

## **Diagnostic optimization of viral detection and characterization for the Michigan seed potato certification program, 2019**

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Potato virus Y (PVY) is one of the primary diseases monitored and tested for in the seed certification process and is one of the top Michigan potato industry research priorities. The industry has expressed interest for in-state screening of tuber testing for seed certification, however, current protocols for viral testing in Michigan are both time and labor intensive. Current methods rely on winter grow out assays which detect viruses in sprouts or plantlets after breaking tuber dormancy. While effective, the current seed certification staff and facilities are limited by the time and space necessary to process large quantities of samples. To enable the Michigan Department of Agriculture and Rural Development (MDARD) to increase handling capacity and efficiency, the Potato and Sugar Beet Pathology (PSBP) program proposed to assist in optimizing the viral detection and diagnostic protocols used in winter testing. Through this work we: 1) investigated improved detection options to identify accurate, timely, and cost-effective methods for use in the Michigan seed potato certification and 2) monitored PVY strain prevalence in Michigan seed potatoes. The results of this work will help develop standard protocols for high-throughput, in-state tuber testing. Future studies will focus on improving virus and vector management in Michigan potato crops.

### **Materials & Methods:**

Tuber testing methods which do not require breaking tuber dormancy to sample from resulting sprouts or plantlets were investigated. General (Mackenzie et al. 2015) and multiplex (Lorenzen et al. 2006) reverse-transcriptase (RT) high-fidelity polymerase chain reaction (PCR) protocols were tested and compared to existing plantlet assays involving enzyme-linked immunosorbent assay (ELISA). Four samples of 300-400 tubers each taken from a single seed potato lot with high levels of visual foliar symptoms of PVY in the field. Samples were divided into 10-tuber subsamples (N=number of subsamples) and subjected to the following tests: 1) dormant tuber (RT-PCR), 2) standard Michigan grow out with leaflets (ELISA), 3) dormant tuber (RT-PCR) and standard grow out (ELISA), and 4) standard Hawaii grow out with leaflets (ELISA). ELISA and RT-PCR methods were compared in leaflets from samples 2, 3, and 4. Sensitivity, accuracy, and agreement of the various methods, as well as the time and labor costs involved, were compared with existing methods. Strain prevalence was determined using preliminary observations collected from nine PVY positive lots (19 subsamples, 265 individual plants) tested in 2018-19. In 2019, testing will be conducted using five seed potato lots. Positive samples will be subjected to strain analysis.

### **Results & Conclusions:**

Dormant tuber and standard grow out methods both identified high levels of PVY (75-87%) in tested subsamples (Table 1). Michigan and Hawaii grow out tests were very similar, 87 and 89% positive for PVY respectively. Testing is in-progress for test method 3, where the tubers and leaflets of the same plants were tested using different methods. Cost estimates are being developed based on these experiments.

**Table 1.** Comparison of ELISA and RT-PCR results based on positive PVY detections (%) using dormant tuber and standard leaflet grow out methods (in-progress).

#	Test method	N	ELISA (+)	RT-PCR (+)
1	Dormant tubers	32	NA	<b>75%</b>
2	Standard leaflet grow out	32	<b>89%</b>	(in-progress)
3	Dormant tubers <u>and</u> leaflet grow out	36	NA; <b>44% (in-progress)</b>	<b>64% (in-progress);</b> (in-progress)
4	Hawaii leaflet grow out	53	<b>87%</b>	(in-progress)

In 2018, 12% of plant samples tested positive for PVY (strains O, N:O, N-Wi) and 2% tested positive for PVY<sup>N</sup> (strains N, NTN). A subset of positive samples from five lots were sent for testing (Karasev); 90% of samples were positive for PVY<sup>N-Wi</sup> and 10% were positive for PVY<sup>N:O</sup>. In 2019 testing, the PVY strain types from potato tubers and leaves were successfully identified using a multiplex RT-PCR. As in 2018, the majority of tested samples were positive for PVY<sup>N:O/N-Wi</sup>, however, testing is ongoing.