Are Californian almond cultivars and rootstocks susceptible to PPV and can almonds be a host for the spread of Sharka in California?

Project No.: **HORT48-Rubio**

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A. Summary

Sharka, a disease caused by *Plum pox virus* (PPV), is one of the most serious limiting factors for *Prunus* production (including apricot, plum, prune and peach) in affected areas. PPV was detected in the USA in 1999, and was declared eradicated by the USDA in October 2019, after 20 years of fighting. Despite the official declaration of eradication, Sharka is still of great importance, mainly because of the huge quarantine efforts and millions of dollars spent on it, even though it has never been described in California. Studies about Sharka on Californian almond cultivars and rootstocks are scarce, and the behavior of these cultivars and rootstocks against Sharka remains unknown. Several years ago, our own results showed a limited potential role for almond as a virus source in Sharka epidemics. However, Sharka is still a global threat for areas of stone fruit production, including almond.

Based on the little information available, it is important to know the level of susceptibility/resistance presented by almond varieties as well as the rootstocks used by the almond industry in California, clarifying the possible role almond trees could play in the permanent threat that Sharka represents.

So far, in the months we have been working on the project, we have selected the first set of almond cultivars and rootstocks widely cultivated and dissemined in California to be phenotyped against PPV. We have had a slight delay in our program, due to Covid-19 and permitions to import plant material from USA to Europe.

B. Objectives *(300 words max.)*

The global objective of this project is to determine the susceptibility to PPV of the most important cultivars and rootstocks used in the almond industry in California, as well as the new promising Californian selections. This global objective includes other main objectives:

1. Plant material selection and collection in California of almond cultivars, rootstocks, new releases and advanced selections from breeding programs. Introduction of these genotypes in the virus evaluation program under controlled conditions at CEBAS-CSIC of Murcia, in Spain.

2. The PPV phenotyping process, including the following: rootstock preparation (stratification, germination, growth in greenhouse, inoculation, grafting of all previously selected plant material); a rest period in a cold chamber; and phenotyping (at least three phenotyping cycles).

3. Sharka susceptibility analysis, elimination of susceptible genotypes and re-grafting of genotypes that show higher resistance to PPV in order to complete a second round of evaluation.

C. Annual Results and Discussion *(This is the core function of this report)*

1. Selection of almond cultivars. The first criteria for selecting varieties was the planted surface area (acreage) of the last 10 years, but we also took into consideration increases and decreases in the percentage of surface area over the last 20 years. In this first set of cultivars, we selected the top 20 cultivars [202004almac.pdf (usda.gov)] and five recent releases: Nonpareil, Monterey, Independence, Wood Colony, Aldrich, Carmel, Fritz, Butte, Sonora, Shasta, Padre, Supareil, Price Cluster, Winters, Bennett, Folsom, Peerless, Ne Plus Ultra, Mission (Texas), Avalon, Kester, Sweetheart, Pyrenees, Matan and Mira. The new realeases and advanced selections will be selected and included in a second set of cultivars next year.
   Regarding the selection of rootstocks, we selected the most widely used, but also some that we consider could be of importance in the near future. We have selected the following rootstocks: Lovell, Nemaguard, Viking, Atlas, Krymsk 86, Hansen 536, Brights 5, Titan, Rootpac R, Empyrean 3 (Tetra), Guardian, Hansen 2168, Nickels (UC 1-82), Marianna 2624, Cornerstone, Nemared, Garrigues, Halford, Alpha, Marianna M40, Ishtara, Adesoto 101, Cadaman, Barrier (Empyrean1), Julior, Kuban, Paramount, Penta(italy)(Empyrean2), GF677, GxN-15 (Garnem), Rootpac20 and GF305 (PPV control).

2. Phenotyping and susceptibility analysis. We have started the rootstock phenotyping with the plant material available in European nurseries, including the following rootstocks: GF305 (PPV control indicator), Garrigues, Adesoto 101, Cadaman, Rootpac R, Rootpac 20, GF677, and GxN-15. Fifteen replicates of each rootstock have already been inoculated by chip budding with PPV. The first evaluation cycle will be performed in the spring of 2021 (end of April). The conventional stratification process is currently ongoing with around 500 seeds.

3. Problems getting the plant material: Different problems have contributed to a slight delay in our proposal.

   There are two main problems. The first is the restriction on importing plant material into the European Union from areas affected by *Xyllela fastidiosa*, and the second is a common problem all around the world in 2020, Covid-19. This virus has totally

limited the presential activitivity in our research center, causing a siginificant delay in most of our activities, including the present project with the ABC.
The availability of plant material will be key for completing the phenotyping objective. With the help of the Foundation Plant Services (UC Davis) we hope solve the problems of importing plant material to Europe.

