Interim Report 2017-2018

Aphid monitoring and virus testing

23 February 2018

Prepared for:

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

Prepared by:

Jennifer Haverstock
Small Fruit Specialist
Perennia Food and Agriculture Inc.

perennia
ideas • innovation • success

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. Government aided in the recovery of the industry by providing assistance from 2013-2016 for virus testing and monitoring of virus insect-vectors, namely 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 most commonly identified two primary viruses in strawberries, Strawberry Mild Yellow Edge Virus (SMYEV) and Strawberry Mottled (SMoV). Other provinces have detected upwards of five different viruses, heightening awareness that viruses may never be completely eradicated. Added to the testing program for 2017-2021 is a virus that had been detected previously in Nova Scotia but not regularly tested, Strawberry polerovirus (SPV-1). Although SPV-1 does not cause visual symptoms or disease by itself, it has been found to be a SMYEV disease transmission component (Thekke-Veetil and Tzanetakis. 2016.) and is thought to be a missing component of the epidemics caused by virus complexes (Xiang et al. 2015.), such as the SMoV and SMYEV disease causing complex found in Nova Scotia.

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 lab reverse transcriptase polymerase chain reaction (RT-PCR) testing methods is required for accurate identification. To better understand this virus-complex and to provide industry with informed recommendations on management of the 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 (i.e. strawberry aphid, *Chitosiphon 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 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: 6-12 yellow pan traps were located in select monitoring plots and examined for winged vector numbers, by species, throughout the growing season.

Raspberry Aphids

Leaf monitoring was used for both 'wingless' and 'winged' large raspberry aphid at both raspberry nurseries and in two fruiting fields. Weekly scouting involved the examination of canes looking for the presence of large raspberry aphids at the tips and undersides of newly expanded leaves where the plant tissue is most tender.

Strawberry and Raspberry Virus

Leaf samples were methodically 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, 20 fields that were carried over (planted 2016) 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. One additional bag of 60 trifoliate-leaves was also collected per field/block from which two random samples of three leaves were used for RT-PCR testing of SMoV and SPV-1. The first fully expanded leaf in either a mother or rooted daughter plant were sampled.

Leaf samples were collected from symptomatic plants 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. Observation of these symptoms triggered virus testing in suspect plantings. A randomized 30-leaf sample was collected from suspect fields/blocks and results communicated directly to the grower.

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 2017 across the province. A total of 19 farms participated with 25 monitoring sites. The farms selected to participate had been monitored the previous 4 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. One new farm was added, while one farm decided to cease participating in the monitoring program. Several of the farms in the northern/eastern part of the province sent in their own leaves for counting but this was quite sporadic and inconsistent.

Strawberry Aphid Egg Counts

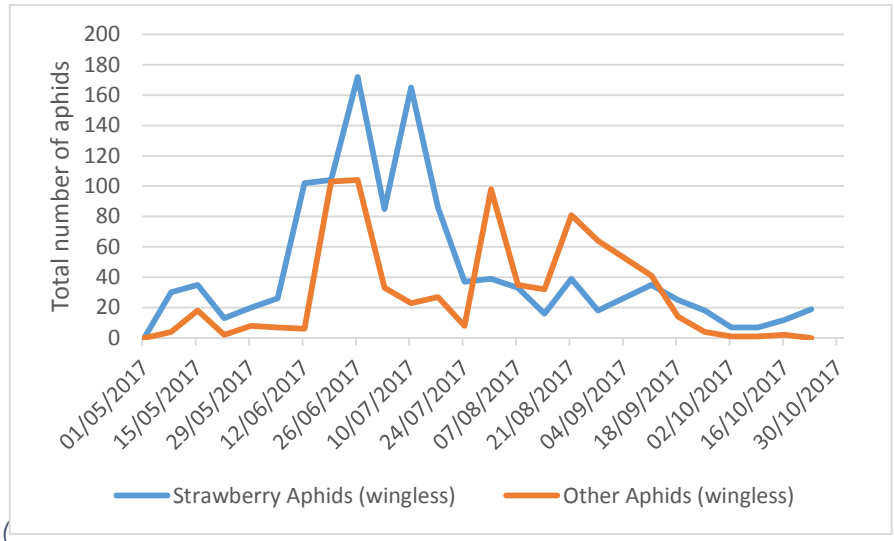
At plot set-up in early May, and for a few weeks following, 30 old overwintered leaf samples were collected for an egg count from each monitoring site. Across all sites no hatched eggs and only one unhatched egg were found (Table 1). To date, few eggs have been found annually as part of these monitoring programs with the exception of the 2016 monitoring year. The notable spike observed in 2016 was thought to be a result of a relatively mild fall and winter. 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. In all monitoring years the overwintered populations of aphid eggs were only found on select farms and 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, 2013-2017.

