



California Citrus Research Board Request for Proposals

For the funding period October 1, 2019 to September 30, 2020

Specific instructions for preparing and submitting new proposal concepts, full proposals, continuing research proposals, and progress and completion reports

2019 Calendar

- **March 1***: Progress Report #1 Due (FY 2018-19 funded projects); RFP is released
- **April 4**: New Project Pre-Proposal Form Due
- **April 26**: Requests for New Project Full Proposals sent out
- **May 23**: New Project Full Proposals Due
- **June 21**: Continuing Project Full Proposals Due
- **July 1***: Progress Report #2 Due (FY 2018-19 funded projects)
- **July 23 & 24**: Oral Presentations of New Project Proposals
- **July 26**: Notify PIs for New Projects Moving Forward
- **August 13-15**: New and Continuing Project Presentations to the Full CRB Board
- **September 24**: Annual CRB Meeting to Finalize Research Project Funding Decisions
- **September 27**: Notify Principal Investigators (PIs) of Award Decisions
- **October 30**: Annual Project Report Due (FY 2018-19 funded projects)
- **November 29**: Completion Reports Due (FY 2018-19 funded projects)

*dates have been shifted due to the IRCHLB Conference in Riverside, California

Last updated: February 26, 2019

Introduction

The mission of the California Citrus Research Board (CRB) is to ensure a sustainable California citrus industry for the benefit of growers by prioritizing, investing in and promoting sound science.

The CRB annually evaluates its research priorities for possible funding. Whereas the Board will consider all proposals submitted for the upcoming fiscal year, the current areas of greatest concern are surviving and finding solutions to Huanglongbing (HLB) and maintaining market accessibility.

The Board is soliciting both new and continuing research proposals for funding consideration in the 2019-2020 Fiscal Year. This document highlights areas of specific industry need, guidelines for the submission of project proposals and the progress reports to be made during the tenure of CRB funding. Final decisions on projects to be funded will occur at the Annual Citrus Research Board Meeting on September 24, 2019.

Scope of Research

Research must be *outcome-based*; that is, the “need” or the end result is determined ahead of time, and research is developed in line with the desired outcomes. Priority may be given to those projects that result directly in a product, technology, solution or method that would benefit the California citrus industry.

Therefore, each researcher who intends to apply for CRB funding must be able to clearly articulate how their project will significantly contribute to the improved production, vitality and economic sustainability of the California citrus industry. Furthermore, researchers must explain how this information will be transferred to or shared with growers. (*NOTE: If your response is through University of California Cooperative Extension [UCCE], then you must already have made arrangements with a UCCE individual and must adequately describe your plan*).

1. Research Categories

There are four research categories that align with the strategies determined by Board and Committee members. These include:

- **5200 – New Varieties Development:** Breeding, development, selection and evaluation of new varieties.
- **5300 – Vectored Diseases:** Including but not limited to HLB, CTV, leprosis, stubborn and citrus variegated chlorosis.
- **5400 – Production and Post-Harvest Technologies:** horticultural factors, food safety, non-vectored, and pre/post-harvest diseases impacting fruit quality and production efficiency.
- **5500 – Pest Management:** Regulatory, endemic and invasive pests including but not limited to Asian citrus psyllid (ACP), fruit flies, thrips, Fuller rose beetle, scales, katydids and mites.

NOTE: Genome projects should be submitted within the category that applies to the organism, e.g., if you intend to propose research on the ACP genome, you would submit your proposal under 5500, the Pest Management category.

Detailed information for each research category is provided below. For new projects, multi-lab, multi-interdisciplinary interactions and industry collaboration are highly recommended, when possible.

5200: New Varieties Development

The goal of this category is to provide the California citrus industry open access to new varieties that will meet the ever-changing market demands, are resistant or tolerant to pests and diseases, and will improve production efficiency. Collaborative efforts are strongly encouraged. Nationwide data collection and evaluation standards were developed in collaboration with other funding agencies and can be found as Addendum 1. All funded projects are expected to follow the agreed upon guidelines.

