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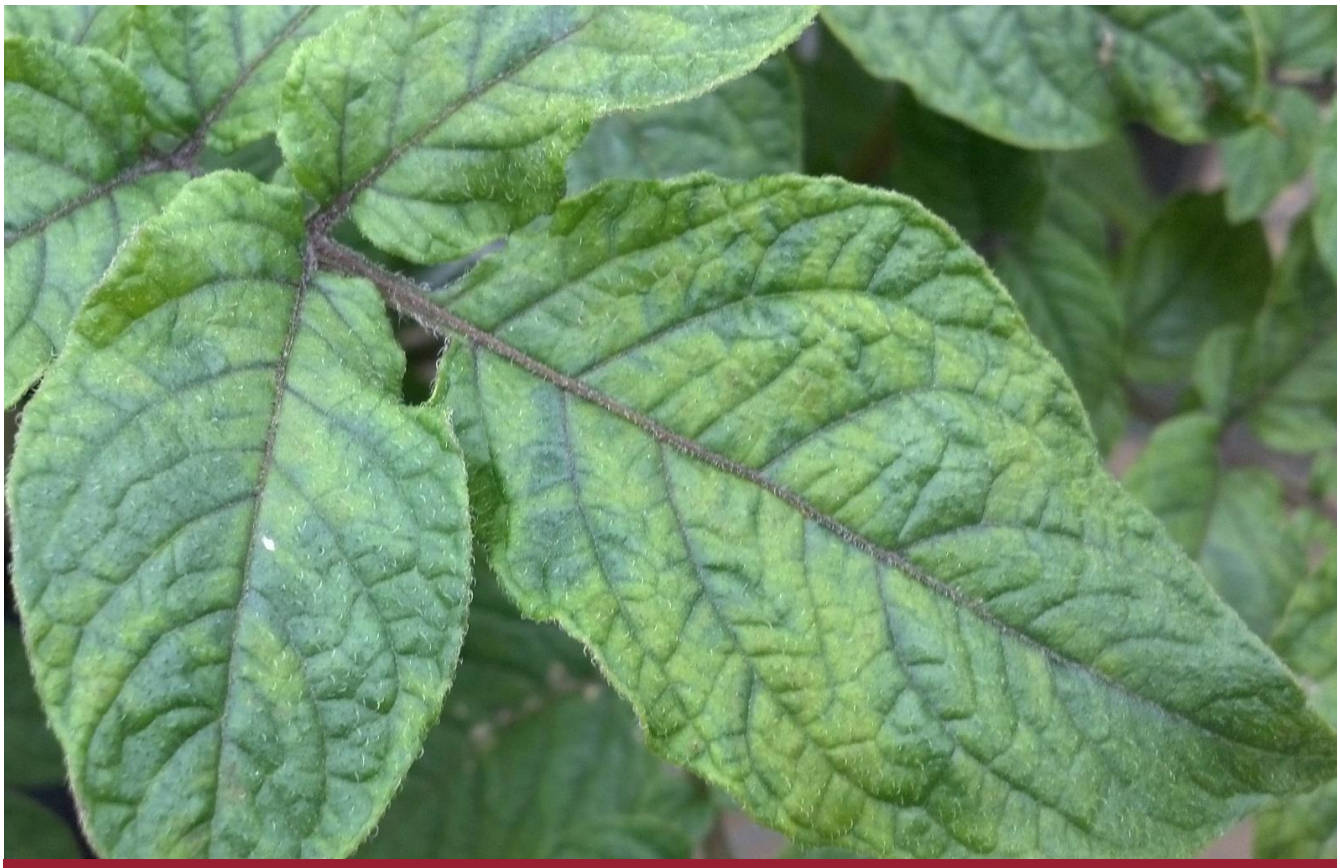

ALTERNATIVE SOURCES OF *POTATO VIRUS Y* IN WESTERN WASHINGTON

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Abstract

While many potato growers in western Washington (WWA) are diligent about managing *Potato virus Y* (PVY) from commonly known inoculum sources, there are various means that are not always recognized by which PVY is able to evade detection and persist in a potato production region. This study investigated potential alternative sources of PVY in WWA by examining: (i) commonly grown specialty potato cultivars which might not express obvious symptoms when infected; (ii) weedy plants that frequently surround potato fields; and, (iii) certified and non-certified seed potato sources locally available for purchase by home gardeners and small farmers. Because all three of these sources have the potential to contribute to the occurrence of PVY in WWA, management recommendations are provided accordingly.

Introduction

Potato virus Y (PVY) is one of the oldest known plant viruses and can cause significant crop and monetary losses (Karasev and Gray 2013). PVY is **tuberborne** and can be transmitted via seed potatoes or **non-persistently** by aphids during the growing season. Many potato growers in WWA are diligent about utilizing common PVY management practices. These include purchasing or producing **blue tag certified seed potatoes**, utilizing insecticide spray programs or **barrier crops** to manage aphid vectors, scouting fields and **rogueing** symptomatic plants during the season, and eliminating volunteer potato plants and cull piles (Crosslin et al. 2008; Nolte et al. 2009). Nevertheless, potatoes in the region still acquire the virus and sustain tuber yield and quality losses. Hence, it is important to ascertain what additional ways PVY is introduced into fields and what supplemental management practices may be achievable. The following questions provided the basis for a research project at WSU Mount Vernon NWREC, and are the topics addressed in this technical bulletin.

Do specialty potato cultivars grown in WWA have symptoms of PVY for which growers are unaccustomed?

In WWA, over 177 cultivars of **specialty potatoes** are grown each year in Skagit and Whatcom Counties. Approximately 12,000 acres are directed for fresh market sales (McMoran 2016), while about 3,335 acres are planted as seed potatoes (Benedict, personal communication). However, the reactions of many of these specialty types to PVY have never been documented. Cultivars with unknown symptomology may escape field inspections and play a role in the spread of PVY, particularly if the cultivars respond differently to different strains of the virus. Given that there are multiple strains of PVY, the reactions of individual potato cultivar(s) infected with different strains are essential to recognize in order to manage this virus.

The oldest known strain of PVY, PVY^{O}, causes characteristic **mosaic** symptoms that are relatively easy to identify on leaves in the field (Karasev and Gray 2013). Other common symptoms of PVY are referred to as **rugose mosaic**, **mild mosaic**, **mottle**, **veinal necrosis**, and **leaf drop** (see Figure 1 A–E, below; and Figure 2 A–E , below). In recent years, recombinant strains of PVY, like PVY^{NTN} and $PVY^{N\text{-}WI}$, have been identified. These strains often elicit mild or **asymptomatic** responses on plant foliage and, as such, confound seed potato certification programs by making visual detection difficult (Gray et al. 2010). Although observed in North America since 1969, these recombinant strains were not reported in WWA until 2012 (Benedict et al. 2015). PVY^{NTN} has been associated with tuber **necrosis** or Potato Tuber Necrotic Ringspot Disease (PTNRD) (Figure 3), and $PVY^{N\text{-}Wi}$ has been associated with tuber cracking, although cracking also can be the result of uneven soil moisture and

temperature or herbicide injury (Figure 4) (Karasev and Gray 2013; Benedict et al. 2015). Both PTNRD and cracking symptoms are highly detrimental to potato tuber quality and marketability.

Can weedy plants in WWA serve as sources of PVY?

PVY has a wide host range, with more than 495 species of plants reported to be capable of becoming infected (Kerlan 2006; Edwardson and Christie 1997). Ditches and riparian buffer zones commonly surround agricultural fields throughout WWA and harbor weedy plants year-round, as well as aphid vectors. Thus, potential non-potato PVY hosts are nearly unavoidable in the region. Over-summering or over-wintering weedy hosts may serve as primary sources of PVY, potentially aiding in the spread of the virus to potato plants in adjoining fields either by aphids or mechanical means (Boquel et al. 2017).

Figure 3. Symptoms of Potato Tuber Necrotic Ringspot Disease (PTNRD) on Yukon Gold potato caused by *Potato virus Y*^{NTN}.

Figure 4. Chieftain tubers exhibiting symptoms of cracking (left), and external (middle) and internal necrosis (right) caused by *Potato virus Y*.

Can seed potatoes, either blue tag certified or non-certified, available at local garden stores and planted in home and market gardens, serve as sources of inoculum for PVY?