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

Wingless Aphids

Wingless aphids were monitored weekly at each monitoring site beginning the second week of May using a 60-bud leaf count method. Consistent with the egg scouting data, leaf counts of wingless aphids in 2017 were observed to be low relative to previous monitoring years. Only select farms were found to have resident populations of strawberry aphids, contributing most to the total counts.



Strawberry aphid populations (

Figure 1) in the Western and Valley regions experienced a spike mid-June through mid-July. A similar spike was observed in the Central and Eastern regions, however it was much shorter, only lasting a few weeks at the end of June. A significant increase in aphid population observed in the Central and Eastern region (Figure 2) from late August to late September is attributed to high aphid counts on one farm. Although aphid populations are believed to spike primarily in the spring, a fall spike has been observed on several farms, necessitating the need for aphid monitoring and management through the fall. Populations across the province had dropped off by late September.

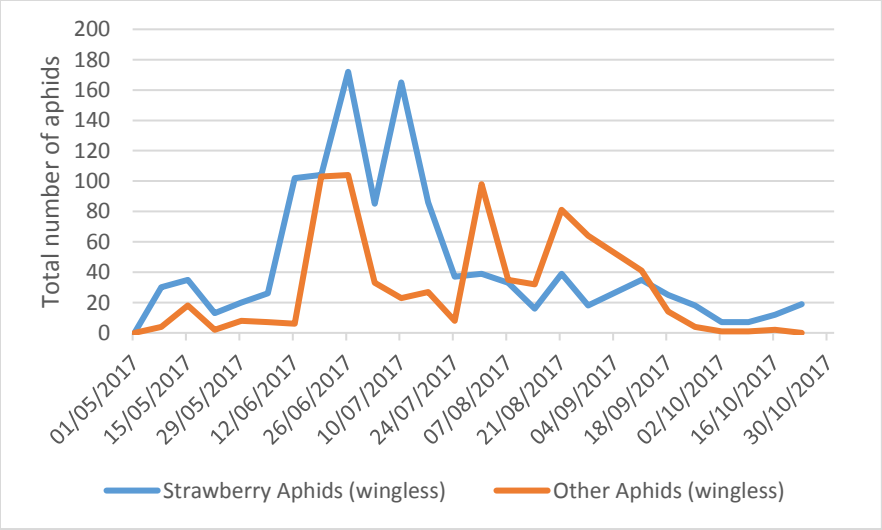


Figure 1. Counts of wingless aphids from all participating farms in the Western and Valley regions of Nova Scotia in 2017.

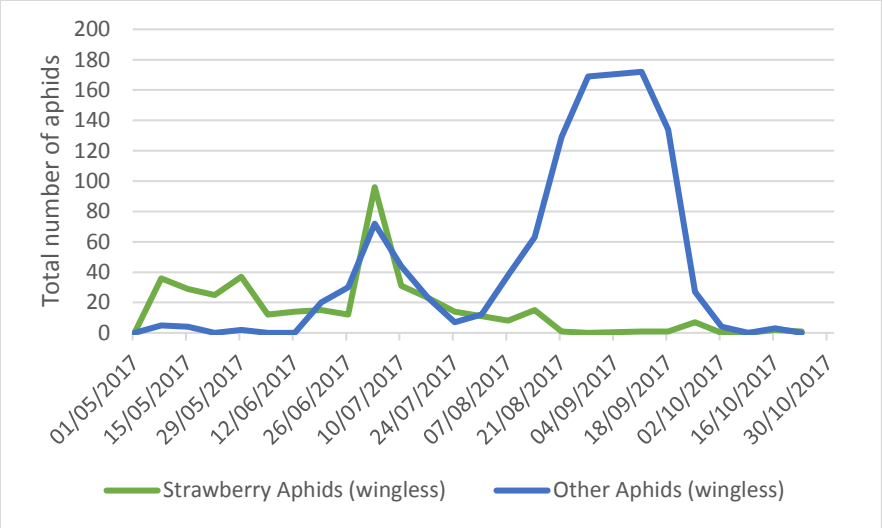


Figure 2. Counts of wingless aphids from all participating farms in the Central and Eastern regions of Nova Scotia in 2017.

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 the end of October 2017.

