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

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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. n orchards worldwide, cherries (P. avium) are either budded or grafted onto rootstocks. Rootstocks provide protection from soil-borne pests and improved tolerance to a biotic 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 Mazza rd 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 cherry rootstocks.

Rootstock cultivars will include GiSelA® 3, GiSelA®5, GiSelA® 6, GiSelA® l2, Krymsk®5, Krymsk® 6, Krymsk®7, EMLA Colt,' MaxMa®l4, Corette'"l, Corette'"2, Corette'"3, Corette'"4, Corette'"5, 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.

Objectives:

1. To test a collection of plants by qRT-PCR to locate infected source material needed for the experiment.

2. To evaluate the effects of LChV-1 and LChV-2 on 16 different popular *Prunus* rootstocks. All rootstocks will be grafted with the same cherry scion cultivar, 'Bing'.

3. To test the inoculated plants in year 2 for the selected viruses and monitor the virus movement and record the symptom observation.

Workplans and Methods: (2 year of project)

Objective 1: To test a collection of plants by qRT-PCR to locate infected source material needed for the experiment.

In the first year of this project, two selections were chosen to serve as the inoculation source for LChV-1 and LChV-2. We identified a single infection source of LChV-1 but were unable to do so for LCHV-2. The inoculation source that we selected is co-infected with CVA.

Objective 2: To evaluate the effects of LChV-1 and LChV-2 on 12 different popular cherry rootstocks. All rootstocks will be grafted with the same cherry scion cultivar, 'Bing'.

We will perform two iterations of this trial, with the objective to represent both industry grafting seasons. We will perform the first iteration of budding in early June of 2018 *(prior year agreement),* and the second in September 2018. Each iteration of the trial will include 50 trees of each rootstock, allocating half towards the evaluation of each of the two viruses. The trial will

include GiSelA®3, GiSelA®5, GiSelA® 6, GiSelA® 12, Krymsk®5, Krymsk® 6, Krymsk®7, EMLA Colt,' MaxMa® l4, Corette"'l, Corette'"2, Corette'"3, Corette'"4, Corette'"5, and seedlings of Mazzard and Mahaleb. We will T-bud 15 trees of each rootstock with virus positive material, five trees with Foundation level material, and five will not be grafted. We will graft two buds to each rootstock. To confirm that scion cultivar does not play a factor, all positive and negative control material will be sourced from the same cherry scion cultivar, 'Bing'.

Rootstock Cultivar	Trees inoculated with LChV-1+ 'Bing'	Trees inoculated with LChV-2+ 'Bing'	Trees budded with FPS 'Bing '	Non-grafted trees
1.eee EMLA Colteee	15	15	5	5
2.eee Brokforest (MaxMa® 14)e	15	15	5	5
3.eee GiSelA® 3eee	15	15	5	5
4.eee GiSelA® 5eee	15	15	5	5
5.eee GiSelA® 6eee	15	15	5	5
6.eee GiSelA® 12eee	15	15	5	5
7.eee Krymsk® 5eee	15	15	5	5
8.eee Krymsk® 6eee	15	15	5	5
9.eee Krymsk® 7eee	15	15	5	5
10.eeMahaleb seedlingeee	15	15	5	5
11.eeMazzard seedlingeee	15	15	5	5
12.eCorette ™ 1eee	15	15	5	5
13.eCorette ™ 2eee	15	15	5	5
14.eCorette ™ 3eee	15	15	5	5
15.eCorette ™ 4eee	15	15	5	5
16.eCorette ™ 5eee	15	15	5	5

Prior to the field trial, we will T-bud inoculate 'Bing' cherry trees sourced from our Foundation nursery. We will collect virus positive material from source trees, confirmed positive as part of objective one by RT-qPCR, and inoculate four trees for each virus treatment. All negative control material will be sourced from the FPS Foundation Orchard.

For the first iteration of the trial previously funded by IAB (funding cycle July 1, 2017 to June 30, 2018), material was collected from negative and positive source trees in late May of 2018 and grafted to container grown rootstocks shortly after. The T-budded and nongrafted control trees will be held in the shadehouse for a short period until they are field planted. The rootstock to be budded in the fall will also be planted in the field at this time. Following the planting, all vegetative growth above the scion buds will be removed to promote scion growth. Observations of budtake and tree performance will be recorded and evaluated. Continuation of this project is contingent upon Program approval and funding.

For the second iteration of the trial, during this funding period of 2018/2019, material will be collected from negative and positive source trees in September of 2018 and T-budded to field planted rootstock. In the spring of 2019, following budbreak, all vegetative growth above the scion buds will be removed to promote scion growth. Observations of budtake and tree performance will be recorded and evaluated. We will periodically evaluate 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 affectedseverely), and tolerant (no symptoms).

***Objective* 3:** *To test the inoculated plants in year 2 for the selected viruses and monitor the virus movement and record the symptom observation.*

All the plants will be tested by RT-PCR for the presence of inoculated viruses to verify the success in transmission. For testing, leaf petioles will be collected from the plants, total RNA will be extracted from each sample and the RNAs will be used in RT-qPCR to test for the presence of the viruses inoculated into each plant. This information will let us correlate the presence of the virus with the symptoms recorded. The symptom development for each treatment will be observed periodically and monitored. 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. Continuation of the project is contingent upon Program approval and funding.