Interim Report 2018-2019

Aphid monitoring and virus testing

19 February 2019

Prepared for:

Horticulture Nova Scotia
32 Main St
Kentville, NS B4N 1J5

Prepared by:

Matthew Peill
Horticulture Specialist
Perennia Food and Agriculture Inc.

Background

An outbreak of two viruses in Nova Scotia strawberries in 2012-2013 caused significant losses to both strawberry nursery and commercial fruiting operations. The commercial fruit industry is estimated to have a farm gate value of $10 million, however a 50 percent crop loss was experienced due to the viruses in 2013. Nova Scotia also has a vibrant strawberry nursery plant industry valued at approximately $9 million. In 2012, two of the five nurseries had a complete crop loss amounting to $3.75 million and one of these nurseries withdrew from production altogether in 2013. Due to the severity of loses, both the federal and provincial government aided in the recovery of the industry by providing assistance from 2013-2016 for virus testing and monitoring of the primary insect-vector of the viruses the strawberry aphid (*Chaetosiphon fragaefolii*). Through support from Canada-Nova Scotia Strawberry Assistance Initiative and the work of Horticulture Nova Scotia, Perennia specialists, and industry, strawberry virus levels appear to have stabilized.

The Nova Scotia strawberry industry is viewed as a leader in virus and vector management, as viruses continue to have a significant effect on production levels across Canada. In Nova Scotia, virus testing has been primarily performed to identify two viruses of strawberries, Strawberry Mild Yellow Edge Virus (SMYEV) and Strawberry Mottle Virus (SMoV). These two viruses in combination were identified to have caused the 2012-2013 virus epidemic in Nova Scotia, though other provinces have detected upwards of five different viruses. Added to the testing program for 2017-2021 is Strawberry Polerovirus 1(SPV-1), which was first identified in eastern Canada from plants tested in 2013 (Xiang et al. 2015). Although SPV-1 does not appear to cause visual symptoms or disease by itself, it is suspected of aiding in the transmission of SMYEV (Thekke-Veetil and Tzanetakis 2016.). Because of this it is thought to be a missing component of recent strawberry epidemics caused by virus complexes, such as SMYEV and SMoV disease causing complex found in Nova Scotia (Thekke-Veetil and Tzanetakis 2016.).

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 specialists in 2014 identified two viruses (Rubus Yellow Net Virus (RYNV) and Raspberry Leaf Mottle Virus (RLMV)) in suspect raspberry and blackberry plantings. A survey of raspberry and blackberry production across Nova Scotia in 2015 and 2016 showed the viruses were more widespread than initially thought. Visual identification of these viruses is exceptionally challenging and verification by the molecular testing method reverse-transcriptase polymerase chain reaction (RT-PCR) is required for accurate identification. To better understand this virus-complex and to provide industry with informed recommendations on management of the primary vector large raspberry aphid (*Amphorophora agathonica*), virus testing and vector monitoring was initiated in 2017.

To allow for the continuation of virus-vector monitoring and virus testing in berry crops an additional four years of funding was secured for the 2017-2021 growing seasons.

Project Objectives

The main objectives of the program are to: (1) aid in the management of strawberry and bramble primary insect virus vectors strawberry aphid (Chaetosiphon fragaefolii) and 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 involvement in the National Clean Plant Program. To meet these objectives, the following activities are proposed:

1. Monitor for strawberry aphid and large raspberry aphid on representative farms across the province. Provide individual monitoring results to cooperating growers and aggregate results to the industry on a timely basis for optimum vector management.

2. 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".
3. 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.

Sampling and Monitoring Protocols

Strawberry Aphids

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: 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: 60 immature 'bud leaves' were collected from each monitoring plot on a weekly basis throughout the growing season.
3. Yellow pan traps monitoring for 'winged' vectors: 6 yellow pan traps were located in select monitoring plots and examined for winged vector numbers, by species, throughout the growing season.

