Final Report

Aphid monitoring and virus testing in strawberries

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Background

An outbreak of two viruses, strawberry mild yellow edge virus (SMYEV) and strawberry mottle virus (SMoV), in Nova Scotia strawberries in 2012-2013 caused significant losses to both strawberry nursery and commercial fruiting operations. The commercial fruit industry was 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 $9 million, which was greatly threatened by viruses. 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.

To aid in the strawberry industry's recovery, Perennia Food & Agriculture and Horticulture Nova Scotia undertook a project to provide assistance in virus testing and monitoring of virus insect-vectors. This project encompassed three growing seasons from April 2014 until March 2017. As a result of this work, strawberry virus levels show initial signs of stabilization.

Project Objectives

The main objectives of the project were to aid in the management of the primary virus vector (i.e. the strawberry aphid, *Chitosiphon spp.)* and to ensure that nursery stock produced in the province met the virus recovery strategy requirements for commercial fruit growers. To meet these objectives the following activities were conducted:

1. Monitor for strawberry 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. Execute the 'virus testing protocol' as outlined in the *Guidelines for growing and inspecting strawberry plants in Nova Scotia*.
3. Conduct a late summer virus survey of all newly planted strawberry fields to assess the progress of virus management efforts in the province. (A similar survey was conducted in 2013 and data from this survey is a suitable benchmark for comparison).

A fourth activity emerged mid-way through Year 1 of the project with the verification of insect-vectored diseases in caneberries and highbush blueberry crops. As such, some project funds were reallocated for scouting and laboratory testing to verify the distribution of these diseases. A two-virus complex in raspberry and blackberry, Rubus Yellow Net Virus (RYNV) and Raspberry Leaf Mottle Virus (RLMV) are transmitted by the large raspberry aphid. They are individually symptomatic and collectively symptomatic, causing reduced yields, smaller leaves and berries, and chlorotic foliage. The stunt phytoplasma in highbush blueberry is spread by the 'sharp-nosed leafhopper' and is a debilitating disease that reduces growth and productivity over time.

Sampling and Monitoring Protocols

Monitoring Sites

The number of farms participating in the project varied from year to year depending on the willingness of cooperators, and ranged from 31 sampling sites on 24 farms to 26 sites on 22 farms. Each of the four

nurseries had two monitoring sites. Figure 1 shows the general distribution of these farms across Nova Scotia. Due to the high concentration of strawberry farms in the Annapolis Valley, nearly half of the farms monitored were within that region of the province (Figure 2). For all cooperating farms, the project sampling block utilized was a "fruiting" field or in the case of nursery, a single nursery block.

