Final report for Virginia Wine Board FY2016

"Establish international collaborations for management of crown gall of grape, caused by *Agrobacterium vitis*"

Mizuho Nita and Akira Kawaguchi
Assistant Professor, AHS AREC, PPWS, Virginia Tech
nita24@vt.edu, 540-869-2560 x33

8 February 2016

Objective 1: The PI (Nita) will visit Japan in March 2015 to visit Okayama Prefectural Technology Center (March 23-24) for Agriculture and Kumiai Chemical Industry (March 27) to discuss details of the use of ARK-1 in VA.

Results: Successfully conducted, and resulted in 1) agreement between Okayama prefecture and the Nita lab on the research collaboration and 2) agreement between Kumiai Chemical Industry and the Nita lab for the use of *R. vitis* ARK-1.

(Please note that this trip is NOT covered by this proposal; however, it is listed as one of objectives of the project because it is very important first step for this collaborations. Neither the Technology Center nor Kumiai Chemical Industry has experiences to collaborate with foreign Universities. Moreover, crown gall is not as big issue in Japan as in VA. Thus, it is very critical for us to make Prefectural Technology Center realized that growers in VA are facing a critical issue, and there is marketing potential for Kumiai Chemical Industry.)

Objective 2: Nita lab has been in contact with APHIS (Animal and Plant Health Importation Service) for the importation process. However it also depends on how the objective #1 turns out. If the Kumiai Chemical Industry intends to market ARK-1 in the US, then they will most likely to cover this objective.

Results: A permit (P526P-15-03305) was issued from APHIS on 18 Aug. 2016

Objective 3: Dr. Kawaguchi has agreed that he will visit VA for VVA meeting in 2016 to present his research results with ARK-1.

Results: Drs. Kawaguchi and Nita provided a joint session at 2016 VA Vineyard Association Meeting at Charlottesville, VA 29 Jan 2016. The title of presentations are: "Overview of Crown Gall of Grape and Known Management Strategies" (Mizuho Nita), and "Biological Control for Grapevine Crown Gall" (Akira Kawaguchi).

Objective 4: Dr. Kawaguchi also agreed that he would train technicians and students at Nita lab for culturing of *R. vitis* isolates so that we can maintain ARK-1 culture, and also be able to re-isolate *R. vitis* when we conduct experiments.

Results: On 24 Sept to 30 Sept 2016, Dr. Kawaguchi visited the Nita lab. He trained our lab technicians for the isolation of *R. vitis*. In addition, we visited six VA vineyards and collected 33 grapevines with crown gall symptoms. Isolation of *R. vitis* was conducted and 100+ individual bacterial colonies have been isolated. We are planning to visit several more in 2016 to increase sample number in our collection. Currently, we are in the process of identifying these bacterial colonies to determine whether they are 1) *R. vitis* or not, and if it is, 2) whether *R. vitis* contains a piece of DNA that cause gall or not. We are in a process of revealing identity of our isolates by utilizing a multiplex PCR procedure developed by Kawaguchi (2005).

Addendum (*Covered by FY17 funding*): A total of 758 bacterial colonies isolated from crown gall diseased vines from vineyards around the state of Virginia. Samples from galls had the bacterium transferred from the plant material to a semi selective media plate to grow colonies of the pathogen. Starting in May of 2016, Single colonies were isolated and then analyzed for genus and pathogenicity by

multiplex polymerase chain reaction. The genus *Rhizobium* was detected by a universal primer set that amplifies a 16S rDNA region of *R. vitis* species. Pathogenicity is detected by a primer set used to amplify the virulence C operon region of the tumor inducing plasmid. Colonies that were identified for being Rhizobium but lacking the tumor inducing plasmid were marked as potential biological control isolates native to Virginia. These isolates will be tested against pathogenic strains shortly to see if they reduce tumor formation rates.

Outcomes and Benefit Expected

There are no means of management against crown gall, other than some cultural practices to avoid wounding events. With restrictions in variety and site selection, unpredictable winter condition, and presence of crown galls in many of our vineyards, it is very clear that we need a tool to fight against this important disease. Therefore, the impact of this collaboration is very high with our stakeholders.

