Final Report

Aphid Monitoring and Virus Testing Program

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Background

In the 2012 growing season, the Nova Scotia strawberry commercial fruit and nursery plant industries were significantly impacted by plant decline associated with plant-borne viruses (Martin and Tzanetakis, 2013). The commercial fruit industry was valued at $10 million at the time, and the nursery plant industry at $9 million. Some growers made the difficult decision to cease strawberry farming activities altogether, while others decided to forgo production that year and to remove fields, resulting in substantial financial losses in 2012-2013. These drastic measures significantly altered the Nova Scotia strawberry farming landscape in the ensuing years. The Nova Scotia and federal governments provided growers with financial assistance and supported collaborative research efforts to mitigate this emerging threat. One such effort was a series of aphid monitoring and virus testing programs led by Horticulture Nova Scotia and Perennia. The critical actions taken in 2013 are believed to be responsible for a sequential decrease in virus numbers in the years that followed.

The Nova Scotia strawberry industry has been viewed as a leader in virus and vector management, as viruses continue to have a significant effect on strawberry production across Canada. In Nova Scotia, virus testing has largely focused on identifying two viruses of strawberries, *Strawberry Mild Yellow Edge Virus* (SMYEV) and *Strawberry Mottle Virus* (SMoV). These two viruses in combination are believed to have caused the 2012-2013 virus epidemic in Nova Scotia, though other provinces have detected upwards of five different viruses (Martin and Tzanetakis, 2013). Additionally, a new strawberry virus, *Strawberry Polerovirus 1* (SPV-1), was first identified in eastern Canada from plants tested as part of a research project by Xiang *et al.* (2015). Although SPV-1 does not appear to cause visual symptoms or disease by itself, it is suspected to aid in the transmission of SMYEV (Thekke-Veetil and Tzanetakis, 2016.), and was randomly screened as part of this program.

Two years following the identification of strawberry viruses (2012), bramble producers, including raspberries and blackberries, noted a decline in production. Preliminary testing conducted by Perennia in 2014 identified two viruses, *Rubus Yellow Net Virus* (RYNV) and *Raspberry Leaf Mottle Virus* (RLMV) in suspect raspberry and blackberry plantings. An initial survey of raspberry and blackberry plantings in 2015 and 2016 revealed that the viruses were more widespread than initially perceived. Visual identification of these viruses is exceptionally challenging and verification by the molecular testing method reverse transcription polymerase chain reaction (RT-PCR) is required for accurate identification. To better understand this virus-complex and to provide the industry with informed recommendations on management of the primary vector large raspberry aphid (*Amphorophora agathonica*), virus testing was initiated in 2017 in new plantings and a final comprehensive industry survey was conducted in 2020.

Recent virus numbers in commercial strawberry fruit fields (2017 - 2020) have shown an increase in both SMYEV and SMoV. This could be caused by several factors including but not limited to significant changes in chemical control options for the virus-vectoring strawberry aphid (*Chaetosiphon fragaefolii* [Cockerell]); annual aphid population dynamics; and/or grower field remediation/rotational practices. The Nova Scotia strawberry industry has shown great signs of rebounding, but continued vigilance is required to safeguard the industry in the long term.

Project Objectives

To allow for continued virus-vector monitoring and virus testing in berry crops, the Nova Scotia Department of Agriculture supported a four-year funding program for the 2017-2020 growing seasons. The main objectives of the program were to: (1) aid in the management of strawberry (*i.e.* strawberry aphid, *Chaetosiphon spp.)* and bramble virus insect-vectors (*i.e.* large raspberry aphid, *Amphorophora agathonica*); and, (2) ensure nursery stock produced in the province meets the recovery strategy requirements for commercial fruit growers, and the industry is prepared for participation in the National Clean Plant Program.

To meet these objectives, the following activities were completed:

1. Monitor for strawberry and large raspberry aphid on representative farms across the province and provide the monitoring results to cooperating growers and the industry as a whole on a timely basis for optimum vector management.
2. Conduct a late summer virus survey of all newly planted strawberry and raspberry fields to evaluate the progress of virus management efforts in the province.
3. Execute the 'virus testing protocol' as outlined in the "Guidelines for growing and inspecting strawberry plants in Nova Scotia" and "Guidelines for growing and inspecting raspberry plants in Nova Scotia".
4. Refine vector management, particularly given the impending loss of an important management tool (*imidacloprid*) from the grower toolbox.

Sampling and Monitoring Protocols

Strawberry Aphid Monitoring

To provide a complete view of the strawberry aphid population, three aphid monitoring methods were employed on cooperating strawberry farms distributed around the province. The monitoring methods were as follows:

1. Early spring leaf monitoring for aphid 'egg' counts (Figure 1A): 30 overwintered horizontal leaves were collected from monitoring plots and examined for aphid egg counts as an early season indicator of overwintered strawberry aphid populations in the study plots.
2. Bud leaf monitoring for 'wingless' and 'winged' vector numbers (Figure 1B): 60 immature 'bud leaves' were collected from each monitoring plot on a weekly basis throughout the growing season.
3. Yellow pan trap monitoring for 'winged' vectors (Figure 1C): 6 yellow pan traps were located in select monitoring plots and examined for winged vector numbers, by species, throughout the growing season.

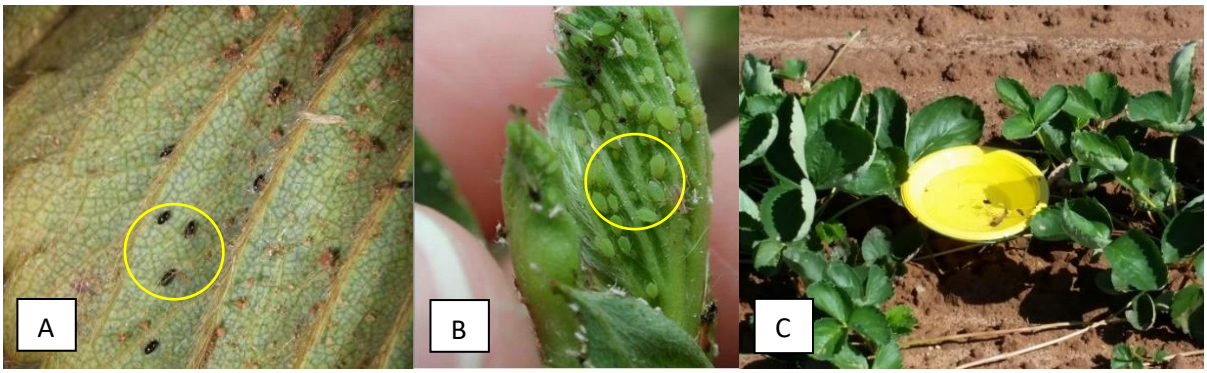

Figure 1. A) Aphid eggs on the underside of an old strawberry leaf (photo courtesy of Dr. Debra Moreau, AAFC, Kentville, NS). B) strawberry bud leaves used in aphid sampling; c) Yellow pan traps used for winged aphid scouting.

While the focus of this report will be presenting the findings from the four-year funding period (2017-2020), where appropriate summarized data from all sampling years (2013-2020) will be presented for context and completeness.

Strawberry Virus Sampling

Leaf samples were collected for virus testing in late summer/early fall from all newly planted commercial fruiting strawberry fields with greater than 1000 plants that would be carried over to the next fruiting year. In addition to sampling newly planted fields, a select few second- and third year (*i.e.*, "carried-over") fields were randomly selected for virus testing.

The sampling methodology employed was designed to identify the level of infection of SMYEV and SMoV in fruiting strawberry fields. The first fully expanded leaf in either a mother or rooted daughter plant was sampled for analysis. Twenty composite samples of 3-trifoliate leaves were collected from individual fields/blocks following a zigzag pattern (Figure 2). Each sample was analyzed for SMYEV using enzyme-linked immunosorbent assay (ELISA). Two 10-leaf composite samples (representing 1 leaf from each of the 20 bags of 3 leaf samples) were used for reverse transcription polymerase chain reaction (RT-PCR) testing of SMoV.