Researchable areas are prioritized for 2019-20 as follows:

1. Develop a solution to the physiological effects of ‘*Candidatus Liberibacter asiaticus*’ (CLas) infection (which may include the role of the ACP in disease epidemiology) with an emphasis on important California citrus scions and rootstocks.
 - a. Develop field-relevant greenhouse evaluation methods for CLas/ACP tolerance or resistance.
 - b. Develop early flowering methods to shorten the breeding cycle.
 - c. Identify targets for gene editing (CRISPR for example). Priority will be given to proposals that have actual potential for resistance.
 - d. Aggressive testing of HLB response in major California citrus varieties to

California CLas strains, including fruit quality assessments. Develop a mechanism to transfer stable tolerance or resistance to the CLas bacterium from rootstocks to scions (with an emphasis on the Carrizo rootstock).

2. Develop new ways to effectively evaluate citrus varieties in multiple locations across California to determine production, HLB resistance and market potential with an emphasis on grower collaboration and real-world approaches.
3. Determine both domestic and export market preferences based on sensory (taste, appearance, feel, smell, etc.) and other factors (such as health benefits). Use this information to evaluate existing varieties and to identify genetic improvement needs within the breeding program.
4. Develop an understanding of the horticultural, handling and genetic levers to influence flavor and other sensory characteristics.
5. Determine quantitative methods to analyze flavor and evaluate post-harvest treatments for their effects on flavor deterioration and storage life.

Principal Investigators should budget time and funding for familiarity/training regarding regulatory requirements of transgenic material for field use and commercialization efforts if part of the research proposal.

5300: Vectored Diseases

The goal of this category is to provide California citrus growers timely and proven information on detection, eradication, control and management strategies and tools for diseases caused by vectored plant pathogens in order to minimize crop damage and economic losses.

High priority researchable areas are equally weighted for 2019-20 as follows:

1. Commercialize and deploy high-throughput Early Detection Technologies (EDTs) for huanglongbing (HLB) affected trees. Evaluate tools to detect CLas/HLB before the development of visual symptoms.
 - a. Cooperative projects (working with USDA-APHIS and CDFA as affecting regulations) to evaluate EDTs in comparison to qPCR methods using field-collected samples.
 - b. Assess the ability of high-throughput technologies (e.g., canines, serological, molecular, host responses) to detect CLas infection in California citrus.
2. Management of existing groves during HLB epidemic.
 - a. Define on farm and regulatory disease management protocols (development of best management practices) from beginning to end in commercial orchards for CLas and ACP before the disease becomes endemic; synchronization of protocols and area-wide control. Based on the locations of infestation and/or infection, different protocols may be needed.
 - i. Define epidemic-based phases of management.

- ii. Develop and recommend appropriate monitoring and sampling protocols for each phase of management. Protocols must include consideration of agronomic and economic realities.
 - iii. Develop monitoring-based economic thresholds to trigger application of specific tactics (insecticides, bactericides, physical barriers, tree removal, block redevelopment, etc.) and transition to the next phase of management.
- b. Develop an effective CLas disease management system, incorporating prophylaxis, therapies and/or delivery systems.
- 3. Develop tools to prevent CLas transmission or suppress HLB disease.
- 4. Research on additional exotic or endemic diseases that pose a threat to the California industry.

5400: Production and Post-Harvest Technologies

The goal of this category is to deliver timely and validated information on horticultural factors, food safety, non-vectored, and pre/post-harvest diseases impacting fruit quality and production efficiency to California growers and processors to keep them competitive in the domestic and international markets. Priority will be given to projects that have a clear path to commercialization.

