

**Sampling Protocol for *Blueberry scorch virus* (BIScV)
in Highbush Blueberry (*Vaccinium corymbosum*)
and Other *Vaccinium* spp.**

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1. Introduction

Blueberry scorch virus (BIScV) is an aphid-borne *Carlavirus* that causes a serious disease of highbush blueberry in North America and Europe. Symptoms of BIScV infection on highbush blueberry range from symptomless to chlorosis, to necrosis of leaves and flowers depending on virus strain and blueberry cultivar (Wegener et al., 2006). In ornamental *Vaccinium* and cranberry (*Vaccinium macrocarpon*), the virus may produce no symptoms yet the plants could be carriers of virus. A virus testing program for propagators of blueberry and other *Vaccinium* spp. is described here.

2. Scope/Purpose

This document outlines the sampling protocol for:

Blueberry Mother plants for BIScV

Fruiting Mother plants

Vegetative Mother plants

Containerized propagation stock for BIScV

Highbush blueberry (*Vaccinium corymbosum*) and other *Vaccinium* spp.

Field growing cranberry vine (*Vaccinium macrocarpon*)

3. Sampling Protocols

3.1. Blueberry Mother Plants (*Vaccinium corymbosum*)

Mother plants are either fruit-producing blueberry plants or densely planted non-fruit bearing plants grown exclusively for their vegetative habit. In both cases, cuttings for propagation are usually taken from the plants in the dormant season and rooted in sawdust beds, either under cover or in the open for several months before being transplanted into containers.

Both fruiting and vegetative Mother plants are grown in open fields with a documented aphid control program including grower spray records. Fruiting Mother plants have restrictions on the number and timing of aphicide applications due to pre-harvest intervals.

All blueberry Mother plants must have had appropriate aphid control during the year prior to shipping. An 'Information Release Agreement' is signed by the grower declaring that appropriate aphid control has been executed and that monitoring and spray records will be provided to E.S. Cropconsult Ltd. These records will in turn be made available to CFIA for confirmation of compliance with the virus testing program for exported *Vaccinium*.

For mature fruiting and vegetative blueberry plants, symptoms of BIScV typically appear in late May when the plants are in bloom. At this time, the virus titre is sufficient in leaves and blossoms for detection of BIScV by ELISA (Enzyme Linked Immunosorbent Assay). The titre remains high through mid-August. Like other members of the genus *Carlavirus*, virus titre is the highest in the middle aged leaves of a branch as opposed to either the youngest leaves at the tip of growth or the older leaves (Dr. Peter Ellis, Phyto

Diagnostics Ltd. North Saanich, BC, *personal communication*). Mother plants are therefore sampled from the onset of symptoms through mid-August.

3.1.1. Sampling Protocol for Fruiting Mother Plants

Fruiting Mother plants are sampled in groups of three. One middle aged leaf from one branch as noted above, is taken from each of three consecutive plants to constitute one sample. Each bush in a Mother block is sampled in this way. If applicable, a buffer of one row of blueberry plants surrounding a Mother block is sampled at the same time. The sequence of activities for field sampling is as follows:

- 3.1.1.1. The field or block to be sampled is mapped by counting the rows and plants per row, including buffer rows.
- 3.1.1.2. A coding system is prepared noting the variety, row, and sample numbers.
- 3.1.1.3. Sample collection bags are prepared (sandwich-sized *Ziploc* bags) and labelled with the codes written in permanent marker. Groups of bags are attached together (20-30) for ease of sampling.
- 3.1.1.4. Sampling locations are marked with wired flags at regular intervals along a row (for instance, every 10 samples). Sample numbers corresponding to the sample taken at the marked spot are written on the flag in order to facilitate finding sampled plants should there be a need to resample.
- 3.1.1.5. On the day of sampling, the field map is updated with sample numbers associated with each Mother bush row or buffer row.
- 3.1.1.6. When leaves are sampled, each bush is visually observed for obvious signs of BScV infection. If symptoms are present, the bush is sampled separately (not in a group of 3), and marked with a wired flag. Details of the sample are recorded on the sampling data sheet.
- 3.1.1.7. Samples are kept cool and out of the sun during sampling, and placed in a cooler when removed from the field.
- 3.1.1.8. Samples are shipped for laboratory testing as outlined in Section 4.
- 3.1.1.9. Wired flags are left in the field to clearly indicate the tested blocks and rows. When Mother plants in a block within a fruiting field are the only plants to be used for propagation, buffer rows are marked with different coloured flags.