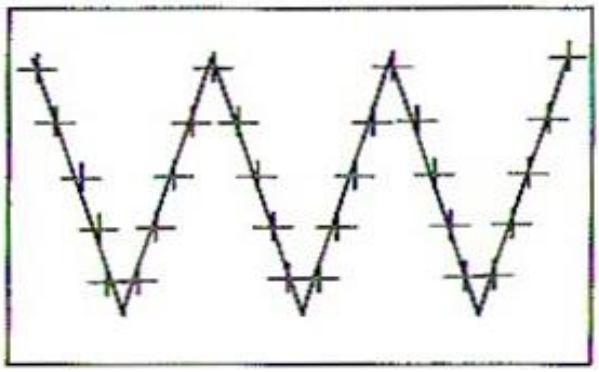

Figure 2. Zigzag pattern employed during virus sampling.

Raspberry Virus Sampling

Due to the perennial nature of raspberry and blackberry production systems, a different virus sampling approach was taken for *Rubus* viruses. Two scenarios would result in virus sampling: 1) new plantings and 2) identification of suspect plants. New plantings were randomly sampled following the procedures described above, while suspect plants were labelled, photo catalogued and sampled.

In the first 3 years of the program, raspberry leaf samples were collected in late summer/early fall from all known newly planted commercial fruiting fields. In the final year of the program (2020), samples were collected from all known commercial fruiting raspberry fields that would be carried over to the next year, to ensure that over the course of the program, all plantings were sampled at least once. Individual fields/blocks (with more than 1000 plants) were randomly sampled so as to have 6 bags with 5-trifoliate leaves each to test for RYNV and RLMV by RT-PCR. Non-senescing leaves were collected for testing approximately one third from the top of the plant on a floricane (if pruned, samples collected from a primocane) (Figure 3).

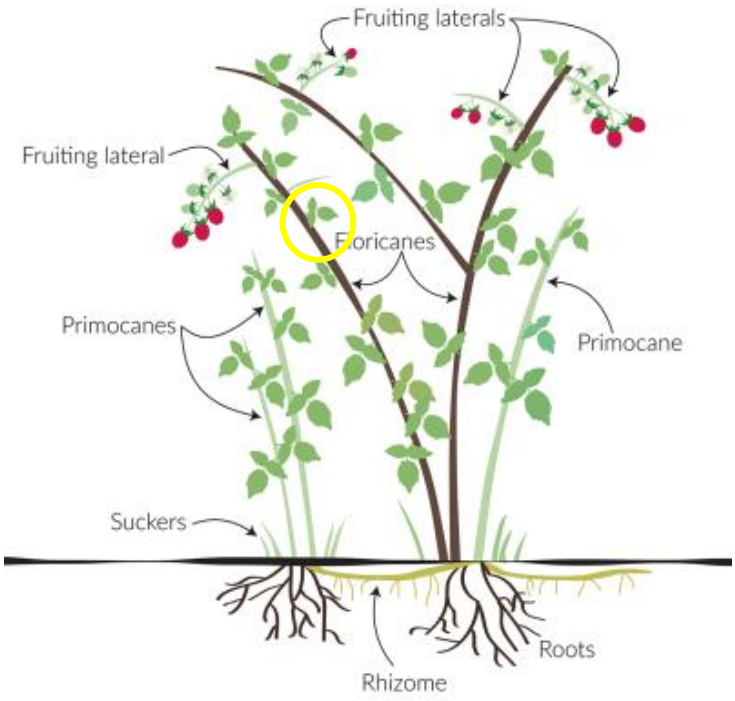

Figure 3. Raspberry plant structure indicating which leaves were collected for virus testing (image credit to: https://extension.umn.edu/fruit/growing-raspberries-home-garden#what-are-primocanes-and-floricanes%3F-331660Strawberry and Raspberry Nursery Virus Sampling).

Nursery Virus Sampling

Leaves from strawberry, raspberry and blackberry nursery stock were collected and submitted by individual nurseries according to the 'virus testing protocols' as outlined in the "Guidelines for growing and inspecting strawberry plants in Nova Scotia" and "Guidelines for growing and inspecting raspberry plants in Nova Scotia" one month prior to plant harvest each year. All samples were shipped to and analyzed for viruses by Phyto Diagnostics Ltd. in British Columbia.

Results and Discussion

Strawberry Aphid Monitoring

Throughout the course of this program, aphid monitoring plots were established starting in the last week of April and were completely deployed by mid-May, depending on growing region. The plots were monitored until approximately the last week of October, but the exact end date was site-dependant. The total number of weeks on average that plots were monitored in any given program year was 25-27 weeks. In years where a monitoring field was removed after harvest, the plot was relocated to the nearest field for the remainder of the season. Locations for aphid monitoring sites were chosen based on geographic location, with the goal of capturing all strawberry producing regions in the province, with most farms concentrated to the Annapolis Valley (Table 1).

Table 1. Number of farms participating in the aphid monitoring program, broken down by region (2014-2020).

Region	*2014*	*2015*	*2016*	*2017*	*2018*	*2019*	*2020*
Western-Valley region	12	12	11	9	10	8	6
Central-Eastern region	9	8	9	8	7	4	5
Provincial nursery fields	6	6	6	6	5	5	6
Nova Scotia total	27	26	26	23	22	17	17

A graphical summary of weekly strawberry aphid counts divided into the Central-Eastern and Western-Valley regions is provided in Figure 4. The earliest date in a season that aphids were detected was 25 April 2016, and the earliest detection of winged aphids was 30 May 2016, both in the Western-Valley region.

The latest day in the season that aphids were detected in a sample was 29 October 2018 in the Central-Eastern region, however, this coincided with completion of annual monitoring, and therefore aphids could have remained unmonitored in fields in both regions after this time. In both regions, a population peak was generally observed around the 12th week of monitoring (mid-July). This peak period spanned several sampling weeks, and aphids were still being detected much later in the fall, which highlights the need for continued monitoring and management of aphids throughout the growing season.

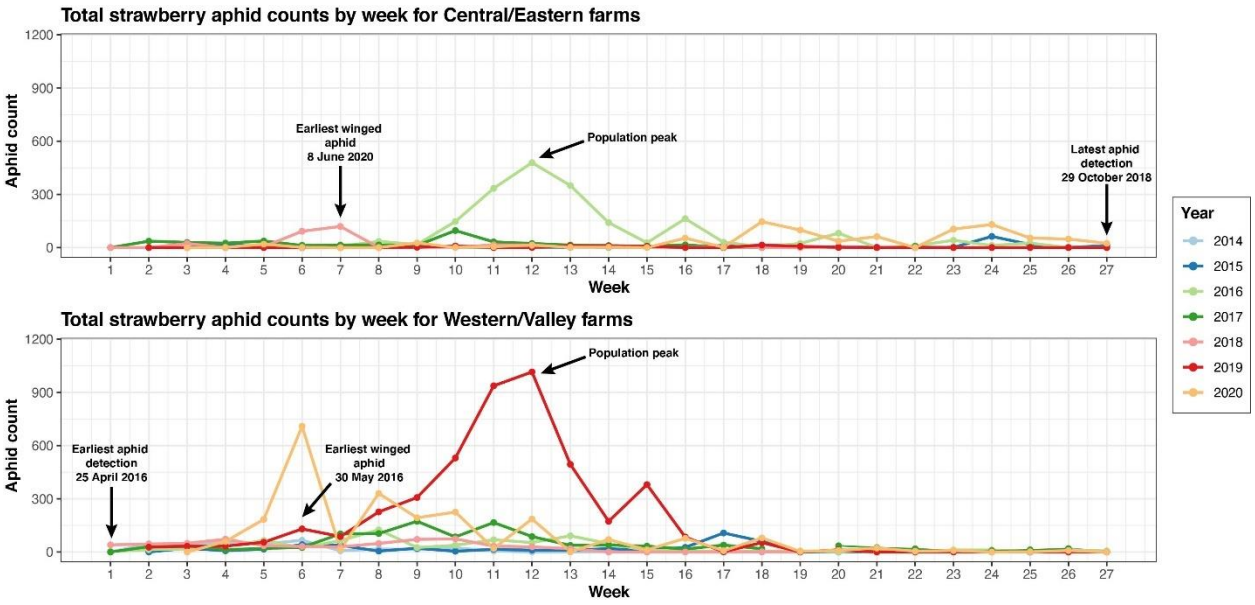

Figure 4. A graphical representation of weekly total strawberry aphid counts broken down by region (2014-2020).

