1. Detection and quantification of *Agrobacterium vitis*, causing crown gall in grapevines, from nursery material (NSERC-Engage)

Objective 1: Identification of suitable primer set for *A. vitis* quantification
We successfully identified suitable primers virAfor/virAref (Eastwell et al., 1995) to detect *A. vitis* in dormant vines.

Objective 2: Develop a ddPCR assay
We successfully developed a droplet digital polymerase chain reaction (ddPCR) assay using *virA* primer to quantify *A. vitis*. The ddPCR assay is fast (1-2 days), allows for absolute quantification of *A.vitis* for the first time and has a higher sensitivity compared to previous detection methods (Johnson et al., 2013). Dormant canes from vines with visible crown gall symptoms were collected, but we were not successful in quantifying *A. vitis* from these samples, although a variety of DNA extraction procedures were tested. It is possible that bacteria overwinter in the root system of grapevines and are pushed up from the roots only in spring with xylem flow as previously reported (Burr et al., 1998). Thus, detection of *A. vitis* in dormant canes is limited. However, we received dormant nursery stock, and were successful in quantification of *A. vitis* from roots (Objective 3).

Objective 3: Detection and quantification of *A. vitis* in dormant vines
Three major Okanagan wineries donated nursery planting material they received from nurseries in the United States, Germany and Canada. Roots of the grapevines were used to quantify *A. vitis* abundance with the method developed in Objective 2. Nurseries had a significant effect on *A. vitis* abundance in roots ($p < 0.001$). Plants originating from Ontario nurseries had fewer *virA* copies (305-932 copies/0.3 g roots) compared to plants from the three Californian nurseries (over 17,000 copies/0.3 g roots). There was considerable variation in copy number among replicates, as indicated by the high standard deviation among samples from the same nursery. A Riesling sample from Germany had 10,278 copies/0.3 g root and a different clone from the same nursery had a 3.6 fold higher concentration. Cherry root samples served as a negative control. Grapevine nursery stocks from the same shipments as the test samples used for ddPCR were planted in spring 2016 in the Okanagan Valley (BC) by the respective wineries. The following winter was cold, with minimum air temperatures frequently reaching less than -20°C during December 2016 and January 2017, which are conducive for disease development. Our study confirms that some nursery material received by local wineries is systemically infected with *A. vitis* before planting. The results of the study were communicated to the wineries involved. Quantification of *A. vitis* in dormant nursery stock before planting is important for growers to determine if nursery material is free of *A. vitis,* as the bacteria can be present systemically, without visible symptoms and lead to disease development after planting.

2. Crown Gall Disease of Grapevines: Quantification of *Agrobacterium vitis* in vineyard soil (NSERC-Engage Plus).

Objective 1: Implementation of a ddPCR assay to quantify *A. vitis* from soil
We have successfully implemented the ddPCR assay developed earlier (NSERC-Engage) to quantify *A. vitis* from soil and we are able to detect a minimum of 100 *A. vitis* cells/g of soil using this assay. To date, there are few reports on *A. vitis* detection in soil, and no other methods exist to quantify the bacterial population in soil. Quantification will be an important aid for growers before establishing a new vineyard or replacing vines, to estimate the potential risk of disease occurrence in a vineyard.

Objective 2: Soil survey to monitor *A. vitis* population in Okanagan vineyards
We have successfully quantified *A. vitis* from rhizosphere soil of two vineyards in the Okanagan valley with crown gall disease. Soil was sampled five times over a growing season in collaboration with a local winery. The abundance of *A. vitis* in soil was assessed with the *virA* ddPCR assay. *Agrobacterium vitis* abundance in vineyard 1 ranged from 1,244 to 64,224 copies/g soil and in vineyard 2 from 85 to 5,827 copies/g soil. Growth stage (bud break, bloom, pea size berries, veraison, after harvest) did not affect *A. vitis* soil abundance, suggesting that timing of replanting is not important, if the goal for growers is to reduce the potential infection of new vines from *A. vitis* residing in soil. Interestingly, the 3-year-old vineyard had significantly less *A. vitis* cells/g of soil compared to the 5-year-old vineyard, suggesting that the bacterial soil population may increase with age of vineyard as more roots containing *A. vitis* degrade and release bacteria into the soil. For growers, this may mean that early removal of vines with crown gall may result in a reduced possibility of infection of replanted vines, but more studies are needed. An additional promising result was the correlation between the carbon/nitrogen ratio (C/N ratio) of vineyard soil and the bacteria; a higher C/N ratio resulted in fewer bacteria. The C/N ratio of soils can be increased by addition of organic amendments, such as compost or wood mulches. Adding organic amendments may be a promising tool for growers to reduce *A. vitis* population in the soil, in addition to other known benefits. The effect of organic amendments on soil *A. vitis* will be studied in detail, if our proposed Growing Forward 3 project receives funding.