Strawberry and Raspberry Virus

Leaf samples were collected in late summer/early fall from all newly planted commercial fruiting strawberry fields with greater than 1000 plants for virus testing. In addition to sampling newly planted fields, 5 fields that were carried over (planted 2017) for a second year of fruiting were randomly selected for virus testing. The sampling methodology employed was designed to identify the level of infection with SMYEV, SMoV and SPV1 in strawberry fields. Individual fields/blocks were randomly sampled so as to have 20 bags with 3 trifoliate-leaves each for SMYEV testing 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 RT-PCR testing of SMoV and SPV-1. The first fully expanded leaf in either a mother or rooted daughter plant was sampled.

Raspberry leaf samples were collected in late summer/early fall from all newly planted commercial fruiting fields. Individual fields/blocks were randomly sampled so as to have 20 bags with 3 trifoliate-leaves each to test for Rubus Yellow Net Virus (RYNV) and Raspberry Leaf Mottle Virus (RLMV). Unlike strawberry viruses, these viruses can be individually symptomatic or collectively symptomatic, causing reduced yields, smaller leaves and berries, and chlorotic foliage. Young fully expanded leaves were sampled from canes.

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" in the fall of each year prior to plant harvest.

All samples were analyzed for viruses by PhytoDiagnostics in British Columbia or Agricultural Services in New Brunswick.

Results

Strawberry Aphid Monitoring

Aphid monitoring plots were established during the first two weeks of May 2018 across the province. A total of 16 farms participated with 19 monitoring sites. Fourteen of the farms selected to participate had been monitored the previous 5 years. These locations had originally been chosen in 2013 based on geographic location in an attempt to capture all strawberry producing regions in the province, with a concentration of farms in the Annapolis Valley. Two new farms with histories of aphids were added, to replace farms that were no longer being surveyed due lack of aphid pressure at the sites.

Strawberry Aphid Egg Counts

At plot set-up in May, and for two weeks following, 30 old overwintered leaf samples were collected from each monitoring site to perform an egg count. Across all sites 792 eggs were found (Table 1). Although this is the highest recorded aphid egg count to date, eggs were only found at 5 of the 16 farms sampled and 2 of those 5 farms had not previously been sampled as part of the monitoring project. This is similar to other monitoring years where overwintered populations of aphid eggs were only found on select farms. The increase could also be related to a relatively mild fall and winter in the previous year (2017), which was also thought to be the cause of the other notable spike observed in the spring of 2016. Another possible reason for the variability between years is because of the highly variable distribution of eggs within a field. Strawberry aphids are colonizers, so will typically be found in defined areas and not spread-out across a field. When populations of aphid eggs were found, a management plan for control was activated once confirmation of strawberry aphid was made at hatch 1-2 weeks later.

Table 1. Eggs counts from regions across Nova Scotia over the monitoring period, 2014-2018.

	2014	*2015*	*2016*	*2017*	*2018*
Western/ Valley region	29	41	257	1	616
Central/ Eastern region	0	45	44	0	176
Nova Scotia total	29	86	301	1	792

Wingless Aphids

Wingless aphids were monitored weekly at each monitoring site beginning the second week of May using a 60-bud leaf count method. Although aphid egg counts were high, leaf counts of wingless aphids in 2018 were observed to be low relative to previous monitoring years. Similar to previous years only select farms were found to have resident populations of strawberry aphids, comprising most of the total counts. The Western and Valley regions experienced a spike mid-May, followed by a dip through early June and a spike again in late-June and early-July (Figure 1). In the Central and Eastern regions, strawberry aphid populations spiked in early June and began to decrease by the end of June (Figure 2). By the beginning of July and until the end of monitoring aphid populations had essentially collapsed across the province, with the exception of a few small temporary increases. This could be partly contributed to the extreme heat experienced through July and into early September. Strawberry aphids develop and reproduce optimally between 18-25^{o}C, and temperatures above this range can supress aphid populations (Cedola and Greco 2010; Krczal 1982). Additionally, monitoring efforts have been very

effective at controlling strawberry aphid populations across the province, and continued management is needed to maintain this.