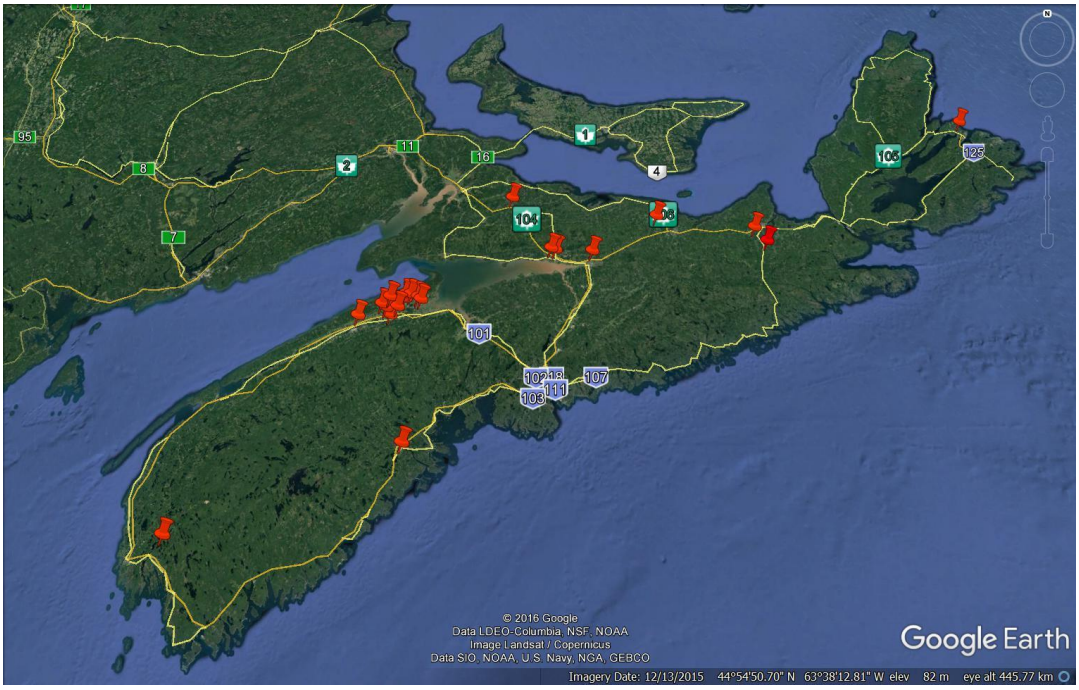

Figure 1 Map displaying the aphid monitoring sites across Nova Scotia through the course of the project.

Figure 2 Map displaying the aphid monitoring sites in the Annapolis Valley through the course of the project.

Aphid Monitoring

Aphid monitoring and sampling began in late April to early May of each year and continued until the end of October. Sampling was conducted by Perennia staff and in some locations, grower cooperators on a weekly basis and analyzed by Perennia student biologists.

Three aphid monitoring and sampling methods were employed on cooperating strawberry farms. These methods were as follows:

a. Early spring leaf monitoring for aphid 'egg' counts – 30 old horizontal leaves were collected randomly from monitoring plots and examined for aphid egg counts as an early season indicator of overwintered strawberry aphid populations in the study plots.
b. Leaf monitoring for 'wingless' and 'winged' vector numbers – 60 immature 'bud leaves' (folded or partially folded trifoliate) were collected randomly from each monitoring plot on a weekly basis throughout the growing season (Figure 3).
c. Yellow stocky traps monitoring for 'winged' vectors – 8-10 yellow sticky traps (6-8 along edges and 2 in the middle) were located in monitoring plots and examined for winged vector numbers, by species, throughout the growing season (Figure 4A).

Figure 3. From left to right, images of strawberry aphid eggs, wingless adults on a bud leaf and, aphid lifecycle.

Virus Sampling

The virus sampling methodology for fruiting fields was designed to identify the level of infection of newly planted strawberry fields. Samples were tested for strawberry mild yellow edge virus (SMYEV), the "canary" virus in Nova Scotia, and 10% of the total samples were tested for the secondary virus, strawberry mottle virus (SMoV).

Individual fields/blocks with more than 1000 plants were randomly sampled annually in late-August to mid-September. Following a zigzag pattern, the first fully expanded leaves in a rooted daughter plant were sampled for virus testing (Figure 4B). To test for SMYEV 60 trifoliate-leaf samples were collected in 20 bags (3 trifoliate-leaves per bag). Due to the excessive cost of analysis, only 2 bags (3 trifoliate-leaves per bag) were collected for SMoV testing.

Nursery stock was tested for virus according to the protocols outlined in the *Guidelines for growing and inspecting strawberry nursery plants in the province of Nova Scotia*. The aim of the nursery sampling protocol is to ensure 95% confidence of less than 2% SMYEV infection and zero SMoV infection.

