| | | |
|---|---|---|
| Approved Budget Line Item: | **510** | Contract No. |
| Board Recommendation Date: | **12/15/22** | **LAU-23B** |

## STANDARD AGREEMENT

This Agreement is made and entered into this 1st Day of (month) January (year) 2023 , in the State of California, by and between the Board, through its duly elected or appointed, qualified and acting

Name of Board

**CALIFORNIA CITRUS NURSERY BOARD** , hereinafter called the Board, and

Contractor's Name

***TEXAS A&M UNIVERSITY - Kingsville*** , hereinafter called the Contractor.

WITNESSTH: That the Contractor for and in consideration of the covenants, conditions, agreements, and stipulations of the Board hereinafter expressed, does hereby agree to furnish to the **Board** services and materials as follows: (*Set forth service to be rendered by Contractor, amount to be paid Contractor, time for performance or completion, and attach plans and specifications, if any.)* The provisions on the reverse side hereof constitute a part of this agreement.

**Contract Title:** "The Effect of Seed Moisture and Storage Temperature on the Viability of Citrus Rootstock Seeds"

**Project Leader:** David Laughlin, Texas A&M - Kingsville Citrus Center

**Contract Amount:** **$28,665**

**Contract Period:** January 1, 2023 through December 31, 2023

**CONTINUED ON _____ SHEETS**

IN WITNESS WHEREOF, this agreement has been executed by the parties hereto, upon the date first above written.

| BOARD | CONTRACTOR | |
|---|---|---|
| Board Name<br>**California Citrus Nursery Board** | Contractor (If not an individual, state whether a corporation, partnership, etc.)<br>**Texas A&M University - Kingsville** | |
| By (Authorized Signature)<br>Don Dillon<br>Don Dillon (Feb 22, 2023 21:10 PST) | By (Authorized Signature)<br>Diana Luna<br>Diana Luna (Feb 23, 2023 07:51 CST) | |
| Printed Name of Person Signing<br>**Don Dillon - Chairman** | Printed Name and Title of Person Signing<br>Diana P. Luna Contracts & Grants/Authorized Official<br>Office of Research & Graduate Studies<br>Texas A&M University-Kingsville | |
| Title<br>**John Gilstrap, Board Manager** | Address:<br>700 University Blvd., MSC 201<br>Kingsville, TX 78363-8202 | |

**1.** It is understood and agreed that neither party to this Agreement shall be responsible for any damages or injuries arising out of the conduct of activities governed by this Agreement, except to the extent that such damages and/or injuries were caused by the negligent or wrongful acts or omissions of its employees, agents or officers.

**2.** The Contractor, and the agents and employees of Contractor, in the performance of this agreement, shall act in an independent capacity and not as officers or employees or agents of the Board.

**3.** The Board and Contractor may terminate this agreement and be relieved of the payment of any consideration to Contractor should Contractor fail to perform the covenants herein contained at the time and in the manner herein provided. In the event of such termination the Board may proceed with the work in any manner deemed proper by the Board. The cost to the Board shall be deducted from any sum due the Contractor under this agreement, and the balance, if any, shall be paid to Contractor upon demand.

**4.** Without written consent of the Board, this agreement is not assignable by Contractor either in whole or in part.

**5.** No alteration or variation of the terms of this contact shall be valid unless made in writing and signed by the parties hereto, and no oral understanding or agreement not incorporated herein, shall be binding on any of the parties hereto.

**6.** The consideration to be paid Contractor, as provided herein, shall be in compensation for all of Contractor's expenses incurred in the performance hereof, including travel and per diem, unless otherwise expressly so provided.

**7.** During the performance of this contract, Contractor and its subcontractors shall not unlawfully discriminate against any employee or applicant for employment because of race, religion, color, national origin, ancestry, physical handicap, medical condition, marital status, age (over 40), or sex. Contractors and subcontractors shall insure that the evaluation and treatment of their employees and applicants for employment are free of such discrimination. Contractors and subcontractors shall comply with the provisions of the Fair Employment and Housing Act (Government Code Section 12900 et seq.) and the applicable regulations promulgated thereunder (California Code of Regulations Title 2, Section 7285.0 et seq.). The applicable regulations of the Fair Employment and Housing Commission implementing Government Code Section 12990, set forth in Chapter 5 of Division 4 of Title 2 of the California Code of Regulations are incorporated into this contract by reference and made a part hereof as if set forth in full. Contractor and its subcontractors shall give written notice of their obligations under this clause to labor organizations with which they have a collective bargaining or other agreement. This Clause shall apply only to the extent that the requirements therein are otherwise applicable to the Federal Government.