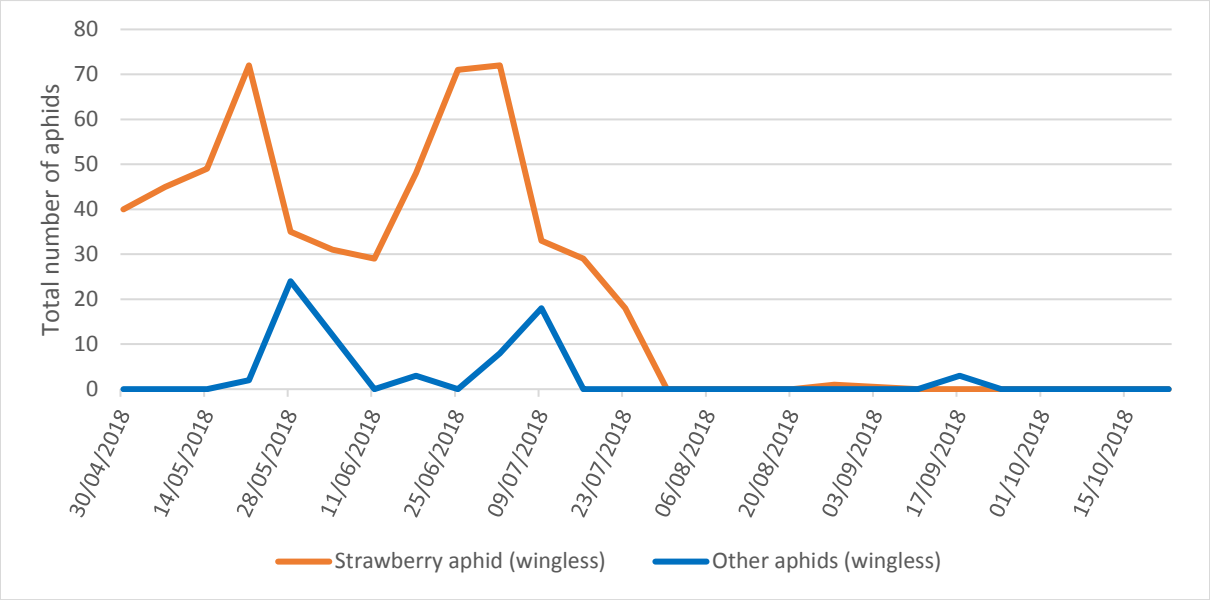

Figure 1. Counts of wingless aphids from all participating farms in the Western and Valley regions of Nova Scotia in 2018.

