Project Report – Year 1

Determining the effect of on-farm management decisions on small fruit viruses in Kings County

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Introduction

The Nova Scotia small fruit industry has faced many challenges in recent years, one of which was the outbreak of strawberry viruses nearly a decade ago. The combined value of strawberry fruit growing and nursery industries at the time was estimated at $19 million. Many growers pulled out fields or left the industry completely as a result of the drastic actions needed to safeguard the industry. Substantial federal and provincial funds were contributed to support the industry recovery at the time, including a program spearheaded by Horticulture Nova Scotia and Perennia Food & Agriculture Inc. to complete annual virus testing of newly planted fruiting strawberry fields, nursery fields and aphid monitoring on strawberry farms in Nova Scotia.

It is understood that the major sources of virus in small fruits is infected plant material, and plant-to-plant spread of virus through feeding by vectors such as strawberry (*Chaetosiphon fragaefolii* [Cockerell]) and large raspberry aphid (*Amphorophora agathonica* Hottes). While a drastic reduction in virus infection rates were observed at the beginning of the program - likely due to many farmers destroying infected fields - a steady increase in rates has been observed since that time. The management of strawberry and raspberry aphids and thus viruses will remain part of growers' annual farm plans for years to come.

The recent uptick in virus infection rates in the province is concerning, and points to management practises as a likely source. Virus pressure in a field can be influenced by several factors such as: age of field, distance of field from older plantings, aphid monitoring and management on farm, and wild plants at field edges harbouring virus and vectors. It would be prudent to further study how management decisions correlate with virus infection levels on farm. One complication, though, is the fact that many virus disease symptoms are easily mistaken for abiotic or biotic stress, or do not appear unless mixed infections are present. This means that rogueing plants by eye remains practically impossible, and the preferred method of diagnosing virus infections is through molecular lab techniques such as polymerase chain reaction (PCR) tests.

Presently, growers need to send plants out of province if they wish to have them tested using molecular approaches. The main small fruit viruses of concern in the province are *Strawberry Mild Yellow Edge Virus* (SMYEV), *Strawberry Mottle Virus* (SMoV), *Rubus Yellow Net Virus* (RYNV) and *Raspberry Leaf Mottle Virus* (RLMV), which can all be screened using PCR tests. In addition to collecting detailed data on farm management practises, this project will also conduct virus screening through Perennia's Plant Health Lab. Many of the resources and equipment accumulated by the lab thus far are directly applicable to small fruit virus testing. We propose using the lab's pre-existing infrastructure and expertise to extend our services into small fruit viruses, providing local growers with an in-province tool for managing virus on farm.

Materials and Methods

Sampling Sites

To determine the effect of on-farm management decisions on small fruit viruses we tested strawberry and raspberry material for their associated viruses. Four fruit producers were chosen that grow strawberries (4 fruiting fields), and two growers were chosen that grow both strawberry and raspberry plants for nursery plant production (4 nursery fields), for a total of 6 growers and 8 sampling sites in Kings County. All the fields used were planted in the spring of 2021. Sampling was repeated across the season to determine when virus titer had increased to the degree that is detectable and fields are being tracked over two sampling years.

Twenty equally spaced groupings of plants were tested for virus in a grid-like pattern across each field starting in the South-west field corner (Figure 1). Plants were marked with nursery tags and flags to ensure they could be resampled across years (all fields) and timepoints (nursery fields). Each grouping consisted of five mother plants for strawberries or five adjacent crowns for raspberries. When sampling for virus testing, one leaf was collected from each of the five plants in the grouping to form a five-leaf composite sample. A five-leaf composite sample was collected from each grouping during virus sampling for a total of twenty composite samples per field. Virus samples were collected in July and September from each of the nursery strawberry and raspberry fields, while fruiting strawberry field samples were only collected in September.

