

**Title:** Development of avocado rootstock varieties resistant to *Phytophthora cinnamomi* and salinity by the implementation of molecular breeding approaches for genomic selection

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**Overall Goal:** The overall goal of this program is to develop and provide elite rootstock material that is highly tolerant to *Phytophthora* root rot (PRR) and salinity. To address this goal we have divided the program into specific areas: 1) Screening for PRR tolerant rootstock varieties, 2) Screening for rootstock varieties with high tolerance to salinity and understanding PRR x salinity interactions to select for both PRR and salinity tolerant rootstocks, 3) Field testing, 4) Implementation of molecular-assisted breeding, and 5) outreach, education, and commercial release of elite rootstocks.

**Milestones proposed:**

- 1- Continue rootstock field evaluation and data tree collection at all active field sites
- 2- Transfer approximately 40% of rootstock germplasm material from Field 6 to Field 7 at UC-SCREC.
- 3- Hold field meeting for growers if appropriate and prepare an article for “From the Grove” regarding program.
- 4- Final report submitted.

**Milestone activities during this period:**

***1) Continue rootstock field evaluation and data tree collection at all active field sites.***

Field data was collected from seven fields of the nine total active field plots. Data collection from Royden #1, #2 and Cornell are schedule for the first and second week of November. These times for rating will be keep consistent in the program.

***2) Transfer new set of microclonals to field 7 SCREC:*** As indicated in the previous report, this milestone will not be done in this period. Four trees corresponding to 101 rootstock lines were ordered from Brokaw Nursery (400 trees). These trees will be clonally propagated onto salinity tolerant rootstocks (2 trees on Dusa and 2 trees on VC801). The trees will be delivery on Spring 2018.

***3) Hold field meeting for growers if appropriate and prepare an article for “From the Grove” regarding program.*** It will appropriate prepare the article for “From the Grove” once we have finished and analyzed all our field and greenhouse interesting data.

**Additional Milestone activities during this period:**

- **Exp. I to determine the effect of salinity on the development of the disease caused by *P. cinnamomi* using clonal rootstocks.**

- Dr. Mauk's research assistant Rue Li, in collaboration with SRA Brandon McKee are conducting this experiment using **clonal material** grafted to Hass and ungrafted. The rootstocks that we are using have known resistance phenotypes to *P. cinnamomi* and salinity (Table 1). A total of 5 treatments are being conducted in the greenhouse: i) Untreated control, ii) Salinity stress (EC 2.0), iii) *P. cinnamomi* inoculation (most virulent strain), and iv) *P. cinnamomi* x salinity.

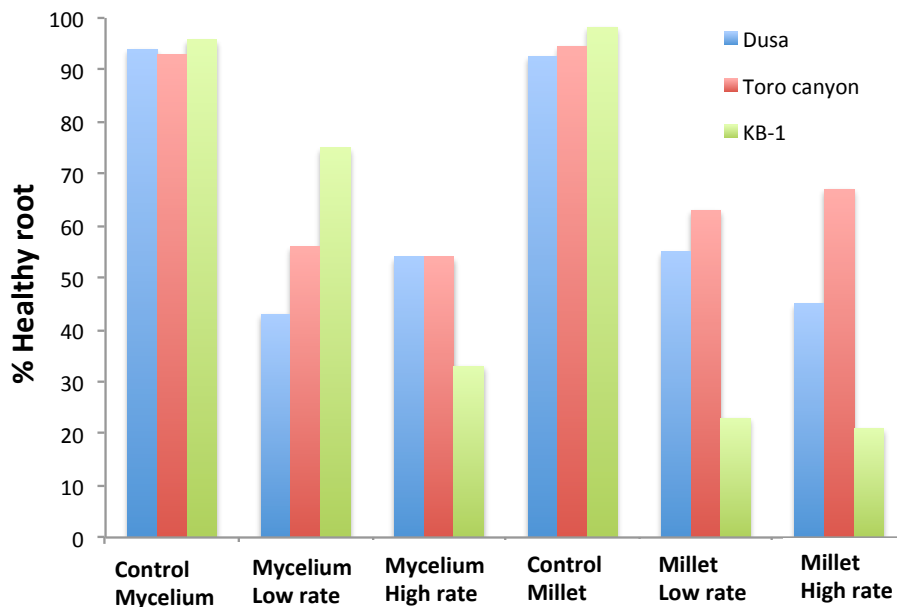
Table 1. Rootstocks being used in the *Pc* x Salinity experiment I.

