Washington Blueberry Commission Research Proposal

Title: Blueberry virus sample pipeline

Year Initiated 2024 Current Year 2024 Terminating Year 2026

Principal Investigators:

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Justification and Background:

BlShV is widespread in Washington. Some fields have recurring symptoms. Blueberry Shock Virus (BlShV) has been widespread in Western Washington blueberry fields for decades. It is currently found throughout western Washington State (Martin and Tzanetakis, 2018). Plants or sections of plants become blighted, losing their production for that season. The conventional wisdom is that plants exhibit BlShV symptoms the season following exposure but subsequently recover and return to full productivity, showing no further disease symptoms. However, numerous growers report plants in some fields exhibiting recurring symptoms.

New viruses found in Washington blueberries, but no clear association with recurring Shock symptoms. In our previous project, we evaluated plants from 10 fields with symptoms of Recurring Shock and found numerous viruses. As expected, most plants tested positive for BlShV. However, we found that Blueberry Scorch Virus (BlScV) accounted for the recurring symptoms in two of the fields. Regarding newly characterized viruses, one of these contained Blueberry Virus S (BlVS) as well, and nearly all the plants tested positive for Blueberry Virus L (BlVL). However, other than the two fields with BlScV, there was no clear association of specific viruses with Recurring Shock symptoms. In British Columbia (BC), most diseased plants (40-50%) test positive for both BlScV and BlShV, with lower frequencies of single

positive plants. In this context, it is possible that Recurring Shock plants have undiagnosed presence of BlScV (see below).

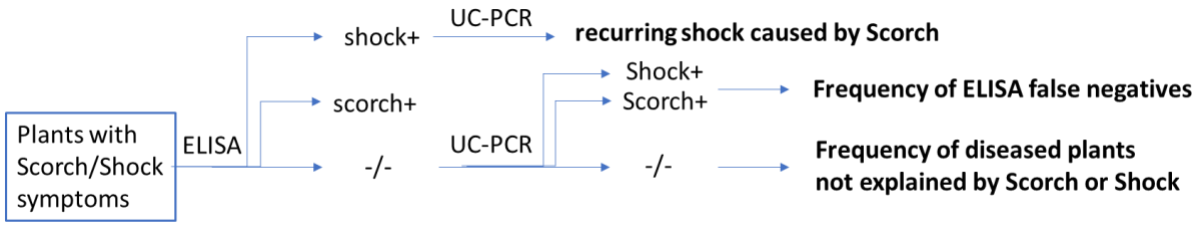
BlScV is more common in Washington than previously understood. In a 2023 survey, we found BlScV in 11 of 49 Washington blueberry fields. This is a much higher incidence of this virus than previously thought. BlScV is eventually lethal to the plant, so management of this disease and preventing its spread is critical for the Washington blueberry industry. So far, the confirmed positives have been in Western Washington, but the numbers of fields surveyed to date in Eastern Washington is small.

BlScV is genetically diverse and current laboratory diagnostic tests do not detect some isolates. Mattsson recently reported considerable genetic variability among BlScV isolates sequenced during recent surveys, in agreement with findings by Dimitre Mollov at the United States Department of Agriculture (USDA) in Oregon. In contrast, the BlShV genome is much less variable and readily detected by PCR. Regarding Scorch, both Mattsson and Mollov have identified BlScV isolates that do not test positive via ELISA based on current antibodies but that do test positive by polymerase chain reaction (PCR). PCR testing in turn also fails when the existing primers (*i.e.*, the molecular sequences used to amplify genetic sequences) do not work for specific viral variants that have either recently evolved or have not been previously identified. Recently, the Mattsson lab developed PCR testing methods to detect small but ultra-conserved (UC) regions of the BlScV genome, avoiding false negatives in BlScV diagnosis. Simply put, if a more universal region of the BlScV genome is used for diagnostics, a broader range of Scorch variants could be detected.

Considering these data, we propose to answer the following questions:

1. Currently-diagnosed BlScV by ELISA and PCR only explains a portion of Recurring Shock symptoms in Washington – would a larger proportion of symptomatic plants in be attributed to BlScV if PCR tests were to target a UC region (*i.e.*, UC-PCR)?
2. Will improved UC-PCR BlScV diagnostics help to understand the cause(s) of Recurring Shock in Washington?
3. How well do improved UC-PCR diagnostics correlate with routine ELISA test results for Washington fields?

Delivering a coordinated "pipeline" (see figure below) of samples from Washington growers' fields to virology and molecular biology labs will help us answer these questions.



We propose to establish this "pipeline" with Walters Ag Research (Walters) collecting large numbers of samples and passing them along to Phyto Diagnostics Company (Ellis) for ELISA testing. Plant symptomology will be shared with the Ellis and Mattsson. The Mattsson lab will test ELISA BlShV-positive plants with Recurring Shock symptoms as well as ELISA double negative plants by UC-PCR for potential presence of BlScV and BlShV. These labs will also be able to choose which samples to evaluate more intensively, based on their symptomology, variety, age, proximity to other samples and ELISA results.