This Contractor shall include the nondiscrimination and compliance provisions of this clause in all subcontracts to perform work under the contract.

**8.** Failure to Comply - "It is hereby mutually agreed that by signing this agreement, Contractor does swear under penalty of perjury, that no more than one (1) final unappealable finding of contempt of court by a Federal court has been issued against Contractor within the immediately preceding two-year period because of the Contractor's failure to comply with an order of a Federal court which orders the Contractor to comply with an order of the National Labor Relations Board (Public Contract Code Section 10296)."

**9**. It is mutually agreed that the Contractor shall be subject to examination and audit of the State of California for a period of three years after final payment under this agreement (Government Code Section 10532). The examination and audit shall be confined to those matters connected with the performance of this contract including but not limited to the cost of administering the contract.

**10**. It is mutually agreed that if the Board's budget authority is repealed by the Legislature in the current year and/or any subsequent years covered under this agreement, this contract shall be of no further force and effect. In this event, the Board shall have no liability to pay any funds whatsoever to the Contractor or to furnish any other considerations under this contract and the Contractor shall not be obligated to perform any provisions of this contract.

**11**. This contract shall not be considered effective until signed by both parties.

**1.** Contractor agrees to perform research in accordance with proposal entitled **"The Effect of Seed Moisture and Storage Temperature on the Viability of Citrus Rootstock Seeds"**, a copy of which is marked Exhibit "A" attached hereto and made a part hereof, to be conducted by **David Laughlin, Texas A&M University, Kingsville Citrus Center,** said research to cover the period **January 1, 2023** through **December 31, 2023**.

**2.** The Board shall pay the Contractor a total of **$28,665** for the work herein described. The Contractor shall submit an invoice in the full amount of the contract and the Board shall have the option of making payment in full or in three installments, the first of which will be for 50% and paid upon final execution of this contract, the second of which will be 40% and paid no later than **October 1, 2023**, and the last of which will be for the final 10%, paid within 15 days of the Board's receipt of the final report.

**3.** If due to factors beyond the control of the Board, funds are not available to the Board for payment to Contractor pursuant to Paragraph 2 of this agreement, Contractor agrees to reduce expenditures for research described in Exhibit "A". Board agrees to provide Contractor the earliest possible written notice of the lack of available funds. In the event of such a cancellation, Board agrees to pay Contractor for all costs incurred to the effective date of cancellation including all uncancelable obligations made in connection with the performance of this agreement.

**4.** If the Contractor finds that as of **December 31, 2023** there are any unexpended balances of the aforesaid funds, said balances shall be shown as carryover funds if this project is to be continued. If the project is completed, excess funds shall be transferred to another project upon approval of the State.

**5.** The Contractor will submit written reports which must be received by the Board no later than **November 1st** of each contract year.

## I. Cover page

1. **Title:** The effect of seed moisture and storage temperature on the viability of citrus rootstock seeds.
2. FY 2023, Year 1
3. **Project leader:** David Laughlin, Assistant Research Professor, Texas A&M University-Kingsville Citrus Center. 312 N International Blvd., Weslaco, TX 78599. david.laughlin@tamuk.edu. 956-447-3360.
4. **Cooperating personnel:** Mark VanNess, Texas Citrus Budwood Program Manager, Texas A&M University-Kingsville Citrus Center 312 N International Blvd., Weslaco, TX 78599. mark.vanness@tamuk.edu. 956-447-3399.
5. There are no current or pending sources of support for this project.
6. This project addresses the CCNB priority #4: Improving management of rootstock seeds and seedlings.

## II. Executive Summary

The foundation of any successful farming business relies on a source of high-quality planting materials. In some cases, these are clonally propagated cuttings but, in most cases, the propagative materials are seeds. This is true of commercial citrus production around the world where seeds of the rootstock of choice are germinated and subsequently grafted or budded with the appropriate scion variety. However, the storage conditions that lead to optimal rootstock seed quality and viability have not been well documented. There are many factors that affect citrus seed quality including fruit ripeness, % moisture, water activity, and temperature. For nurseries that often receive orders year-round it is of utmost importance to have a reliable source of high-quality rootstock seed and to know under which conditions to store that seed to maximize viability over time.