Researchable areas are equally prioritized for 2019-20 as follows:

1. Conduct field trials to maximize fertigation and water use efficiencies in citrus orchards.
2. Conduct field trials in citrus orchards to explore the causes of post-bloom and preharvest fruit drop.
3. Develop mechanical harvest technology and/or robots to reduce production cost and increase productivity. Develop accurate mechanical crop estimation approaches.
4. Secure adequate data to support industry positions dealing with trade activities and barriers to the citrus export industry.
5. Evaluate existing and newly developed chemistries to minimize pre- and post-harvest fruit deterioration.
6. Develop data on food safety issues of citrus to mitigate regulatory action or to satisfy new regulations.
7. Determine the potential introduction of exotic non-vectored or graft-transmissible pathogens of citrus that would threaten production and/or market access both domestically and internationally.
8. Develop new, non-chemical technologies to mitigate and/or minimize losses due to non-vectored or graft-transmissible pathogens, e.g., irradiation, variable radio frequency, electromagnetic methods.

5500: Pest Management

The goal of this category is to provide California citrus growers timely and scientifically valid information on eradication, control and management strategies and tools against pests to minimize crop damage and economic losses and to maintain market accessibility of foreign and domestic markets. Priority will be given to projects that have a clear path to commercialization and fit within these priorities.

Researchable areas are prioritized for 2019-20 as follows:

Identify new and/or improve currently developed control strategies, including chemical, biological and cultural controls for both conventional and organic citrus.

1. Asian citrus psyllid & other exotic pests.
 - Improve detection methods, sampling methods and economic thresholds.
 - Determine pesticide efficacy for both organic and conventional situations.
 - Develop non-pesticide methods of control (biological & cultural).
 - Evaluate the efficacy of the California ACP treatment programs and develop year-round regional IPM programs for ACP in California.
 - Monitor for and manage pesticide resistance.
2. Endemic pests including (but not limited to) thrips, scales, mites, earwigs and katydids.
 - Improve detection methods, sampling methods and economic thresholds.
 - Pesticide efficacy (pests) and selectivity (natural enemies).
 - Innovative, non-pesticide technologies to minimize losses.
3. Regulatory pests including (but not limited to) Asian citrus psyllid, Fuller rose beetle, bean thrips, mites and fruit flies.
 - Develop pre- and post-harvest methods of disinfesting citrus fruit.

2. New Pre-Proposals

A novel, innovative research project submitted to the CRB that addresses the needs of the California citrus industry can be considered in one of two ways: a well-defined, full-scale project or a scaled-down project that intends to develop a proof-of-concept. The CRB may request that a project demonstrate the potential applicability of a concept before considering a larger-scale project. The CRB process for new pre-proposals includes:

- Completion of a pre-proposal application, to be submitted online by 5 pm PDT on April 4, 2019.
- Review of pre-proposals by the Research Priority Screening Committee during April 2019.
- If the pre-proposal is accepted, the researcher will be invited by April 26, 2019 to submit a full project proposal.

Submission Instructions:

Researchers should complete a pre-proposal form online for each new project concept. This pre-proposal will include basic information such as project title, name and contact information for the PI and collaborator(s). Researchers also need to provide information regarding:

1. **Purpose and Rationale** (1,600 characters*) a description of the project in layperson's terms with background information to support the reason for conducting this research – what prompted this project.
2. **Objectives, Timelines and Milestones** (4,000 characters*) a workplan including goals and objectives; and a timeline of milestones for when each will be accomplished.
3. **Expected Results and Practical Applications** (3,000 characters*) a description of how this project will benefit the California citrus industry, including what potential products the industry will receive.

NOTE: For the 2019-20 RFP process, the CRB continues to use an online proposal submission format. Much of the formatting and questions are similar to the application in previous years, but rather than submitting a PDF or Word document, information provided in the Pre-Proposal and Full Proposal forms only will be accessible online. Taking extra time to familiarize yourself with the online submission procedure is strongly recommended.

An FAQ (Frequently Asked Questions) document will be available on our website (<http://www.citrusresearch.org/research>) to address these and other commonly encountered questions. Researchers are Strongly Encouraged to review this document while preparing pre-proposals and full proposals.