When total annual aphid counts were analyzed across years (Figure 5), substantial variation was observed across years, and regions. These drastic fluctuations, especially those observed in 2018 to present in the Western-Valley region, suggest that strawberry aphid management continues to be an important management consideration for growers across the province, regardless of population numbers in the proceeding year.

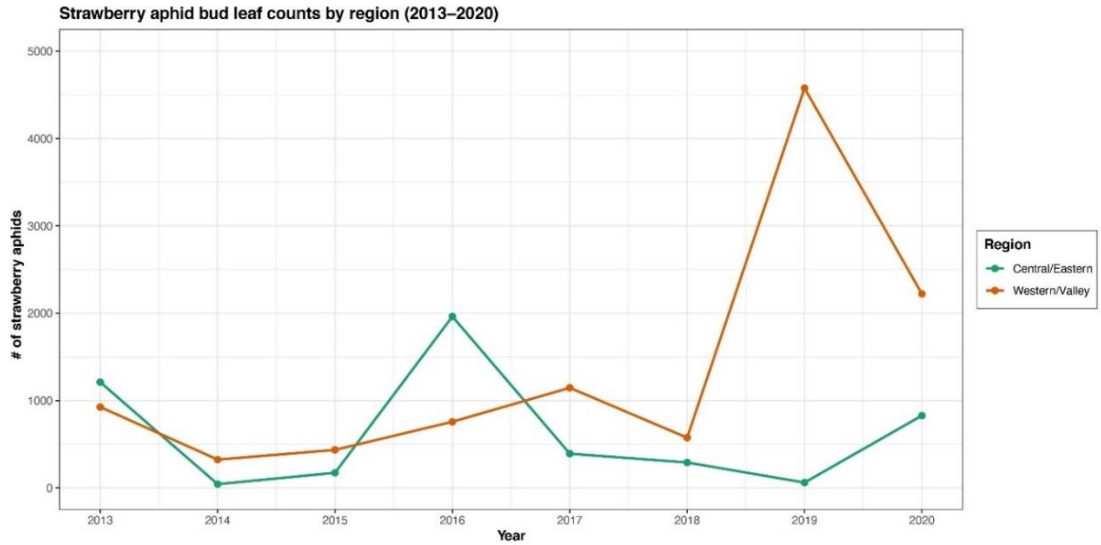

Figure 5. Annual total strawberry aphid counts broken down by region (2013-2020).

A detailed breakdown of annual sampling trends can be found in Figure 6, which details the wingless and winged strawberry aphid counts for both regions in 2020. Aphid counts again varied throughout the growing season, with wingless counts peaking earlier in the growing season for the Western-Valley region, and later for Central-Eastern sites. The 2020 data suggest that in this year, timing of aphid control strategies would have varied based on region, and that Central-Eastern sites were still detecting substantial wingless aphid numbers well into the fall. The large counts and fluctuations in numbers observed across 2020 can be attributed to farms with heavy population levels, and subsequent management. It should also be noted that the method of monitoring winged aphids did not detect substantial populations, and therefore it is not recommended that management be based on pan traps alone. It is recommended that regular bud leaf sampling be used to monitor populations on farm and to make informed management decisions.

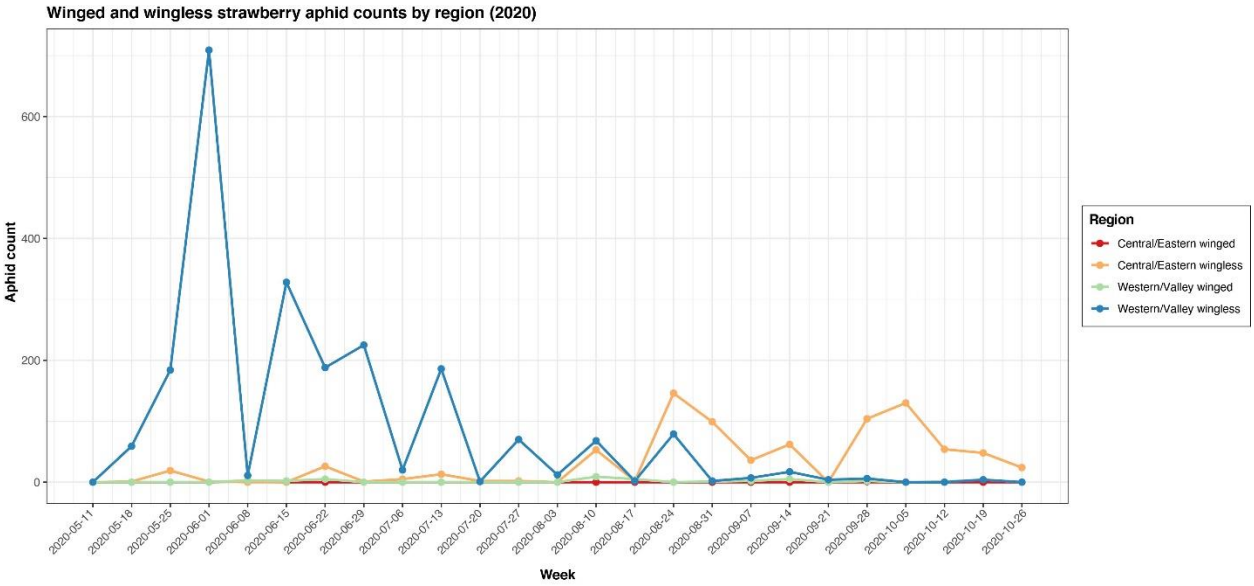

Figure 6. Weekly winged and wingless strawberry aphid counts broken down by region (2020).

Strawberry Aphid Egg Counts

At plot set-up in April and May and for two weeks following, samples of 30 overwintered leaves were collected from each monitoring site to perform an egg count, with the exception of 2020 when COVID-19 restrictions limited sampling activities early in the season (Table 2). The variability noted in egg counts between years was likely due to the uneven distribution of eggs within a field. Strawberry aphids are colonizers and are typically found in defined areas, not spread out across a field (personal communication Dr. D. Moreau, AAFC, 2016). When aphid eggs were detected in leaf samples, samples were incubated for 1-2 weeks until hatching. Samples were then analyzed under a high magnification microscope, and if identified as strawberry aphid, respective growers were advised of an aphid control strategy.

Table 2. Egg counts from regions across Nova Scotia over the monitoring period, 2014-2020.

Region	2014	2015	2016	2017	2018	2019	2020
Western-Valley region	29	41	257	1	616	198	*NA**
Central-Eastern region	0	45	44	0	176	0	*NA*
Nova Scotia total	29	86	301	1	792	198	*NA*

*NA** = COVID-19 inhibited the ability to collect egg samples due to the timing of the sample collection and provincial lock down

Several methods of aphid monitoring were assessed during this monitoring program (*i.e.*, pan traps, bud leaf counts, and egg counts), with varying levels of success. Overall, it would appear that aphid populations persist from year to year and warrant active management, although this can vary between site and year due to a number of factors. It is especially important that growers monitor aphid populations on their own farms, and ideally across different fields. While all monitoring methods proved to be informative from a research approach, it is recommended that growers collect several bud leaf samples from each field in production on their farm to track aphids. Growers may wish to sample fields on a weekly basis, especially around historical periods of population peaks in their growing region or employ a crop scout. Monitoring of aphids will continue to be an important part of strawberry and nursery growers' annual farm plans, especially in light of our virus screening results presented below.