3.1.2. Sampling Protocol for Vegetative Blueberry Mother Plants

The growth pattern of vegetative Mother plants makes it difficult to define individual plants. Therefore a middle aged leaf is taken from each apparent bush with the direction to err on the side of taking too many samples than too few. Because of this, some plants may be sampled twice. The field protocol is as follows:

- 3.1.2.1. The field or block to be sampled is mapped by counting the rows, approximate number of plants per row, including buffer rows (if applicable).
- 3.1.2.2. A coding system is prepared noting the variety, row, and sample numbers.

- 3.1.2.3. Sample collection bags are prepared (sandwich-sized *Ziploc* bags) and labelled with the codes written in permanent marker. Groups of bags are attached together (20-30) for ease of sampling.
- 3.1.2.4. Sampling locations are marked with wired flags at regular intervals along a row (for instance, every 10 samples). Sample numbers corresponding to the sample taken at the marked spot are written on the flag in order to facilitate locating specific plants should there be a need to resample.
- 3.1.2.5. As a back up numbering system, permanent marker is used to write the sample number directly on a leaf of the sampled branch remaining on the Mother plant when it is especially difficult to differentiate plants.
- 3.1.2.6. On the day of sampling, the field map is updated with sample numbers associated with each Mother bush row or buffer row.
- 3.1.2.7. When sampling leaves, each bush is visually observed for obvious signs of BISCv infection. Because BISCv symptoms are most apparent on flowers, vegetative plants may not have obvious symptoms. However, unhealthy plants or plants showing symptoms such as chlorosis and/or blighted leaves are sampled individually (not in a group of 3) and marked with a wired flag, flagging tape or marker directly on the bush and recorded on the sampling sheet.
- 3.1.2.8. If there are other blueberry plants around the vegetative Mother rows, a buffer row will also be sampled as described above.
- 3.1.2.9. Samples are kept cool and out of the sun during sampling, and placed in a cooler when removed from the field.
- 3.1.2.10. Samples are shipped for laboratory testing as outlined in Section 4.

3.1.3. Procedure for BISCv-positive Blueberry Mother Plants

- 3.1.3.1. The sample of 3 consecutive plants that had the positive ELISA test is traced using sampling records and the farm map.
- 3.1.3.2. Each individual plant from the group of three is re-sampled by collecting 3 leaves from each plant and bagging them separately in labelled *Ziploc* bags.
- 3.1.3.3. Samples are shipped as described above to Phyto Diagnostics Ltd. for testing.
- 3.1.3.4. When the positive plant is identified, it is marked for removal.
- 3.1.3.5. Plants around the infected plant are suspect and will be targeted for testing in the following year.
- 3.1.3.6. If none of the plants test positive in the re-test, a second set of individual samples will be taken as long as the last date for sampling Mother plants has not passed. If it has, the plants will be marked for sampling the following spring and to indicate that they should not be used for cuttings.
- 3.1.3.7. The implications of a BISCv-positive Mother plant are discussed with the grower in order to develop a plan to minimize the possibility of infected cuttings.
- 3.1.3.8. Growers are advised to develop a plan for tracking cuttings from Mother plants.

3.2. Containerized Stock (highbush blueberry and other *Vaccinium* spp.)

Approximately 450 species of *Vaccinium* exist worldwide. A variety of *Vaccinium* spp. are propagated in nurseries in British Columbia for export to the USA as plugs or in containers. In many cases, *Vaccinium* spp. other than highbush blueberry, are grown from seed. In some cases, they are vegetatively propagated. Virus testing requirements apply to both.

Large scale propagation of cranberry for fruit production involves mowing or pruning an established bed during the dormant season to obtain cuttings. Cuttings are rolled into bails which are kept cool and moist until planting. A separate sampling protocol for large scale (field) cranberry propagators is outlined below.