The Washington State Department of Agriculture (WSDA) and the Washington State Seed Potato Commission (WSSPC) operate the state's seed potato certification program which is designed to help ensure cultivar purity and vigor, and disease-free seed potatoes, including diseases caused by tuberborne viruses such as PVY (WSDA 2008). Seed potatoes certified in this way receive a blue tag. While commercial potato growers contract directly with certified seed potato producers to obtain quantities of seed tubers large enough for hundreds of acres, both certified and non-certified seed potatoes can be purchased in small quantities from local garden stores by home owners or other individuals (Beissinger et al. 2017). These seed potatoes often originate from areas outside of WWA and are planted in gardens and small plots which sometimes immediately adjoin commercial potato fields. As a result, these small plantings may provide a means of introducing already known or new strains of PVY into the region.

Objectives and Methods

Cultivar by strain trial—This study identified symptoms associated with PVY^{O}, PVY^{NTN}, and $PVY^{N\text{-}Wi}$ on ten specialty potato cultivars commonly grown in WWA.

Experimental set-up. PVY inoculations were done in two experiments each consisting of five cultivars (Experiment 1 = Austrian Crescent, Cal White, Purple Majesty, Rose Finn Apple, and Russian Banana; and, Experiment 2 = All Blue, Chieftain, French Fingerling, Russet Burbank, and Yukon Gold). Three strains of PVY (PVY^{O}, PVY^{NTN}, and $PVY^{N\text{-}Wi}$) and one non-inoculated buffer control were always included. There were four replications of three plants per each cultivar/strain combination, making for 12 inoculated plants per treatment or a total of 240 plants per experiment.

Virus-free seed tubers were kindly provided as **mini-tubers** by Lori Ewing at the University of Idaho Tissue Culture Laboratory, except for Purple Majesty which was only available as virus-free **tissue-culture plantlets**. All seed tubers were shipped overnight express to WSU Mount Vernon NWREC and immediately planted in early March 2016 into 1-gal pots with Sunshine potting mix (SunGro Horticulture, Agawam, MA). Pots were arranged on greenhouse benches using a split-plot design, where replicate served as a block, PVY strain/buffer control was a whole plot factor, and potato cultivar was a subplot factor. The greenhouse was maintained at 68°F, and lighting with incandescent lamps allowed exposure to 16-hour days and 8-hour nights. Plants were watered daily with a fertigation ratio of 1:100 using 20-20-20 fertilizer. Fine nylon mesh fabric was hung on all doors leading into the greenhouse bay to allay insect entry, and insecticide sprays were regularly applied weekly or biweekly for further protection against potential aphid vectors.

White burley tobacco leaves infected either with PVY^{O}, PVY^{NTN}, or $PVY^{N\text{-}Wi}$ (kindly provided by Dr. Alex Karasev, University of Idaho, Moscow, ID; Table 1) were used for virus inoculations three to four weeks after planting (late March or early April) following standard techniques. Plants were rated for symptom development at least twice per month between March and May. The ratings recorded the presence or absence of viral symptoms (see Figure 1, below; and see Figure 2, below, for more information). At the end of May, vines were killed by clipping stems at soil line using a sanitized metal shears dipped into a diluted bleach (1:10) solution. Selected leaf samples also were submitted to the Karasev Lab for testing by **RT-PCR** and ELISA to confirm that the integrity of each PVY strain had been maintained.

Table 1. Isolates of *Potato virus Y* (PVY) used for inoculating the 2016 cultivar by strain inoculation trials, courtesy of Dr. Alex Karasev, University of Idaho.

Strain	Isolate	Serotype	GenBank genome sequence
PVY^{O}	Oz	O	EF026074
PVY^{NTN}	HR1	N	FJ204166
$PVY^{N\text{-}Wi}$	N1	O	HQ912863

Prior to harvest, pots were kept in the greenhouse at 66 to 68°F for two more weeks to allow the skins of the new tubers to mature. During this period, any stem regrowth was cut with sanitized metal sheers. Near the end of June, each pot was emptied, and tubers were gently rinsed with tap water and dried, visually inspected and sliced, and rated as either asymptomatic, or cracked and necrotic if greater than 50% of the tuber exhibited symptoms (Figures 3 and 4). The total number of tubers in each category were counted and weighed.

All statistical analyses regarding tuber yield were done with SAS version 9.2 (SAS institute, 2010, Cary, NC) using a value of $\alpha = 0.05$. Total tuber weight (healthy plus cracked) per plant was analyzed using the SAS GLM procedure. Yield data were normally distributed; thus, no transformation was performed.

Pairwise comparisons were carried out among cultivars within each strain. Due to zero-rich data for cracking and internal necrosis categories (Figure 5), these categories were not normally distributed despite transformation. Since pairwise comparisons were not possible, only the means are reported.

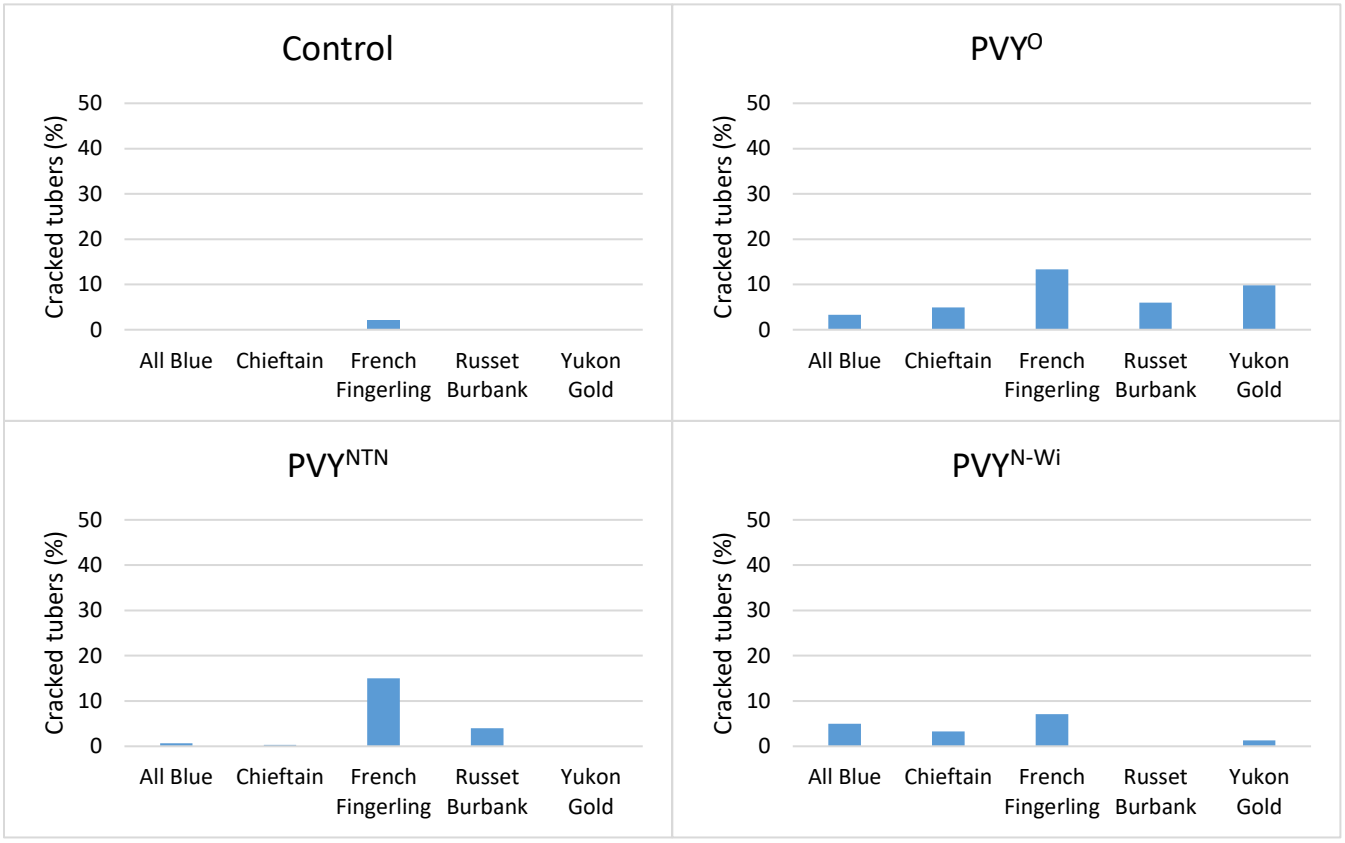

Figure 5. Percent cracked progeny tubers (by weight in grams) in Experiment 2, suspected to be caused by *Potato virus Y*. Only Cal White inoculated with PVY^{O} and $PVY^{N\text{-}Wi}$ had cracked tubers in Experiment 1, thus those data are not shown.