**Character count limits. Maximum character limits have been included in parenthesis for your reference. The Citrus Research Board reserves the right to cut any response longer than the allowed character limit.*

Pre-Proposal forms must be completed and submitted online by **April 4, 2019** by **5 pm PDT**. You will find the link to begin this process on our website: <http://www.citrusresearch.org/research>

To start the submission process, you must have an account with the CRB Proposal System. If you have submitted a proposal since 2017, please use your login credentials. For new users, a valid email address and current contact information will be needed to create an account. After account creation, you immediately will receive notification to verify your email address. (Do not use a general email account. You will want to use your specific email address when setting up your account. e.g. jane.smith@citrusresearch.org). If your e-mail address has changed, contact support@citrusresearch.org to request your e-mail address be updated.

NOTE: If you add collaborators to your Pre-Proposal and that individual has not previously created an account on the system, a new account will be created at this time for them. Once you submit their names and e-mail addresses, each collaborator you add will IMMEDIATELY be notified by email that you have initiated an account on his/her behalf.

Once you have verified your email address and logged into the system on the CRB website, you can create and edit your pre-proposal(s). Once you have completed all edits and are ready to send the pre-proposal for review, click SUBMIT.

NOTE: Once you click SUBMIT, you will be unable to make any further changes. Your pre-proposal will be collected and provided to the CRB Research Priority Screening Committee who will meet and review all pre-proposals after the submission window has closed. Following committee review, an email will be sent to the PI to notify him/her of the committee's decision regarding the pre-proposal.

3. New Full Proposals

Researchers invited to submit a full proposal for a new project must complete and submit a full proposal for each project online according to the guidelines specified in **Section 5. Guidelines for Full Research Proposal** (below). The CRB review process for new full proposals is as follows:

- New, full proposals are received by the CRB Research Department, reviewed by both the Research Priority Screening Committee and an Ad Hoc Scientific Review Panel.
- The PI gives an oral presentation by telephone and WebEx to the Research Priority Screening Committee on either **July 23** or **24, 2019**.
- The Research Priority Screening Committee reviews and evaluates full proposals and presentations.
- If approved, PIs will give an oral presentation to the full Citrus Research Board, including Research Committees, during **August 13-15, 2019**, in Bakersfield, California.
- Proposals are reviewed by Research Committees who make a funding recommendation to the full board.
- A final funding decision is made by the full board at the annual meeting on **September 24, 2019**.

Submission Instructions: All new full proposals must be completed and submitted **online** at <http://citrusresearch.org/research>.

4. Continuing Project Proposals

Regardless of the anticipated duration of the project, all researchers currently receiving CRB funding must re-apply each year. The Full Project Proposal Form for 2019-20 should be completed and submitted **online** at: <http://citrusresearch.org/research> for each project according to the guidelines specified in Section 5 below. The window to submit a continuing project proposal will open on or about **May 21, 2019** and close on **June 21, 2019** at 5pm PDT.

If an ongoing CRB funded project is currently in its final year of funding, the CRB strongly supports that the project reach completion as scheduled. Any related new projects will need to go through the new project proposal pathway.

Researchers currently funded by the CRB with an ongoing project that has not yet reached its final year are invited to submit a Full Project Proposal Form for each project online according to the guidelines specified in **Section 5. Guidelines for Full Research Proposal** (below). The CRB review process for continuing project proposals is as follows:

- PIs complete and submit online a Full Project Proposal Form by 5 pm PDT on **June 21, 2019**. The Full Project Proposal Form can be found at: <http://citrusresearch.org/research>.
- PIs present a 10-minute oral presentation of proposed research to the full board, including Research Committees, during **August 13-15, 2019**, in Bakersfield, California.
- Proposals are reviewed by Research Committees who make a funding recommendation to the full board.
- A final funding decision is made by the full board at the annual meeting on **September 24, 2019**.