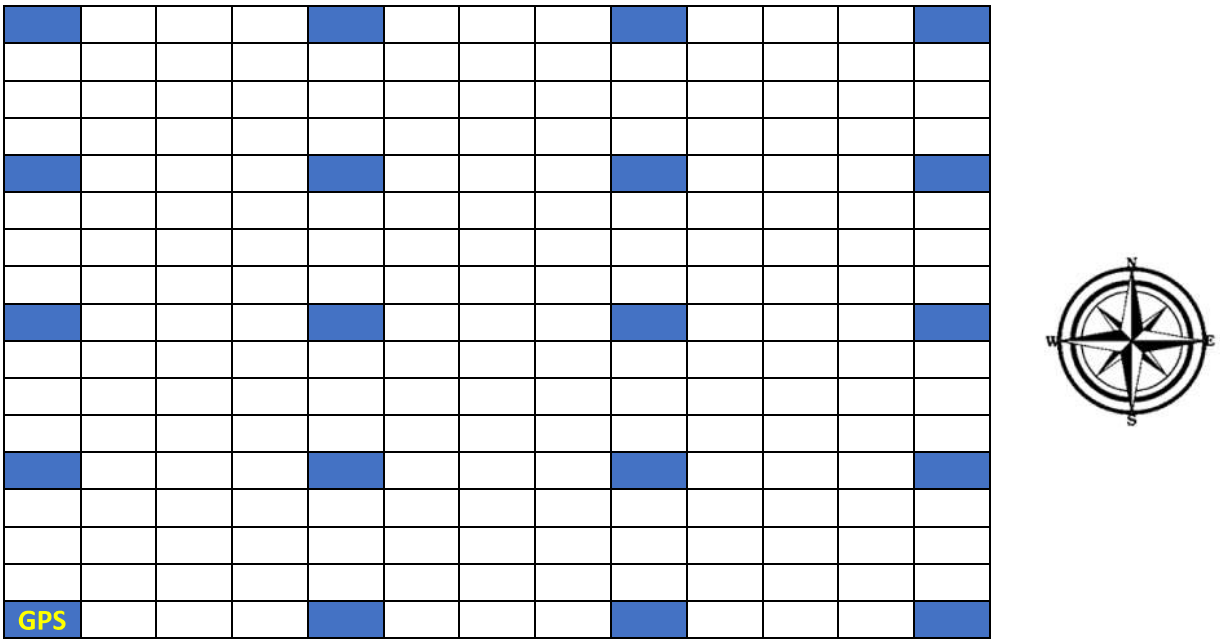

Figure 1 *Virus sampling strategy. Each blue square identifies a series of five adjacent mother plants or crowns for strawberry and raspberry plants, respectively.*

Wild Host Virus Screening

Virus testing was also conducted on wild hosts of strawberry and raspberry viruses and vectors. Wild *Fragaria*, *Rubus*, *Potentilla*, and *Rosa spp*. were sampled to determine virus levels in the field edges to a distance of approximately twenty feet into surrounding brush/forest. One to fifteen samples were collected at each site throughout September with each sample consisting of a composite leaf sample collected from plants of a single wild host species in the field edge. Images and GPS locations were also collected for each sample. Each wild host sample was tested for SMYEV, SMoV, RLMV and RYNV using the same protocol as for the cultivated strawberry and raspberry samples. Additional testing was also performed with internal controls to ensure sample integrity.

Virus Screening Protocol

Once strawberry or raspberry leaves were collected, they were processed for long-term storage at -80°C as soon as possible. The screening protocol, based on Rowhani et al. (2000), consisted of macerating strawberry or raspberry petiole tissue in extraction buffers and amplifying RNA and DNA material from small fruit viruses using an RT-PCR/PCR approach, followed by visualization of amplified products using gel electrophoresis. All 160 strawberry composite samples were tested for SMYEV and SMoV, and all 80 raspberry composite samples were tested for RYNV and RLMV. In cases where faint results were generated using gel electrophoresis, samples were re-tested using a different set of RT-PCR/PCR primers to confirm infection.

To develop our small fruit virus testing protocol, forty raspberry and twenty strawberry split leaf composite samples were collected from fields with known small fruit virus infections and sent to Phyto Diagnostics to validate our methodology and confirm positive controls. Our results for SMYEV, SMoV, and RLMV were consistent with Phyto Diagnostics, but none of the raspberry samples sent tested positive for RYNV. Outside the scope of this project, considerable effort was then put into to developing the RYNV PCR protocol and finding a positive control. Development of the RYNV PCR test required the addition of a DNA digestion step (removes DNA from samples) to the standard PCR protocol (Poudel et al. 2013). The DNA digestion step was added because in some cases RYNV DNA has integrated into raspberry genomic DNA, which can produce false RYNV positives (Diaz-Lara et al. 2020). By performing the DNA digestion step, the raspberry genomic DNA in the sample is broken down ensuring that you are only amplifying RYNV RNA from the sample during PCR. An additional four raspberry split leaf composite samples were sent to both Phyto Diagnostics and University of Guelph Laboratory Services to validate the modified protocol. Once the RYNV protocol was validated a positive control was found from raspberry material that had previously been confirmed to be infected with RYNV by Phyto Diagnostics.

