Study of the Effects of Little cherry virus-1 and Little cherry virus-2 on Different Cherry Rootstocks*

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This is an ongoing project from a prior year Agreement.

Project Summary/Abstract

Briefly describe the long-term objectives for achieving the stated goals of the project.

Little cherry disease (LCD), associated with Little cherry virus-1 (LChV-1) or -2 (LChV-2), is a common problem of cherries (Prunus avium) which occurs worldwide, causes unmarketable fruit and often results in tree or orchard removal (Jelkmann and Eastwell, 2011). Most of the new cherry rootstocks used in cherry production are interspecific Prunus hybrids which introduces an increased risk of an adverse reaction (hypersensitivity) to some viruses (Lang and Howell, 2001). Hypersensitive reactions exhibit graft union gum exudation, premature abscission, and tree death within one or two growing seasons and have been shown to occur in Prunus when infected with Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) (Howell and Lang, 2001, Lang and Howell, 2001, Lang et al., 1998). We propose to evaluate the effects of LChV-1 and LChV-2 on 16 different popular Prunus rootstocks. All rootstocks will be grafted with a scion variety from the same accession. Observations of budtake and tree performance will be recorded and evaluated for two years. Rootstocks will be rated for sensitivity to LChV-1 and LChV-2 and this information will be shared with growers and nurseries to assist in making rootstock selection decisions.

Scope of Work

Describe the goals and specific objectives of the proposed project and summarize the expected outcomes. If applicable, describe the overall strategy, methodology, and analyses to be used. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate. Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the goals and objectives.

Project's Benefit to Nursery Industry

In the US, sweet cherry fresh market production totaled 254,906 tons and was valued at $703 million in 2015 (NASS, 2017). Washington, California and Oregon account for more than 90% of sweet cherry industry in the US, with 34,786, 34,742, and 13,416 acres planted to sweet cherries in 2012, respectively (NASS, 2017). Interest in sweet cherry production has increased in recent years due to the high value of fresh market cherries and the increasing availability premium quality varieties and new rootstocks with exciting horticultural traits (Lang and Howell, 2001).

Little cherry disease is a concern to growers wherever cherries are grown. LCD is associated with LChV-1 or LChV-2, which can be found in single and mixed infections. Trees with LCD produce cherries of small size and poor color making fruit unmarketable. The problem results in unpicked limbs or trees, tree removal and even orchard removal. The disease is readily transmitted by grafting and LChV-2 is vectored by mealybugs (Jelkmann and Eastwell, 2011). To date, no breeding programs have been successful in finding resistance to the disease.

In orchards worldwide, cherries (P. avium) are either budded or grafted onto rootstocks. Rootstocks provide protection from soil-borne pests and improved tolerance to abiotic stresses, such as heavy soils, drought conditions, salinity, and cold winter temperatures, thus, increasing the survival of the scion material. Traditionally, cherries in the US were grown on Mazzard or Mahaleb rootstocks or clonally-propagated 'Colt' which are generally tolerant of infection by pollen-borne viruses, PDV and PNRSV (Lang et al. 1998). It has been increasingly well-documented that new Prunus rootstock selections can show hypersensitive reactions to viruses that have been typically well tolerated by traditional rootstocks (Lang et al. 1997, Lang et al. 1998, Lang and Howell 2001, Howell and Lang 2001). These new rootstock selections are derived from species other than or are hybrids with P. avium which offers genetic diversity and novel horticultural traits, but with an increased risk of hypersensitivity. Hypersensitive (rapid and lethal) reactions exhibit graft union gum exudation, premature abscission, and tree death within one or two growing seasons. Viruses with documented hypersensitivity include PNRSV and PDV (Howell and Lang, 2001). It is not currently known if LChV-1 and LChV-2 can cause similar hypersensitive reactions in the common Prunus rootstocks.

We plan to conduct a field trial to investigate hypersensitivity reactions to LChV-1 and LChV-2 in the top c*herry* rootstocks. Rootstock cultivars will include GiSelA®3, GiSelA®5, GiSelA®6, GiSelA®12, Krymsk®5, Krymsk®6, Krymsk®7, EMLA Colt,' MaxMa®14, Cass, Clare, Clinton, Crawford, Lake, and seedlings of Mazzard and Mahaleb. We will assess the sensitivity of these rootstocks to LChV-1 and LChV-2 and share the results of our research.