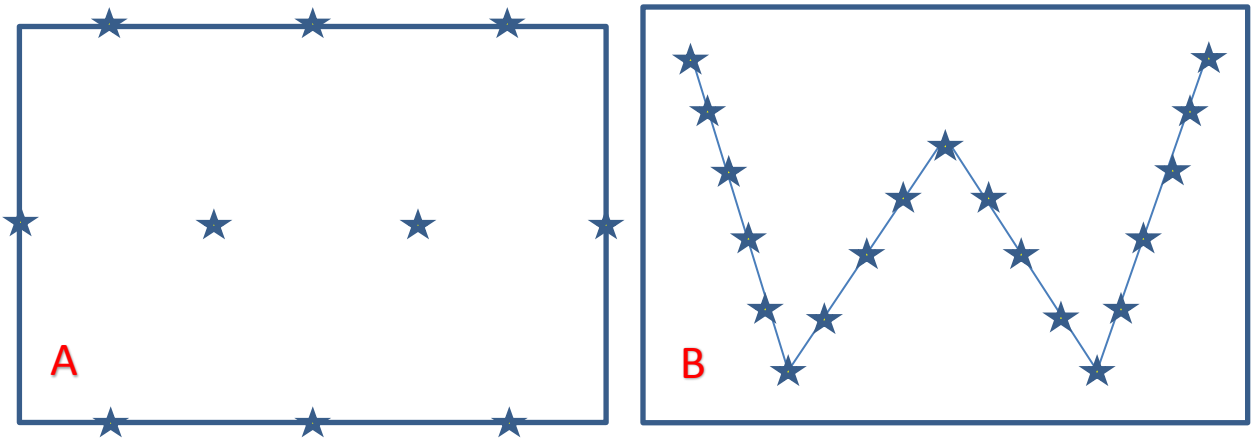

Figure 4. (A) Random leaf sampling for both aphids and virus followed a W pattern. (B) Configuration of yellow sticky traps in fields/ blocks.

Results

In 2013, the year immediately following the strawberry virus outbreak, a one-year project was initiated under the Canadian Agricultural Adaptation Program (CAAP) much like the one being reported on here. During the initial CAAP project, sampling and monitoring protocols were developed, which were slightly modified and employed in the current project. The results found during the CAAP project were used as a baseline for aphid and virus monitoring in this project, and are therefore referenced in the results.

Aphid Monitoring

Aphid monitoring plots were established in late-April/early-May annually on select farms across Nova Scotia to monitor three aphid forms – eggs, wingless (apterae), and winged (alatae) forms. The farms selected to participate were 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.

At plot set-up in each monitoring year and for a few weeks following, 30 leaf samples were collected for an egg count from each field. Results of the CAAP project in 2013 reported high levels of aphid eggs found in cooperating producer's fields. However, in the consecutive 2 years the overwintering population was found to be low throughout the province (Table 1). In 2016 a notable spike was observed, which was thought to be a result of a relatively mild fall and winter. In all project 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 course of the project.

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

The higher population of overwintered eggs found in spring 2016 was directly reflected in wingless aphid leaf counts by a population spike in June, as presented in Figure 5. In contrast, low leaf counts of wingless apterae were found in 2014 and 2015. The farms that were found to have high resident populations of strawberry aphids effectively managed the aphids, minimizing winged aphids.

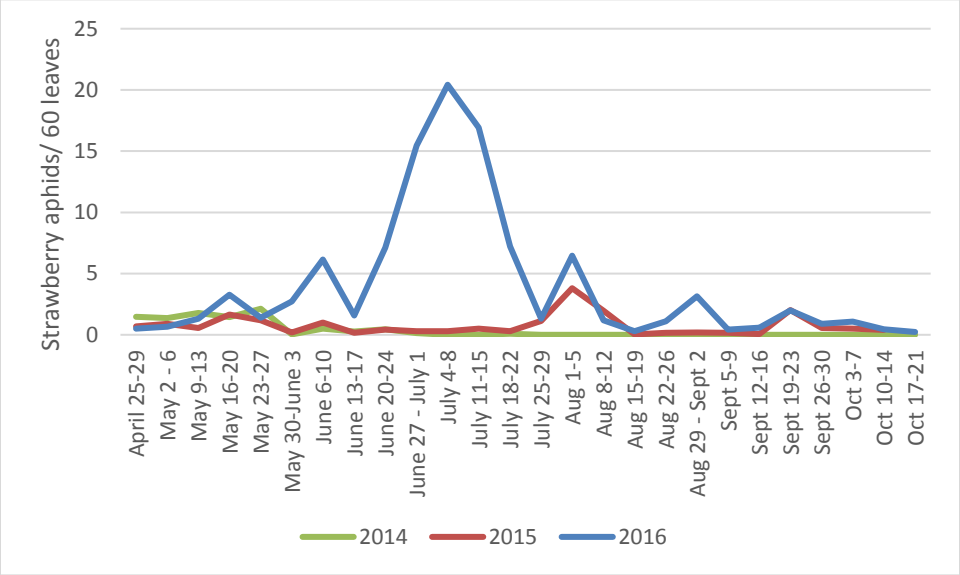

Figure 5. Average wingless strawberry aphid population from leaf counts on participating farms.