The sampling protocol for containerized blueberry stock and containerized ornamental *Vaccinium* spp. is based on the 2002 Oregon protocol. According to this protocol, sampling and analysis of non-dormant (green) plant material must take place within 60 days of the date of shipment. Plants destined to be shipped when dormant are to be sampled and tested between July 1st and September 15th during the season prior to shipping. For operational purposes, field sampling is completed by August 31st in order to allow the lab sufficient time to complete testing.

The following table is used to determine the number of samples required for laboratory testing of containerized blueberry for *Blueberry scorch virus*: The same numbers are used for other containerized *Vaccinium* spp.

Lot Size *	Number of samples to be tested per variety (cultivar)
1-49	All plants
50-1000	50 plants
1001-9000	5% of plants
>9000	450 plants

* A Lot is defined as a single cultivar grown at a single location. For example, if a grower has 5 cultivars at one site there would be 5 lots for testing purposes. If a grower has 5 lots at each of three sites there would be 15 lots for testing purposes.

All containerized plants must have had appropriate aphid control during the year prior to shipping. An 'Information Release Agreement' must be signed by the grower declaring that appropriate aphid control has been executed and that monitoring and spray records will be provided to E.S. Cropconsult Ltd. These records will in turn be provided to CFIA for confirmation of compliance with the virus testing program for exported *Vaccinium*.

3.2.1. Sampling Protocol for Containerized Stock

3.2.1.1 In discussion with the grower, the source of propagation material is determined (i.e. seed vs. cuttings), and any lots destined for export to the USA are identified with the numbers of plants per lot and the

locations. The number of samples taken from each lot is determined according to Table 1, and if propagative material was taken from Mother plants, the number of Mother plants for sampling is also determined. A few extra samples are added to the required number to err on the side of caution.

- 3.2.1.2 The farm is mapped with the location of each lot to be sampled. Individual beds and rows are counted and the number of plants in each is estimated. Row lengths are measured in paces.
- 3.2.1.3 A sampling plan is established to ensure samples are taken evenly from all areas of each lot. This includes how many paces between samples, how many rows to be sampled, and which side of a row to sample from.
- 3.2.1.4 A coding system comprised of the variety and sample numbers is determined for each lot.
- 3.2.1.5 Sample collection bags (sandwich-sized *Ziploc* bags) are prepared and labelled with the code written in permanent marker. Groups of 20-30 bags are attached together for ease of sampling.
- 3.2.1.6 Wired flags are used to mark the sample number at the end of each row in each bed, and at regular intervals along the sampled row (for instance, every 5-10 samples). Sample numbers corresponding to the sample taken at the marked spot are written on the flag. This facilitates finding sampled plants should there be a need to resample.
- 3.2.1.7 On the day of sampling, the field map is updated with the sample numbers associated with each lot.
- 3.2.1.8 Sample locations are selected based on a predetermined number of paces between samples.
- 3.2.1.9 At each sample site, single leaves that are at least 50% expanded are collected from five different plants and combined in one sample bag to constitute a sample. These leaves are collected from plants on one side of the row (pre-determined in sampling plan) but from plants that are not consecutive (i.e. from plants as spread out as the sampler can reach).
- 3.2.1.10 Virus symptoms rarely appear on young plants as they are rapidly growing. However, unhealthy plants and/or plants showing symptoms of chlorosis or leaf blight are noted and sampled individually (not in a group of 5) and marked with a wired flag or flagging tape.
- 3.2.1.11 Samples are kept cool and out of the sun during sampling and placed in a cooler when removed from the field.
- 3.2.1.12 The field map is updated with any notes from the sampling plan.
- 3.2.1.13 Samples are shipped for laboratory testing as outlined in Section 4.

3.2.2. Procedure for BScV-positive Containerized Blueberry and *Vaccinium* Stock

- 3.2.2.1. The sample of 5 plants with the positive ELISA test is traced using sampling records and the farm map.
- 3.2.2.2. It is not always possible to identify individual plants for retesting. The grower may choose to destroy all plants in the area of the positive, or

resample individual plants. Should the grower decide to destroy plants rather than resample, an area of approximately 20 plants surrounding the positive sample are identified for removal.