Strawberry Virus Testing

The second major activity of this monitoring program was to execute an annual virus testing program in all newly planted strawberry fields and select carried-over fields. The number of blocks sampled across the province varied across years (92 - 155), but this was largely due to an enormous sampling effort at the onset of widespread strawberry decline in the province (*i.e.*, 2013-2014). Of the two testing approaches, ELISA is less costly, and therefore more subsamples were collected, which is reflected by the higher sample numbers across all years (Table 3). In order to assess virus status in carried-over fields, in 2017-2020 several two- and three-year old fields were also indexed for virus. Figure 7 breaks down sample numbers by year and region, and confirms that regions were consistently sampled each year, with the majority taken from the Valley and Central regions.

Table 3. Virus testing sample numbers across all newly planted and a subset of carried-over fields (2017-2020).

	Total Blocks Sampled		*ELISA Samples*		*RT-PCR Samples*	
Sampling Year	**Newly Planted**	**Carried-Over**	**Newly Planted**	**Carried-Over**	**Newly Planted**	**Carried-Over**
2017	97	10	1811	172	185	19
2018	87	5	1650	100	161	10
2019	80	16	1474	299	152	30
2020	89	28	1459	454	152	47

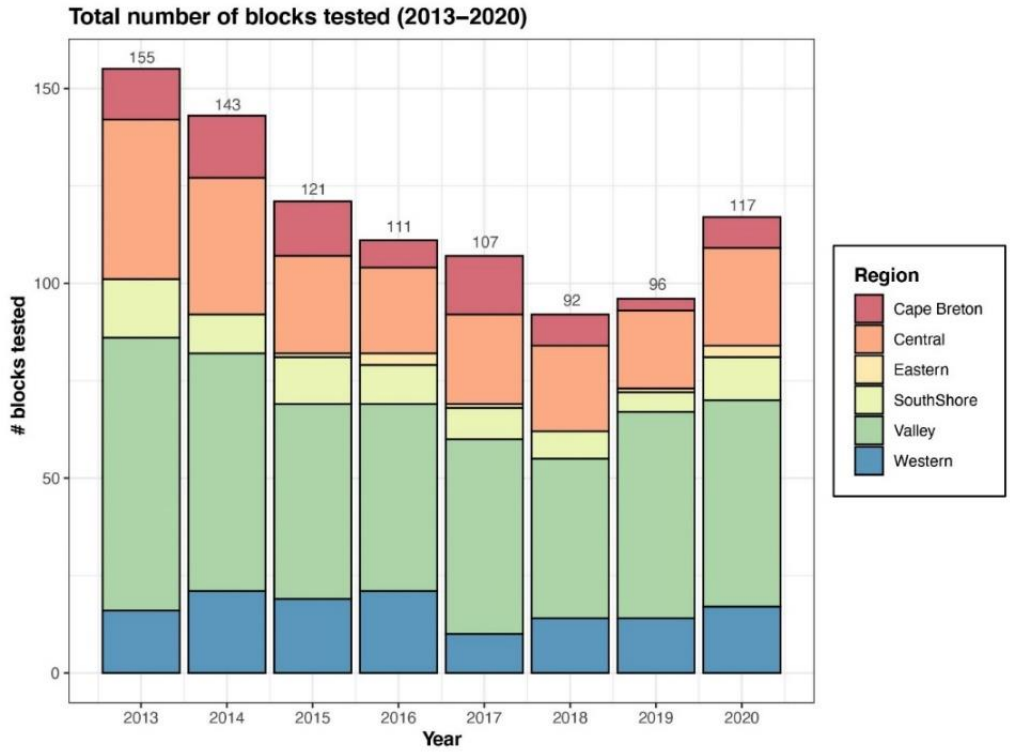

Figure 7. A breakdown of total number of blocks sampled by year and region (2013-2020).

This program has allowed for monitoring of virus levels and the recovery progress in Nova Scotia following substantial losses nearly a decade ago. Over the eight years of testing, 942 blocks were sampled, and 16,017 ELISA and 1,639 RT-PCR tests were completed. As more farms transition to holding fruiting fields over for a second year of picking, it was important to examine these fields as a possible source of inoculum. In the final 4 years of the program, 5-28 carried-over blocks were sampled, totalling 100 - 454 and 10 - 47 SMYEV and SMoV tests, respectively (Table 3). This enormous effort by Perennia and NSDA staff provides a cross section of virus incidence across newly planted and carried-over fields across years that has proven a crucial resource for growers.

The breakdown of virus incidence across years reveals several interesting trends. Testing during the strawberry decline outbreak showed elevated numbers of both SMYEV and SMoV (2013). After aggressive field remediation, it would appear that virus inoculum declined in subsequent years, but viruses still persisted (Figure 8). In the last four years of this program, though, virus numbers have started to climb again, especially SMoV. In the final year of this program, infection rates were 6.6% and 17.1% in newly planted fields for SMYEV and SMoV, respectively. A more worrying trend, though, is the number of blocks testing positive for both viruses, as both viruses must be present for plants to exhibit symptoms of decline. While a drastic reduction was observed after the first year of this program, the percentage of blocks positive for both viruses has increased in recent years to a rate of 15.7% in 2020.

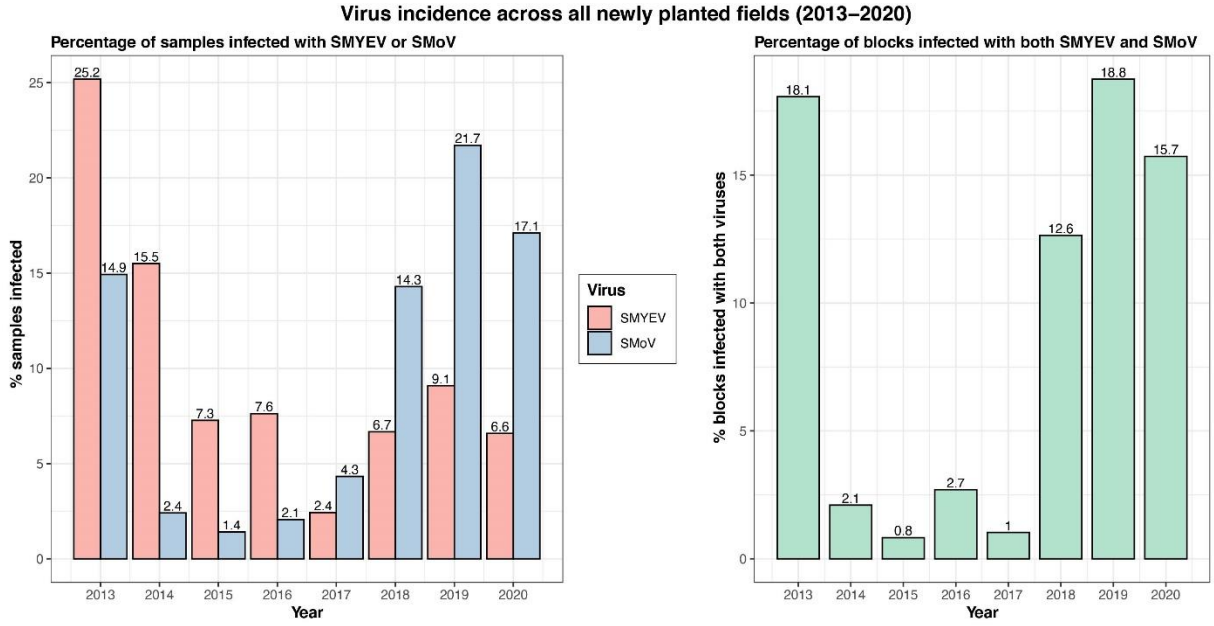

Figure 8. A breakdown of virus incidence across newly planted fields (2013-2020). The left plot shows percentage of samples infected with SMYEV (red bars) and SMoV (blue bars). The right panel shows of all blocks sampled, the percentage of which were infected with both viruses (green bars).