Compared to the first year of monitoring in 2013, all study years from 2014-2016 found low counts of winged strawberry aphid. It has been shown that when winged aphids are found, it is an indication of a well-established aphid population in the crop (Rondon and Cantliffe. 2005. http://edis.ifas.ufl.edu). Therefore, it was not surprising to see few winged aphids given the low numbers of wingless aphids. Not only were there fewer strawberry aphids in 2014-2016 compared to 2013 but they represented a much smaller fraction of the total aphid population, as shown in Table 2. Despite these lower numbers, it is important to continue monitoring for aphids and not overlook the importance of non-resident aphids arriving by jet stream or on imported plants. The risk of aphids travelling from the United States, Ontario, or Quebec into Nova Scotia is that they may carry alternative viruses not yet found in Nova Scotia.

Table 2. Total winged strawberry and other aphid counts throughout the monitoring period in the two monitoring regions of Nova Scotia, 2013 to 2016.

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

It was observed in all monitoring years that total aphid flight started in late-May and died out by the end of September (Figure 6). The only year that strawberry aphid flight was apparent was in 2014, which followed a similar trend to that observed in 2013. As depicted in Figure 7, strawberry aphid flight lasted for approximately 6 weeks beginning in early June. In all other monitoring years the flight period for strawberry aphid could not be deduced as monitoring counts were insufficient to clearly identify its initiation and termination. Throughout the course of the project it became very clear that there is little to no pattern in aphid populations in a commercial production field. It is thought that this variability is correlated to effective integrated pest management and weather conditions during both the winter and growing season. The fluctuation and unpredictability in flight increases the need for monitoring aphid populations, so as to adequately alert farms when to control aphids.

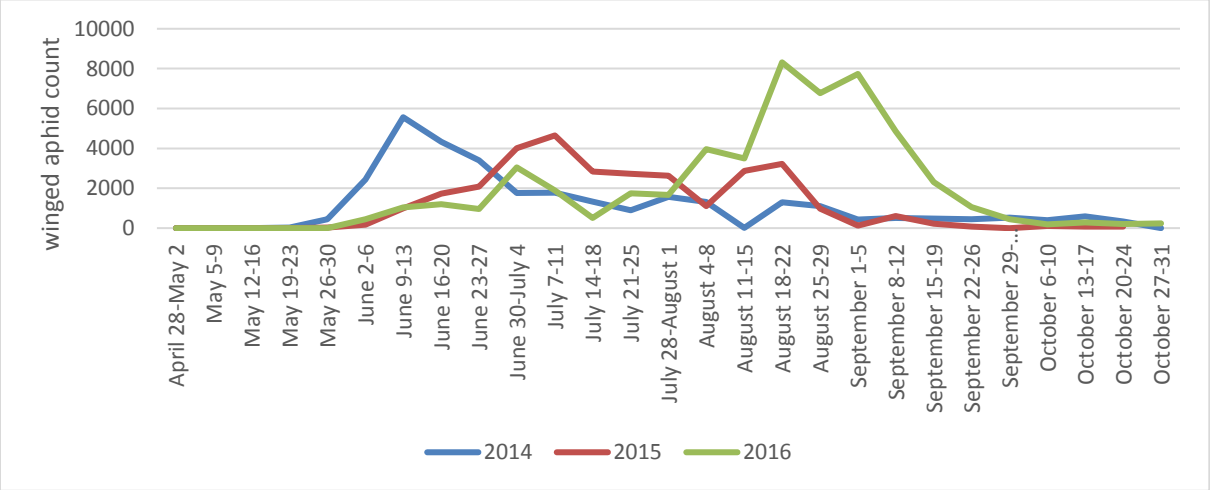

Figure 6. Cumulative counts of total winged aphids (yellow sticky traps) on all participating farms.

