Diagnostic optimization of viral detection and characterization of Potato virus Y for the Michigan seed potato certification program, 2022

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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. The MSU Potato and Sugar Beet Pathology (PSBP) program continues to work with the Michigan Department of Agriculture and Michigan Seed Potato Association seed inspectors to: 1) investigate improved detection options to identify accurate, timely, and cost-effective methods for use in Michigan seed potato certification, 2) monitor PVY strain prevalence in Michigan seed potatoes, and 3) investigate PVY strain by chipping potato variety responses.

Materials & Methods:
Tuber testing methods, which do not require breaking tuber dormancy to sample sprouts or plantlets, were used. 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 will be compared to existing plantlet assays involving enzyme-linked immunosorbent assay (ELISA) to validate. In 2022, we selected six seed lots for validation of dormant tuber methods. Samples of 200 tubers were taken from each seed lot. Each variety was sampled from two lots where visual PVY was either present or absent in summer field inspections (Table 1). Dormant tuber RT-PCR testing was conducted on all tubers in 10-tuber subsamples. After treatment with Rindite to break dormancy, subsamples were planted and grown out for standard leaflet ELISA. Subsets of positive samples (from research and commercial testing) will be subject to PVY strain confirmation by RT-PCR.

We also conducted growth chamber assays using characterized PVY strains with elite potato germplasm using previously reported methods by Gundersen et al. (2019). Based on our assessment of PVY strain populations in Michigan, we have selected four characterized strains (N:Wi, NTN, N:O, O) and obtained them from collaborators at the University of Idaho. Six varieties were selected for preliminary experiments: Snowden, Lamoka, Mackinaw, Lady Liberty, Petoskey and MSZ242-13. These entries represent current chip varieties used in Michigan and elite experimental varieties originating from the MSU Potato Breeding and Genetics program.

Results & Conclusions:
In 2022, dormant tuber methods identified higher levels of PVY than estimated from the summer field inspections in selected research lots, however, absence and presence of virus observed was equivalent between methods (Table 1). This could be due to in-field spread, varietal expression, strain differences, or variety by strain interactions. Currently, we are repeating validation experiments to verify agreement between dormant tuber and standard grow out methods.

Table 1. RT-PCR results from seed lots assessed for PVY incidence based on summer field inspections. Results are based on positive PVY detections (%) using dormant tuber methods in 2022 (N=number of 10-tuber subsamples tested).

Variety	Typical Symptom Expression	N	Visual Summer (Jun-Jul)	Present (+) Absent (-) (Jun-Jul)	Dormant Tuber RT-PCR (Oct-Nov)	Leaflet ELISA Greenhouse (Jan)
A	Unreliable	20	0.04	+	6.70	*In progress*
A	Unreliable	20	0.00	-	0.00	*In progress*
B	Unreliable	20	0.20	+	14.87	*In progress*
B	Unreliable	20	0.00	-	0.00	*In progress*
C	Reliable	20	0.76	+	9.97	*In progress*
C	Reliable	20	0.00	-	0.00	*In progress*

We continued to assess the strain types prevalent in Michigan seed growing regions (N = approx**.** 7,150 tubers tested in 2022-23). In 2022-23 dormant tuber tests, three major PVY strains were detected, and strain O was not observed this year. Observations from the past three years suggest that $PVY^{N\text{-}Wi}$ remains most prevalent, however, PVY^{NTN}, $PVY^{N:O}$ and PVY^{E} continue to be detected. Strains NTN and E are tuber necrotic strains, and their frequencies must be closely monitored to best inform the seed potato industry of potential risks. We also detected one case of Tobacco rattle virus (TRV) from variety trials samples during field season.

In preliminary experiments, potato variety responses of daughter plants were measured after mechanical infection of mother plants with four PVY strains. We observed mild to severe foliar symptom depending on strain and variety. Across varieties, reductions in total tuber weight relative to the mock-inoculated control were observed for all PVY strains and greatest (mean of 20% reduction) in plants infected with strain NTN (Figure 1). Growth chamber space and conditions limited the quality and number of plants assessed and will require further optimization and repeated experiments. Dormant tuber tests of daughter tubers showed average 91.7% (range of 85.7 to 100%) of tubers originating from PVY-infected mother plants were infected with virus except resistant varieties, Mackinaw and Lady Liberty, where no detectable levels of virus were observed in daughter tubers.