This research has a great benefit to the cherry growing industry as the results will assist growers and nurseries in rootstock selection for new plantings. Informed rootstock selection will result in healthier, more productive cherry trees.

Workplans and Methods:

This project was initiated in 2017. Two iterations of the trial outlined in Table 1, with the objective to represent spring and fall industry grafting seasons, were performed. The first iteration occurred in early June of 2018, and the second in September 2018. Each iteration of the trial included 40 trees of each rootstock, allocating 15 towards the evaluation of each of the two viruses. We T-bud grafted 15 trees of each rootstock with two buds of virus positive material, five trees with 2 buds of Foundation level clean material, and five were not grafted. (Table 1).

Table 1. Rootstock cultivar and inoculation treatments.

Rootstock Cultivar1	# Trees LChV-1+ 'Bing'	# Trees LChV-2+ 'Bing'	# Trees FPS 'Bing'	# Non-grafted

1. EMLA Colt	15	15	5	5
2. Brokforest (MaxMa 14 ®)	15	15	5	5
3. Gisela 3	15	15	5	5
4. Gisela 5	15	15	5	5
5. Gisela 6	15	15	5	5
6. Gisela 12	15	15	5	5
7. Krymsk 5	15	15	5	5
8. Krymsk 6	15	15	5	5
9. Krymsk 7	15	15	5	5
10. Mahaleb seedling	15	15	5	5
11. Mazzard seedling	15	15	5	5
12. Cass	15	15	5	5
13. Clare	15	15	5	5
14. Clinton	15	15	5	5
15. Crawford	15	15	5	5
16. Lake	15	15	5	5

The potted rootstock plants were planted after grafting in a randomized field block. The first iteration of grafted material was planted in October 2018, the second iteration of grafting was planted in April 2019.

These trees were tested by RT-PCR for spread of LChV-1 and LChV-2 from the infected bud grafts into the rootstocks in 2019. We obtained 36% and 46% successful LChV-1 and LChV-2 transmission, respectively, into the sixteen selected rootstock cultivars (Fig. 1). In trees with failed virus transmission, grafting of infected buds was repeated in the 2019/2020 funded year.

