Strain-Specific Resistance to *Potato virus Y* (PVY) in Potato and Its Effect on the Relative Abundance of PVY Strains in Commercial Potato Fields

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Abstract

Potato virus Y (PVY) is a serious threat to potato production due to effects on tuber yield and quality, in particular, due to induction of potato tuber necrotic ringspot disease (PTNRD), typically associated with recombinant strains of PVY. These recombinant strains have been spreading in the United States for the past several years, although the reasons for this continuing spread remained unclear. To document and assess this spread between 2011 and 2015, strain composition of PVY isolates circulating in the Columbia Basin potato production area was determined from hundreds of seed lots of various cultivars. The proportion of nonrecombinant PVY^O isolates circulating in Columbia Basin potato dropped ninefold during this period, from 63% of all PVY-positive plants in 2011 to less than 7% in 2015. This drop in PVY^O was concomitant with the rise of the recombinant PVY^{N-Wi} strain incidence, from less than 27% of all PVY-positive plants in

2011 to 53% in 2015. The proportion of the PVY^{NTN} recombinant strain, associated with PTNRD symptoms in susceptible cultivars, increased from 7% in 2011 to approximately 24% in 2015. To further address the shift in strain abundance, greenhouse experiments were conducted and revealed that three of the four most popular potato cultivars grown in the Columbia Basin exhibited strain-specific resistance against PVY^O. Reduced levels of systemic movement of PVY^O in such cultivars would favor spread of recombinant strains in the field. The negative selection against the nonrecombinant PVY^O strain is likely caused by the presence of the *Ny_{ibr}* gene identified in potato cultivars in laboratory experiments. Presence of strain-specific resistance genes in potato cultivars may represent the driving force changing PVY strain composition to predominantly recombinant strains in potato production areas.

In the past 15 to 20 years, *Potato virus Y* (PVY) has been a major problem for potato in the United States (Gray et al. 2010; Karasev and Gray 2013) due to the gradual spread of recombinant strains often associated with the potato tuber necrotic ringspot disease (PTNRD) (Crosslin et al. 2002, 2006; Gray et al. 2010; Karasev et al. 2008; Lorenzen et al. 2006a, 2008; Piche et al. 2004). PVY exists as a complex of at least nine strains that differ in host specificity in various solanaceous species, and cause a range of symptoms in different potato cultivars (Karasev and Gray 2013; Singh et al. 2008). PVY affects both potato yield and tuber quality, resulting in substantial economic losses for both seed potato producers and ware potato producers supplying the fresh and processed market (Beczner et al. 1984; Benedict et al. 2015; Hane and Hamm 1999; Le Romancer et al. 1994; Nolte et al. 2004).

Originally, strains of PVY were classified based on the induction of hypersensitive resistance (HR) response in a standard set of potato (*Solanum tuberosum*) cultivars with known genetic backgrounds (Cockerham 1970; de Bokx and Huttinga 1981; Jones 1990; Singh et al. 2008). The HR response in *S. tuberosum* was triggered due to the presence of three strain-specific *N* resistance genes (*Ny*, *Nc*, and *Nz*), and four strains were genetically defined: PVY^O (triggering *Ny_{ibr}*), PVY^C (triggering *Nc_{ibr}*), PVY^Z (triggering *Nz_{ibr}*), and PVY^N (overcoming all three *N* genes without the HR response) (Chikh-Ali et al. 2014; Cockerham 1970; Jones 1990; Singh et al. 2008). Molecular characterization of PVY strains revealed that PVY^O, PVY^N, and PVY^C had nonrecombinant genomes that formed three separate

phylogenetic clades (Glais et al. 2002; Karasev and Gray 2013; Lorenzen et al. 2006a; Singh et al. 2008). PVY^Z, on the other hand, was found to have a recombinant genome built of PVY^O and PVY^N parental sequences that was classified as either PVY^{NTN} or PVY^{NTN-NW} recombinant based on molecular characteristics (Chikh-Ali et al. 2010, 2014; Hu et al. 2009a; Karasev and Gray 2013; Kerlan et al. 2011). Recently, PVY^Z was proposed to include other, nonrecombinant isolates as well (Jones and Kehoe 2016; Kehoe and Jones 2016). There are multiple other recombinants, most often built of PVY^O and PVY^N parental sequences, named PVY^{N-Wi}, PVY^{N-O}, PVY-NE11, and others (Karasev and Gray 2013), but these were not defined genetically and were classified only based on molecular properties.