PVY confirmation. In mid-May, a trifoliate leaf section with the most representative symptoms was removed from one potato plant per each cultivar by strain replication and stored separately in plastic bags temporarily (Experiment 1) or at -112°F (Experiment 2) until needed. A total of 80 trifoliate leaves per experiment were tested in the laboratory using **TAS-ELISA** to confirm the PVY infections (Ellis et al. 1996). PathoScreen kits (Agdia, Elkhart, IN; Item number: PSA 20001/0096) specific to all strains of PVY were utilized for this testing.

Weedy plant survey—This study determined if weedy plants surrounding potato fields in Whatcom and Skagit Counties are infected with PVY and possibly serve as alternative hosts.

*Selection and establishment of **sentinel** plot sites*. Weedy plants were surveyed during Summer and Fall 2015, and again in Winter, Spring, Summer and Fall of 2015 and 2016. In mid-June 2015, three plots each were established in Whatcom and Skagit Counties. Location of plot sites was chosen based on proximity to potato fields, characteristic weed species, and representative landscape diversity typically surrounding potato fields. For set-up, the potato field perimeter was scouted for an area of high incidence of diverse weeds. A plot of approximately $30ft^2$ was measured, marked with semi-permanent wooden stakes, and flagged. GPS coordinates at each stake were taken using a GeoXH unit (Trimble Navigation Limited, Sunnyvale, CA). In 2016, plots in both counties were relocated in order to be near fields that had PVY positive plants during the 2015 growing season; in Skagit County, two of the plots were established at WSU Mount Vernon NWREC.

Weedy plant sampling. Plant sampling took place in 2015: June (Summer 1), August (Fall 1); and in 2016: February (Winter), March (Spring), May (Summer 2), and August (Fall 2). Each plot was visually assessed for the total number of weedy plants. Samples of all species were cut using sanitized metal shears, placed into gallon-sized plastic Ziploc bags, and stored in Styrofoam coolers with ice packs. Weeds were either identified in the field by Jean Gauthier (WSU-Skagit County Extension entomology coordinator) or by Dr. Tim Miller (WSU-Mount Vernon NWREC weed scientist). After identification, three leaf samples from each temporary storage bag were removed at random, placed into new sample bags, and stored at -112°F until tested in the laboratory using TAS-ELISA as explained above (Ellis et al. 1996).

Locally available seed potato survey—This study established whether PVY is present in certified and non-certified seed potato sources, locally available for purchase by home owners and small farm operations.

Seed potato selection. In March and April 2016, seed potatoes were collected from two commercial outlets in Skagit County and three in Whatcom County. Both blue tag certified and non-certified seed was purchased in Skagit County, while only blue tag certified seed was available for purchase in Whatcom County. Ten cultivars of blue tag certified seed potatoes, and five cultivars of non-certified seed potatoes, were obtained. At least one asymptomatic seed tuber was selected from each cultivar, and at least one seed tuber (if available) with the symptom of cracking was selected and purchased.

Seed potato grow-out. The collected seed tubers were planted in late March (Skagit County samples) and mid-May 2016 (Whatcom County samples). Once removed from storage, they were placed in a paper sack and warmed on a bench at room temperature (approximately 77°F) for 24 hr. Each seed tuber was labeled with a felt-tipped pen, photographed, and cut in half with a sterile knife so that the cracking symptom (if present) and at least two eyes were part of each seed piece. One-half seed piece was planted in a 1-gal pot with Sunshine potting mix. Plants were maintained in a greenhouse, as stated above, and observed at least once during the greenhouse grow-out for virus symptoms on foliage. At 11 and 9 weeks after planting, vines for the two respective experiments were clipped at the soil line to allow for tuber periderm maturation. After another 2 weeks, tubers from each plant were rated for symptoms of virus, and counted, weighed, and archived.

PVY detection. Trifoliate leaf sections on each plant showing the most representative viral symptoms were removed and stored in bags. Each sample was tested with Agdia ImmunoStrips for PVY as described above. If a plant tested negative for PVY, the sample was retested in the laboratory using TAS-ELISA as stated above. Positive leaf samples were sent to the Karasev Lab for PVY strain typing.

Results

Cultivar by strain trial—*PVY infection*. When tested with TAS-ELISA, PVY infection varied depending on strain and cultivar (Table 2). No control plants tested positive for PVY in either experiment. In Experiment 1, Austrian Crescent infection was low when inoculated with PVY^{O} and $PVY^{N\text{-}Wi}$, Cal White and Purple Majesty were infected by all strains of PVY, while Rose Finn Apple and Russian Banana had low infection by $PVY^{N\text{-}Wi}$. In Experiment 2, All Blue was not infected by PVY^{O}, and had slightly lower foliar infection with PVY^{NTN}. Chieftain, French Fingerling, and Russet Burbank were infected by all three strains. Yukon Gold infection was slightly lower when inoculated with PVY^{NTN} and $PVY^{N\text{-}Wi}$ than PVY^{O}.

Table 2. Results of TAS-ELISA testing on ten specialty potato cultivars inoculated with three strains of *Potato virus Y* during cultivar by strain trials at WSU Mount Vernon NWREC. Each entry shows the number of reps that tested positive for PVY.

Potato cultivar	PVY^{O}	PVY^{NTN}	$PVY^{N\text{-}Wi}$	Buffer control
Experiment 1				
Austrian Crescent	1/4	4/4	1/4	0/4
Cal White	4/4	4/4	4/4	0/4
Purple Majesty	4/4	4/4	4/4	0/4
Rose Finn Apple	4/4	4/4	1/4	0/4
Russian Banana	4/4	4/4	2/4	0/4
Experiment 2				
All Blue	0/4	3/4	4/4	0/4
Chieftain	4/4	4/4	4/4	0/4
French Fingerling	4/4	4/4	4/4	0/4
Russet Burbank	4/4	4/4	4/4	0/4
Yukon Gold	4/4	3/4	3/4	0/4

Foliar symptoms. Symptom expression varied by PVY strain (see Figure 1, below; and see Figure 2, below, for more information). For all cultivars in Experiment 1, mosaic was most frequently observed on plants infected with PVY^{O} or PVY^{NTN} (Table 3). Mottle was most frequently observed when plants were infected with $PVY^{N\text{-}Wi}$. Veinal necrosis was most frequently observed on plants infected with PVY^{O} or PVY^{NTN}. Leaf drop was infrequently observed on most cultivars, except for Austrian Crescent when plants were infected with PVY^{O}. Cal White symptom reactions were the most consistent and expressed nearly identical symptoms of mosaic and veinal necrosis across all three strains. In Experiment 2, mosaic was the most frequently observed symptom for all combinations and mottle was more infrequent, likely because mosaic reactions were strong. Veinal necrosis was evident, except on French Fingerling and Russet Burbank infected with PVY^{NTN}, and Russet Burbank infected with $PVY^{N\text{-}Wi}$; Chieftain had a low number of plants with veinal necrosis when infected with PVY^{O}. Leaf drop was apparent primarily on Russet Burbank and Yukon Gold infected with PVY^{O}, and Yukon Gold infected with PVY^{NTN}. The other PVY^{NTN} and $PVY^{N\text{-}Wi}$ infected plants, for the most part, had low levels of leaf drop.

Table 3. Heat maps of foliar symptoms expressed on 10 specialty potato cultivars inoculated with three strains of *Potato virus Y* at WSU Mount Vernon NWREC in 2016. Each box represents the frequency[a] of a given symptom[b] out of a total of 12 plants per potato cultivar[c] by PVY strain combination. Note: All control plants were healthy (0), thus those data not shown.