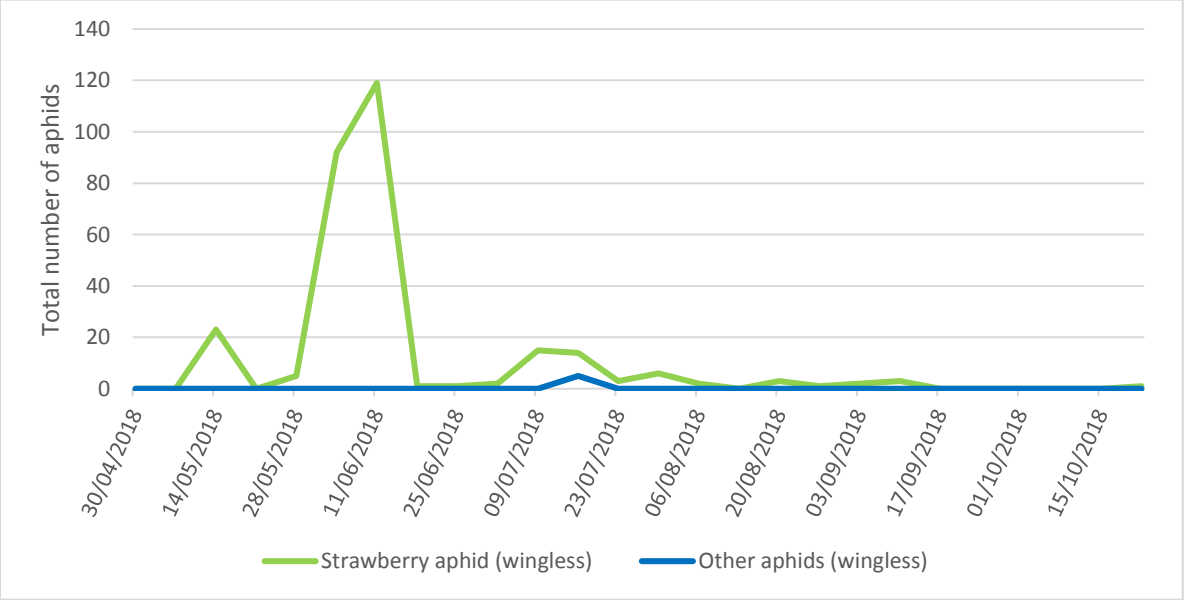

Figure 2. Counts of wingless aphids from all participating farms in the Central and Eastern regions of Nova Scotia in 2018.

Winged Aphids

Targeted monitoring of winged aphids was accomplished through the use of pan traps, with occasional winged aphids picked up during weekly bud leaf counts. Pan traps were deployed in monitoring plots

during the last two weeks of May across the province and monitored until late-October 2018. Similar to previous monitoring years winged strawberry aphids made up only a small portion of total winged aphid catches, representing 0.21% of total catches in the Western and Valley region and 0% in the Central and Eastern region (Table 2). The total number of winged "other" aphids caught across the province has also continued to drop.

Table 2. Total winged strawberry and winged other aphid counts throughout the monitoring period in the two monitoring regions of Nova Scotia, 2013-2018.

	Western and Valley Region						Central and Eastern Region					
	2013	**2014**	**2015**	**2016**	**2017**	**2018**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Total winged strawberry aphids (count)	596	24	12	10	3	2	493	43	3	4	1	0
Total winged other aphids (count)	19459	27138	27191	50484	4497	948	10774	3801	4138	1894	262	144
Winged strawberry aphids as a % of total winged aphids	3.1	0.09	0.04	0.02	0.07	0.21	4.6	1.1	0.07	0.21	0.39	0.0

The aphid flight in the 2018 season was quite sporadic, with may dips and spikes throughout season making it difficult to determine a single high risk flight period for the Western and Valley region (Figure 3) or Eastern and Central region (Figure 4). The aphid flight died out across the province by early October. Overall total winged aphid number were low compared to previous monitoring years. A flight period for winged strawberry aphids alone could not be deduced in either region as monitoring counts were insufficient to clearly identify its initiation and termination. An extremely low population of winged and wingless strawberry aphids indicate that control measures have been employed at appropriate times, allowing for little strawberry aphid establishment.