Epidemiological data on PVY strain prevalence in potato is available for different production areas and sometimes over multiple years (Anfoka et al. 2014; Baldauf et al. 2006; Chikh-Ali et al. 2010, 2016a,b; Crosslin et al. 2006; Djilani-Khouadja et al. 2010; Ellis et al. 1997; Galvino-Costa et al. 2012a; Gray et al. 2010; Karasev et al. 2008; Kerlan et al. 1999; Lorenzen et al. 2006a, 2008; Schubert et al. 2007, 2015). In Western Europe (Kerlan et al. 1999; Schubert et al. 2007), Brazil (Galvino-Costa et al. 2012a), the Mediterranean (Djilani-Khouadja et al. 2010), the Middle East (Anfoka et al. 2014; Chikh-Ali et al. 2010, 2016a), and Indonesia (Chikh-Ali et al. 2016b), there was a consistent trend over the past 30 years in which recombinant strains such as PVY^{NTN} and PVY^{N-Wi} replaced nonrecombinant PVY^C, PVY^O, and PVY^N isolates as the most common in potato production. Until recently, one of the few remaining strongholds for PVY^O was the United States, where it was the most abundant strain found in the 2004-to-2006 PVY strain survey (Gray et al. 2010). However, recombinant strains were identified in the United States as well (Baldauf et al. 2006; Crosslin et al. 2002, 2006; Gray et al. 2010; Karasev et al. 2008; Lorenzen et al. 2006a, 2008), and there was a general concern that recombinant strains may continue to spread as they had in other countries (Gray et al. 2010).

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Various explanations were suggested for this observed transition from nonrecombinant to recombinant strains of PVY, including marketing of new susceptible potato cultivars, more efficient transmission by aphids, more efficient translocation into tubers, strain-specific resistance, influx of new, exotic isolates of PVY, and others (Karasev and Gray 2013). Experimental evidence for any of these factors being involved in the observed shift to the recombinant strains of PVY in potato production was limited and not convincing. The problem was due partly to the shortage of studies on the (strain-specific) HR induction in U.S. potato cultivars and partly to the lack of consistent epidemiological surveys addressing PVY strain composition in an area representing a substantial segment of the potato production in the United States. Nevertheless, PVY isolates from strains PVY^O and PVY^Z were studied for their ability to elicit HR in several potato cultivars harboring *Ny_{thr}* and *Nz_{thr}* genes, respectively, and grown in the United States (Chikh-Ali et al. 2014; Karasev et al. 2011; Kehoe and Jones 2016; Kerlan et al. 2011; Rowley et al. 2015). The virus determinant involved in interaction with the *Ny_{thr}* gene in potato and, hence, in triggering the HR response was mapped within the HC-Pro cistron of the PVY genome (Tian and Valkonen 2013, 2015). The HR response was found to provide partial resistance against PVY^O and PVY^Z strains of PVY (Jones 1990; Kerlan et al. 2011; Rowley et al. 2015), restricting virus strains mostly to cultivars in which they did not evoke hypersensitivity (Cockerham 1970; Jones 1990; Karasev and Gray 2013).

Potato production in the Columbia Basin takes place in seven counties of the south-central state of Washington and in two counties of the north-central state of Oregon, along the Columbia River. This area is responsible for almost 30% of U.S. potato production (NASS 2015). Here, we present data for the field survey of PVY strains circulating in the Columbia Basin potato production area between 2011 and 2015, reflecting the dynamic nature of the PVY strain composition there. These data may be viewed as a good estimation of the

PVY strain composition changes in the entire United States, particularly because the hundreds of seed lots tested were also shipped and used throughout North America. We also conducted a controlled study of the PVY strain changes in four potato cultivars widely grown in the Columbia Basin and observed a similar strain composition shift apparent within a single growing season. This shift in the proportion of PVY strains circulating in potato was directly linked to the strain-specific HR response associated with the presence of *N* genes in three of the four studied cultivars: ‘Alturas’, ‘Umatilla Russet’, and ‘Ranger Russet’.