It should be noted that across farms and blocks substantial variation in infection rates has been observed, which is not evident by pooling testing results. Some farms' newly planted fields had very low to zero virus incidence, while others were more variable. Also, given that SMoV samples are pooled for testing to reduce cost, the SMoV incidence reported here may be slightly inflated, but any amount of virus detected in a field should be a concern for growers. Several new fields with high infection rates were observed close to second- or third-year fields with virus infections, which act as a source of inoculum. Although carried-over fields were only tested in the last four years of the program, and the number of blocks tested varied, a very high incidence of infection was reported across all years (Figure 9). To explore the relationship between infection rates and management practises, incidence across years was explored for a case farm (Figure 10), and a comparison was made between farms that did and did not have a monitoring program (Figure 11).

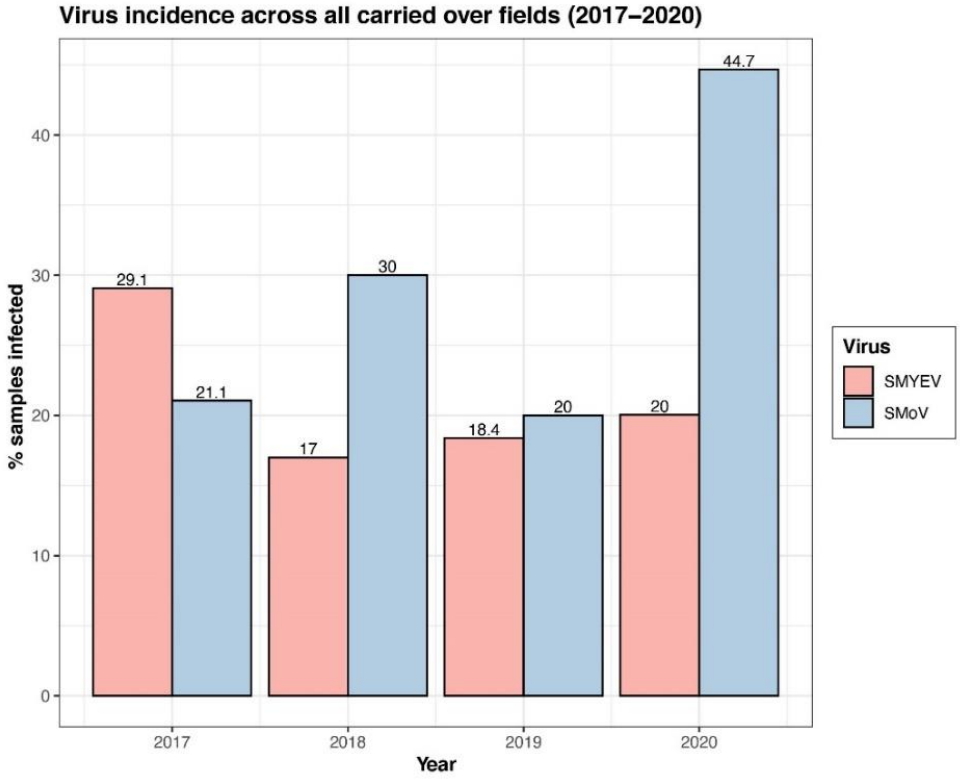

Figure 9. A breakdown of virus incidence across carried over fields, with the percentage of samples infected with SMYEV (red bars) and SMoV (blue bars) (2017-2020).

At the beginning of this program, infection rates on the case farm for the two viruses were initially low, but in recent years there was a dramatic increase (Figure 10). While the data are partially skewed due to the testing of carried-over fields in the last four years of the project (2017-2020), a more alarming trend was observed in the final year of testing. In 2020 five newly planted fields were tested on the case farm and the per block infection rate for SMYEV varied from 0-90%, while all five blocks were positive for SMoV. Of the three carried-over fields tested, the per block infection rate for SMYEV varied from 15-40%, while all blocks were positive for SMoV. These results suggest that for 7/8 blocks tested on the case farm, the virus complex of SMYEV and SMoV was present, and strawberry decline could possibly occur. It is impossible to prove with this data that carrying over fields is increasing virus infection rates, but this case farm example and the carried-over field infection rates (Figure 9) highlight the potential risk of doing so, and the need for on-farm monitoring of viruses and vectors.

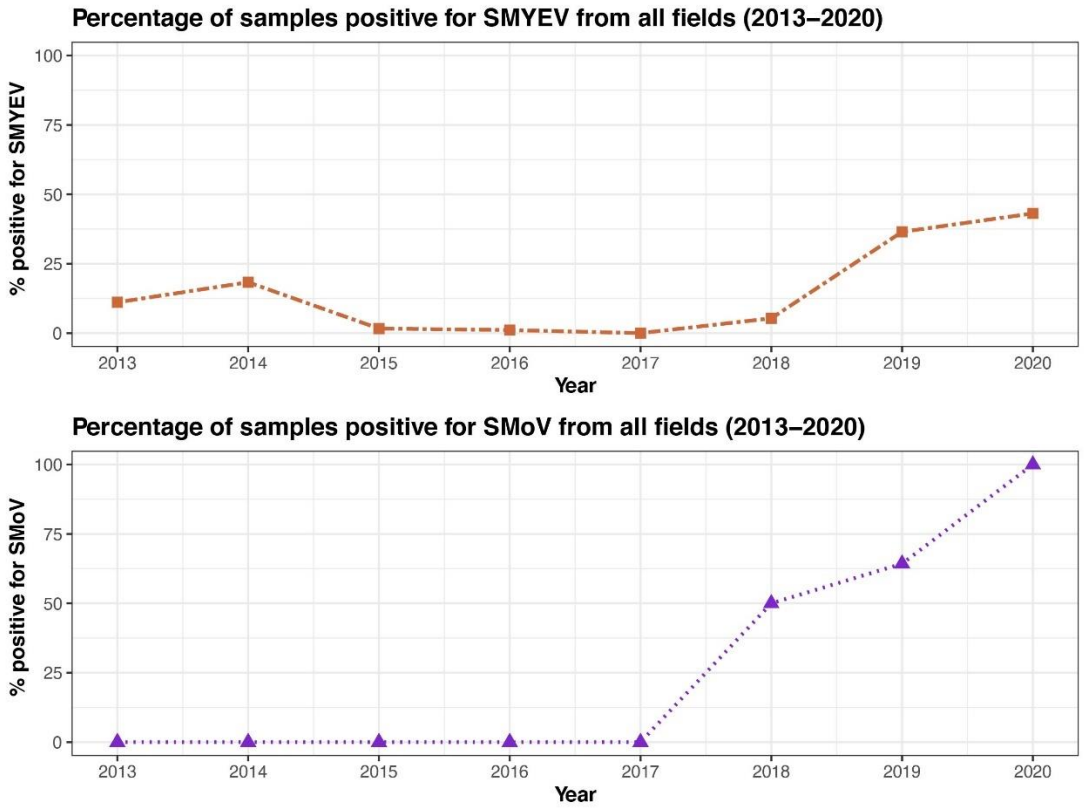

Figure 10. Case farm with increasing virus levels over time as a result of carrying over fruiting fields and limited rotation.

To explore whether a monitoring program could benefit local growers, all farms were divided by participation in a locally provided monitoring service (Figure 11). Although it is impossible to confirm with these data that a monitoring program reduced inoculum and vector pressure, a general trend of reduced virus incidence was observed on farms that did engage in a monitoring program. While a monitoring program is undoubtedly beneficial for a local grower, the data below would suggest that further vigilance is required, as infection rates in the last four years of the program also increased on monitored farms.