Production Field Survey

To determine the effect of production practices on small fruit viruses detailed Information related to on-farm land management practices such as placement, age, and rotation of fields as well as aphid and field edge management was collected for each field site. An online based survey was sent to participating growers in December of 2021 to collect management information. Results from the survey are being

correlated with virus testing results for each site, to determine what effect different management practices had on small fruit viruses.

Results and Discussion

Fruiting and nursery field virus screening

To determine the effect of on-farm management on small fruit viruses four fruiting strawberry fields, and two nursery strawberry fields were tested for SMYEV and SMoV, and two nursery raspberry fields were tested for RYNV and RLMV. Of the four fruiting strawberry field sites, site 1 and 2 were positive for both viruses, site 3 was positive SMYEV, and site 4 was negative for both viruses (Table 1). Site 1 had the highest rate of infection and was the only field with a sample infected with both SMYEV and SMoV (Table 1). Site 2 and 3 both had low infections rates, and although site 2 had both viruses present, individual samples were only infected with a single virus (Table 1). Co-infections of SMYEV and SMoV are of special concern because strawberry plants show viral disease symptoms when infected with two or more viruses (Martin and Tzanetakis 2015). Across all the fruiting strawberry field samples, SMYEV had the highest rate of infection and there was a low incidence SMoV infected samples and SMYEV and SMoV co-infected samples (Figure 2). The overall fruiting strawberry field virus results are comparable to what has previously been found from provincial virus surveys (Haverstock et al. 2021).

Table 1. Virus screening results for fruiting strawberry fields in 2021. Samples were collected from all field sites in September 2021.

Site	SMYEV	SMoV	SMYEV and SMoV	No. of samples
Site 1	14	0	1	20
Site 2	2	3	0	20
Site 3	3	0	0	20
Site 4	0	0	0	20
Total	20	3	1	80
%*	25	3.75	1.25	

*Percent of total samples infected with strawberry viruses.

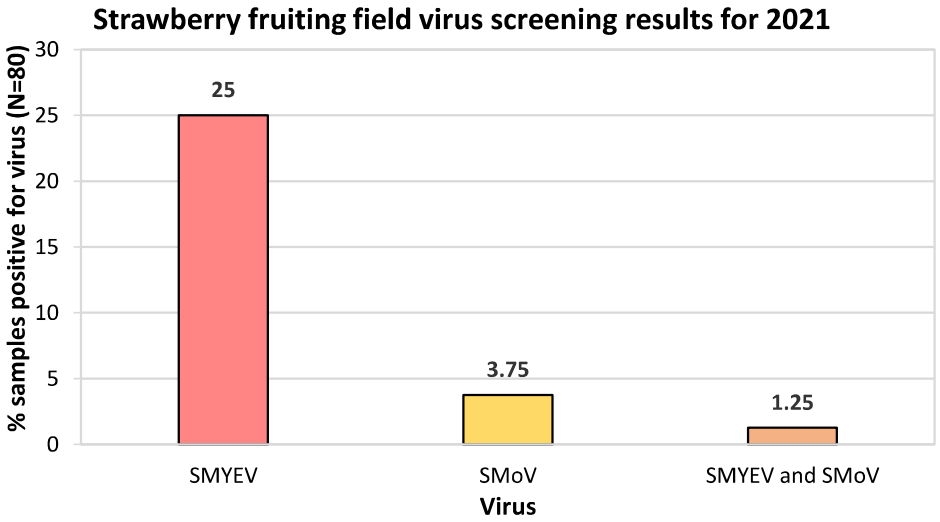

Figure 2 *Virus infection rates for 2021 strawberry fruiting field samples.*

Nursery strawberry and raspberry fields were tested over two time point (once in July and again in September), to see if virus infections were detectable earlier in the season and to track the spread of infections within a season. Strawberry nursery field site 1 and 2 tested negative for both SMYEV and SMoV at both time points (Table 2). Raspberry nursery field site 1 and 2 both had samples that tested positive for RLMV, but no samples tested positive for RYNV (Table 3). There was also no additional spread of viral infections between timepoints at either raspberry nursery field sites (Table 3). The low rate of virus infection and spread is expected within nursery fields as nursery production uses intensive management practices to ensure that plants are free of viruses and other pathogens and pests (Martin and Tzanetakis 2015). Additionally, based on our results, raspberries can be sampled effectively for virus in July. Previous testing has generally been done no earlier then August.

