

**Virginia Wine Board Grant  
Final Report**

6/30/2022

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*Title:* Application of a biocontrol agent *Rhizobium vitis* ARK-1 at the time of grafting

*Proposal Number:* P2DA2WQW

*Project Type:* ☒ Research ☐ Education ☐ Marketing

Is this a multi-year grant? ☒ Yes ☐ No

If yes, which year does this report address? 1<sup>st</sup> of a 3-year grant

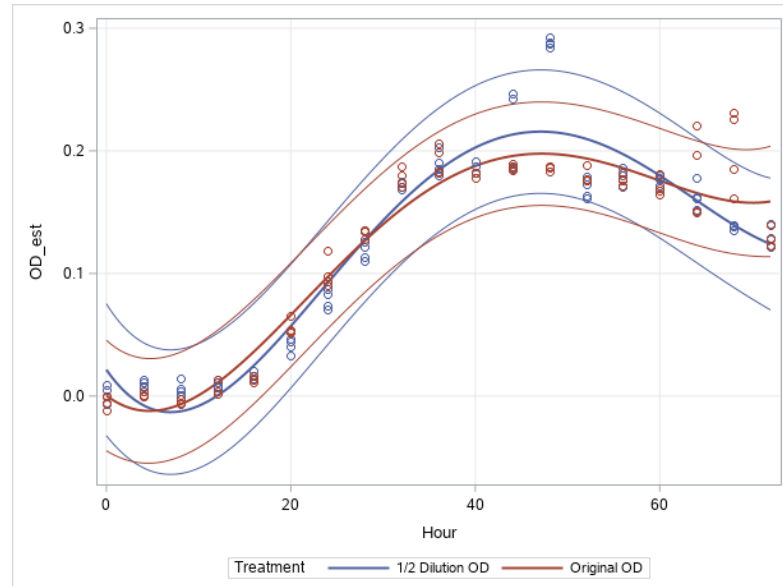
*Original Funding Amount:* \$45,516

*Current Balance:* \$1,011

*Objectives and Results:*

1. Evaluate the efficacy of application of ARK-1 at the time of grafting
  - a. A trial was conducted in July 2021, but unfortunately, none of the graft wounds survived the duration of the experiment. We will repeat it in Summer 2022 via green graft.
2. Evaluate root dipping and soil application methods as after-grafting treatment
  - a. We are preparing for these two experiments as of June 2022. We expect to start root dipping experiments in July 2022, followed by soil application experiments.
3. Continue screening for VA native non-pathogenic *R. vitis* strains for potential use as biological agents.
  - a. We have screened additional 30 isolates in 2021-22, unfortunately, none were shown to be candidates for biological control.
4. Visualize the quantity of ARK-1 and a pathogenic isolate with a sensitive molecular assay for the rapid detection of pathogenic *R. vitis*.
  - a. A new real-time PCR procedure protocol was established, and we have conducted two trials in 2021. The preliminary results indicated that both ARK-1 and a pathogenic strain can move from the point of inoculation. We are currently conducting a series of experiments that expand the range of time and distance to understand how the pathogen and ARK-1 moves in grapes.
  - b. **NEW** sub-objectives that needed to be addressed due to a new equipment that replaced an old one. We conducted a series of experiments to determine how the bacterium increase their cell numbers over time at the time of inoculum preparation for experiments. We also revisited our procedures to accurately estimate how many of them are viable. We found that our isolates peaked out around 48 hours, which was what we expected (figure below). At the peak of activity (= at the highest rate of growth, which happens before the peak in the figure below, approximately at 30 hours), the viable cell count was  $\sim 1 \times 10^8$  cell/mL, which was close to what was previously published, but

nearly twice as higher as our old estimate using the retired equipment. These results will help us refine our future inoculation experiments.



*Problems and Delays:* A student who was supposed to be on board for the fall 2021 declined the offer a few weeks before the semester. After additional search and interviews, Mr. Mahadi Redoi joined the lab in January as a Ph-D student. Due to the delay in the process, some of experiments were postponed as noted above, and we focused on the real-time PCR procedure.

#### *Overall Benefit for Virginia Wine Industry:*

Crown gall of grape, caused by *Rhizobium* (= *Agrobacterium*) *vitis*, is a chronic issue among Virginia vineyards. Due to extreme temperature fluctuations during winter (e.g., 2013-14), some of the vineyards experienced more than 50% of their vines infected with crown gall in VA. Unfortunately, we do not have a useful means of control for *R. vitis* other than cost-prohibitive cultural practices. Cultural management strategies, such as the hilling of the graft union during the winter, are the predominant methods used for the control of crown gall. However, many of these strategies are not feasible or sustainable due to their cost and labor requirements (Burr et al. 1998). We believe our research will contribute to provide an effective solution to this economically important disease.

#### *Publications and Activities Associated with Project:*

Nahiyani, A. and Nita, M. (2021) "Rhizobium vitis ARK-1 reduces grapevine crown gall formation against higher cell density of tumorigenic isolates and over a wide application timings" Plant Health, American Phytopathological Society, 8 August 2021

Nahiyani, A. and Nita, M. (2021) "Rhizobium vitis ARK-1 reduces grapevine crown gall formation against higher cell density of tumorigenic isolates and over a wide application timings" American Phytopathological Society Potomac Division Meeting, 10-12 March 2021: Won the first place in a graduate student oral presentation competition

Wong, A. T., Kawaguchi, A., & Nita, M. (2021). Efficacy of a biological control agent *Rhizobium vitis* ARK-1 against Virginia R. Vitis isolates, and relative relationship among Japanese and Virginia R. vitis isolates. *Crop Protection*, 146, 105685. <https://doi.org/10.1016/j.cropro.2021.105685>

*Future Work:*

In addition to the proposed studies, we have begun: 1) an experiment to induce gall formation using clean plants that are planted in infested soil to understand the event that can trigger the formation of galls, and 2) a study with a new (and non-patented) strain VAR03-1 to evaluate its efficacy against the US R. vitis isolates.

*Final Budget and Justification:*

Item Type	Original Awarded Amount	Final Amount Spent
Personnel	\$26,327	\$13,446
Fringe	\$2,524	\$1,356
Travel	[\$0.00]	[\$0.00]
Supplies & Materials	\$1,600	\$22,250
Contractual	[\$0.00]	[\$0.00]
Other	\$15,065	\$7,514
Total	\$45,516	\$44,505

Since we could not allocate a graduate associate until January and also we made progress with a real-time PCR part (which requires many reagents that tend to be expensive), we transferred personnel and other budget to Supplies and Materials. A budget adjustment proposal was submitted and approved by the Wine Board in March 2022.

*References:* Wong, A. T., Kawaguchi, A., & Nita, M. (2021). Efficacy of a biological control agent *Rhizobium vitis* ARK-1 against Virginia R. Vitis isolates, and relative relationship among Japanese and Virginia R. vitis isolates. *Crop Protection*, 146, 105685. <https://doi.org/10.1016/j.cropro.2021.105685>