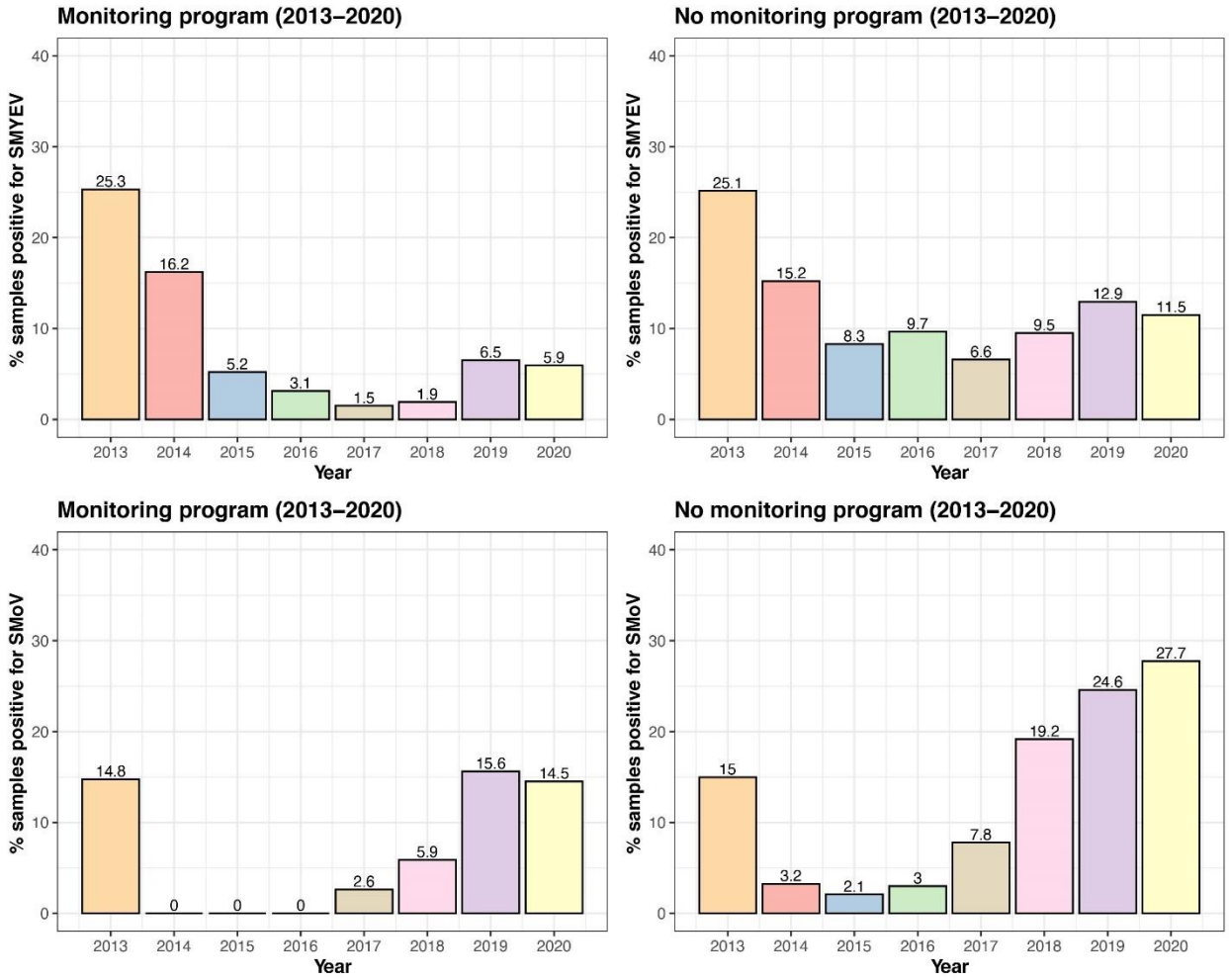

Figure 11. A comparison of virus infection rates across farms that did and did not have a monitoring program (2013-2020).

Despite a sequential decline in virus infection rates during the first four years of monitoring, in the last four years, infection rates have started to climb, especially those for SMoV (Figures 8 and 12). There are several possible explanations for this observation, and it is likely that a combination of factors are contributing. Anecdotal evidence from this project suggests that carrying over fields for multiple years can create sources of inoculum on farm that can infect adjacent newly planted and carried-over fields. Aphid numbers from the monitoring program fluctuated depending on farm, region, and year but a general trend of an increase in the number of strawberry aphids counted per 60 leaf sample was observed, and when overlayed with the infection rates, presents a possible contributing factor (Figure 12). These fluctuations in numbers are likely due to a combination of seasonal population dynamics, and management by growers. Further complicating this is the changes by the PMRA to products available for aphid control during key phenological stages. While it would appear that participating in a local monitoring service may have benefits for the grower, it must be used in combination with other aggressive management techniques (Figure 11).

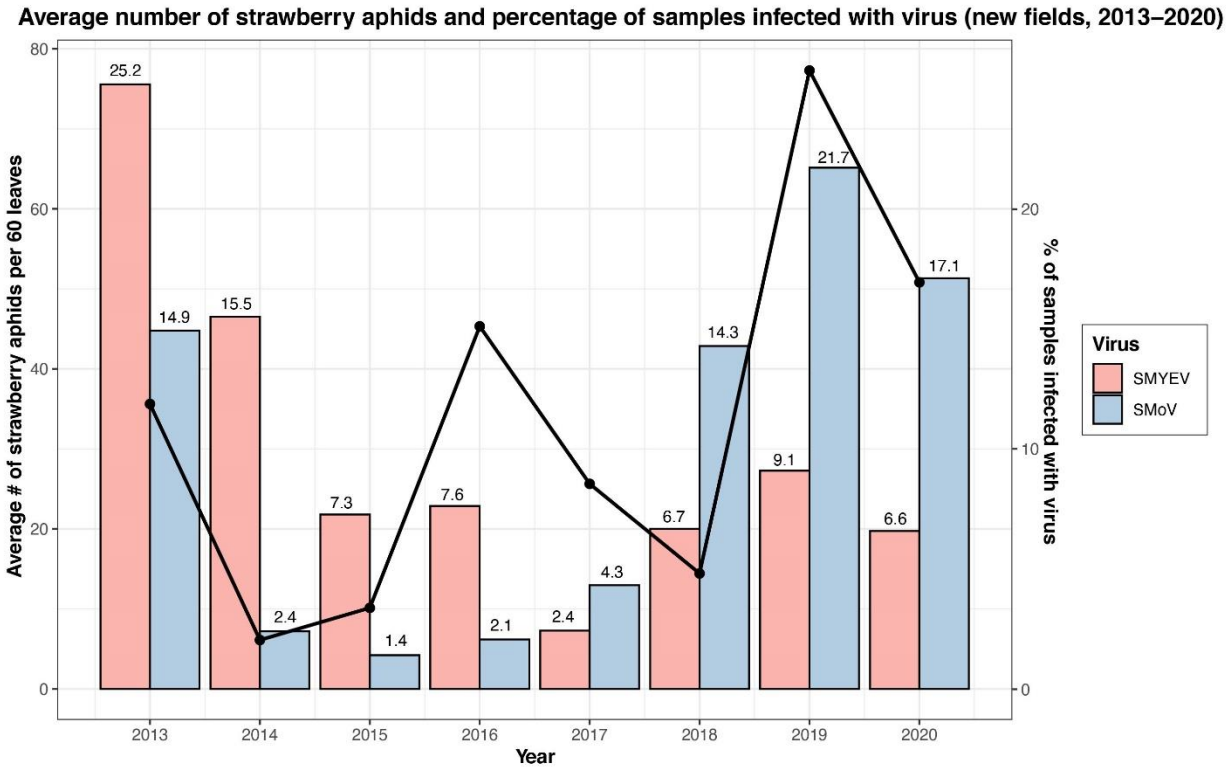

Figure 12. An overlay of infection rates for newly planted fields and the average number of strawberry aphids recorded per 60 bud leaf sample (2013-2020).

As this monitoring project concludes, and the PMRA re-evaluates products that contribute to aphid management, the onus of aphid monitoring and management will only increase for growers. Adding to this complication is the fact that some growers have begun to hold fields over for additional years of fruiting. Growers must be aware that by carrying over fields for multiple years, they are putting other neighbouring fields at risk if they have virus on their farm and are not vigilant with strawberry aphid management. If virus is present, then high aphid populations will increase the risk of virus spread. Therefore, some key recommendations for growers include: 1) to plant certified virus free stock if possible, 2) to avoid carrying over fields for more than 2 years, 3) to monitor aphid populations on their farm using bud leaf counts throughout the growing season, with increased monitoring during historical periods of population peaks, 4) to contact a Perennia specialist for management options when strawberry aphid populations are detected on their farm, 5) to annually test fields for SMYEV and SMoV, and 6) to rotate placement of newly planted fields on farm.