5. Guidelines for Full Research Proposals

Every full proposal must include the following contact information for each PI, collaborator(s), contracts officer and financial officer:

- name,
- affiliation (physical address including Department),
- email address,
- telephone number, and
- mailing address if different from physical location.

For each identified collaborator, you must provide a letter from that person, submitted as an online attachment, certifying that he/she:

- has read the proposal,
- has outlined their specific role as collaborator in the proposed research,
- has been adequately briefed about the proposal, and
- can meet the milestones of this project.

A brief CV (*two pages maximum*) should be submitted as an attachment for the PI and each identified collaborator. The CV should include name, affiliation and relevant experience (employment, relevant publications, etc.). Any CV longer than two pages will be cut off after the second page.

An appropriate contact outside of this project should be identified as a reference for additional information and/or an explanation of this type of research. The contact should be able to adequately address scientific questions regarding the project but should not be a current co-PI or collaborator in any other CRB-funded project(s).

Project Impact Statement (*700 characters**) The impact statement:

- briefly identifies the issue or problem,
- describes how the work proposed will significantly help solve the issue or problem, and
- highlights the benefit of the proposed research to California citrus growers.

Executive Summary (*3,500 characters**) Provide a clear and concise executive summary of the project in layperson's language. If your project is funded, this summary will be included on the Citrus Research Board website to describe to growers and to the general public the research work being conducted. Include the following:

- overall goal and specific objectives of the project,
- summary of the work plan or methodology, and
- expected outcomes and/or functional product or solution.

Background Information/Relevant Literature (*2,000 characters**) Provide background information and a brief review of pertinent literature. What prompted you to propose this research? List relevant peer-reviewed publications, including your own.

Pertinent Questions Provide answers to the following questions:

- Who will be the end user of the results of this project? (*400 characters**)
- How will California citrus growers or others benefit from your results? (*500 characters**)
- Will this research result in a product requiring commercialization or further development by another entity? If so, describe. (*250 characters**)

Gantt Chart of Progress Complete the Gantt Chart template found at: <http://citrusresearch.org/research>. The “Objectives” and “Tasks” you intend to complete should be clearly and succinctly stated. You must assign “Milestones” to each task which indicate the time that will be necessary to complete each task. Accuracy in work plan development is recommended as this will be a major criterion used by the board when evaluating projects for continued funding. Once completed, the Gantt Chart must be uploaded as a PDF to your proposal.

Work Plan/Procedures (6,000 characters*) Elaborate on your stated objectives, tasks and milestones in the “Work Plan and Procedures” section. You must explain how they will be achieved, describe your experimental design and, if appropriate, list site locations for proposed trials.

NOTE: For projects involving basic or discovery research, include any potential issues concerning intellectual property rights (including background patents currently associated with this proposal) and identify the steps needed for ultimate use by growers (e.g., federal approval to test a genetically-modified plant in the field for evaluation, licensing background, patents, or the registration of a new chemical). Identify any potential involvement of UCCE personnel and/or resources.

Budget Section While the Research Proposal form allows investigators to include budget requests for up to three years, the PI is still required to submit an annual Full Proposal Form and fulfill progress reporting milestones. Complete the budget form as specified.

- For projects involving multiple researchers who will be receiving a sub-award, the PI must prepare a project budget to include both PI and collaborator funding.
- For continuing projects, include funding levels for the current year (FY 2018-19). Indicate whether you expect to have any carry-over funds from the current funding year in the Budget Justification section.
- Board and the Committee Members prefer that PIs give oral presentations in person rather than by telephone. To accommodate the additional expense, a separate budget line for travel to CRB-related meetings is provided.
- The Lindcove Research and Extension Center (LREC) provides Land, Labor and Facilities (LLF) for projects approved by the UC Research Advisory Committee. On the ‘LREC Charges’ budget line of the CRB proposal, the PI should estimate the expenses that the research project will occur at LREC.