D. Outreach Activities

1. Please describe outreach activities including the event description (date, location, topic of the presentation, approx. number of participants and type of audience)

   NONE SO FAR.

E. Materials and Methods *(500 word max.)*:

1. Outline materials used and methods to conduct experiment(s)

   Materials: The plant material used (cultivars, rootstocks, seeds) has aready been described in point C (Annual Results and Discussion).

   Methods: The PPV phenotyping process is a long procedure that we are carrying out following two independent methodologies depending on the plant material used, varieties or rootstocks.

   Grafting cultivars/rootstocks onto GF305 seedlings: The first step of the process is to obtain rootstock seedlings from GF305 peach, a biological indicator of PPV, by stratifying seeds at 7 °C for 16 weeks. The first stratification is currently ongoing (12 weeks) with around 500 seeds. At the begininig of February, germinated seeds will be sown in pots. After two months of growth, seedlings will be inoculated by grafting with a piece of bark of infected GF305 seedlings showing severe Sharka symptoms. A month later, several replicates (at least 10) of each selected scion will be grafted onto the inoculated GF305 peach seedling. See figure 1.

   Grafting of own-rooted rootstocks: Inoculation was performed directly on the own-rooted rootstocks we have thus far by grafting a bud from an infected GF305 peach seedling. When the bud sprouts, the presence of symptoms on leaves is used to confirm the efficiency of inoculation (figure 2). The first evalution cycle of the eight rootstocks already inoculated will be performed at the end of April 2021.

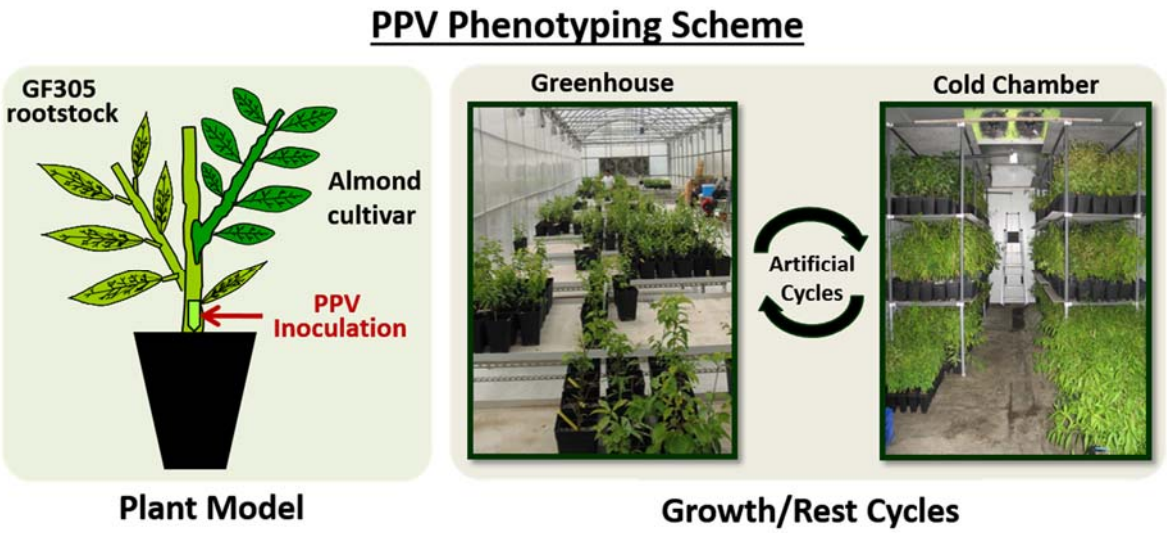

Figure 1. PPV phenotyping process. Plant model: GF305 rootstock infected with PPV and grafted with the genotypes to be tested. Pictures show the greenhouse where the evaluation will be carried out and the cold chamber, where the plants will be submitted to artificial rest periods.

Figure 2: Commercial own-rooted Rootpac-R rootstocks (left). Sprouted GF305 fully infected with PPV on a GF677 rootstock (right).

2. So far, the availability of plant material has been limited. As we mentioned before, the restriction on importing plant material into the European Union from areas affected by *Xyllela fastidiosa*, and Covid-19, have contributed to a slight delay in our schedule.
   On other hand, some rootstocks are usually sold own-rooted, so will be impossible to bring to Europe from USA. So we will collect rootstock scions instead and phenotype them by grafting onto infected GF305, like we do with cultivars..
   We will try to solve the permission issue in order to import live scions from USA with the collaboration of the Foundation Plant Services of UC Davis. In the meantime, we have started with the plant material and resources that we have found in Spain and Europe.

F. Publications that emerged from this work

1. List peer review publications in preparation, accepted or published
2. Other publications (e.g. outreach materials)
3. Please provide copies of publications

   NONE SO FAR