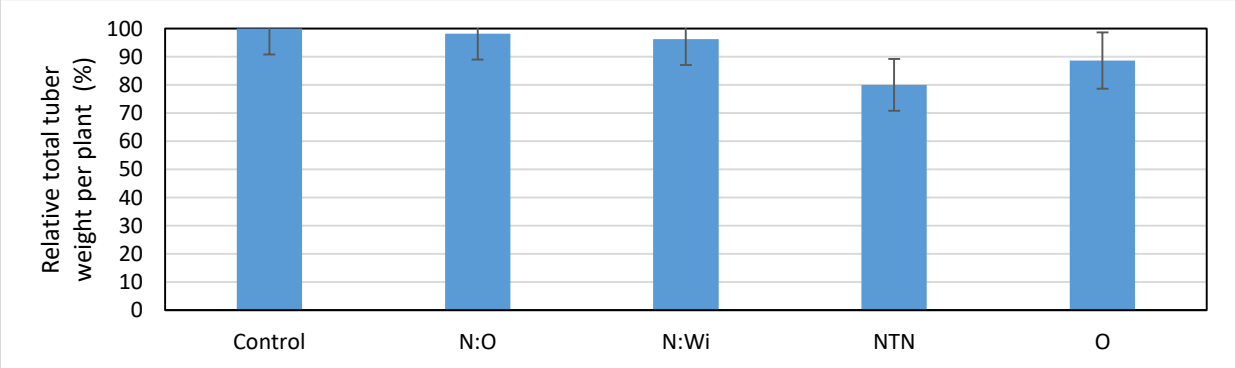

Figure 1. Total tuber weight per plant for mechanically-inoculated first-generation mother plants infected with PVY strains N:O, N:Wi, NTN, and O relative to the mock-inoculated control. Means across six chip potato varieties: Lady Liberty, Lamoka, Mackinaw, MSZ242-13, Petoskey, and Snowden. Bars represent two preliminary replicate plants and error bars represent standard error.

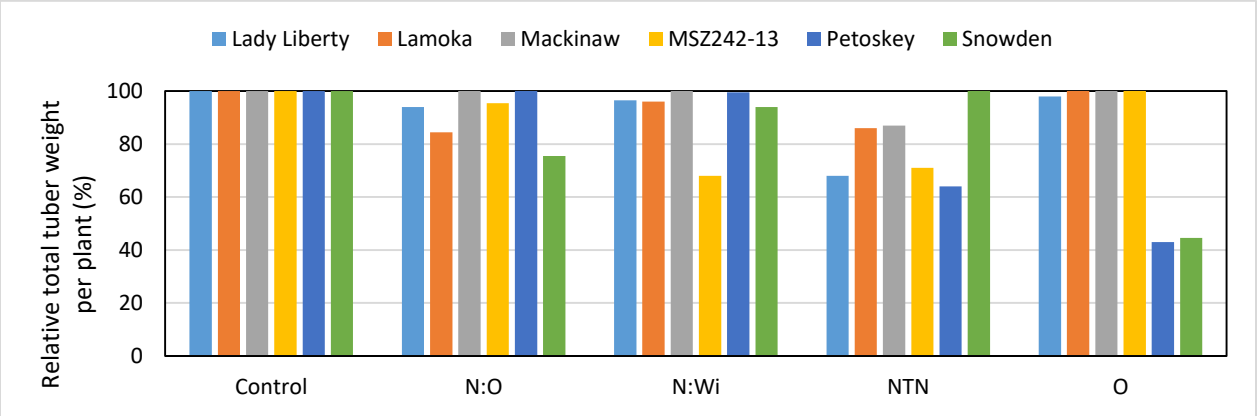

Figure 2. Total tuber weight per plant of mechanically-inoculated first-generation mother plants infected with PVY strains N:O, N:Wi, NTN, and O relative to the mock-inoculated control. Means shown for six chip potato varieties: Lady Liberty, Lamoka, Mackinaw, MSZ242-13, Petoskey, and Snowden. Bars represent two preliminary replicate plants.

In preliminary experiments, yield of some varieties appear generally less impacted by PVY infection of mother plants, such as Mackinaw, while others appear more sensitive to certain strains such as MSZ242-13 to strains N:Wi and NTN, Petoskey to strains NTN and O, and Snowden to strain O (Figure 2). Second-generation observations indicate infected Lamoka and Snowden seed resulted moderate to severe foliar symptoms for strains O and N:Wi whereas Mackinaw showed minimal symptoms (Figure 3, next page). Strain by variety experiments will inform the seed potato industry of the yield and quality impacts that current PVY strains may have on prevalent chip varieties. We will repeat and plan to increase the number of varieties and modify the strain panels used based on current growing practices and strain population assessments. This information will also confirm robust PVY resistance to multiple strains, further informing and directing future breeding efforts.