In the face of abiotic stressors such as drought and soil salinity and biotic stressors such Phytophthora foot rot or citrus Huanglongbing (HLB), new rootstocks are being developed and released that may require specific storage conditions that are distinct from traditional rootstocks such as sour orange or rough lemon. Standards for the storage of both new and traditional citrus rootstock seeds need to be developed.

## III. Benefit to the Citrus Nursery Industry

In order to provide citrus nurseries with a continuous supply of high-quality rootstock seeds, some basic seed handling and storage standards should be defined. Many other industries, especially annual row crops such as soybean, corn, or cotton have strict seed handling protocols that are met so that the farmer receives the highest quality seeds that they feel confident will result in uniform germination rates and high-quality crops (International Seed Testing Association, 1993). The volume and rigor demanded by these sectors may be excessive to produce citrus rootstock seeds, nonetheless it is important to develop seed

handling and storage standards. The percent moisture within a seed is related to the water activity of the seeds which is amount of water available for microorganism growth and development that result in a loss of quality and viability. Water activity can be measured more easily using dedicated equipment but must be correlated with the corresponding percent moisture (Hay et al. 2022). Testing a range of seed moisture levels to identify the optimal moisture content at which to store citrus seeds is critical to maintain seed viability.

Seed viability is also a function of temperature (Wawrzniak et al. 2020). Many seeds can be stored at below freezing and maintain excellent viability. However, citrus evolved in tropical and subtropical Asia so the seeds may not be adapted to maintain viability at temperatures below 0 °C. Therefore identifying the ideal storage temperature in combination with the appropriate percent seed moisture will lead to seeds that maintain uniform viability over long term storage.

Citrus production around the world is varied and consists of many different varieties and scions and rootstocks. Therefore, any study that is done on citrus rootstock seed viability should include traditional rootstocks like sour orange, alemow, rangpur lime, and rough lemon as well as trifoliate hybrid rootstocks such as Carrizo citrange and Swingle citrumelo, and more modern rootstocks that are currently in use that may confer tolerance to citrus HLB such as US-942.

This project would address all three of the issues discussed and would have beneficial impacts for the California citrus nursery industry. The results of this study would also potentially have positive impacts for the citrus nursery sectors in other citrus-producing states such as Texas, Florida, Arizona, Louisiana and Georgia as well as in other citrus-producing nations.

## IV. Objectives

This study proposes three main objectives:

1. Test and identify different seed moisture percentages and correlated water activities to determine the optimal seed moisture for long term seed storage and viability.
2. Test and identify different storage temperatures to determine the optimal conditions for long term seed storage and viability.
3. Test different moisture treatments and storage conditions on multiple important citrus rootstock varieties.

## V. Workplan and Methods

To ensure delivery of seed viability this project will be divided into a number of tasks.

**Task 1. Determine the average moisture content for the citrus rootstock varieties to be tested at a single harvesting point.**

Fresh seed will be harvested from the citrus rootstock variety trees at the Texas A&M University-Kingsville Citrus Center in Weslaco, Texas. The fruit will be harvested in January of 2023 and °Brix will be measured for fruit harvested to ensure fruit ripeness uniformity for any future studies. The fruit will then be sent for seed extraction to a local nursery that is equipped with a seed extractor. First, the average initial percent moisture for all varieties tested will need to be determined using the following formula (Figure 1).

$$\text{Seed moisture content (\%)} = \frac{\text{fresh seed weight - dry seed weight}}{\text{dry seed weight}} \text{ X } 100$$

Figure 1.

This will be done by taking 5 subsamples of 50, well formed, non-viviparous seeds and recording the mass before and after desiccation in a drying oven. Once this baseline is established more fresh seed will be harvested to perform the study. The completion date of this task will be the first week of February 2023.

**Task 2. Test and identify different seed moisture percentages and correlated water activities to determine the optimal seed moisture for long term seed storage and viability.**

The study will be set up in a factorial design for each variety tested where both temperature and seed moisture treatments will be tested. Potential interactions between both factors will be analyzed throughout the course of the project. For each variety the experiment will be set up in the following manner. The fresh wet seeds will then be immediately taken to the laboratory to be dried to five different moisture percentages which will be determined by weighing wet seeds followed by desiccation in a drying oven after which percent seed moisture can be determined. The proposed moisture percentages to be tested are 60%, 45%, 30%, 15%, 5%. The target moisture percentages will be achieved by serially testing samples until the target moisture content is achieved.