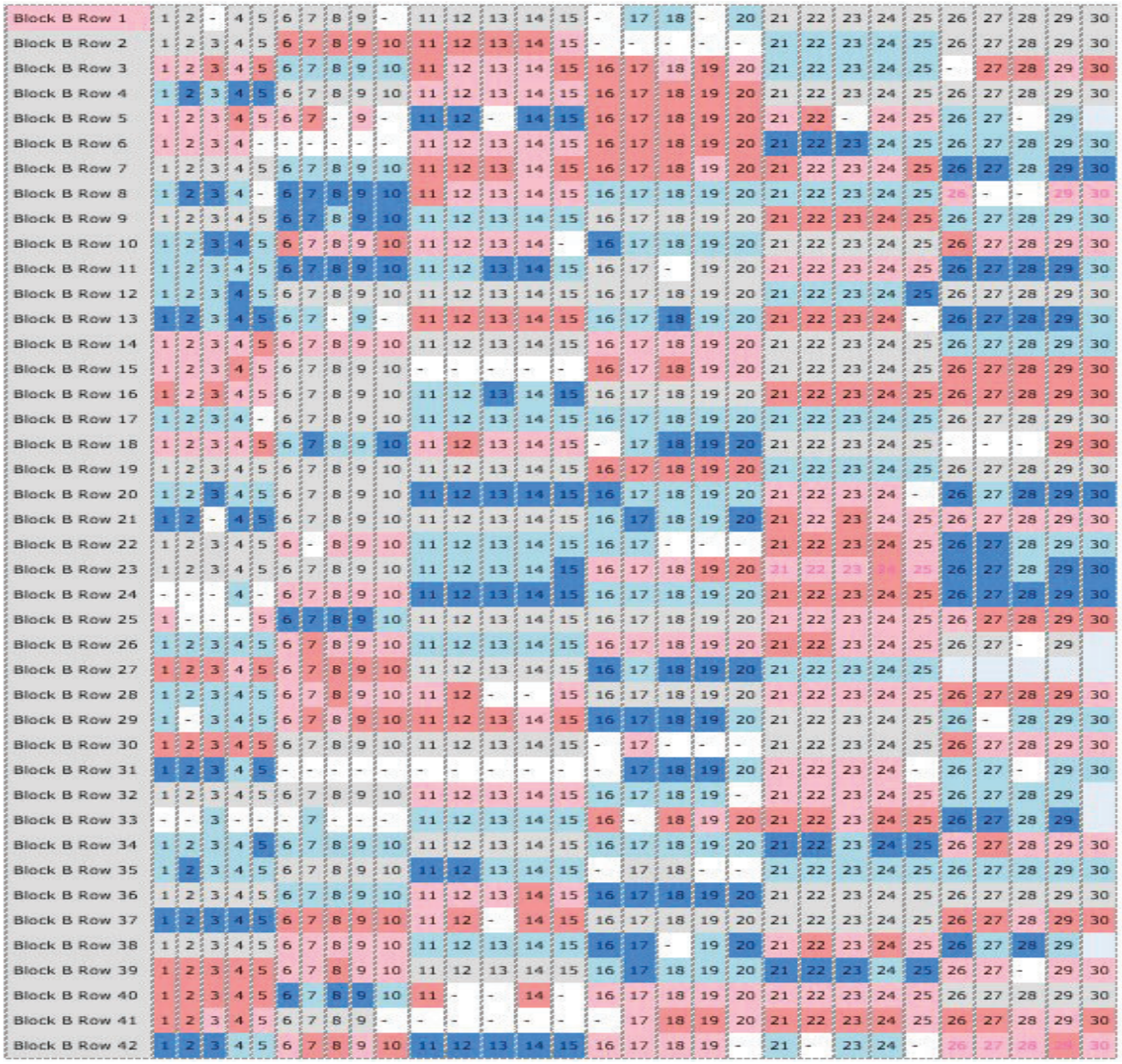

Figure 1. The randomized block of rootstocks grafted with healthy, LChV-1 or LChV-2 infected Bing scion in the spring and fall iterations. The grey boxes represent plants grafted with healthy Bing scion or ungrafted controls. The dark blue boxes represent plants with successful grafting and transmission of LChV-1. The light blue boxes represent plants with unsuccessful grafting and/or negative RT-qPCR results for LChV-1. The dark salmon boxes represent plants with successful grafting and transmission of LChV-2. The light pink boxes represent plants with unsuccessful grafting and/or negative RT-qPCR results for LChV-2. White areas represent plants that died.

Scope of work for this year of this project

- Grafting done in the 2019/2020 funded year will be monitored for growth of the scion buds. To encourage scion growth in all grafted trees in the field, two prunings of the rootstocks in the randomized block trial will be done. Once during winter dormancy and again in early spring.
- For trees with failed graft growth (and failed virus spread as seen in Fig. 1, light blue and light pink boxes), grafting of LChV-1 and LChV-2 buds will be repeated.
- To test the inoculated plants for the selected viruses and monitor the virus movement. All the plants that previously tested negative for LChV-1 or LChV-2 will be tested by RT-qPCR for the presence of the inoculated virus to verify the success of transmission. This information allows us to correlate the presence of the virus with the symptoms recorded.

- Symptom development for each treatment will continue to be observed. We will evaluate all of the field planted trees for visual symptoms of tree death, gumming, leaf distortion, leaf color and vigor. Based on these observations, the reaction of the rootstocks to the virus will be categorized as lethal (died in the presence of the virus), severe (strong adverse response, but non-lethal within two years), sensitive (mild virus symptoms noted but the tree not affected severely), and tolerant (no symptoms). If deemed necessary some plants with suspicious symptoms will be sacrificed, the trunk will be autoclaved to remove the bark and examine the wood for wood marking symptoms.
- Orchard training and maintenance. Weed and pest control maintenance will continue through this funding cycle.