Raspberry Aphid Monitoring & Virus Testing

Large raspberry aphid monitoring was executed annually by Perennia and in conjunction with a local pest monitoring company, APM Agricultural Pest Monitoring Consulting Ltd. Due to the sporadic distribution of large raspberry aphids in fields (Lightle *et al*., 2014), the main monitoring method was examining the underside of newly expanded leaves for aphid presence (Figure 13). The main objective of monitoring was to identify when the first large raspberry aphid was detected in fields, so as to alert growers when to start their management program for the season.

Figure 13. Large raspberry aphid as seen without magnification. Photo Credit: Erika Bent, APM

In the final year of this project, limited large raspberry aphid monitoring was performed by Perennia, but scouting performed by APM Agricultural Pest Monitoring Consulting Ltd. identified large raspberry aphids in early-June 2020. This first sighting was earlier than previous years, when first sightings occurred in late June and early July.

In addition to large raspberry aphid monitoring, plants were also tested for virus. Virus testing involved screening for the two predominant raspberry/blackberry viruses in Nova Scotia: *Rubus Yellow Net Virus* (RYNV) and *Raspberry Leaf Mottle Virus* (RLMV). To confirm that these were the only viruses present in Nova Scotia raspberry/blackberry plantings, random samples were screened for an additional six viruses (*Raspberry Latent Virus*, *Black Raspberry Necrosis Virus*, *Blackberry Yellow Vein associated Virus*, *Blackberry Virus*, *Blackberry Chlorotic Ringspot Virus*, and *Raspberry Bushy Dwarf Virus*).

Initially, the main triggers for sampling raspberry and blackberry plantings was a grower noting decline in plant vigour/health or notifying Perennia of newly planted fields. Due to the perennial nature of bramble production some plantings did not meet these criteria, which resulted in some raspberry plantings not being sampled. As a result, in the final year of the program (2020), a comprehensive survey

of all known raspberry plantings in Nova Scotia was conducted and samples were analyzed for RYNV and RLMV. In 2020, a total of 13 farms compiled of 169 blocks (6 composite samples from each block) were sampled for RYNV and RLMV (Table 4). Of the 845 composite samples analyzed, 26.6% were positive for RYNV, 21.9% were positive for RLMV, and 1.4% were determined to have both viruses.

Table 4. Raspberry virus testing sample numbers and infection rates for RYNV and RLMV (2020).

			RYNV	*RLMV*
Sampling Year	**# Blocks**	**Samples**	**Percent Infected**	**Percent Infected**
2020	169	845	26.6%	21.9%

Unlike strawberry viruses, raspberry viruses can be individually symptomatic or collectively symptomatic, causing reduced yields, smaller leaves and berries, and chlorotic foliage (Delbridge and Hildebrand presentation). They are also more difficult to distinguish in field because they can be confused for other issues, such as herbicide injury, mite damage, poor crop nutrition, late spring frost damage or powdery mildew, which cause poor vigour and yellowing or mottled leaves. When growers noted a decline in plant vigor/health, a series of diagnostic tests were performed to rule out other causes before looking at viruses. Leaves of virus-infected plants may not always show virus-like symptoms and the appearance of symptoms may fluctuate through the season as the virus moves within the plant. To increase the chance of detecting virus, young leaves and shoot tips were sampled in the spring and fall when plants were actively growing, and the weather is cooler (Martin *et al.* 2013). Hotter weather can suppress virus disease symptoms.

Figure 14. Plants suspected to be infected with raspberry viruses, where symptoms appear similar to those attributed to mite damage (A) or nutrient deficiencies (B).

Through this program, it was determined that two viruses of concern and their vector, the large raspberry aphid are present in Nova Scotia, suggesting that bramble producers should be vigilant with monitoring and management programs. Martin *et al.* (2013) found that RYNV will spread rapidly in areas with high populations of the large raspberry aphid. Once a plant has become infected, it will harbour and be a source of the virus for the remainder of its life.

A crucial opportunity for limiting virus spread is presented by testing new plantings for virus. In a young raspberry planting, there is an opportunity to remove infected plants once virus has been confirmed to slow the spread of virus throughout the planting. In multi-year production systems such as raspberries, virus inoculum levels can build-up over time and insect vectors can pick up virus in one field and transmit it to adjacent fields or even nearby plots/farms if winged forms are present. Therefore, some key recommendations for growers include: 1) planting certified virus free stock if possible, 2) virus testing suspect plants, particularly in new fields, 3) rogueing out virus positive plants, 4) monitoring aphid populations through regular field scouting throughout the growing season, 5) contacting a Perennia specialist for management options when substantial aphid populations are detected on their farm, 6) managing wild raspberry plantings at field edges, and 7) rotating placement of newly planted fields on farm at a distance from older plantings.

Nursery stock virus testing

The third major activity of this program was to conduct testing of G4 strawberry nursery stock, previously referred to as "certified" stock, on all Nova Scotia strawberry plant nurseries. This testing was completed approximately one month prior to plant harvest/tip plugging. For the majority of fields, this resulted in sampling late August for 'southern' stock and mid-October for 'northern' stock. A testing protocol with low tolerances for SMYEV and SMoV was executed successfully according to "Guidelines for growing and inspecting strawberry plants in Nova Scotia". A 'Blue Tag' was issued to strawberry and raspberry producers as an indication of nursery stock meeting all guidelines, including virus level requirements.

Additionally, certified raspberry stock was tested in the fall of each year, approximately one month prior to plant harvest to test for presence of RYNV and RLMV. The sampling protocol follows that which is laid out in the "Guidelines for growing and inspecting raspberry plants in Nova Scotia".

Grower Communications

A crucial deliverable of the aphid and virus program was grower communication, which was delivered through a variety of formats and throughout the year (examples found in the Appendices). These included:

1. Annual updates on the program's research findings and progress in the form of a written report published on the Horticulture Nova Scotia and Perennia websites, and a presentation at Hort Congress
2. Aphid monitoring results communicated via text or email to cooperating growers (private)
3. Industry-wide aphid monitoring alerts via email and Perennia's Strawberry Blog
4. Development and publication of strawberry aphid and large raspberry aphid fact sheets

5. Individual communication of grower virus test results annually
6. Development and distribution of an aphid management program, updated annually to reflect product changes

Continued Vector-Virus Management

The final activity of this program was to assist with the refinement of strawberry aphid management strategies and to set industry on a virus-vector management path beyond this program. Based on the results and observations of this program, the recommended steps that growers should continue to follow as part of their best practices for vector-virus management are:

Start Clean

Nova Scotia strawberry and raspberry nursery plants are tested extensively every year as part of the provincial inspection program. Many nurseries across North American do not sell plants that go through third party certification and some certification programs do not test for viruses. The Nova Scotia inspection program ensures that plants sold from Nova Scotia nurseries are clean, as indicated by the 'Blue tag'.

Reduce inoculum

Remove older strawberry fields directly after harvest, as they may be harbouring both aphids and viruses. It is generally recommended not to hold fields for more than two harvest years as virus levels generally increase dramatically after this time. Care should be taken to remove wild populations of strawberries surrounding strawberry fields. These have been shown to be a reservoir for virus and aphids.

In raspberry and blackberry plantings, rogue out plants that were screened and found to be positive for viruses.

Monitor for Vectors

At the time of mulch removal, over wintering eggs (Figure 15) can be found on the underside leaves of strawberry plants. It is too early to spray at this time as the eggs have not hatched but assessing egg numbers at mulch removal does give an indication of aphid levels in the field.