Materials and Methods

Field plot design and locations. The Washington seed lot trials were planted at the Washington State University research farm near Othello and the Oregon seed lot trials were planted at the Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston. These trials were conducted from 2011 to 2015 using certified seed lots imported into Washington and Oregon from a number of U.S. states and Canadian provinces (Pavek and Holden 2016). Whole tubers were submitted by the growers in the Columbia Basin and planted in Oregon and Washington in single rows at 200 tubers/seed lot. Plant spacing was 25 cm and rows were 85 cm apart. Fertilization, irrigation frequency, and insect, weed, and disease control practices were consistent with commercial potato production practices in the Columbia Basin. Planting dates varied each year, depending on receipt of seed lots, but ranged from late April to late May. The designs of the field plots and locations were the same as described previously (Crosslin et al. 2006).

Plant sampling, PVY testing, and serotyping. Each year in early to mid-June, seed lot trials were visually assessed by a group of Pacific Northwest (Washington, Oregon, Idaho, and Montana) potato pathologists, extension specialists, and volunteers for abiotic and

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May 27, 2015

Fig. 1. A, Screenhouse experiment layout, showing the dimensions of the plot and randomized block design of the experiment. Letters indicate individual potato plants: B = Russet Burbank, U = Umatilla Russet, R = Ranger Russet, and A = Alturas. Shading color designates the *Potato virus Y* (PVY) strain used for inoculation: green means PVY^O (isolate Tb60), blue means PVY^{N_W} (isolate N1), orange means PVY^{N_{TR}} (isolate HR1), and no shading means uninoculated. **B**, Picture of the screenhouse plot just prior to inoculation with PVY isolates.

biotic abnormalities, and each plant displaying an abnormality was flagged. Specifically, each plant showing mosaic symptoms attributed to virus infection was marked with a yellow flag. Sample collection for the PVY testing and typing was conducted on the day of the first reading and flagging, not more than 2 h after this reading was completed. Samples were collected from each seed lot having at least one yellow flag but not more than 10 samples per lot, due to limited testing and typing resources. The number of samples collected varied between 222 and 431 for Othello, WA and between 101 and 215 for Hermiston, OR, and depended on the number of seed lot samples submitted for the testing each year and the relative incidence of infection for any given season. Three leaves per flagged plant were collected and placed in a sealable plastic bag labeled with the seed lot number. Bags were kept in coolers on ice or in a cold room at approximately 4°C until processing for PVY strain typing, usually within 2 to 3 days after collection.

In the 2011 to 2015 seasons, all Othello samples were initially tested by triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA), as described previously (Karasev et al. 2010; Nikolaeva et al. 2012), with some modifications. Four monoclonal antibodies were routinely used in this assay to determine the serotype of the PVY strain present: Asc5, 1F5, SASA-N, and Mab2 (occasionally, SASA-O was used in place of Mab2). Asc5 is a strain-nonspecific mouse monoclonal antibody cocktail, produced against purified PVY isolate Mont (PVY^N) through a series of intramuscular injections into a mouse, with subsequent fusion of spleen and myeloma cells, selection of hybridoma cell lines producing PVY-specific immunoglobulins, and production of ascites in mice (O. V. Nikolaeva and A. V. Karasev, unpublished). Monoclonal antibodies 1F5 (Ellis et al. 1996) and Mab2 (McDonald and Kristjansson 1993) were obtained from Agdia (Elkhart, IN) or from Phyto Diagnostics (North Saanich, BC, Canada). 1F5 is specific to PVY^N, PVY^{NTN}, and PVY^{O5}, and Mab2 is specific to the PVY^O, PVY^{N-Wi}, and PVY^C strains. Monoclonal antibodies SASA-N and SASA-O were obtained from the Scottish Agricultural Science Agency (SASA, Edinburgh). SASA-N is specific to PVY^N and PVY^{NTN}, and SASA-O is specific to the PVY^O, PVY^{N-Wi}, and PVY^C strains.