The seeds will then be packaged in permeable paper envelopes and then in plastic bags, then stored using a completely randomized design to account for incubator variation at six different temperatures using temperature-controlled incubators. Percent relative humidity within each incubator will be monitored.

Every four weeks for 12 months, beginning on the day that the seeds are packaged and stored, the first 5 subsamples of 50 seeds per variety will be planted in flats containing Berger BM6 soilless substrate and germinated in a temperature-controlled chamber. Percent viability will be evaluated after 3-4 weeks following sowing. Extra seeds will be stored for monitoring beyond the scope of this project. The completion date of this task will be December 2023.

**Sub-task 2.2 Correlate water activity of citrus rootstock seeds with percent moisture.**

Once the seeds to be tested are dried down to the appropriate moisture contents they will then be analyzed using a water activity meter. These data will be used to build a correlation table that can be used to rapidly determine appropriate moisture contents. The completion date of this subtask will be in February 2023.

**Task 3. Test and identify different seed storage temperatures to determine the optimal conditions for long term seed storage and viability.**

The study will be set up in a factorial design for each variety tested where both temperature and seed moisture treatments will be tested. Potential interactions between both factors will be analyzed throughout the course of the project. Fresh wet seeds will be dried to the previously mentioned percent moistures and incubated at 5 different temperatures.

The seeds will then be packaged in paper envelopes and stored using a completely randomized design to account for incubator variation at six different temperatures using temperature-controlled incubators. The temperature treatments that will be tested will be 30 °C, 20 °C, 10 °C, 4 °C, 0 °C and -20 °C.

Every four weeks for 12 months, beginning on the day that the seeds are packaged and stored, the first 5 subsamples of 50 seeds per variety will be planted in flats containing Berger BM6 soilless substrate and germinated in a temperature-controlled chamber. Percent viability will be evaluated after 3-4 weeks following sowing. Extra seeds will be stored for monitoring beyond the scope of this project. The completion date of this task will be December 2023.

**Task 4. Test different moisture treatments and storage conditions on multiple important citrus rootstock varieties.**

Using the same temperature and moisture treatments, viability will be tested using at least five different rootstock varieties and up to ten. The varieties to be tested are to be decided and are contingent on seed availability if this project is selected for funding.

Every four weeks for 12 months, beginning on the day that the seeds are packaged and stored, the first 5 subsamples of 50 seeds per variety will be planted in flats containing Berger BM6 soilless substrate and germinated in a temperature-controlled chamber. Percent viability will be evaluated after 3-4 weeks following sowing. Extra seeds will be stored for monitoring beyond the scope of this project. The completion date of this task will be December 2023.

## VI. Project Management and Evaluation

The project leader, Dr. David Laughlin, will design and coordinate the experiments or each objective. A research technician will be assigned to this project and will execute each project objective including fruit harvesting, seed extraction, seed drying, moisture reading, and germination assays. The cooperator, Mark VanNess, will provide the fruit from which seed will be harvested and will provide practical input on conditions and varieties selected.

## VII. Literature Review

Much research has been done on the handling of and storage of seeds for row crops and horticultural crops (Hartmann et al. 2011)). There also exist standard methods for the handling of some tree species. However, there is a paucity of information related to the handling of citrus rootstock seeds to maximize viability. Some studies have been performed that examine the viability of some different scion varieties. Some variables that have been studied are the effects of variety and fruit ripeness. Some of these studies have shown that fruit ripeness is important but harvesting fruit over the harvest season has little impact unless the fruit presents large numbers of precociously germinating seeds. Interestingly it appears that different varieties contain varying initial moisture percentages and different moisture tolerances related to viability (Orbović et al. 2013). Several studies have been performed in Brazil that study the effects of long-term cold storage and seed moisture among different varieties (de Carvalho 2013). One particular study showed that seed viability of Volkamer lemon, Rangpur lime, and Sunki mandarin maintain high viability when stored at 5 °C for 10 months compared to trifoliate orange and citrumelo. Therefore, there exists precedent that indicates that variety is strongly related to long term viability which justifies the need to perform evaluations on numerous rootstock types and create protocols for each. Seed moisture was not controlled or monitored in this study. Despite numerous studies having been performed, the practical recommendations resulting from these studies are unavailable or difficult to find.

## VIII. Literature cited


de Carvalho, S.A. (2013). Monitoring the viability of citrus rootstocks seeds stored under refrigeration. *Revista Brasileira de Fruticultura*. 35, 238-245.

de Carvalho, D.U., Boakye, D.A., Gast, T., Leite Jr., R.P., and Alferez, F. (2021). Determining seed viability during fruit maturation to improve seed production and availability of new citrus rootstocks. *Front Plant Sci*. 12:777078.