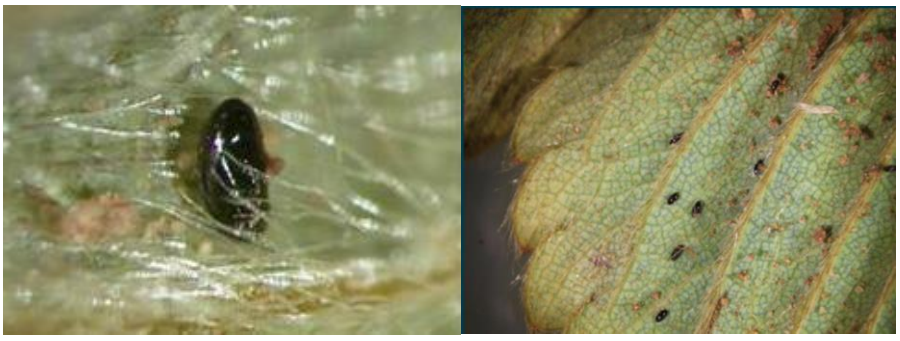

Figure 15. Over wintering eggs found on older strawberry leaves in spring. Photo credit: D. Moreau

Continue to monitor for wingless strawberry aphid (Figure 16) populations in the field. A threshold has been established to trigger spraying of one aphid in 16 leaves, but some growers may want to decrease this threshold if there is known elevated levels of virus in the field.

Figure 16. Wingless strawberry aphid, noting bulbs at end of body hairs as key species identifier. Photo credit: D. Moreau

As the season progresses, winged aphids (Figure 17) form as a result of increased population numbers and seasonal conditions. Winged aphids will move out of or into fields from nearby wild or cultivated plantings, in what is generally termed "flight". The flight of strawberry and the large raspberry aphid normally occurs in June (Figure 6) depending on location and will continue into the fall at varying levels depending on management practices and local conditions. Flight is a key management time, as winged strawberry aphids present a significant risk for virus spread. Leaf sampling or trapping with pan traps or sticky yellow traps can be used to indicate when aphid flight is occurring. It is suggested that a treatment may be necessary when there is one winged aphid caught per ten traps in strawberries. Care should be taken to continue monitoring for aphids within the field. It is possible that there are localized high levels of wingless aphids within the field that will not be caught in the traps. It is important to monitor until cool weather shuts down aphid activity.

Figure 17. Mature winged strawberry aphid (left) and raspberry aphid (right). Photo credit: D. Moreau

Control Vectors

There are a number of chemical control products that can be used for the control of strawberry aphids. Care should be taken not to overuse any one particular insecticide group so that resistance to an insecticide group does not develop. Treatment should be applied when monitoring counts exceed 15 nymphs per 60 leaf sample (Lewis, 2015).

Foliar Applications

Whenever employing chemical control options, be sure to read and follow all labels carefully. Chemical control options should be rotated appropriately between chemical groups for resistance management.

Group	Product	Rate / ha	REI	PHI	Note
1	Cygon 480-AG Lagon 480 E	2.25 L/ha	48 hrs	7 days	Do not apply when bees are actively foraging.
4A	Admire 240	175 ml/ha	24 hrs	7 days	Maximum of 2 applications per season. Do not apply pre-bloom or during bloom or when bees are actively foraging. Apply post-bloom only.
	Assail 70 WP	86-86 g/ha	12 hr	1 day	
4D	Sivanto Prime	500-750 ml/ha	12 hrs	0 days	Toxic to certain beneficial insects. Maximum 2 applications per season. Where possible, rotate with products outside of Group 4.
4A+15	Cormoran	500-750 ml/ha	12 hrs	1 day	Do not apply more than once every 10-15 days. Do not apply when bees are active. Maximum 2 applications of a group 4A per season.
28	Exirel	0.501.5 l/ha	12 hrs	1 day	Use Hasten or MSO adjuvant. Do not tank mix of make sequential applications with Group 11 fungicides, Copper fungicides, Captan Supra, Meastro, Folpan, Bravo or Echo.
NC	Kopa Insecticidal Soap	2% v/v in 700-1,900 l/ha	12 hrs	0 days	Reapply every 1-2 weeks. Applying within 3 days of sulfur may increase sulfur burn.
NC	Vegol Crop Oil	2 %v/v in 700-1,900 l/ha	12 hrs	0 days	Thorough coverage is essential. Tolerance has not been established in all varieties. Do not apply within 48 hours of a front or high temperatures. Do not apply with 14 days of Folpan, Captan Supra, Meastro and 30 days of Sulfur.
29	Beleaf 50SG	120-160 g/ha, min. 94 L/ha water	12 hrs	0 days	Max 3 applications per year.

Pre-Harvest Management

At first report or identification of winged aphids (flight) in your area, begin alternating foliar applications of Cygon 480-Ag/ Lagon 480E, Assail up to 1 day before expected harvest.
Harvest Management
At the onset of harvest, alternate products with short or 0-day pre-harvest intervals, such as Beleaf 50SG, Exirel, Cormoran or Sivanto Prime at recommended intervals until harvest is complete. Pay close attention to pre-harvest and re-entry intervals indicated on the product labels. Continue alternating applications until the flight collapses or the maximum number of applications/amounts of product has been reached.
Post-Harvest Management
After harvest is completed, growers can switch to products with longer pre harvest intervals and those products which can be a little harsher on pollinators as bloom has finished.
Suppression Products
Group NC products such as Purespray Green Oil (feeding deterrent) or Vegol Crop Oil (aphid suppression) are important rotational products during the harvest period. They can be used at any point leading up to the aphid flight as well as part of a spray rotation with other foliar applied products. ** Do not use chlorothalonil, captan, sulphur or dimethoate in a spray program with these products.* ** Do not use when temperatures are high.*

This publication was compiled by representatives from Perennia using information from the Pest Management Regulatory Agency of Health Canada, specific pesticide labels and manufacturer's information. This information is continuously changing and therefore it can cease to be current and accurate. Pesticide labels are the best source of information and should always be consulted prior to using a product.

Information on large raspberry aphid management can be found in the *Perennia Raspberry Management Schedule: A guide to insect, mite and disease management in raspberries in Nova Scotia* (https://www.perennia.ca/portfolio-items/caneberries/), which is updated annually.

Test fields to be over wintered

Strawberry fields that are to be over-wintered should be virus tested in late summer/early fall. This will allow the grower to make decisions on whether the virus levels are low enough to be considered for fruiting the following year. Results from a late summer/fall viruses test can be a good indication to a grower what their virus load is going into the next season and help inform whether they remove the field following harvest or not. Fields with high virus levels should be taken out to ensure that virus does not spread to other over-wintering fields and fields to be planted the following spring. If virus levels are present at acceptable levels, special attention should be paid to controlling aphid levels in order to control virus amplification in the field or virus spread to newer or neighbouring fields.

Summary

Following completion of an eight-year intensive recovery and monitoring program for Nova Scotia strawberry and bramble producers, much has been learned and several questions remain. Viruses of economic concern are present in Nova Scotia, as well as vectoring insects that can aid their spread. While an initial decline was observed in strawberry virus infection rates, likely due to drastic field remediation in 2012-2013, in the past four years rates have climbed. While it is difficult to pinpoint the main source of virus infection, it is known that the primary vector, the strawberry aphid, is present on Nova Scotia strawberry farms. Additional grower management practises can affect virus levels such as monitoring of aphid populations on farm and carrying over fields for multiple production years. Several raspberry viruses of economic concern have been detected in Nova Scotia as well as the vector, the large raspberry aphid. While raspberry virus levels have yet to reach pandemic levels, the perennial nature of bramble production, and the 2020 virus levels in Nova Scotia (Table 4) should be a concern for growers.

Management of viruses and vectors on farm is a complex task that requires vigilance at several levels. Primarily, growers should be planting virus-free material. Once planted, growers would ideally screen a subset of plants for virus each year, and manage fields based on inoculum levels. Vector management is important for limiting spread, and farmers should be monitoring their presence, either themselves or through a crop consultant, and controlling them when appropriate. Up to date management options can be provided by Perennia specialists by request. Insufficient management of blocks with high virus incidence not only threatens production at the block level, but also that of the farm and neighbouring farms due to spread via vectors. Blocks should be rotated on farm to avoid planting new blocks adjacent to older fields with high virus incidence, and ideally such older blocks should be pulled out of production. While the recent surge in infection rates is disheartening, it is likely that virus management will be a mainstay in Nova Scotia fruit production, and combined efforts across all growing regions can help bring numbers down to a manageable level.

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