Following the trend of previous monitoring years (**Error! Not a valid bookmark self-reference.**), winged strawberry aphids accounted for only a minute (0.07% Western and Valley region and 0.39% Central and Eastern region) portion of total winged aphids found across the province. A noteworthy drop in winged 'other' aphids was also observed compared to previous monitoring years. The possible cause for this variation is that prior to the 2017 monitoring year winged aphid trapping was accomplished through the use of yellow sticky traps. Although yellow sticky traps are a recommended method of aphid trapping, they have been found to be a less accurate method of trapping compared to other methods. With this knowledge, it was decided that through the course of this project (2017-2021) yellow pan traps would be deployed for winged aphid trapping.

Table 2. Total winged strawberry and winged other aphid counts throughout the monitoring period in the two monitoring regions of Nova Scotia, 2013-2017.

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

The high risk aphid flight period for all aphids in the Western and Valley region started mid-late June in the 2017 growing season, approximately two weeks later than observed in previous years (Figure 3). Although not as extreme with significantly lower numbers, total aphid flight in the Central and Eastern regions followed a similar pattern as in the Western and Valley regions (

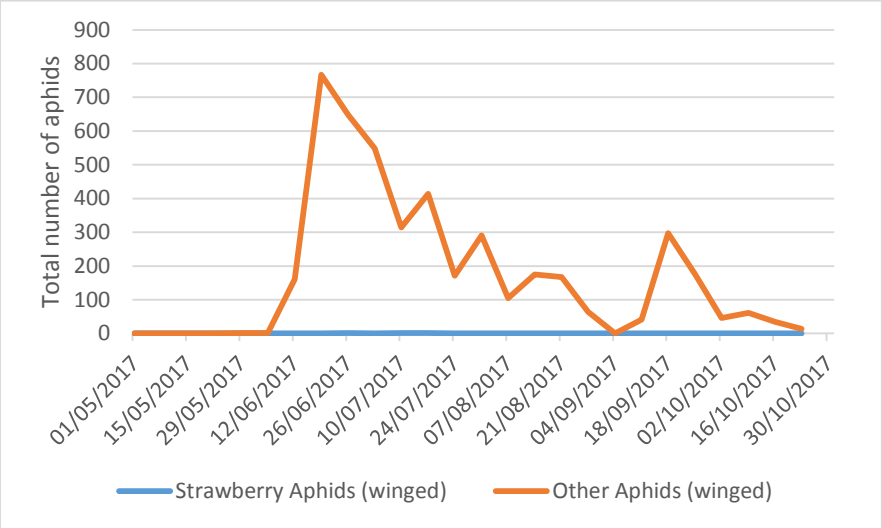


Figure 3. Counts of winged aphids (yellow pan traps) from all participating farms in the Western and Valley regions of Nova Scotia for the 2017 season.

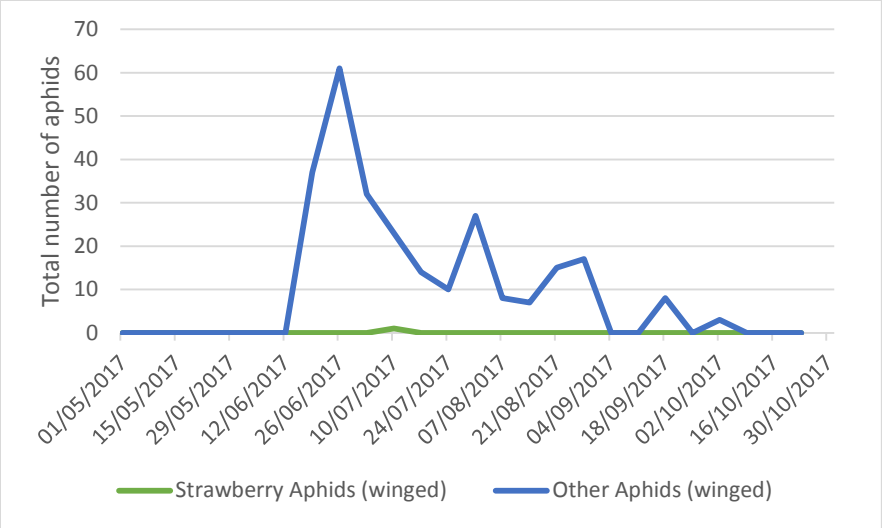


Figure 4). Aphid flight across the province flight died out by early October.

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 colonization.

