

Diagnostic optimization of viral detection and characterization of Potato virus Y for the Michigan seed potato certification program, 2023

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The MSU Potato and Sugar Beet Pathology (PSBP) program continues to work with the Michigan Department of Agriculture and Michigan Seed Potato Association 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 and other tuber necrotic virus prevalence in Michigan seed potatoes, and 3) investigate PVY strain by chipping potato variety responses.

Materials & Methods:

In 2023, we selected two seed lots for validation of direct tuber testing methods (Chikh-Ali et al. 2013; Lorenzen et al. 2006, 2010; Mackenzie et al. 2015). Samples of 400 tubers were taken from each seed lot. Positive samples from three additional lots were tested to further confirm accuracy. Direct tuber RT-PCR testing was conducted in 25-tuber subsamples. After treatment with Rindite to break dormancy, subsamples were planted and grown out for standard leaflet ELISA. Results from summer visual inspection, direct tuber, and leaflet ELISA methods were compared (Table 1). Subsets of positive samples (from research and commercial testing) will be subject to PVY strain confirmation by RT-PCR.

We also are repeating assays to assess PVY strain by variety responses (Gundersen et al. 2019). Based on Michigan survey observations, four strains (N:Wi, NTN, N:O, O) were obtained from collaborators at the University of Idaho. Six varieties were selected for repeat growth chamber experiments: Snowden, Lamoka, Mackinaw, Lady Liberty, Petoskey, and MSZ242-13 (Dundee). In 2023, three varieties were added, MSW474-1, NY163, and Manistee, and screened using three Michigan PVY isolates (N:Wi, NTN, N:O) in a greenhouse assay. These entries represent current chip varieties used in Michigan and elite experimental varieties originating from the MSU Potato Breeding and Genetics program. These experiments are currently in progress for 2023-24.

Results & Conclusions:

Validation of direct tuber testing methods

In 2023, our experiments indicated 100% accuracy between positive samples from direct tuber tests and positives from leaflet ELISA tests. In addition, there was 90% agreement in these methods for the extra lot samples tested. This year, the conventional leaflet ELISA methods identified higher PVY incidences than corresponding direct tuber tests. The qPCR thresholds used will be re-evaluated and adjusted accordingly. In-progress are several tests evaluating immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) (Chikh-Ali and Karasev, 2015) methods used by Montana, Idaho and Wisconsin certification programs. These methods will be further compared for accuracy, efficiency, and cost for adoption in Michigan.

Table 1. RT-PCR and ELISA results from seed lots assessed for PVY incidence based on summer field inspections. Results are based on positive PVY detections (%) using direct tuber methods in 2023 (N=number of 25-tuber subsamples tested).

Variety	Typical Symptom Expression	N	Visual Summer (Jun-Jul)	Direct Tuber RT-PCR (Oct-Nov)	Leaflet ELISA Greenhouse ^a (Jan)
A	Reliable	16	0.00	0.53	1.16
B	Reliable	16	0.00	1.86	2.84

^aLeaflet ELISA test was performed 10 leaf subsamples (N=40).

Prevalence of PVY strain types in Michigan seed growing regions

In 2023-24, PVY strains were characterized from approximately 10,200 tubers tested in 2023-24 (Figure.1). Observations from the past five years suggest that PVY^{N:Wi} remains most prevalent, however, PVY^{NTN} continues to be detected and we observed one sample with co-infection of PVY^{N:Wi} and PVY^O strains this year. NTN and E, as well as some N:Wi isolates, are known tuber necrotic strains. Thus, their frequencies must be closely monitored. Strain typing in leaf material received from Hawaii is currently in progress. Survey of seed lots for tuber necrotic viruses Potato mop-top virus (PMTV) and Tobacco rattle virus (TRV) in Michigan is ongoing.

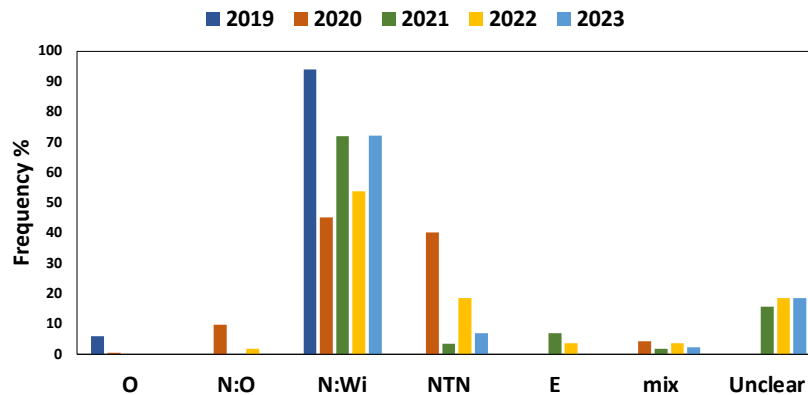


Figure 1. Representative PVY strains collected from Michigan potato seed certification program postharvest tests. In 2020-21, N = 212 positives in 17,752 total samples. In 2021-22, N = 57 positives in 21,600 total samples. In 2022-23, N= 54 positives in 7,150 total samples. IN 2023-24, N= 62 positives in 10,200 total samples. *In 2023, nine positive samples were included as N:Wi; Chikh-Ali et al. (2013) primers indicated suspect N:Wi but confirmation using Lorenzen et al. (2006) multiplex primers did not distinguish between N:O and N:Wi strains.

Screening of PVY strain x variety responses

In repeat bioassay experiment, potato variety responses of daughter plants were measured after mechanical infection of mother plants with four PVY strains for growth chamber and three strain for greenhouse assay. We observed mild to severe foliar symptoms depending on strain and variety. Across varieties, reductions in total tuber weight relative to the mock-inoculated control were observed with N:Wi strains reaching 23% and 38% in growth chamber and greenhouse assays, respectively. Direct tuber tests of daughter tubers showed an average 94.2% (range of 83.0 to 100%) of positive tubers originating from PVY-infected susceptible mother plants. In resistant varieties, Mackinaw and Lady Liberty, no detectable levels of virus were observed in daughter tubers.

In these experiments, yield of PVY-resistant varieties, Mackinaw and Lady Liberty, appear less impacted by seedborne infection while others appear more sensitive to certain strains (e.g., Snowden and Lamoka to N:Wi and NTN, Petoskey and Snowden to strain O, and NY163 to strain N:Wi and NTN). Confirmation of these observations is in progress, and will identify PVY resistance to multiple strains, further informing variety selection and breeding efforts.

Overall Summary:

- Direct tuber methods continue to agree with grow out methods. These tools aim to complement observations made in summer and winter field inspections and to offer a rapid option for use in seed certification testing, particularly in latent varieties, and early decision making.
- Observations from the past five years suggest that PVY^{N:Wi} remains most prevalent, however, tuber necrotic strain PVY^{NTN} also continues to be detected.
- Bioassay results of variety by strain screening efforts suggest tuber yield impacts and foliar symptoms may be observed from seed infected with common Michigan strains.

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