Relationship to WBC Research Priority:
This proposal relates to the WBC research priority 8: "Management of viruses".

Objective:

1. Establish a pipeline from growers' fields to researchers' labs for samples and information to address questions about blueberry viruses in Washington,

Procedures:

This project is anticipated to last three years. This research is investigative in nature as we are attempting to determine what is going on with viruses in Washington blueberry fields. Consequently, our proposed methodologies are likely to shift as we gain more information. To this end, Gerbrandt (BC Blueberry Council) will coordinate between collaborators (Walters, Ellis, Mattsson) to host semi-annual or quarterly meetings via Zoom to coordinate sample collection/testing and to share the status of their findings, informing more targeted sampling efforts for the subsequent year. In Year 1 (2024), an emphasis will likely be on fields with Recurring Shock, BlScV, and nearby symptomatic fields that tested negative for BlScV. Another emphasis will likely be to increase the range and intensity of testing in Eastern Washington fields.

Samples will be collected from May through August. Each sample will be documented via photographs of the plant, a description of symptoms, if any, and the plant's location, variety, and age . Grower identities and exact geographical locations of samples will be kept confidential. Samples will be divided into two identical portions. One of these will be shipped to Ellis (Saanich, BC) where they will be tested via ELISA for BlScV and BlShV. The same leaves will be tested by Mattsson via UC-PCR to avoid discrepancies based on uneven virus distribution in plants. The second portion will be retained by Walters who will be responsible for communicating sample data, photographic documentation, and ELISA results to Ellis and Mattsson.

Based upon the ELISA results, sample data and photos, Mattsson will request samples for further analysis. Samples destined for Mattsson will be shipped by Ellis.

Results will be shared with growers through presentations at grower meetings and through the Small Fruit Update newsletter.

Describe how this research will benefit Washington blueberry growers:

The above "pipeline" will map the emerging threat of BlScV and at the same time shed light on the frequency of BlScV positive plants that are not detected by ELISA. To the central point of this proposal, we aim to improve our understanding of BlScV and BlShV in Washington fields to inform field management (*e.g.*, aphid control, removal of sick plants) so that virus issues can be kept at manageable levels. The study will also find out if current ELISA testing yields an acceptable number of false negatives. Considering that ELISA testing is inherently less sensitive than PCR, a low frequency of false negatives is expected (as in Covid at-home testing). However, a high frequency of false negatives by ELISA indicates that the assay needs to be updated in form of new antibody development to detect novel virus variants.

References:

Finn CE, Mackey TA, Postman JD, Martin RR. 2017. Identifying blueberry germplasm that is slow to get blueberry shock virus in the Pacific Northwest United States. *Acta Hortic* 1180: 423-429

Martin RR, Tzanetakis IE. 2018. High risk blueberry viruses by region in North America; implications for certification, nurseries and fruit production. *Viruses* 10(7): 342

Budget:

	2024	2025	2026
Salaries[1/]	**$17000**	**$17000**	**$17200**
Walters	*$2000*	*$2000*	*$2200*
Kannangara	*$15000*	*$15000*	*$15000*
Time-Slip	**$**	**$**	**$**
Operations (goods & services) [2/]	**$928**	**$ 1100**	**$ 1300**
Travel[3/]	**$ 819**	**$ 850**	**$ 850**
Walters	*$ 491*		
Mattsson,Kannangara	*$ 328*		
Meetings	**$**	**$**	**$**
Other[4/]	**$2990**	**$3000**	**$3000**
Shipping	*$ 990*	*$ 1000*	*$ 1000*
Mattsson supplies	*$2000*	*$2000*	*$2000*
Equipment	**$**	**$**	**$**
Benefits	**$**	**$**	**$**
Total	**$21,737**	**$21,950**	**$22,350**

[1/] $2,000-Walters, 2% FTE, benefits included. $15,000- 50% FTE, PhD stipend, Sachi Kannangara

[2/] ELISA testing, Phyto Diagnostics. 500 samples at $4.10 per sample, plus setup charges: 6X at$52.20

[3/] Walters: 750 miles, $0.655 per mile, Mattsson and student: 500 miles, $0.655 per mile

[4/] $990-Shipping (Walters, 6 shipments to Phyto Diagnostics@$150 each, plus 3 shipments to US labs at $30 each). $2000-Mattsson lab: Enzymes (reverse transcriptase and Taq-polymerase, DNase), part cost of TaqMan probes, primers, 96-well plates for UC-qPCR, user fee for qPCR machine

Researchers should report on all funding (cash or in kind) that has been secured that could support this project or for which funding has been sought.

Gerbrandt's time in coordinating this project will be provided as in-kind support.

Walters also receives funding from the Northwest Center for Small Fruit Research.

For work in the Mattsson lab, funding provided to the BCBC for work conducted in BC will be submitted for matching funding from a Canadian organization called Mitacs with the potential to provide a 1:1 match if the proposal is accepted.