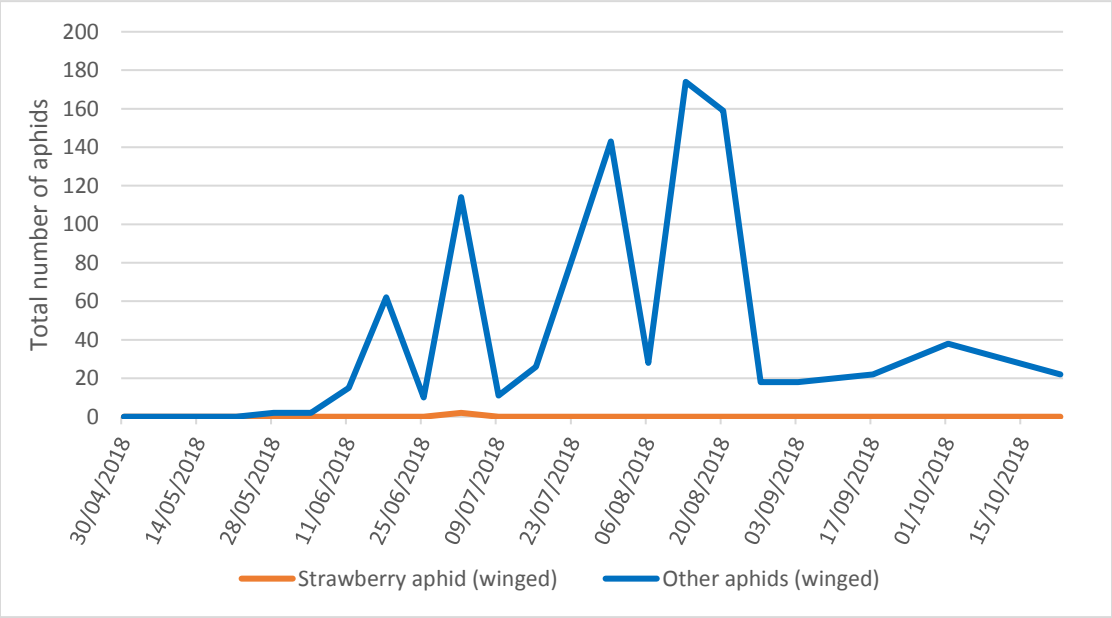

Figure 3. Counts of winged aphids (yellow pan traps) from all participating farms in the Western and Valley regions of Nova Scotia for the 2018 season.

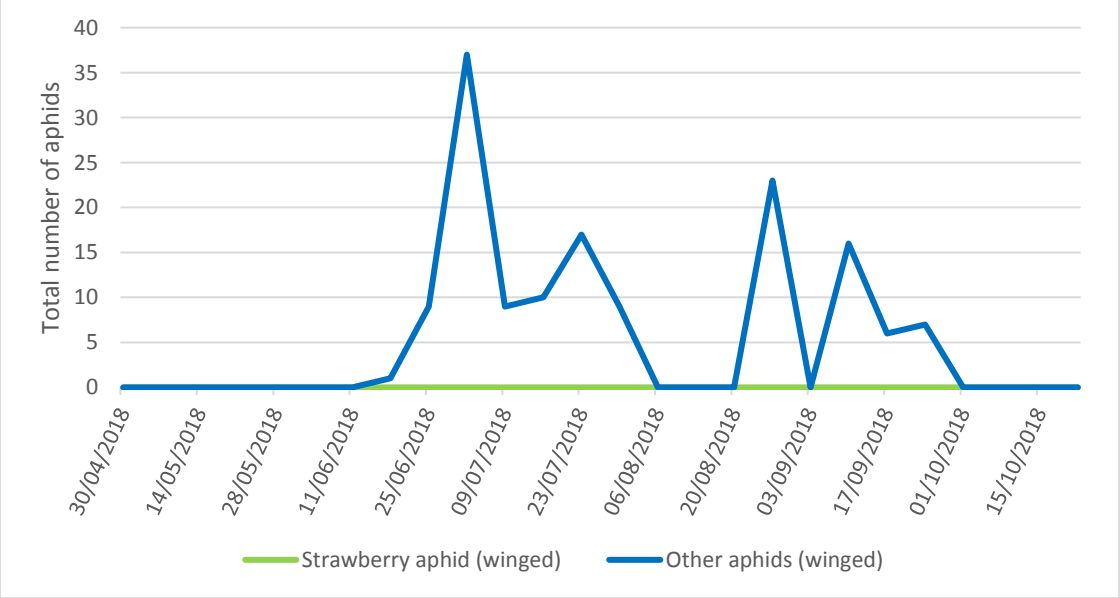

Figure 3. Counts of winged aphids (yellow pan traps) from all participating farms in the Central and Eastern regions of Nova Scotia for the 2018 season.

Strawberry Virus Testing

The continued strawberry virus sampling has allowed for monitoring of virus level trends and recovery progress of the two primary viruses responsible for significant crop loss in Nova Scotia. Virus sampling

occurred on 43 Nova Scotia strawberry farms in 2018. A total of 87 newly planted fields/blocks were sampled, for a total of 1650, 161, and 6 samples submitted for SMYEV, SMoV and SPV-1, respectively. As more farms transition to holding fruiting fields over for a second year of picking, it was important to sample a cross section of these farms. In total, 5 second year fruiting fields were virus sampled, totalling 100, 10 and 4 samples submitted for SMYEV, SMoV and SPV-1, respectively.

