum of 8 trees is required for yield data collection. Death of observation trees should be noted in trial data summary reports each calendar year and if possible similar trees should be selected at random within the experimental unit (plot) for evaluation.
    g. The trial should incorporate appropriate rows (2) of buffer around the trial and within the trial. Buffers between plots minimize the competition effect of removing genotypes due to poor performance.
    h. The objective of the advanced field trial is evaluating candidate varieties for commercial relevance. Therefore total yield, fruit and juice quality traits must be measured every season after cropping. Early production is an important goal for new plantings due to higher production costs and shorter life expectancy for trees/groves. This situation has made it imperative that growers make an income as soon as possible so the field trials must be harvested each year until the trial is retired.

i. Trial tree spacing and grove design should be specified taking into account tree size data collected from preliminary field trials if it is available.
j. Horticultural practices: The trial should be managed using appropriate best management commercial practices for plant fertilization irrigation, pest management, pruning, hedging or topping. Grower cooperators will communicate horticultural practices annually. The incidence of wind, freezes, drought or flood or other phenomena that impact the trial should be recorded and reported. Pre-commercialization trials should not serve as a location for overlaying cultural practice treatments or to evaluate any other variable treatments which would interfere with the primary purpose of the trials.

5. Trial establishment : Tree survival should be monitored after planting, and a protocol defined if replanting or removing genotypes is necessary.
6. Location characteristics: Soil type, pH, organic matter content, irrigation water quality, incidents of drought, freezes, hurricanes or other phenomena which affect the field trial should be noted and evaluated. Trials that are affected negatively should be retired if the validity of the data collected is questionable.

**Pre-commercial field trial evaluations**

**Environment**

**Evaluate candidate variety performance based on the following conditions if applicable:** Data collected from grid soil sampling and testing. Soil samples to be collected within each block at the drip line to account for block and block × treatment interaction

1. Salinity
2. pH
3. Soil type
4. Organic matter content
5. Asian Citrus Psyllid infestation- relevant to HLB testing.

**Horticultural Performance**

**Means of propagation**: For each candidate variety, describe means and methods of propagation through seed, cuttings or tissue culture.
**Tree height (m):** Measured from the base of the tree to the top of uniform canopy ignoring errant vigorous branches. Pruning of vegetation as hedging, topping or skirting should be noted.
**Tree skirt height (m):** Measured from the base of the tree to the bottom of uniform canopy ignoring errant vigorous branches.
**Canopy Diameter (cm):** Two perpendicular diameters measured between trees along the row and perpendicular to the row.
**Trunk diameter (cm):** Two perpendicular trunk diameters measured with calipers on each of the observation trees. Diameters will be measured 10 cm above the bud union, a different criteria is used due to branching please describe the selected distance above the bud union.
**Leaf nutrient content:** Follow established sampling guidelines.
**Bloom**: Observations of bloom time and environmental conditions.
**Yield and maturity date:** Fruit maturity date and the total weight of fruit harvested per experimental unit.
**Yield/acre**: Calculation per experimental unit based on yield/experimental unit and planting density.

**Juice quality:** Juice weight, total soluble solids, brix/acid ratio, color rating and flavor profile measured on a random pre-determined sub-sample of fruit per experimental unit at harvest.
**Fruit quality**: Fruit size and color measured on a pre-determined random sub-sample of fruit per experimental unit at harvest.
**Post-Harvest fruit evaluation:** Where applicable in scion trials evaluate post-harvest handling traits, especially for the fresh-fruit market, such as diseases, bruising, degreening, etc.

**Diseases and Pests**

**Huanglongbing (HLB):** PCR testing annually for bacterial titer is required at least once a year during the highest stress period. Leaf tissue samples should be collected from each observational (sampling) unit.

**HLB:** Visual rating of disease incidence and severity (HLB Decline Index) adjusted for tree size.

Tree canopy decline index (DI) score: For each quadrant visually assess HLB symptoms on a scale of 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.

1. Small trees (Year 1 and 2): Rate the entire canopy as one unit. The maximum score per tree will be 5.
2. Medium canopy trees (Canopy volume ≤ $3m^3$) divide canopy into bilaterally. Apply ratings to each sector. The maximum score per tree will be 10.
3. Mature trees, each canopy hemisphere is 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 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. Trees that were severely declined with initial DI scores greater than 32 (average DI 4 x 8 = 80% declined with symptoms) will not be chosen as measurement trees within each plot.

Evaluation of other pests and diseases based on incidence and severity.

1. Blight: Die-back and quick decline ratings in canopy sections developed for HLB DI ratings by tree age and size.
2. *Phytophthora nicotianae.*
3. *P. palmivora/Diaprepes* weevil complex and burrowing nematodes
4. Other citrus nematodes.
5. Citrus Tristeza Virus: Die-back and quick decline ratings the same as used for HLB disease rating
6. Post-bloom fruit drop: Count the number of buttons in canopy sections accounting for variability.
7. Citrus Canker: Percent lesions on leaves and fruit in canopy sections developed for HLB DI ratings by tree age and size.
8. Other: Incidence and severity of other pests and diseases should be recorded, and evaluation criteria developed and when necessary.

**Guidelines specific to pre-commercial rootstock field trials.**

Scion selection: For simplicity of trial design, data collection and interpretation, one scion clone is recommended for each replicated, multi-location rootstock trial.This leads to separate trials for each 'Valencia,' 'Hamlin', 'Mid-sweet', 'Grapefruit', 'Tangerine', 'Mandarin' etc. scion clone selected for each trial. Complex designs utilizing more than one clone for any scion type, for example, two Valencia clones, must be balanced and take into the effect of scion and scion/rootstock interaction on the validity of data collected.

**Guidelines specific to pre-commercial scion field trials.**

Rootstock selection: For simplicity of the trial design, data collection and interpretation, one rootstock variety and one scion variety-type (multiple scion genotypes) of similar maturity (sweet orange, grapefruit, etc.) is recommended for each replicated, multi-location trial. For example, Valencia candidate scions budded on Swingle only (Valencia1/Swingle, Valencia2/Swingle). This leads to separate trials for each 'Swingle, 'Sour orange, 'Carrizo'etc. scion/rootstock combination selected per trial. However, if the decision is made to test the candidate scions on more than one rootstock (e.g., valencia1/Swingle, Valencia2/Swingle, Valencia1/Carrizo, Valencia2/Carrizo) the following considerations should be made:

1. Each experimental unit (split-plot) should contain every scion/rootstock combination.
2. If known, from preliminary data, the effect of rootstocks on scion maturity should be taken into account.