NOTE: If you anticipate that additional time will be necessary to complete the proposed research, you will be required to complete and submit a “request for unused funds” form by August 1, 2019. **The extension period will be decided by the Citrus Research Board based upon funds remaining and progress made toward proposed research goals.**

Budget Justification (2,000 characters*) Describe how funds will be used. You may want to include details such as type of equipment necessary and staffing needs, travel for conferences or contract research efforts required to complete the proposed work.

Other Funding Sources List any in-kind contributions for the proposed CRB-supported project and describe how other funds may enhance and/or impact the timing of this project or if CRB project funding can be used as matching funds for other funding sources.

Additionally, in order to evaluate how the proposed work fits with other potential or funded projects, the PI and any collaborator requesting funding needs to identify the source and amounts of all current and potential or pending non-CRB sources of support for citrus related projects for which you currently have funds, have applied for funds or intend to apply for funds for 2019-20 fiscal year support.

Information provided should be limited to the following information:

- Principal Investigator/Collaborator of CRB-funded project proposal
- Principal Investigator of externally funded or proposed project
- Project Title
- Funding Agency
- Amount (to PI/Collaborator)
- Start and End Dates

6. Guidelines for Written Reports

There will be two written progress reports and one annual report due for all projects funded by the CRB in FY 2019-20. A *Citrograph* article may also be requested based on project maturity. Additionally, PIs with projects in their final year will be requested to submit a Completion Report summarizing final project results and PIs with projects that have commercialization potential may be contacted by CRB staff and consultants to provide additional information to assist in commercialization efforts.

Progress reports will be due **March 2, 2020** and **July 1, 2020** and the annual report will be due **October 30, 2020**. Reports must be prepared following the report guidelines and forms to be made available at <http://www.citrusresearch.org/research>.

The goal of the progress, annual and completion reports are to document progress and accomplishments made:

- Progress Reports – since previous progress report submission (or project start).
- Annual Reports – over the entire course of the fiscal year.
- Completion Reports –over the length of the entire project.

Reports should restate project objectives and milestones as described in the funded proposal, summarize activities that were undertaken to accomplish each objective, and explain progress made toward each goal. Include comments on collaboration and provide data summaries and

graphs as appropriate. Reports must relate accomplishments to practical application for the California citrus industry.

7. Guidelines for Oral Presentations

It is expected that PIs with ongoing research projects will give an in-person oral presentation to the Citrus Research Board and Research Committees in August 2019 to explain intended research plans for the following fiscal year. **Presentations must follow the “Presentation Template,”** which will be made available on the CRB website. Presentations must accurately indicate the planned objectives and timeline necessary to complete each. Participation in the entire subject session is strongly encouraged, as it provides an opportunity for collaboration among research groups and helps prevent duplication of efforts.

8. Expectations of Funded Principal Investigators

It is expected that every funded PI will do the following:

- Give an in-person oral presentation to the Board and Research Committees during August 2019 to present intended research plans for the following fiscal year.
- Submit two written progress reports using the CRB Progress Report Form – one in March and one in July (see Guidelines for Written Reports). This report is used by the CRB Research Committees and the Board to review the status of all projects and guide future funding efforts.
- Submit one written annual report using the CRB Annual Report Form at the conclusion of the fiscal year. This report is used to inform California citrus growers of the work conducted through the CRB on their behalf.
- Submit a Project Completion Report Form when projects complete their final year or when funding has been terminated. This report is used by CRB staff, board and committee members for the review of project accomplishments and to guide future funding efforts.
- For each CRB-funded project, you may be requested to submit one full-length Progress Report article (1,500-word maximum) written in **layperson language** for publication in *Citrograph* or as a Research Report in *Citrograph* online. A Final Report article (2,000 words) will be requested once the project is completed and may take the place of a Progress Report article. **Adherence to *Citrograph* Author Guidelines is required.** Guidelines are available at: <http://citrusresearch.org/citrograph/guidelines>. *Citrograph* is the CRB’s quarterly magazine written specifically for the California citrus growers to share with them the purpose and actionable results of the research that their assessment dollars funded. This report is NOT geared toward other researchers, and *Citrograph* is NOT a peer-reviewed journal. *Citrograph* editorial staff will inform you when your article is due.