Table 2 Virus screening results from nursery strawberry fields in 2021. Samples for time point 1 were collected in July 2021, and in September 2021 for time point 2.

Time Point 1				
Site	SMYEV	SMoV	SMYEV and SMoV	No. of samples
Site 1	0	0	0	20
Site 2	0	0	0	20
Total	0	0	0	40
Time Point 2				
Site	SMYEV	SMoV	SMYEV and SMoV	No. of Samples
Site 1	0	0	0	20
Site 2	0	0	0	20
Total	0	0	0	40

Table 3 Virus screening results from nursery raspberry fields in 2021. Samples for time point 1 were collected in July 2021, and in September 2021 for time point 2.

Time Point 1				
Site	RYNV	RLMV	RYNV and RLMV	No. of samples
Site 1	0	2	0	20
Site 2	0	1	0	20
Total	0	3	0	40
Time Point 2				
Site	RYNV	RLMV	RYNV and RLMV	No. of samples
Site 1	0	2	0	16
Site 2	0	1	0	20
Total	0	3	0	36

Wild Host Virus Screening

Wild host samples were collected in September 2021 to determine if small fruit viruses were present in the edge of small fruit crop fields. In total 66 wild host samples were collected across all eight sites for virus testing (Figure 3). Samples were collected from wild *Fragaria*, *Rubus*, *Potentilla*, and *Rosa spp*., with the majority of samples being from *Rubus* spp. (Figure 3). Virus testing for wild host samples is still ongoing and results will be given in the final report.

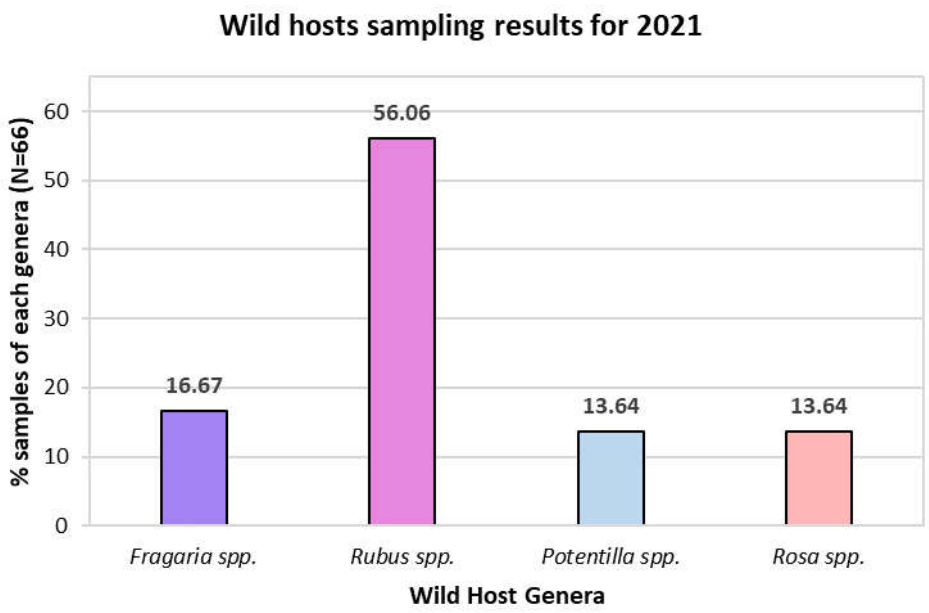

Figure 3 Wild host sampling results for 2021. Wild host samples were collected in September of 2021.

Production Survey

To determine the effect of production practices on small fruit viruses detailed Information related to on-farm land management practices was collected from participating producers. Survey results are still being collected and the effect of management decisions will not become fully apparent until the second year of sampling. Further data collection is still needed to elucidate the effects of management practices on small fruit viruses.

Conclusions

Increases in virus infection rates in the province are concerning, and management practises are a possible cause. In 2021, eight sites were screened for viruses to determine the effect of management practices on small fruit viruses. Virus screening from 2021 will provide a baseline to compare the management decisions used at each site to determine best practices. Wild host sample were also collected for virus testing, which is on going. A survey to collect production information have also been sent out to participating growers and in the process of being processed. Virus testing protocol for SMYEV, SMoV, RLMV, and RYNV has now been developed and validated in the lab, and will be available as service line at a later date. Further data collection, planned for next season, is still needed to determine the effects of management practices on small fruit viruses.

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