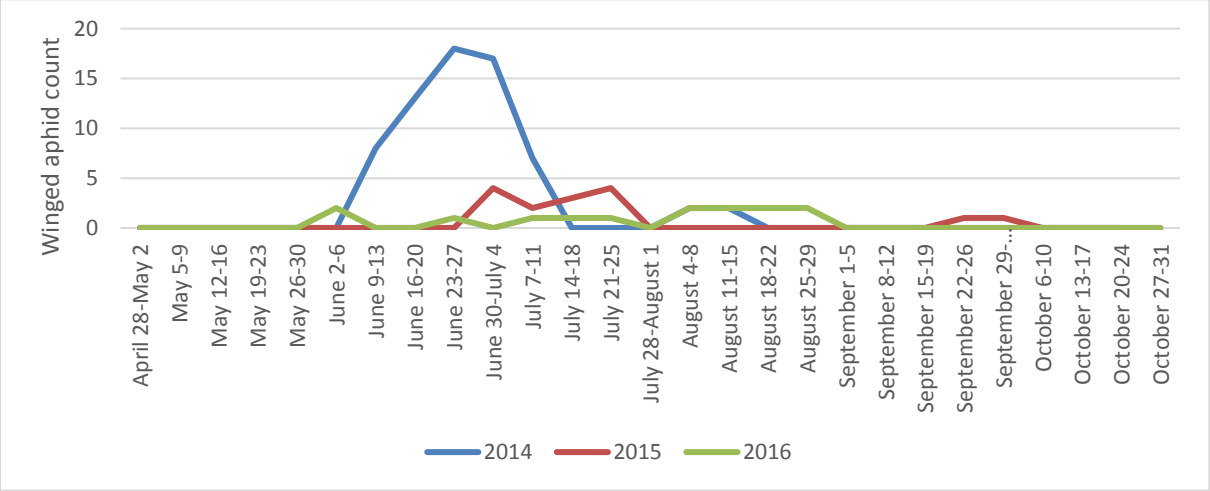

Figure 7. Cumulative counts of winged strawberry aphids (sticky traps) on all participating farms.

In 2014, the melon aphid (*Aphis gossypii*), a secondary vector for SMoV, was confirmed to be distributed across the province in numbers comparable to that of the primary vector, strawberry aphid. However, further study in 2015 indicated that melon aphid was very difficult to distinguish from other *Aphis* species and that the 2014 results may have overestimated the importance of it as a contributor to the virus epidemic. Due to the difficulty of confidently identifying this vector it was decided to focus only on the primary vector, the strawberry aphid for the remainder of the project.

Regular monitoring updates were communicated by email to the general industry and other stakeholders. Moreover, individual monitoring results were communicated to the 25 cooperating farms on a weekly basis to optimize their aphid management. The primary method of aphid control was the use of insecticides. By alerting growers to the presence of aphids, they were able to effectively target sprays at the appropriate time. Effective and timely control of the strawberry aphid was essential due to the aphids' ability to develop large populations in a short period of time (Rondon and Cantliffe. 2005 http://edis.ifas.ufl.edu). As a result of diligent and effective monitoring and spray programs, strawberry aphids were limited in their ability to vector SMYEV and SMoV.

Benchmark Virus Testing 2014-2016

Benchmark testing of newly planted strawberry fields was first conducted in late summer of 2013 and repeated at a similar time in 2014, 2015, and 2016. The purpose of this sampling was to monitor virus level trends and the recovery progress. In 2013 the levels of the two viruses, SMYEV and SMoV, were 25% and 15% respectively but dropped to 15% and 2.5% respectively in 2014. This trend continued in 2015 with a further drop of SMYEV infections to 7.3% and SMoV to only 1.4% of total samples collected (Figure 8). This is excellent evidence of the success of the recovery program and is further supported by the exceptional crop reported for the 2015 growing season. Although, there was a slight increase in 2016 of virus levels, it does appear that virus infection levels are showing signs of stabilization.