Experiment 1

Cultivar	PVY^{O}			
	M	Mo	VN	LD
Aust. Cres.	0	7	6	9
Cal White	12	0	10	0
Purple Maj.	9	1	12	4
R.F. Apple	11	1	6	1
R. Banana	6	6	4	0

Cultivar	PVY^{NTN}			
	M	Mo	VN	LD
Aust. Cres.	8	3	10	2
Cal White	12	0	9	0
Purple Maj.	11	1	7	0
R.F. Apple	7	3	1	1
R. Banana	8	4	2	0

Cultivar	$PVY^{N\text{-}Wi}$			
	M	Mo	VN	LD
Aust. Cres.	0	9	1	1
Cal White	12	0	12	0
Purple Maj.	3	8	6	0
R.F. Apple	2	5	4	0
R. Banana	0	11	3	0

Experiment 2

Cultivar	PVY^{O}			
	M	Mo	VN	LD
All Blue	10	9	10	7
Chieftain	9	1	3	4
F. Fingerling	12	0	9	0
R. Burbank	10	3	7	11
Yukon Gold	10	3	11	12

Cultivar	PVY^{NTN}			
	M	Mo	VN	LD
All Blue	12	10	12	2
Chieftain	12	0	11	0
F. Fingerling	12	0	0	1
R. Burbank	12	9	0	3
Yukon Gold	11	7	9	9

Cultivar	$PVY^{N\text{-}Wi}$			
	M	Mo	VN	LD
All Blue	12	2	12	4
Chieftain	12	0	10	0
F. Fingerling	12	0	9	0
R. Burbank	12	12	0	3
Yukon Gold	11	4	7	3

[a] White boxes=0 plants observed with symptom; light gray boxes=1–5 plants observed with symptom; dark gray boxes=6–12 plants with symptoms.

[b] M=mosaic, light and dark islands on leaf surfaces; Mo=mottle, faint and mild light and dark green islands on leaf surfaces; VN=veinal necrosis, necrotic veins on leaf surface or underside; LD=leaf drop; wilted and dying petioles and leaves, not yet detached from plant.

[c] Cultivar abbreviations: Aust. Cres.=Austrian Crescent; Purple Maj.=Purple Majesty R.F. Apple=Rose Finn Apple; F. Fingerling=French Fingerling; R. Burbank=Russet Burbank.

Tuber symptoms and yield. Frequency of tuber symptoms in both greenhouse experiments was low. Only Cal White in Experiment 1 had tubers that displayed cracking and internal necrosis upon inoculation with PVY^{O} and $PVY^{N\text{-}Wi}$ (data not shown) while almost all cultivars in Experiment 2 except for Chieftain and Yukon Gold inoculated with PVY^{NTN} and Russet Burbank with $PVY^{N\text{-}Wi}$ had some progeny tubers with cracks (Figure 5). Some French Fingerling tubers from the control plants also had cracks, but greenhouse conditions frequently lead to cracked French Fingerling tubers (D. Inglis, personal communication). Yield of tubers that were healthy (asymptomatic) differed by cultivar and strain (Figure 6). In both trials, plants infected with $PVY^{N\text{-}Wi}$ had the lowest yield of healthy tubers.

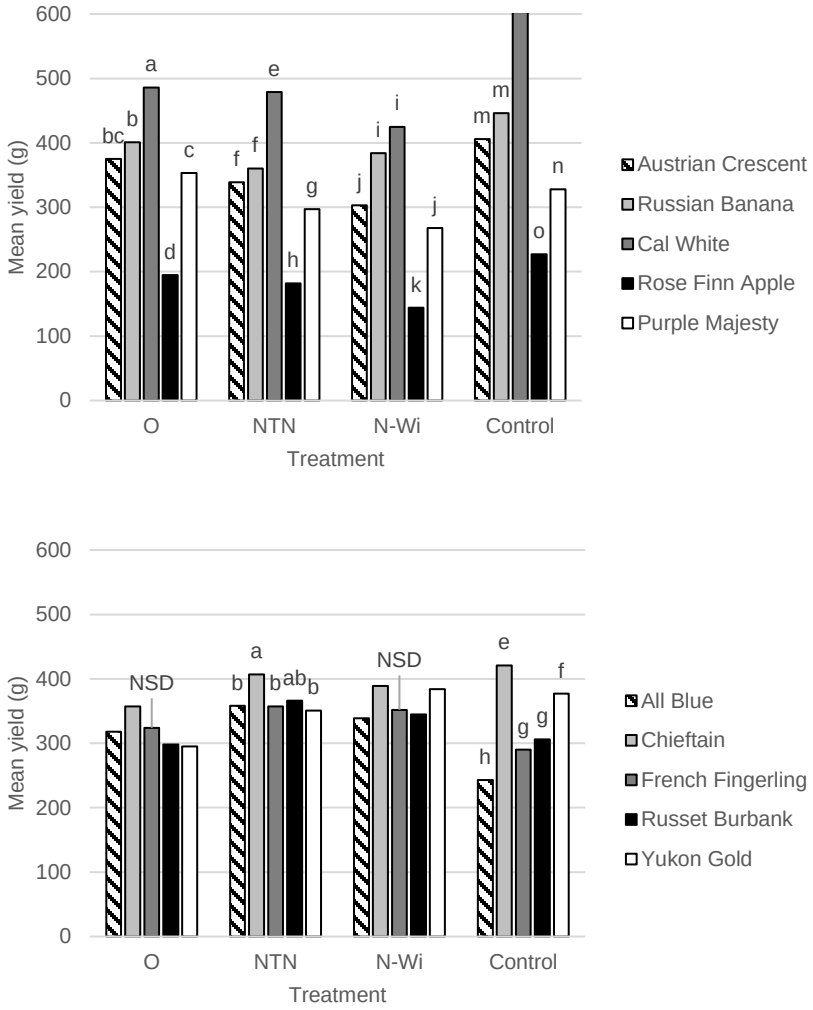

Figure 6. Yield data for healthy (asymptomatic) tubers harvested from potato cultivar by *Potato Virus Y* strain experiments. Each bar represents the mean yield of each cultivar across all replications. Bars with the same letter are not statistically different (NSD) by Fishers LSD test (α = 0.05). Experiment 1 (top); Experiment 2 (bottom).

Weedy plant survey:

Throughout the sentinel plots in Skagit and Whatcom counties, 183 samples of weedy plants were collected across 55 plant species (Table 4). No sample tested positive for PVY by TAS-ELISA, regardless of season tested or weed life cycle type.

Table 4. Weedy plants collected at five sampling times[a] in Skagit and Whatcom Counties, WA during 2015–2016, and tested with TAS-ELISA to determine *Potato virus Y* status.

Scientific name	Common name	Season tested	Location[b]	No. plants tested	ELISA
Summer annual					
Amaranthus powellii	Pigweed	S1, F1, F2	Ska, Wha	6	-
Brassica rapa	Field Mustard	S1, F1, Sp, S2, F2	Ska, Wha	7	-
Chenopodium album	Lambs quarters	S1, F1, S2, F2	Ska, Wha	9	-
Erodium cicutarium	Redstem fillaree	S2, F2	Wha	3	-
Fagopyrum esculentum	Buckwheat	S2	Ska	1	-
Gnaphalium uliginosum	Marsh cudweed	F1	Wha	1	-
Lapsana communis	Nipplewort	F1, S2	Ska, Wha	2	-
Matricaria discoidea	Pineapple weed	Sp, S2, F2	Ska, Wha	4	-
Persicaria maculosa	Lady's thumb	F1	Wha	1	-
Polygonum lapathifolium	Smartweed	S1, F1, S2, F2	Ska, Wha	8	-
Solanum dulcamara	Bittersweet nightshade	S1	Wha	1	-
Solanum sarrachoides	Hairy nightshade	F1	Wha	1	-
Solanum tuberosum	Potato	F1, Sp, S2	Ska, Wha	10	-
Veronica hederifolia	Veronica ivy	Sp	Ska	1	-
Vicia tetrasperma	Four-seeded vetch	S1	Ska, Wha	2	-
Winter annual					
Capsella bursa-pastoris	Shepard's purse	Sp	Ska	1	-
Galium aparine	Cleavers	S1	Ska	2	-
Lamium amplexicaule	Henbit	Sp	Ska	1	-
Lamium purpureum	Purple deadnettle	Sp	Ska	1	-
Senecio vulgaris	Grounsel	W, Sp	Ska, Wha	2	-
Stellaria media	Chickweed	F1, W, Sp	Ska, Wha	3	-
Biennial					
Geranium dissectum	Cutleaf geranium	S2, F2	Wha	4	-
Melilotus officionalis	Sweet clover	S1	Ska	1	-
Onopordum acanthium	Scotch thistle	S2, F2	Ska, Wha	4	-
Sisymbrium officinale	Hedge mustard	S2	Wha	1	-
Perennial					
Alnus rubra	Red alder	S1, F1	Ska	3	-
Chamerion angustifolium	Fireweed	S1, F1, S2, F2	Ska, Wha	8	-
Chamerion latifolium	Dwarf fireweed	S1, F1	Ska, Wha	5	-
Cirsium arvense	Canada thistle	S1, F1	Ska, Wha	4	-
Cirsium vulgare	Bull thistle	S1	Ska	1	-
Convolvulus sepium	Bindweed	S1, F1	Wha	2	-
Crataegus douglasii	Douglas hawthorn	S1, F1	Wha	2	-
Equisetum arvense	Common horsetail	S1, F1	Wha	2	-
Geum macrophyllum	Large-leaved avens	F1	Ska	1	-
Geum urbanum	Herb bennet	S1	Ska	1	-
Hypericum perforatum	St. John's wort	S2	Wha	1	-
Lonicera involucrata	Twinberry	S1, F1	Ska	2	-
Lotus corniculatus	Birdsfoot trefoil	F1, F2	Ska, Wha	3	-
Phalaris arundinacea	Reed canary grass	S1, F1	Ska, Wha	9	-
Plantago lanceolata	English plantain	S1, F1, S2, F2	Ska, Wha	6	-
Plantago major	Common plantain	F1, S2, F2	Wha	4	-
Populus balsamifera	Black cottonwood	S1, F1	Wha	2	-