- **Failure to submit timely and quality reports may result in premature termination of the project and cessation of funding.**
- Publication of CRB-funded work in any other magazine outside of *Citrograph*, apart from peer-reviewed journals, *must receive prior official written permission from CRB Staff.*
- The PI must acknowledge the Citrus Research Board as the funding source along with the CRB research project number in all relevant presentations, press releases and publications where research findings are disclosed.
- Any publications, presentations and other public releases directly resulting from this work should be provided to the CRB Research Department for review at least 30 days prior to release.
- All patentable inventions will be disclosed to the CRB Research Department. Please refer to contract Exhibit C, Section 21 “Patents”, for further terms and conditions on patent rights.
- As part of its mission, the CRB is interested in moving research developed through CRB-funded research projects out to the California citrus growers. As such, those projects with commercial interest may be requested to meet with CRB staff and consultants or provide additional information to assist in commercialization efforts.
- As part of its mission, the CRB sponsors informational meetings for members of our industry and the general public. As a benefit of attending these meetings, we offer California Department of Pesticide Regulation (DPR)-approved Continuing Education (CE) Units of value to certain licensed professionals working in our state. As a CRB-funded researcher, you may be asked to make a presentation of your research at one or more of these meetings. To receive DPR approval of the CE units, we must describe how your presentation will discuss pest management or pesticide-related topics. Acceptable topics also may include pesticide laws and regulations. Upon award of your research contract, you will be asked to describe in 150 words or less in layperson’s terms how your proposed research is directly relevant to pesticide laws and regulations, pesticides, or the management of one or more citrus pests. Note that these descriptions may be subject to revision prior to the date of your actual presentation.
- Sign a contract of funding acceptance and abide by all terms, including all bullets above. The CRB reserves the right to modify the deliverables indicated in this RFP and will so state in the research contract.

Addendum 1: Guidelines for data/metrics collection for citrus HLB greenhouse and early-stage field 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, 2018 in Denver, Colorado.

The guidelines are not intended to be absolute and final as new and improved procedures are likely to emerge in the months and years to follow. As available resources, goals, and approaches to research vary among different research groups, it is expected that some of the suggested guidelines will have to be modified for particular research projects. Programs seeking funding for new projects should follow these guidelines when developing new project proposals. Justification for modifications from the suggested guidelines should be provided by continuing project proposals. The guidelines are also not intended to restrict researchers from conducting additional analyses, it is recommended to follow suggested practices as best as possible.

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 CLAs, respectively. Number of psyllids and tissue pieces may be adjusted based on the size of experimental plants. Budstick 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 CLAs used and reference to any previous publications or work with the same source.
- Researchers must do one of the following:
 - Use a CLAs 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 CLas 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 CLas detection.
- If CLas testing of roots is to be part of the experiment, fibrous roots ($\leq 2\text{mm}$) should be used.
- Use the “Li primers” (Li et al., 2006) for real-time PCR detection of CLas. 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 CLas, the cutoff should be clearly indicated on reports.
- Duration of experiment and time intervals for disease ratings will vary depending on 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 randomized or randomized blocked. Design will depend on 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 purpose and space available, 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 purpose, 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 $[\text{circumference}/\pi]$. Report trunk cross sectional area (TCSA) using the formula $[\pi \times (\text{diameter}/2)^2]$.
 - 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: $[(\text{diameter parallel to row} \times \text{diameter perpendicular to row}) \times \text{height}]/4$, modified from Wutscher and Hill (1995).
- Determine leaf macro and micronutrient concentrations annually during July-August from 12 mature, 4-6-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 CLas 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 CLAs:
 - 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 ($\leq 2\text{mm}$) 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.
 - 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. 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.