In 2018, the levels of the two primary viruses, SMYEV and SMoV, were 6.7% and 14.3% respectively in newly planted fields. Although it appears from these figures that virus levels have increased significantly from last year, the majority of new fields/blocks tested were virus free. The dramatic increase can be contributed to a few select sites that had not been tested for a several years (due to no new plantings) and had high levels of both viruses. When provincial virus levels are adjusted for these sites the percentage of SMYEV and SMoV drops to 3.6% and 11.0%. Increases in the incidence of SMoV could be contributed to the new testing protocol used, which is more representative.

As growers have begun to hold fields over for additional years of fruiting, aphid management becomes even more important. In the 5 second-year fruiting fields that were sampled it was determined that both viruses were present at higher levels (17% SMYEV and 30% SMoV) than in newly planted fields. This indicates that the low level of virus initially detected in the newly planted fields is being further transmitted and increasing virus levels within the field. As fields are kept longer the risk of virus levels building within the field increases as well as the risk of these older fields causing infections in nearby new fields.

SPV-1 was only tested for at select sites where it was suspected there would be infections, because of this of there was a 70% incidence SPV-1 from the 10 samples tested. The effects of SPV-1 infections are still not well understood, or its suspected role as a helper virus to SMYEV.

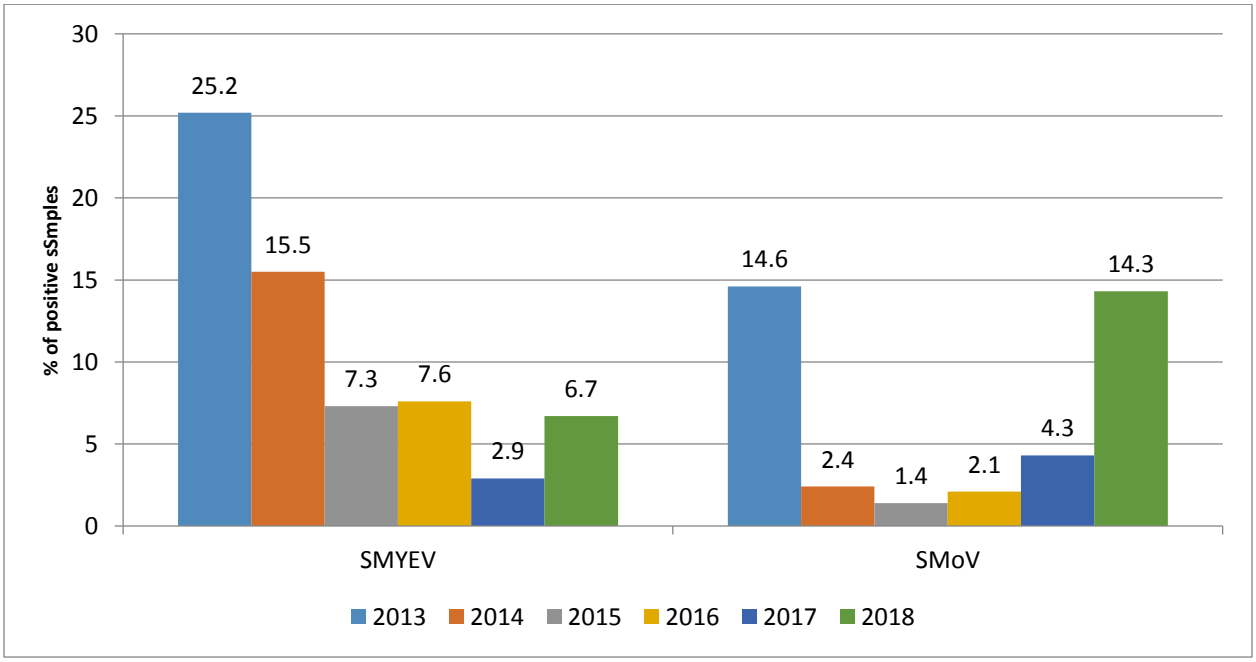

Figure 4. Infection levels (Strawberry Mild Yellow Edge Virus (SMYEV) and Strawberry Mottle Virus (SMoV)) of all newly planted fields with over 1000 strawberry plants from 2013-2018 (excludes nursery testing).