Prunus laurocerasus	Cherry laurel	S1, F1	Ska	2	-
Rhamnus purshiana	Cascara buckthorn	S1, F1	Wha	2	-
Rubus armeniacus	Himalayan blackberry	S1, F1, S2, F2	Ska, Wha	12	-
Rubus laciniatus	Evergreen blackberry	S2	Wha	1	-
Rubus spectabilis	Salmonberry	S1, F1	Ska, Wha	4	-
Rumex crispus	Curly dock	S2	Ska, Wha	2	-
Rumex obtusifolius	Broadleaf dock	S1, S2, F2	Ska, Wha	5	-
Sonchus hierrensis	Sow thistle	S1, F1	Wha	3	-
Spirea douglasii	Douglas spirea	S1, F1	Wha	2	-
Taraxacum officinale	Dandelion	S1, F1, Sp, S2, F2	Ska, Wha	8	-
Trifolium pratense	Red clover	S1, F1, S2, F2	Wha	5	-
Trifolium repens	White clover	S2, F2	Ska, Wha	2	-
Urtica diocia	Stinging nettle	S1, F1	Wha	2	-

[a] Plants were collected at five sampling times during 2015 and 2016 based on stages in the potato production system. S1=Summer (emergence) 2015; F1=Fall (harvest) 2015; W=Winter 2016; Spring (pre-emergence) 2016; S2=Summer (emergence) 2016; F2=Fall (harvest) 2016, [b] Ska=Skagit County; Wha=Whatcom County.

Locally available seed potato survey:

All cultivars that initially expressed PVY symptoms of cracking in the seed tuber produced plants that proved positive for PVY. In addition, one French Fingerling plant from an asymptomatic tuber also was positive (Table 5).

No conventionally produced blue tag certified seed potato plants were positive for PVY, while 14% of organically produced blue tag certified seed potato plants were positive for PVY. Moreover, 54% of organically produced non-certified seed potato plants were positive for PVY. Strain typing revealed infections by PVY^{O}, PVY^{NTN}, and $PVY^{N\text{-}Wi}$ in positive plants. Incidence of tuber cracking was relatively low, but the cracked progeny tubers that were identified originated from plants and cultivars that expressed PVY symptoms. Yellow Finn had the most severe mosaic and yielded 2% cracked progeny tubers whereas Red Pontiac had notable mosaic and yielded 18% cracked progeny tubers (Figure 7).

Figure 7. Severe plant mosaic on Yellow Finn (left) and tuber cracking on Red Pontiac (right), both originating from organically produced, non-certified seed purchased from a local garden store.

Table 5. Blue tag certified and non-certified seed potatoes with cracking symptoms obtained from local garden stores in Skagit and Whatcom Counties, WA in 2015, then grown-out in the greenhouse and tested for *Potato virus Y* using TAS-ELISA.

Cultivar	Seed tuber generation	Production system[a]	No. cracked/no. planted seed pieces	Foliar symptoms[b]	No. cracked progeny tubers	No. PVY+/ no. planted seed pieces	PVY strain
Blue Tag Certified Seed Potato Tubers							
Cal White	G3	Conv	0/2	None	1/12	0/2	---
Caribe	G3	Org	1/2	Mos	1/11	1/2	NTN
Chieftain	*	Org	1/7	None	0/40	0/7	---
French Fingerling	G2	Org	2/4	None	0/26	1/4	O
Kennebec	*	Org	1/2	None	0/8	0/2	---
Russian Banana	G1	Org	0/3	None	0/63	0/3	---
Russet Burbank	*	Org	4/9	Mos	1/33	2/9	O, NTN
Viking Purple	G4	Conv	2/3	None	0/16	0/3	---
Yukon Gem	G3	Conv	2/3	None	0/20	0/3	---
Yukon Gold	G4	Org	0/2	Mos, VN, LD	0/5	1/2	N-Wi
Non-Certified Seed Potato Tubers							
Huckleberry Gold	*	Org	0/2	None	0/32	0/2	---
Ozette	*	Org	1/3	Mos	0/79	1/3	O
Red Pontiac	*	Org	1/2	Mos	4/22	2/2	O, NTN
Rose Finn Apple	*	Org	1/2	None	0/22	0/2	---
Yellow Finn	*	Org	3/4	Mos	1/41	4/4	O, N-Wi

*Unknown. [a] Conv=conventional and Org=organic potato production system. [b] Mos=mosaic (light and dark islands on leaf surfaces); VN=veinal necrosis (necrotic veins on leaf surface or underside); LD=leaf drop (wilted and dying petioles and leaves, not yet detached from plant).

Discussion

Cultivar by strain trial:

Specialty potato cultivars that display mild symptoms can serve as sources of inoculum of PVY. Thus, growers of specialty potatoes need to be aware of the specific symptoms that occur on the cultivars they grow and manage infected plants accordingly. Further, it is important that specialty potato growers are aware of the adverse effects that different strains of PVY cause on the tubers of some cultivars. Unlike potatoes grown for processing, those sold competitively on the fresh market must be of highest quality and free of defects. The same standard pertains to seed potatoes for which high quality, disease- and defect-free seed is essential for blue tag certification.

Foliar symptoms on specialty potatoes may differ depending on the cultivar and strain of PVY. Although mosaic can be an expected reaction of all three strains on All Blue, Cal White, Chieftain, French Fingerling, Russet Burbank, and Yukon Gold, it may not be easily detectable on Austrian Crescent, Purple Majesty, Rose Finn Apple, or Russian Banana plants infected with $PVY^{N\text{-}Wi}$. Leaf drop may not be a consistently reliable symptom, especially with PVY^{NTN} and $PVY^{N\text{-}Wi}$ infections. Hence, over-reliance of leaf drop as a key PVY indicator symptom can lead to missed detections. The website potatovirus.com provides information on foliar symptoms that can be referenced when scouting for PVY in field and greenhouse settings (USDA ARS 2018).

Even when symptoms are documented for a certain cultivar though, PVY infected plants in the field can present as mildly affected or asymptomatic; thus, are a challenge to identify depending on time of day and weather conditions. Mottling in particular is a subtle symptom that can mimic nutritional problems and therefore remain undetected in the field. In Experiment 1, mottling was most frequently observed on Austrian Crescent, Purple Majesty, and Russian Banana plants infected with $PVY^{N\text{-}Wi}$, and in Experiment 2 on All Blue infected with PVY^{O}, All Blue and Russet Burbank infected with PVY^{NTN}, and Russet Burbank infected with $PVY^{N\text{-}Wi}$.

Interestingly, plants infected with $PVY^{N\text{-}Wi}$ had the lowest average tuber yields and highest incidence of tuber symptoms. Regular scouting plants in fields for PVY symptoms and examining developing tubers, in addition to regular laboratory testing, is a crucial part of an effective PVY management program. While there are options for testing plants rapidly in the field (i.e., Agdia Immunostrips), ELISA is a more reliable testing method for PVY, especially when plants are senescing or stressed (Beissinger and Inglis 2018). To differentiate among the strains, however, more sophisticated molecular and serological laboratory tests are required.