Overall Summary:

Dormant tuber methods continue to agree with relative absence and presence observations made in summer field inspections and offer an option for seed certification testing where results are available 3-4 months sooner than the typical winter grow out. Final validation experiments are in progress. Coordination between MSU, MDARD, and MSPA has enabled regular monitoring of Michigan PVY strains and indicate strain N-Wi remains most prevalent, however, tuber necrotic strains NTN and E, and other tuber necrotic viruses, require further monitoring. Furthermore, preliminary results of variety by strain screening efforts suggest tuber yield impacts and foliar symptoms may be observed in seed infected with common Michigan strains.

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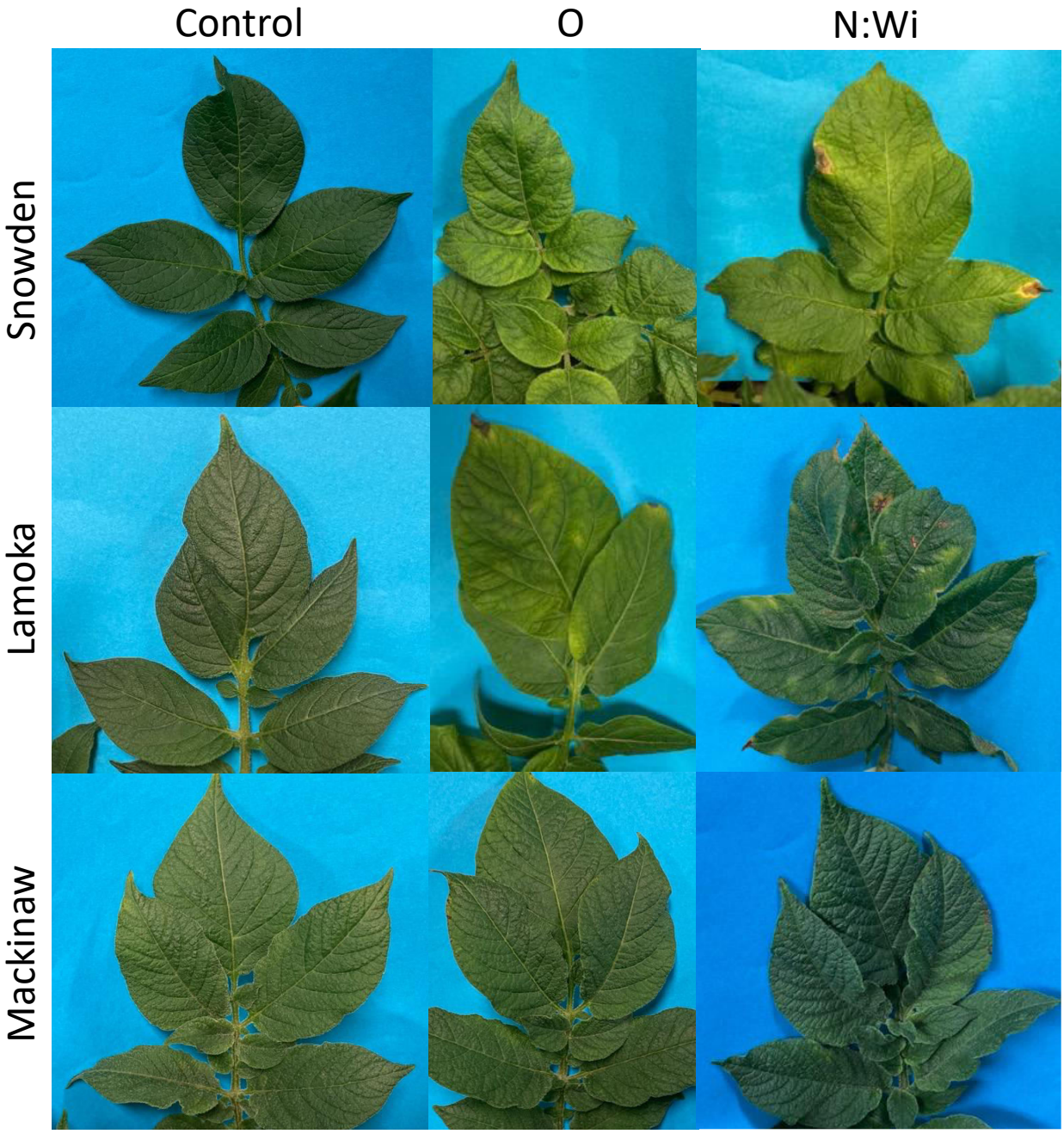

Figure 3. Foliar symptom images from second-generation daughter plants following grow out from mechanically-inoculated first-generation mother plants infected with PVY strains O, N:Wi and mock-inoculated control. Three potato varieties: Snowden, Lamoka and Mackinaw. Images are not scaled.