Variety	Phenotype
Zutano	Susceptible to <i>Pc</i> and tolerant to salinity
Dusa	Resistant to <i>Pc</i> moderate sensitive to salinity
Zentmyer	Resistant to <i>Pc</i> and very susceptible to salinity
VC801	Resistant to both <i>Pc</i> and salinity
PP9	Susceptible to <i>Pc</i> and good for salinity

- Six months old microclonals (after being received by Persea Tree Nursery) were inoculated with the most virulent *P. cinnamomi* strain on July 15<sup>th</sup>. The salinity treatment was applied gradually on July 15<sup>th</sup> until the EC value reached to 2.0 (2 weeks after inoculation).
- Data collected is being analyzed but we have been observed interesting trends during the course of this experiment:
  - *In some cases we are observing differences on how the different rootstocks perform under these treatments when they are grafted to Hass. These results argue for a Rootstock:Scion interactions in response to salinity, Phytophthora root rot (PRR) disease, and both (PRR x salinity). These experiments need to be repeated.*
  - *The development of the phytophthora root rot (PRR) disease is lower when rootstocks are challenge with *P. cinnamomi* and salinity at the same time.*
- **Experiments for refinement of *Pc* inoculation methods (Brandon McKee and Rodger Belisle)**
  - Experiments conducted in the greenhouse using different concentrations of pathogen inoculation and different methods (millet and mycelium) were terminated. Data is being analyzed to determine statistically significant differences among the treatments.
  - **Testing Mycelium inoculation vs Millet inoculation and two concentration of pathogen inoculum using clonal rootstocks.** Ungrafted clonal rootstocks corresponding to Dusa, Toro Canyon, and the susceptible control RO.54 (KB1) were obtained from Brokaw Nursery. These clonal plants were subjected to five treatments of 5 replications per treatment: i) uninoculated, ii) Millet inoculation

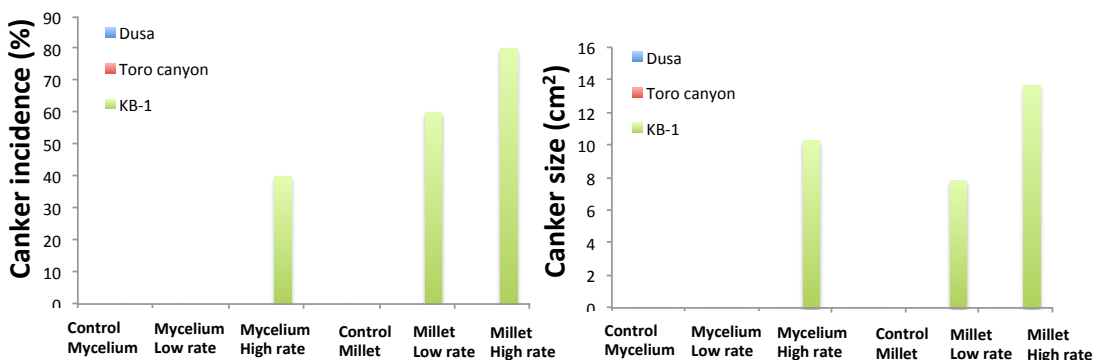
4.5 gr (millet plus mycelium)/pot, iii) Millet inoculation 9 gr (millet plus mycelium)/pot, iv) Mycelium inoculation (0.6 gr/L.), and v) Mycelium inoculation (1.2 gr/L.). Plants were inoculated on July 28, 2016 with a **mixture of 3 Southern isolates of *P. cinnamomi*** that are the most virulent strains. **Note.** *We have been changed the way of millet application in the soil during inoculation based on the results of my student Rodger Belisle and his fungicide greenhouse trials. This new application improved the results of inoculations.*

**Fig. 1. Preliminary analyses. Root health was scored after 8 weeks post inoculation.**



Two weeks after inoculation visual *stem lesions (cankers)* were observed only in the susceptible rootstocks (RO.54) independent of the method of inoculation (Fig. 2 and 3). Stem lesions were not observed previously so new experiments will be conducted to confirm these results. We calculated the canker incidence as the amount of plants developing this lesion and also we determined the stem lesion size (Canker size).

**Fig. 2. Stem lesions (Cankers) were developed only in the susceptible KB-1 rootstock.**



**Fig. 3. Susceptible KB-1 rootstock inoculated with the most virulent isolates using millet inoculation with the higher rate of inoculum concentration 8 weeks after inoculation.**



- Initiate screening of VCs for PRR resistance in GH:** UCR rootstocks PP35, PP40, PP80 and a subset of VC rootstocks with some indication of *P. cinnamomi* resistance (VC225, VC802, VC803, and VC804) were inoculated on September 29. These clonal plants were donated by Brokaw Nursery and were grafted to Hass. Dusa (Industry standard), RO.54 (KB1, as susceptible control) and RO.05, a new rootstock from South Africa, which has good levels of *Pc* and salinity resistance, were used as controls. We used **millet inoculation in this experiment with the higher rate of inoculum** and have 5 replications per treatment: i) uninoculated control, ii) A mixture of isolates representing the diversity of the *Pc* population (6 isolates), iii) The most virulent isolate *Pc* 2109. The experiment is undergoing.