Weedy plant survey:

Weeds surrounding potato production areas in Skagit and Whatcom Counties did not serve as important sources of inoculum of PVY, at least in 2015 and 2016. However, nearly 50 species of aphids are capable of transmitting PVY (Ragsdale et al. 2008; DiFonzo et al. 1997). Despite the fact that all of the weedy plant samples tested negative in this study and did not appear to function as overwintering sources of PVY, aphids were regularly seen colonizing weeds in the various sampling areas, especially during the summer and fall sampling times. It is possible that by providing habitat for aphids, weedy plants can serve the role of a barrier crop where aphids clean their stylets before entering a potato field. Nevertheless, some of the most common weeds that were tested in this study, like dandelion and lambs quarters, are reported hosts of PVY. Hence, it is advisable that weedy plants surrounding fields are managed either by mowing or chemical means, and a PVY resistant barrier cover crop like a sorghum x Sudangrass mix be planted when possible instead. In this way, the barrier crop would not become infected with PVY or serve as a source of inoculum of PVY in adjoining potato fields.

Locally available seed potato survey:

This survey was initiated because seed potato tubers from the Pacific Northwest that exhibited cracking symptoms were observed at several local garden stores in Whatcom and Skagit Counties. After growing out the blue tag certified seed tuber collection, 14% (5 of 37) yielded PVY positive plants, all of which had been organically produced. Unfortunately, some of these seed tubers were sold in Whatcom County, which is designated as an isolation district for seed potato production (WSDA 2008). Further, 54% (7 of 13) organically produced seed tuber collections that were not blue tag certified, yielded PVY positive plants. Seed potatoes, whether blue tag certified or not, or organically produced or not, can pose a risk to Washington's potato industry if they are infected with PVY and planted in close proximity to commercial potato fields (not uncommon for the rural and urban interface communities of WWA).

The rules for the blue tag certification of seed potatoes in Washington enforce different standards for seed lots grown on less than 0.25 acre (WSDA 2008). While all Generation 1 lots must be submitted for post-harvest testing, seed lots grown on less than 0.25 acre and replanted on the same farm are exempt. In addition, lots grown on less than 0.25 acre submit a minimum of four tubers per total hundred-weight with a minimum of 50 tubers, instead of the 400 tubers required of seed lots grown on higher acreages. Thus, the industry may need to re-assess the rules concerning which seed potato field sizes are exempt from post-harvest testing and how the testing should be done.

Conclusion: Management Recommendations

Based on this work, the following strategies could help to enhance management of PVY in WWA.

1. Recognize the symptoms of PVY that occur on different potato cultivars and know which PVY strains have been identified in your area. Refer to potatovirus.com and bulletins like this one when choosing cultivars to plant. Avoid planting cultivars with known mild to asymptomatic foliar reactions to PVY infections unless active field scouting with PVY detection and mitigation practices are in place. Asymptomatic foliar infections can lead to surprise symptoms on tubers at harvest time, and infected progeny tubers can transmit PVY to subsequent seed potato generations.
2. Scout fields regularly and often for PVY. Symptoms can change rapidly, and early observation and testing of PVY plants is crucial for limiting spread of the virus.
3. Rogue and destroy (do not compost) all plants exhibiting PVY foliar symptoms and ones that have tested positive for PVY in order to limit inoculum spread in fields and regions. Rogued potato plants left lying in the field can be a source of PVY for aphids for several days, so the plants always need to be bagged and removed (Boquel et al. 2017).
4. Control weedy plants in ditch banks and roadways surrounding potato growing areas to minimize habitat for aphids. Consider planting a non-PVY host crop around potato growing fields to serve as stylet cleaning sites for aphids.
5. Educate local garden stores to be aware of symptoms of PVY tuber cracking, so as not to unintentionally sell PVY infected seed tubers. Avoid purchasing cracked seed tubers that may be positive for PVY and notify garden centers if you see them for sale.
6. Increase public awareness about the existence of a seed potato isolation district in Whatcom County, and the importance of planting certified seed potatoes that are free of PVY to help protect the county's seed potato industry.
7. Explore modifying WSDA seed potato certification rules concerning winter grow-out testing as related to current field acreage size requirements.
8. Encourage laboratory analyses for PVY during winter grow-out testing so that mild or asymptomatic reactions are not overlooked, and that faster and more accurate results can be obtained.

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Figure 1, A–E

Foliar symptoms expressed on five cultivars of specialty potatoes inoculated with three strains of *Potato virus Y* (Experiment 1).

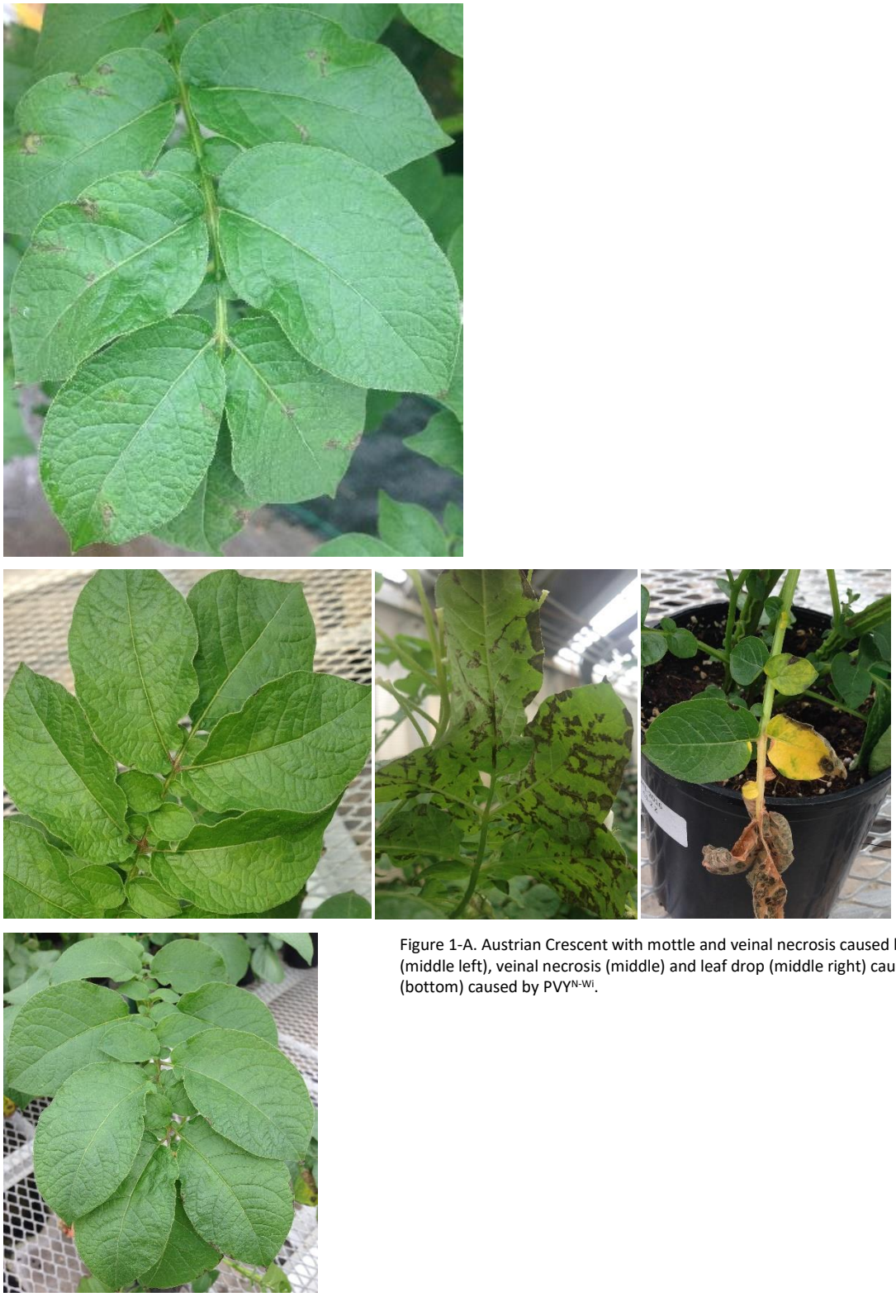

Figure 1-A. Austrian Crescent with mottle and veinal necrosis caused by PVY^{O} (top left), and mottle, (middle left), veinal necrosis (middle) and leaf drop (middle right) caused by PVY^{NTN}, and mottle (bottom) caused by $PVY^{N\text{-}Wi}$.