3.2.2.3. If individual plants are re-sampled, three leaves are collected from each plant to constitute one sample. At least 20 individual plants are re-sampled. The re-sampled area is marked using flags of a different colour than the flags used to mark the original samples. Samples are sent for laboratory testing as described in section 4.

3.2.2.4. A separate report is provided to the grower. This lot will not be included in the report for export eligible plants.

3.3. Field Growing Cranberry/Cranberry Mother Blocks (*Vaccinium macrocarpon*)

Cranberry plants do not typically show any symptoms of BISCv infection, however virus titre can be high in new leaves in summer. Fields destined for cuttings of material for export, will therefore be sampled in July and August as for containerized *Vaccinium*.

3.3.1 Sampling Protocol for Field Growing Cranberry/Cranberry Mother Blocks

3.3.1.1 Depending on the size and growth stage of the cranberry field, a sampling pattern is determined using the solid set sprinkler system to mark the sampling path. When possible, samples of uprights are collected while walking a "Z" pattern through the field. Five hundred individual uprights are collected. When two cultivars are planted in one field and both are to be exported, cultivars will be sampled separately.

3.3.1.2 An upright is collected approximately every 20 paces in large fields, or less in small fields (less than 2 acres) and pooled in a *Ziploc* bag labelled with the farm name, field information and date of collection.

3.3.1.3 For lab testing, one sample consists of one leaf from each of five different uprights. (e.g. 100 uprights = 20 samples).

3.3.1.4 Samples are shipped for laboratory testing as outlined in Section 4.

3.3.2 Procedure for BISCv-positive cranberry

3.3.2.1 If BISCv is detected in a growing field, due to the nature of the cranberry vines and the method used for propagation, the grower will be advised not to export vines from the tested field. A letter will be provided stating that BISCv was detected in the Mother cranberry block.

3.3.2.2 If several fields are tested and some are positive while others are negative, two letters are provided, one listing fields from which vine may be exported, and one listing fields from which vine should not be exported.

4. Delivery of samples to Phyto Diagnostics Ltd.

4.1 Samples collected from the field are kept at 4°C until sampling has been completed for the individual farm. Once sampling has been completed, leaf

samples are shipped to the testing lab (Phyto Diagnostics Ltd. North Saanich, BC).

- 4.2 Prior to shipping, samples are checked to ensure the shipment is complete and that no samples are missing. A packing sheet is prepared with information about each sample (codes, number of samples, any individual plants sampled or bags with fewer than 3 leaves, variety, field, and grower) and this is placed in the box with the samples. This information is also emailed to Phyto Diagnostics Ltd. at the time of shipping.
- 4.3 Samples are either ground-couriered or shipped by air freight from the Vancouver International Airport to Phyto Diagnostics Ltd.

5. Receiving results from Phyto Diagnostics Ltd.

- 5.1 Phyto Diagnostics Ltd. uses a Double Antibody Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA) to detect BISCv.
- 5.2 Once testing is complete, results are sent directly to both the grower and E.S. Cropconsult Ltd. Results are normally received 1-2 weeks after submission.
- 5.3 The laboratory report is checked to ensure there are no discrepancies between the results and the samples that were sent.

6. Reporting of Results

- 6.1 Reports to the grower state the sampling protocol used, dates and lots sampled, number of samples per lot, codes as indicated on field flags, and results of lab tests. Samples of reports for blueberry Mother plants and containerized *Vaccinium* are attached.
- 6.2 If the grower has both positive and negative results, two reports will be provided.
- 6.3 Scorch-negative reports will describe lots which may be exported to the USA.
- 6.4 Scorch-positive reports will include re-sampling records and/or recommendations for removal of the infected plant(s) and state that the affected lot(s) may not be exported to the USA.

7. Confidentiality

- 7.1 Grower records are confidential and are kept with field maps, notes, reports, personnel records, spray records and lab results in a file designated for each farm and year. Grower records will be retained on file for a minimum of 7 years.
- 7.2 Entire files will be made available to CFIA as per the "Prior Sampling Authorization Permission Form".

8. References

Wegener,L.A; Martin, R.R; Bernardy,M; Macdonald,L and Z.K Punja. 2006. Epidemiology and strain identification of blueberry scorch virus on highbush blueberry in British Columbia. Canadian Journal of Pathology. 25:114