Diagnostic optimization of viral detection and characterization for the Michigan seed potato certification program, 2020

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Potato virus Y (PVY) is a major concern throughout the US, including the North Central region, and is one of the primary diseases monitored and tested for in the seed certification process. Cost-effective and efficient detection of PVY in early generation potato seed lots will help prevent infected material from entering the production chain and will prevent unnecessary yield and profit loss. Since 2018, Michigan Department of Agriculture and Rural Development (MDARD) and MSU Potato and Sugar Beet Pathology (PSBP) have been collaborating to increase handling capacity, efficiency and optimizing the viral detection and diagnostic protocols used in winter testing. Through this work we continue to: 1) investigate improved detection options to identify accurate, timely, and cost-effective methods for use in the Michigan seed potato certification and 2) monitor PVY strain prevalence in Michigan seed potatoes. The results of this work will help develop standard protocols for high-throughput, in-state tuber testing.

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, 2010; Chikh-Ali et al. 2013) reverse-transcriptase (RT) high-fidelity polymerase chain reaction (PCR) protocols were compared to existing plantlet assays involving enzyme-linked immunosorbent assay (ELISA). In 2019, four samples of tubers were taken from a single seed potato lot with high levels of visual foliar symptoms of PVY in the field (4.9%). Samples were divided into 10-tuber 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). Sensitivity, accuracy, and agreement of the various methods, as well as cost of each test, were compared with existing methods.

In 2020, samples of 400 tubers were collected from seed lots with three levels of visual foliar symptoms of PVY in the field. We investigated high (0.9%), medium (0.23%) and low incidence (0.01%) lots and conducted RT-PCR tuber testing. All of the tubers were tested then planted and grown out for standard leaflet ELISA. Subsets of positive samples will be subject to PVY strain confirmation by RT-PCR.

Results & Conclusions:
In 2019, both dormant tuber and standard grow out methods identified high levels of PVY (12.9-100%) in in a visually high-incidence lot (Table 1). In treatment 3, where the tubers and leaflet grow outs of the same plants were tested using different methods, 30.1 and 16.4% PVY was detected, respectively. The majority of tested samples were positive for PVY^{N-Wi} (Figure 1). Overall, all tested methods validated high-incidence visual inspection results (4.9%) and would result in rejection of this lot for certification.

In 2020, more than 90% of tested samples were positive for the PVY strain N:Wi, however, N:O, NTN, and O strains were also present (Figure 1). Dormant tuber methods validated summer and winter visual inspection results for the high-PVY lot (Table 2). However, our methods detected higher levels of PVY in the low and medium lots than estimated from the summer field inspections (though more similar to the winter visual). This could be due to in-field spread, variety differences, strains differences (Figure 1), or variety by strain interactions. Overall, our results suggest that PVY^{N-Wi} is the prevalent strain in Michigan, and dormant tuber testing is a viable and informative option for our seed certification program.

Table 1. Comparison of ELISA and RT-PCR results from a seed lot assessed at 4.9% visually positive for PVY in the field. Results are based on positive PVY detections (%) using dormant tuber and standard leaflet grow out methods in 2019 (N=number of 10-tuber subsamples tested).

#	Test method	N	ELISA (%)	RT-PCR (%)
1	Dormant tuber	32	-	**12.9**
2	Leaflet grow out	14	**23.2**	**38.9**
3	Dormant tuber and leaflet grow out	36	- **16.4**	**30.1** **>27.8**[a]
4	Hawaii leaflet grow out – tested in MI	53	**18.3**	-
5	Hawaii leaflet grow out – tested in HI	56	**15.8**	-

[a] All 10-tuber subsamples tested positive in this sample. Result adjusted to better reflect actual PVY incidence.

Table 2. Comparison of ELISA and RT-PCR results from seed lots assessed for high, medium, and low incidence based on field inspections. Results are based on positive PVY detections (%) using dormant tuber and standard leaflet grow out methods in 2020 (N=number of 10-tuber subsamples tested).

Sample	Gas	N	Visual Summer (Jun-Jul)	Visual Winter (Jan)	Dormant Tuber RT-PCR[a] (Oct-Nov)	Leaflet ELISA Greenhouse[a] (Jan)	Leaflet ELISA Field – Florida (Jan)
High	-	24	0.90	>25.0	**>27.2**[b]	**29.6**	-
High	+	24	0.90	>25.0	**>27.2**[b]	**22.4**	-
Medium	+	40	0.23	4.40	**6.70**	**5.62**	**3.98**
Low	+	40	0.01	0.45[c]	**1.61**	**1.12**	**1.84**

[a] Dormant tuber RT-PCR and leaflet ELISA greenhouse results from the same 10-tuber subsamples.
[b] All 10-tuber subsamples tested positive in this sample. Result given for greater than 23 out of 24 subsamples to better reflect actual PVY incidence.
[c] Symptoms were very mild in the field and actual PVY incidence was suspected to be higher than 0.45%.

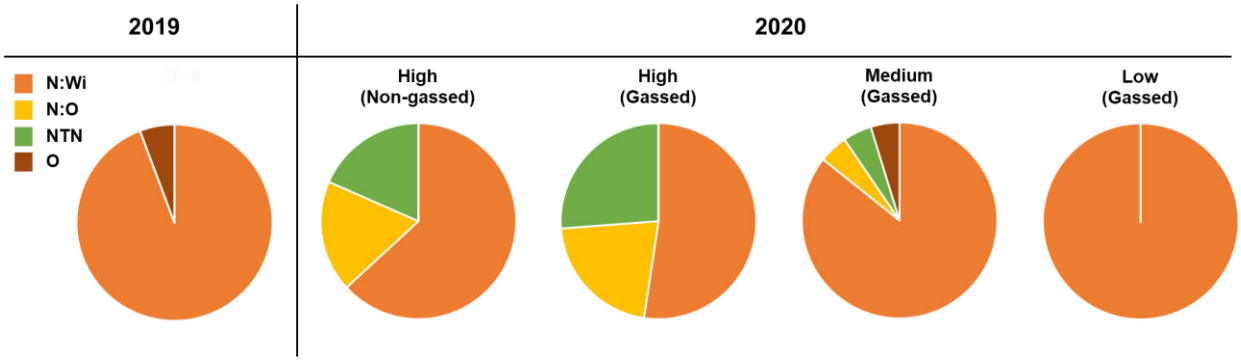

Figure 1. PVY strains present in seed certification pathology experiments conducted in 2019 and 2020.