Figure 1-B. Cal White with mosaic (top left) and mosaic and veinal necrosis (top right) caused by PVY^{NTN}, and with mosaic (bottom left) and veinal necrosis (bottom right) caused by $PVY^{N\text{-}Wi}$.

Figure 1-C. Purple Majesty with mosaic (top left) and veinal necrosis (top right) caused by PVY^{O}, and with mosaic (middle left) and veinal necrosis (middle right) caused by PVY^{NTN}, and with mottle (bottom) caused by $PVY^{N\text{-}Wi}$.

Figure 1-D. Rose Finn Apple with mosaic (left) caused by PVY^{O}, and with mottle (middle) caused by PVY^{NTN}, and with mottle (right) $PVY^{N\text{-}Wi}$.

Figure 1-E. Russian Banana with mosaic caused by PVY^{O} (top left), and with mottle (top middle) and mosaic (top right) caused by PVY^{NTN}, and with veinal necrosis (bottom) $PVY^{N\text{-}Wi}$.

Figure 2, A–E

Foliar symptoms expressed on five cultivars of specialty potatoes inoculated with three strains of *Potato virus Y* (Experiment 2).

Figure 2-A. All Blue with mosaic (left) caused by PVY^{NTN}, and with mosaic (middle) and veinal necrosis (right) caused by $PVY^{N\text{-}Wi}$.

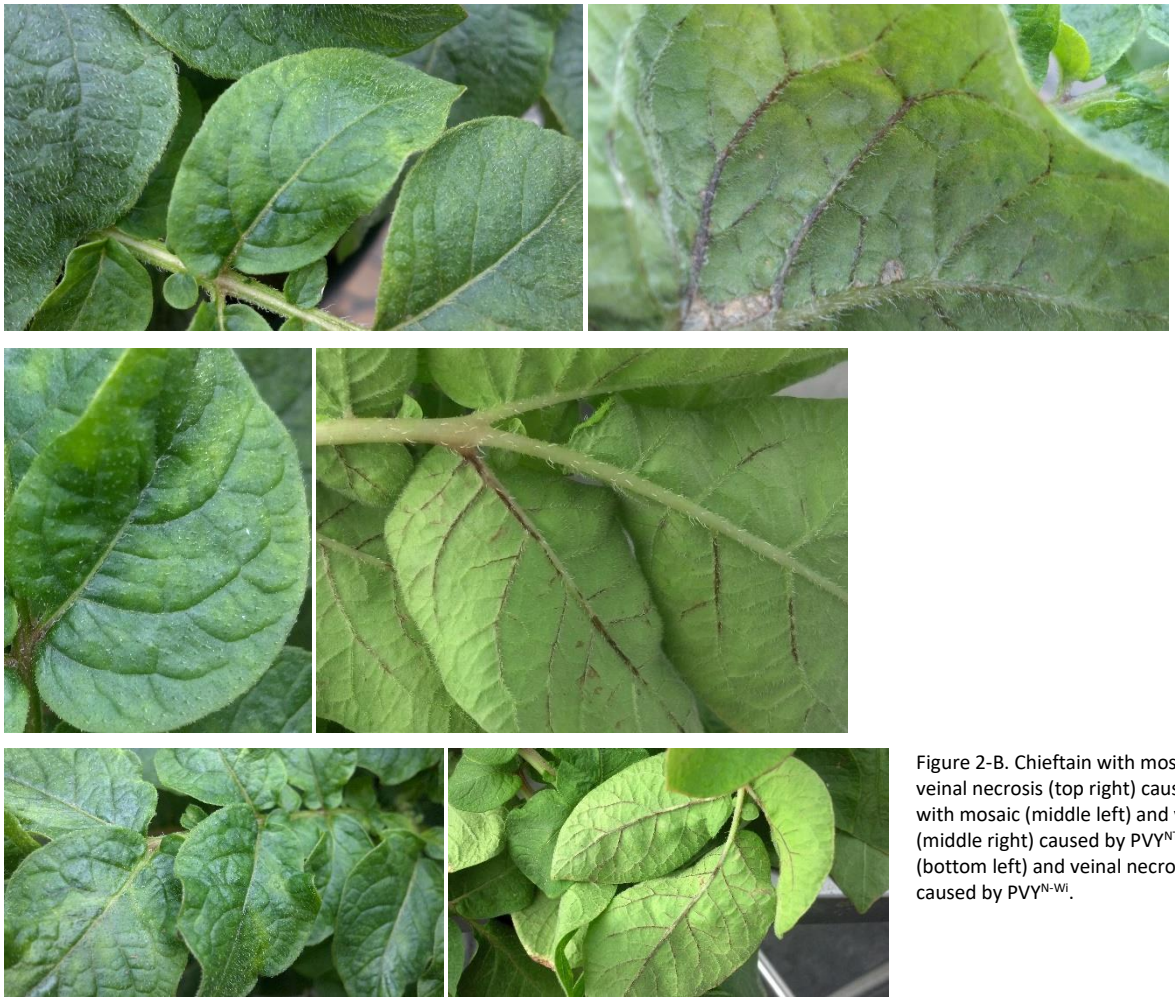

Figure 2-B. Chieftain with mosaic (top left) and veinal necrosis (top right) caused by PVY^{O}, and with mosaic (middle left) and veinal necrosis (middle right) caused by PVY^{NTN}; and, with mosaic (bottom left) and veinal necrosis (bottom right) caused by $PVY^{N\text{-}Wi}$.

Figure 2-C. French Fingerling with rugose mosaic (top left) and veinal necrosis (top right) caused by PVY^{O},and with rugose mosaic (middle) caused by PVY^{NTN}, and with mosaic (bottom left) and veinal necrosis (bottom right) caused by $PVY^{N\text{-}Wi}$.