Raspberry Aphid and Virus Monitoring

There was limited large raspberry aphid monitoring performed this year, but scouting performed by a local pest management company found large raspberry aphid in early-July. This first sighting of large raspberry aphid is consistent with 2017, when the first sighting occurred in late June.

Given the perennial nature of the raspberry/blackberry production cycle, virus sampling for the *Rubus* viruses is not conducted as systematically as strawberry. 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 2018, all new raspberry plantings that were tested came back negative for RYNV and RLMV.

Nursery stock virus testing

Testing of G4 strawberry nursery stock, previously referred to as "certified" stock, was conducted on the three Nova Scotia strawberry plant nurseries in late August for 'southern' stock and mid-October for 'northern' stock. A testing protocol with low tolerances for strawberry mild yellow edge virus and zero tolerance for strawberry mottle virus was executed successfully again in 2018 according to Guidelines.

Additionally, certified raspberry stock was tested in fall 2018 for the two raspberry viruses and found to be clean. As such, Nova Scotia raspberry fruit growers can be assured that, like strawberries, their raspberry nursery stock does not carry virus disease.

Summary

Monitoring results clearly indicated that numbers of the primary disease vector, the strawberry aphid, remained quite low 2018 after relatively high populations were observed in 2013. It can be speculated that effective management is the most probable explanation. Although a strawberry aphid flight was undetectable there were still low levels of infections found in newly planted fields. This indicates that aphids are moving another way between transmission sites, possibly 'hitching rides' or for fields that are in close proximity to each other, simply walking or being blown by wind gusts.

Virus testing of newly planted fields conducted in late-summer/early-fall showed slight increases in virus levels. The SMYEV and SMoV that was found appears to be persisting in pockets on select farms and efforts will be made to reduce it further by targeted monitoring and management in the coming growing season. Original virus management recommendations encouraged growers to only fruit fields for one year and then plough them down to prevent the accumulation of virus in their plantings. This was with good reason, as we determined again this year that virus levels were found to be higher in carried over fruiting fields. If growers choose to carry-over fields for an additional year of harvest they also need to be aware of the increased risk of transmitting virus.

Finally, efforts will be continued to assess and monitor for the two insect-vectored diseases found in *Rubus* species spread by the large raspberry aphid. Management of RYNV and RLMV is much like strawberry in that the vector needs to be managed to avoid virus transmission to other plants or blocks. Although the large raspberry aphid is easier to spot with the naked eye, it has shown to be more challenging to monitor. New monitoring protocol will be implemented in 2019 so that more accurate and timely notifications to can be given to growers about vector presence.

References

Cédola, C., and Greco, N. 2010. Presence of the aphid, *Chaetosiphon fragaefolii*, on strawberry in Argentina. J. Insect Sci. **10**(1): 9. doi:10.1673/031.010.0901.

Krczal, H. 1982. Investigations on the biology of the strawberry aphid (*Chaetosiphon fragaefolii*), the most important vector of strawberry viruses in West Germany. Acta Horticult. **129**: 63–68. doi:10.17660/ActaHortic.1982.129.11.

Thekke-Veetil, T., and Tzanetakis, E. 2016. First report of strawberry polerovirus in the United States. Plant Disease. **100**: 867

Xiang, Y., Bernardy, M., Bhagwat, B., Bouthillier, M., DeYoung, R., and Wiersma, P.A. 2015. The complete genome sequence of a new *Polerovirus* in strawberry plants from eastern Canada showing strawberry decline symptoms. Arch. Virol. **160**: 55