Objective 3: Greenhouse assay to determine the *A. vitis* density in soil and its effect on crown gall disease severity
In June 2017, dormant rooted grapevines were donated by an Ontario nursery, planted in pots and the soil inoculated with varying concentrations of *A. vitis* cells. However, we have not observed crown gall symptoms to date. This is because the natural development of galls in greenhouse conditions is not easily achieved, and previous studies showed that up to 24 months incubation time may be needed before bacteria can be detected in xylem sap (Pu and Goodman, 1993). To decrease the incubation time, dormancy was induced in three replicates of each *A. vitis* soil inoculum concentration (ranging from 10^8-10^1cells/g soil). The dormant vines were moved into a controlled environment chamber and subjected to freeze-thaw cycles at -5°C, similar to the environmental conditions leading to crown gall disease in vineyards. Plants were removed from the chamber and are currently in a greenhouse. We hope that galls will develop with re-growth of the vines. Results of this experiment are important to determine the threshold in soil of *A. vitis* that will lead to crown gall disease in the vineyard. This will allow us to estimate the risk of disease development in a vineyard before planting and may lead to recommendations to growers regarding soil disinfestations before planting.

Other positive outcomes:

- We have submitted a manuscript to the journal Plant Disease reporting on the results obtained through NSERC-Engage/NSERC-Engage Plus and the BCWGC. We expect a decision on acceptance by the end of March 2018.

- Tanja Voegel was awarded a UBC Postdoctoral Travel Award to give an oral presentation titled: "Quantification of *Agrobacterium vitis* in nursery planting material" at the Canadian Phytopathological Society – B.C. Regional Meeting October 27-28, 2016.

- Louise Nelson attended the 17th Annual Enology & Viticulture Conference July 18-19, 2016 in Penticton, where we presented a research poster titled: "Crown gall of grapevines: detection of *Agrobacterium vitis* in soil" to representatives of the industry.

- Tanja Voegel attended the 18th Annual Enology & Viticulture Conference July 17-18, 2017 in Penticton, where we presented a research poster titled: "Crown gall of grapevines: quantification of *Agrobacterium vitis* in grapevine nursery stock" to representatives of the industry. Our crown gall research was of interest to many growers and a representative of CFIA in Victoria.
  In addition, Tanja participated in the Grape Pest and Disease Identification and Information Session, where she presented a poster titled: "Crown Gall Disease", crown gall specimen, education and general information to many growers. The industry liaison person for the NSERC-Engage Plus grant, Sue de Charmoy, was contacted at the conference.

- Results of our research were presented in the BC Fruit Grower magazine (fall/winter edition, page 8) and because of this, we were contacted by a former BC Ministry of Agriculture employee interested in our research.

- Tanja Voegel presented the results of our research at the 38th Annual Crown Gall Conference October 7-8, 2017 in Corvallis, Orgeon. The conference was very beneficial to the research project, because of the attendance of many crown gall experts, especially Dr. Tom Burr, the worldwide expert of crown gall disease in grapevines, who gave valuable information and advice to the project.

- We are in continuing contact with the vineyard manager of the two vineyards we sampled to discuss crown gall research and possible management strategies, and have provided the results of our study to the Okanagan wineries involved.

References:

Burr, T.J., Bazzi, C., Süle, S., Otten, L., 1998. Crown gall of grape: biology of Agrobacterium vitis and the development of disease control strategies. Plant Disease 82, 1288-1297.

Eastwell, K.C., Willis, L.G., Cavileer, T.D., 1995. A rapid and sensitive method to detect Agrobacterium vitis in grapevine cuttings using the polymerase chain reaction. Plant Disease 79, 822-827.

Johnson, K.L., Zheng, D., Kaewnum, S., Reid, C.L., Burr, T., 2013. Development of a magnetic capture hybridization real-time PCR assay for detection of tumorigenic Agrobacterium vitis in grapevines. Phytopathology 103, 633-640.

Pu, X.-A., Goodman, R.N., 1993. Effects of fumigation and biological control on infection of indexed crown gall free grape plants. American journal of enology and viticulture 44, 241-248.