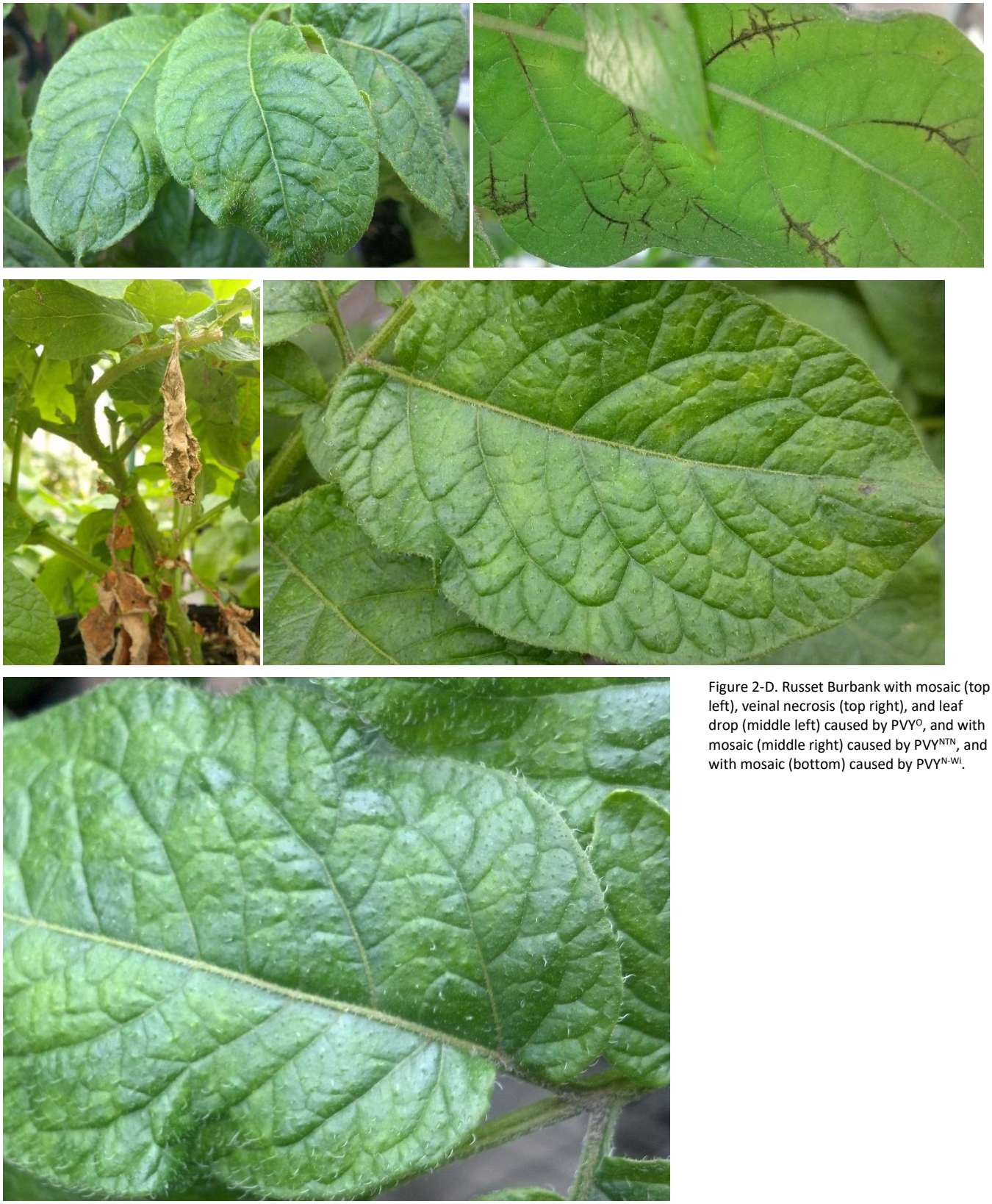

Figure 2-D. Russet Burbank with mosaic (top left), veinal necrosis (top right), and leaf drop (middle left) caused by PVY^{O}, and with mosaic (middle right) caused by PVY^{NTN}, and with mosaic (bottom) caused by PVY^{N-Wi}.

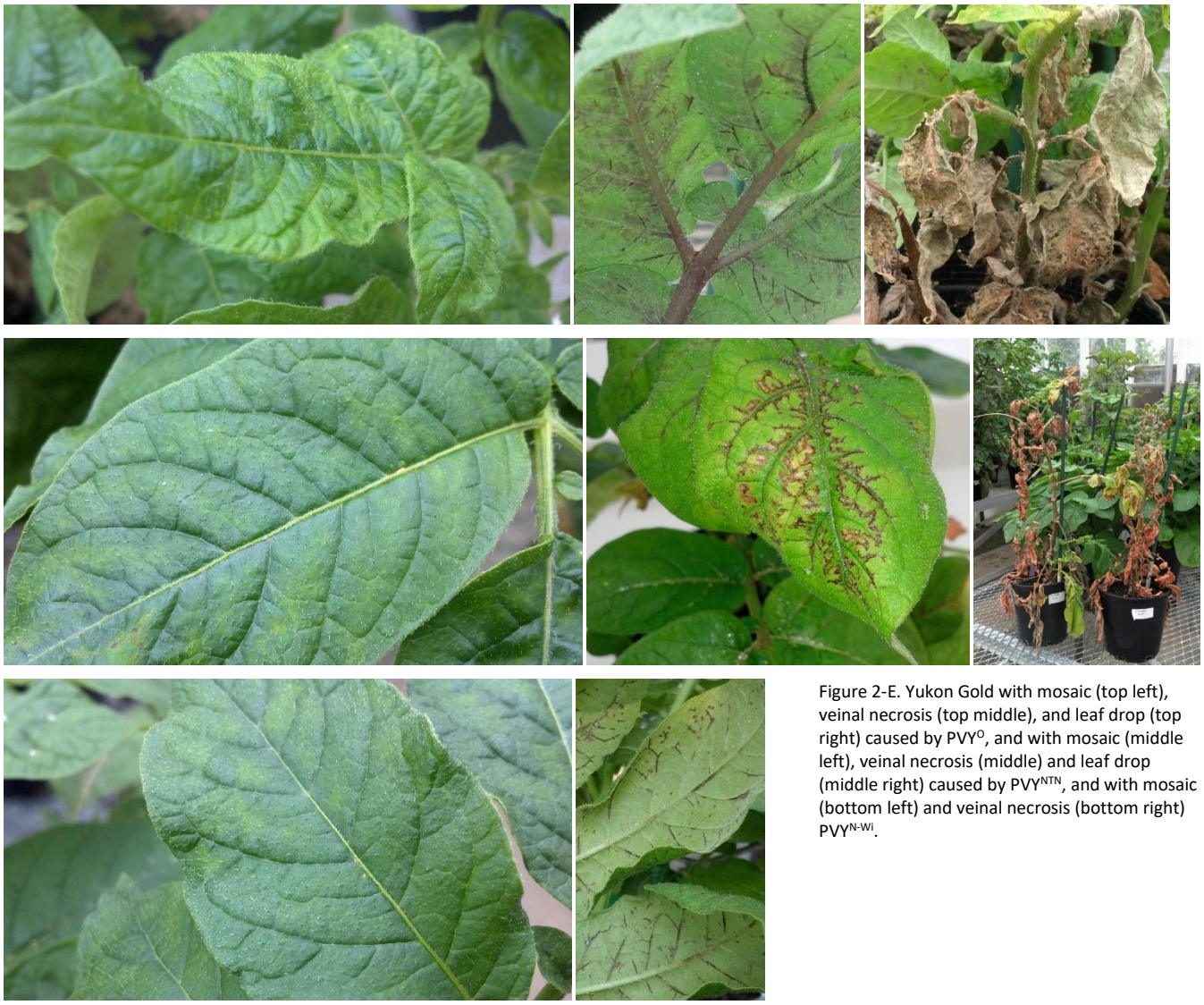

Figure 2-E. Yukon Gold with mosaic (top left), veinal necrosis (top middle), and leaf drop (top right) caused by PVY^{O}, and with mosaic (middle left), veinal necrosis (middle) and leaf drop (middle right) caused by PVY^{NTN}, and with mosaic (bottom left) and veinal necrosis (bottom right) PVY^{N-Wi}.

Glossary

asymptomatic. A plant that expresses no visual symptoms.

barrier crops. A cultural control strategy used to reduce the spread of aphid-vectored non-persistent plant viruses. Non-host crops are planted to surround a virus-susceptible crop, in an effort to attract the vector to the barrier crop instead of to the host.

blue tag certified seed potatoes. Potatoes that have been produced according to strict inspection protocols and regulations to help ensure that they meet standards for size, condition, and physical defects, and that the subsequent generations of tubers are disease-free.

leaf drop. Necrosis and death of one or more lateral stems that remain attached to the main stem of the potato plant.

mild mosaic. A classic symptom caused by plant virus infection. Mild mosaic presents as faint and sometimes difficult to discern light and dark green islands on the surface of plant foliage.

mini-tubers. The progeny tubers produced by tissue-culture plantlets (see tissue-culture plantlets).

mottle. A difficult to discern symptom, especially in the field. Often presents as a very faint mosaic symptom.

necrosis. The death of plant or tuber tissue. Presents as blackening of the affected area.

non-persistent. A plant virus that can be spread by its vector in a matter of seconds to minutes. Generally, insecticidal sprays are not a good option for non-persistent virus control, because the aphids transmit the virus too quickly, before the insecticide can act.

rogue. To remove and dispose of an individual plant or group of plants due to a plant pathogen infection or other undesirable plant characteristic.

rugose mosaic. A severe symptom indicative of a PVY infection. Rugose mosaic presents as leathery, rough or bunched leaves, with light and dark green islands on the surface of plant foliage.

RT-PCR. Reverse Transcriptase Polymerase Chain Reaction. A common molecular test used in the laboratory to detect the nucleic acid of plant viruses.

sentinel. An area of weedy, native, or invasive plants that has not been cultivated. Sentinel plots commonly surround agricultural fields and often are used for research purposes.

specialty potatoes. Potato cultivars that are generally grown and sold for the fresh market or marketed directly to consumers. Some examples include red, white, yellow, purple, and fingerling varieties.

TAS-ELISA. Triple Antibody Sandwich-Enzyme-Linked Immunosorbent Assay. A common serological test used in the laboratory to detect the coat proteins of plant viruses.

tissue-culture plantlets. The beginning of the seed certification process. Small cuttings of healthy plants are grown in laboratory culture with specific nutrients to produce genetic clones of the mother plants. The process helps to insure virus-free plants.

tuberborne. When plants are infected with a virus, the virus accumulates in the tubers. Because potatoes grow as vegetative propagates and produce clonally, the infected tubers produce plants already infected. Thus, the infection is tuberborne.

veinal necrosis. Darkening or blackening of veins on the underside of plant foliage.

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