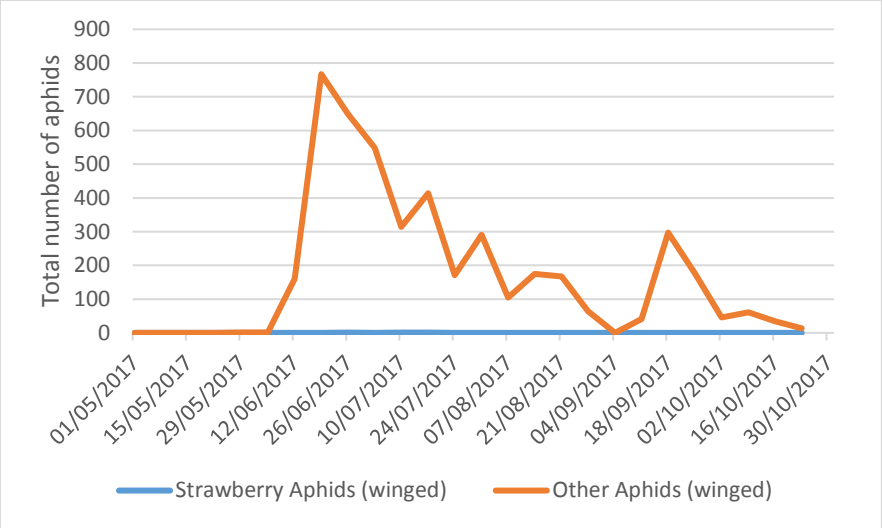


Figure 3. Counts of winged aphids (yellow pan traps) from all participating farms in the Western and Valley regions of Nova Scotia for the 2017 season.

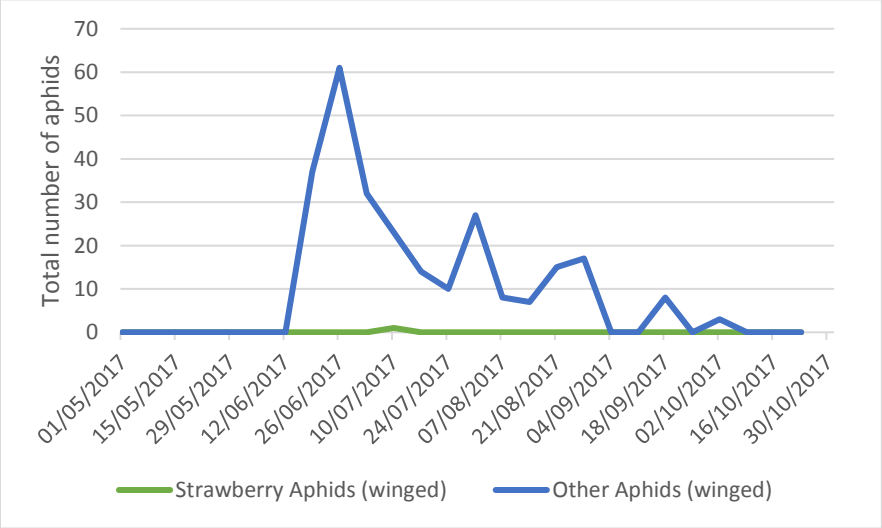


Figure 4. Counts of winged aphids (yellow pan traps) from all participating farms in the Central and Eastern regions of Nova Scotia for the 2017 season.

Strawberry Virus Testing

The continued strawberry virus sampling has allowed for monitoring virus level trends and recovery progress of the primary two viruses responsible for significant crop loss in Nova Scotia. Virus sampling occurred on 47 Nova Scotia strawberry farms in 2017. A total of 97 newly planted fields/blocks were sampled, for a total of 1530, 185, and 58 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, 10 second year fruiting fields were virus sampled, totalling 172, 19 and 6 samples submitted for SMYEV, SMoV and SPV-1, respectively.

In 2017, the levels of the two primary viruses, SMYEV and SMoV, were 2.9% and 4.3% respectively in newly planted fields. These show a continued decrease of SMYEV levels over the last 5 years of virus recovery monitoring, but a slight increase in SMoV. Although the viruses appear to be stabilizing, it is important to be vigilant in aphid monitoring and management to ensure virus levels remain at a minimum in Nova Scotia. As growers have begun to hold fields over for additional years of fruiting, this aphid management becomes even more important. In the 10 second-year fruiting fields that were sampled it was determined that both viruses were present at much higher levels (29% SMYEV and 21% 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.