The major benefit was the achievement of collaborations among VT Grape Pathology lab, Okayama Prefectural Technology Center, and Kumiai Chemical Industry. Also having Dr. Kawaguchi at the VVA meeting was well received among our growers who have issues with crown gall. In addition, several of nurseries showed their interests at the VVA meeting. Since our lab has not been working on *R. vitis* in the past, this partnership has been a great opportunity for us to expand our knowledge. At the same time, having collaborator in the US will help both Okayama Prefectural Technology Center and Kumiai Chemical Industry to have options to test out their products in the area where crown gall is a serious issue.

In the near future, we would like to achieve is to demonstrate the effectiveness of ARK-1 isolate against *R. vitis* isolates in VA. If ARK-1 or other strain of *R. vitis* can have an efficacy even after infection by pathogenic strain of *R. vitis*, it will provide a tool to manage crown gall on infected vines. These are some of experiments that have been discussed, and some of them were proposed for 2016-17 funding.

1) Field validation of ARK-1 under VA condition as a rescue application to crown gall infested vineyards

We have a 0.25 acre of Chardonnay vineyard located in AHS AREC planted in 2013. Inoculation study will be conducted at this site where 50-60% of vines are already naturally infected with crown gall. ARK-1 will be applied 1) to plant surface via spray, 2) to plant tissue via injection, results will be visually measured as overall health of the vine, as well as molecularly by detecting *R. vitis* DNA (of both wild type and ARK-1) from the treated vine.

2) Field validation of ARK-1 under VA condition as a preventative application to healthy vines

At AHS AREC, we created five 4 x 12 ft raised beds that contains a mix of mushroom compost and sands, which were not exposed to grapes in the past. Grapevine (cv. Chardonnay) will be planted in a meshed root bag. Treatments will be 1) ARK-1 amended in the soil, 2) Vines dipped in water containing ARK-1 for 24 hours prior to the planting, 3) inoculated control, and 4) non-inoculated control. Each block that contains all treatments will have three vines of the same treatment placed randomly, and there will be 6 replications of the block. Thus, a total of 72 vines will be used for the experiment.
After 30 days of planting, cultured wild-type *R. vitis* from vines at AHS AREC will be inoculated onto treatment vines. Treatment vines will be artificially wounded by cutting the bark to create 0.5 mm deep, 1 cm wide wound. Then a suspension with *R. vitis*, which is grown on an artificial medium for 5 days, will be applied onto the wound using an atomizer. The surface of the wounds will be sealed with Parafilm to provide protection for desiccation. The plastic containers will be used in the experiment in order to minimize the movement of *R. vitis* between vines. Soil surface in between containers will be covered with straws to minimize splash dispersal of the pathogen. After 30 days, the vines will be visually examined for gall formation, and also re-isolation of *R. vitis* will be made for DNA-based confirmation.

3) Screening of native *R. vitis* in VA soil for their ability to compete or interfere with pathogenic *R. vitis*.

Although ARK-1 has been showing very promising results, it would be ideal if we can identify more isolates of *R. vitis* with similar inhibitory or antagonistic activities against *R. vitis*. The difference among *R. vitis* population in Japan and US may be large enough that it may affect the efficacy of ARK-1. If that is the case, other isolates in our native soil may have a better efficacy or use of two or more strains of antagonistic *R. vitis* strain may provide better results.

For the pathogenicity test, strains isolated from grapevines around the state of VA will be tested on young tomato plants (other host for *R. vitis*) or grapevines. Isolates are prepared from cultures grown on PSA plates at 30°C for 2 days. The stems of 1- to 2-mo-old plants grown in a greenhouse will be inoculated by the needle prick method. Tomato plants will be examined regularly for gall production over a 4-week period; the period will be extended to 12 weeks in the case of grapevine. Isolates that failed to induce galls on all test plants are regarded as nonpathogenic.

II. Problems and Delays

Although the APHIS permit was issued in August, shipment of ARK-1 from Japan has been delayed until April 2016, due to a product development schedule.

III. Future Project Plans

We have submitted our proposal for field and lab experiments to the Wine Board

IV. Funding Expended To Date

We have utilized 100%.