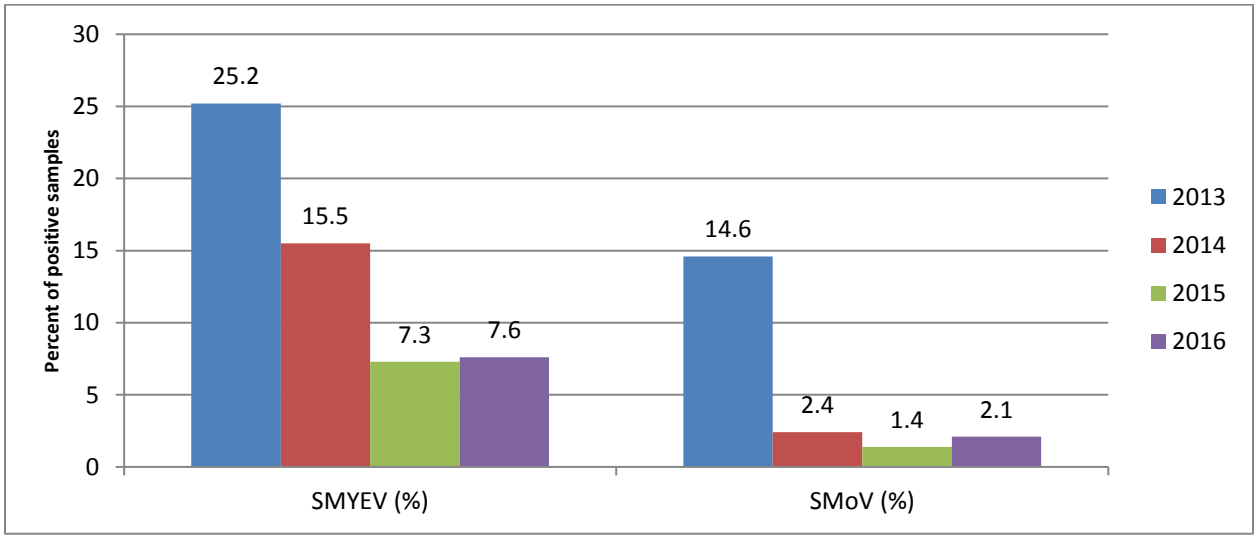

Figure 8. Infection levels (Strawberry Mild Yellow Edge Virus (SMYEV) and Strawberry Mottle Virus (SMoV)) of all newly planted fields with over 1000 strawberry plants throughout the duration of the project (excludes nursery virus testing).

Virus Testing of G4 Nursery Stock

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 SMYEV and zero tolerance for SMoV was executed in all monitoring years.

Additionally, certified raspberry stock was tested in the fall of 2015 and 2016 for the two raspberry viruses (RYNV and RLMV) identified in commercial fruiting fields and found to be clean.

Raspberry Virus Complex and Blueberry Stunt Phytoplasma

In addition two plots were set-up to monitor for raspberry aphid and sharp-nosed leafhopper respectively, on two additional farms. In addition, several other farms were tested in 2015 within the Valley for both the raspberry and highbush blueberry insect-vectored diseases. This testing confirmed that they are both relatively widespread in the Annapolis Valley. In 2016, additional surveys were conducted across Nova Scotia, confirming that the viruses are not confined to the Valley. The key to avoiding spread and increase in future will be the execution of competent monitoring programs for the vectors as has been successfully demonstrated for the strawberry viruses. To this end, monitoring plots were established in the two raspberry nurseries and in infected farms during 2015 and 2016 to help optimize vector management and to add to the knowledge of these pests.

Summary

Monitoring results clearly indicated that numbers of the primary disease vector, the strawberry aphid, remained quite low in 2014 through 2016 after relatively high populations were observed in 2013. It can be speculated that effective management and perhaps two difficult winters are the most probable explanations. Clearly there was a flight as there were low levels of infections found in newly planted fields but it appeared that the yellow sticky traps used for monitoring the winged form of strawberry aphid were unreliable as a scouting tool, particularly when aphid populations were low. In future studies, bud leaf counts will be used as the primary indicator of aphid presence and populations.

Interprovincial contributions to the strawberry aphid population via jet stream and imported plant product appeared to be minimal. This is encouraging, given that several additional viruses are being reported across North America, causing concern that they may be transmitted to Nova Scotia by in-flying alatae. In saying that, there is one additional virus (strawberry polerovirus-1) that has recently been detected in Nova Scotia strawberry fields. If future monitoring programs were to be conducted, this virus would be added to the testing regime.

Virus testing of newly planted fields conducted in late-summer/early-fall has shown a significant drop in new infections from the 2014 season, to the extent that a normal crop forecast is again being made for the coming growing season. Strawberry mild yellow edge virus, the more common of the two viruses causing the epidemic of 2012/2013, still remains at a level above the 5% target threshold and efforts will continue to further reduce levels of this virus. Although strawberry mottle virus was found to be at very low levels overall, it persists in pockets on several farms and efforts will be made to reduce it further by targeted monitoring and management in the coming growing season.

The success of the virus recovery program to date is a direct results of the diligent efforts of growers and the virus management strategy developed by John Lewis, which included:

- Reduce inoculum by removing older strawberry fields that are infested with viruses.
- Use clean planting stock from an accredited plant grower, and isolate new fields from sources of virus such as older fields, and wild strawberries.
- Monitor for insect-vectors – aphids and whiteflies.
- Control strawberry aphids during their flight period.

Finally, efforts will be continued to assess and monitor for the two new insect-vectored diseases, a two-virus complex in raspberries spread by the large raspberry aphid, and a phytoplasma disease in highbush blueberries spread by the sharp-nosed leaf hopper.