Hartmann, H.T., Kester, D.E., Davies, F.T., Geneve, R.L. (2011). Hartmann and Kester's Plant Propagation: Principled and Practices. 163-164.

Hay, F.R., Rezaei, S., and Buitink, J. (2022). Seed Moisture Isotherms, Sorption Models, and Longevity. *Front Plant Sci*. 13:891913.

International Seed Testing Association. (1993). Rules for testing seeds: Rules 1993. *Seed Sci. Technol.* 21(suppl.):1B259.

Orbović, V., Dutt, M., Grosser, J.W. (2013). Evaluation of the germination potential of citrus seeds during the harvesting season. *HortScience*. 48(9):1197-1199.

Wawrzyniak, M., Michalak, M. and Chmielarz, P. (2020). Effect of different conditions of storage on seed viability and seedling growth of six European wild fruit woody plants. *Annals of Forest Science* 77:58.

**BUDGET PROPOSAL**

**Project Title**: The effect of seed moisture and storage temperature on the viability of citrus rootstock seeds.

**Project Leader:** David Laughlin

**Proposed Fiscal Year:** FY 2023

**A. PERSONNEL:**

**Salaries, Wages and Fringe Benefits ($ total):** Funds are requested for one month of salary equaling **$5,000** and fringe/benefits equaling **$1,765** for Dr. David Laughlin. Funds are also requested to provide a salary to support a research assistant for **$16,000** to assist the PI in data collection. Total funds requested for personnel are **$22,765.**

**B. EQUIPMENT/SUPPLIES**

**Nonexpendable Equipment**: **$3,000** in funds are requested for the purchase of a water activity meter to correlate seed moisture levels with water activity. **$2,000** in funds are requested for the purchase of an additional incubator to perform seed viability assays. Total funds requested for nonexpendable equipment are **$5,000**.

| Seed viability assay supplies | Justification | Cost |
|---|---|---|
| Seed drying trays | Mesh trays to dry seeds | $150 |
| Plastic/paper bags | Plastic and paper bags to store seeds for viability assays | $50 |
| Germination flats | 1-inch trays for gemination/viability assays | $100 |
| Substrate | Berger soilless substrate for germination assays | $200 |
| Racks | Metal rack for stacking trays in germination chamber | $200 |
| Seeds and extraction service | Rootstock seeds and extraction fee | $200 |

Total: **$900.00**

**C. TOTAL REQUESTED: $28,665**

Signature of requestor:  Date: 11/26/2022

Signature of cooperator:  Date: 11/26/2022

Signature of dept chair: 

Date: 11/26/2022

# LAU-23B

Final Audit Report 2023-02-23

| | |
|---|---|
| Created: | 2023-02-22 |
| By: | JD Allen (jdallen@tabcomp.com) |
| Status: | Signed |
| Transaction ID: | CBJCHBCAABAAT_Rej0exf8nDht8nNeWL891TjzcGgGxo |

## "LAU-23B" History

Document created by JD Allen (jdallen@tabcomp.com)
2023-02-22 - 5:54:43 PM GMT- IP address: 67.182.126.228

Document emailed to dfdillon831@gmail.com for signature
2023-02-22 - 5:56:04 PM GMT

Email viewed by dfdillon831@gmail.com
2023-02-22 - 5:56:05 PM GMT- IP address: 66.249.92.12

Signer dfdillon831@gmail.com entered name at signing as Don Dillon
2023-02-23 - 5:10:46 AM GMT- IP address: 69.106.239.204

Document e-signed by Don Dillon (dfdillon831@gmail.com)
Signature Date: 2023-02-23 - 5:10:48 AM GMT - Time Source: server- IP address: 69.106.239.204

Document emailed to Diana Luna (diana.luna@tamuk.edu) for signature
2023-02-23 - 5:10:50 AM GMT

Email viewed by Diana Luna (diana.luna@tamuk.edu)
2023-02-23 - 1:51:34 PM GMT- IP address: 104.47.74.126

Document e-signed by Diana Luna (diana.luna@tamuk.edu)
Signature Date: 2023-02-23 - 1:51:55 PM GMT - Time Source: server- IP address: 139.94.88.248

Agreement completed.
2023-02-23 - 1:51:55 PM GMT