In its first year of testing SPV-1 was found to have a presence of 31%. This is not an unreasonable number when compared to the level of virus reported by Thekke-Veetil and Tzanetakis in 2016. From 118 samples, randomly collected from nursery and commercial settings in the Midwest and Midsouth during the 2013 and 2014 growing seasons, 50% were confirmed positive for SPV-1 (Thekke-Veetil and Tzanetakis. 2016). Although SPV-1 is thought to be a helper virus of SMYEV, it was difficult to correlate the presence of SPV-1 with SMYEV given the sampling methodology used. In the next sampling season, methods of sampling for SPV-1 will be re-evaluated to enable better correlation with SMYEV.

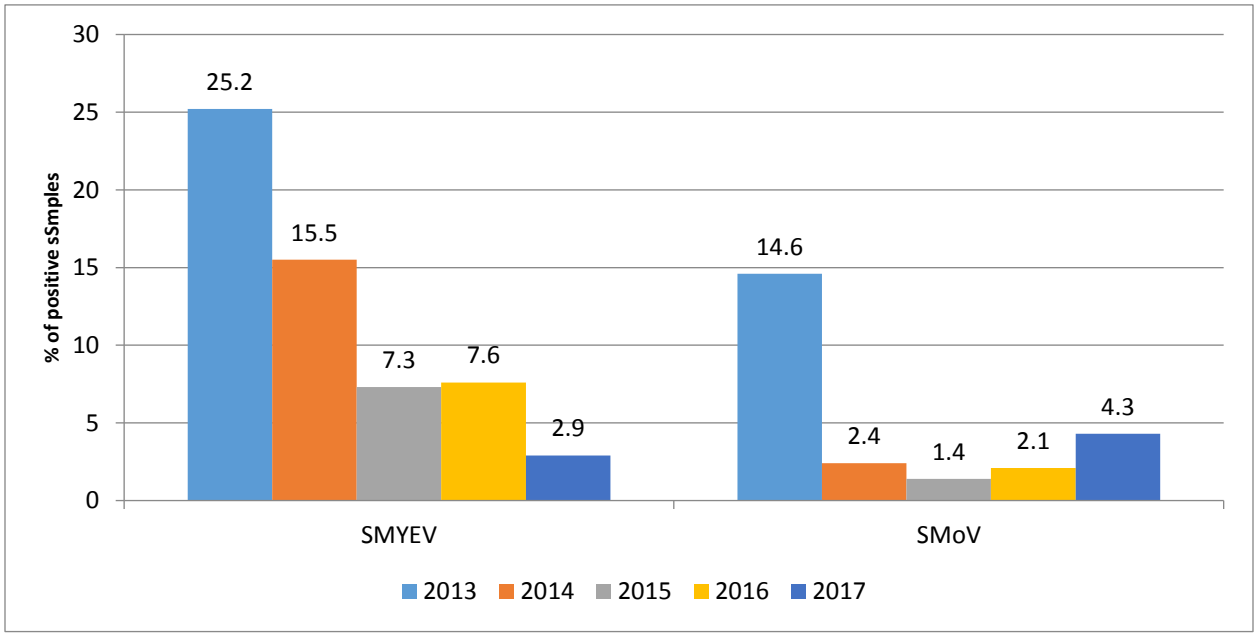


Figure 5. Infection levels (Strawberry Mild Yellow Edge Virus (SMYEV) and Strawberry Mottle Virus (SMoV)) of all newly planted fields with over 1000 strawberry plants from 2013-2017 (excludes nursery testing).

Raspberry Aphid and Virus Monitoring

Plots were set-up to monitor for large raspberry aphid on two farms in the Valley region of Nova Scotia. Despite weekly field scouting, few large raspberry aphids were detected in monitoring plots. The first sighting of large raspberry aphid in 2017 occurred in late June and correlated with sightings by a local pest management company.

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. Again in 2017, RYNV and RLMV were found on several farms throughout the Valley and Western regions of the province, in both new and older plantings.

Nursery stock virus testing

Testing of G4 strawberry nursery stock, previously referred to as "certified" stock, was conducted on the four 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 2017 according to Guidelines.

Additionally, certified raspberry stock was tested in fall 2017 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 2017 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 continued to show signs of virus level stabilization. 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 this year that virus levels were found to be significantly higher in carried over fruiting fields. If growers choose to carry-over fields for an additional year of picking 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. This program will further refine the monitoring program for large raspberry aphid to provide growers with accurate and timely notifications about vector presence.

References

Thekke-Veetil, T, and Tzanetakis, I.E. 2016. *Phytopathology.* 100:4 https://doi.org/10.1094/PDIS-09-15-1044-PDN

Xiang, Y., et al. 2015. *Arch. Virol*. 160:553. https://doi.org/10.1007/s00705-014-2267-0