Reverse-transcription polymerase chain reaction-based PVY strain typing. In the 2011 to 2015 seasons in the Hermiston, OR trials, PVY strain typing was conducted using the reverse-transcription polymerase chain reaction (RT-PCR) methodology developed by Lorenzen et al. (2006b). The same method was also used in the 2011 season on samples originated from the Othello, WA trials. In both cases, samples identified by TAS-ELISA as positive were subjected to a separate total nucleic acid extraction, and then used in RT-PCR assays according to Lorenzen et al. (2006b). With this methodology, nonrecombinant PVY^O and PVY^N strains can be distinguished from recombinant PVY^{NTN} and PVY^{N-Wi/N:O} strains; however, no distinction can be made between PVY^{N:O} and PVY^{N-Wi} strains, and no distinction is possible between PVY^{NA-N} and PVY-NE11 strains (Chikh-Ali et al. 2013a; Lorenzen et al. 2006b). In 2012, PVY-positive samples from Othello trials were subjected to an additional RT-PCR typing according to an improved methodology (Chikh-Ali et al. 2010), with the same total nucleic acid extracts used for the Lorenzen et al. (2006b) method as well. In 2013 to 2015, the samples from Othello were typed using the immunocapture (IC) RT-PCR methodology developed recently (Chikh-Ali et al. 2013a). In this method, the extraction of the sample was the same as for ELISA tests, the samples were loaded onto the IC plates at the same time as ELISA samples, and RT-PCR typing was conducted using both Lorenzen et al. (2006b) and Chikh-Ali et al. (2010) protocols. In this combined typing, more than nine strains of PVY can be distinguished, including nonrecombinant PVY^O, PVY^N, and PVY^{NA-N} and recombinant PVY^{NTN}, PVY^{N-Wi}, PVY^{N:O}, and PVY-NE11 (Chikh-Ali et al. 2013a).

Layout of the 2015 screenhouse experiment. In spring to summer 2015, a screenhouse experiment was conducted at Hermiston, OR to assess the infection rate of three strains of PVY in the four

most common potato varieties grown in the Columbia Basin. The screenhouse was used to both mimic the field conditions for potato production and minimize any current-season spread of the virus by excluding aphids, by thorough insecticide application, and by the use of mechanical inoculation. In all, 160 plants each of 'Russet Burbank', 'Alturas', 'Umatilla Russet', and 'Ranger Russet' were divided into four groups: infected with PVY^O (isolate Tb60), PVY^{N-Wi} (N1), or PVY^{NTN} (HR1), or left uninfected. On 16 April 2015, nuclear seed minitubers of the four potato varieties, the most widely planted cultivars used in the Columbia Basin, were planted into a 22-by-11-meter screenhouse in a randomized complete block design in Hermiston (Fig. 1). The experiment had 16 treatments: four varieties × three strains of PVY plus one untreated check (UTC) × 10 plants × four replications = 640 plants. Hence, each of the four potato varieties was represented by 160 plants, with 40 plants inoculated with each of the three PVY strains plus 40 plants of UTC. Prior to inoculation, all plants were sampled and tested for possible PVY presence. Leaves from each plant in each row were sampled, bulked (10 plants/group), and assayed for PVY using RT-PCR (Cating et al. 2015). No PVY-positive plants were detected prior to inoculation. Potato plants were then mechanically inoculated with the PVY^O (isolate Tb60), PVY^{NTN} (HR1), and PVY^{N-Wi} (N1) strains of PVY on 27 May 2015, when plants were approximately 20 to 76 cm in height (Fig. 1). Each virus strain was inoculated one at a time to avoid potential contamination. After inoculation, each plant was rinsed with water to remove excess inoculum from the leaves. Five weeks later (on 1 July 2015), individual plants were sampled; upper, uninoculated leaves were assayed for PVY; and PVY-positive samples were typed to strain using ELISA and RT-PCR according to the previously published methodology (Chikh-Ali et al. 2013a; Nikolaeva et al. 2012).

Potato cultivar sources and plant maintenance. 'Maris Bard' potato was originally received from the National Potato Germplasm Collection in Sturgeon Bay, WI as tissue culture plantlets. Three other cultivars ('Desiree', 'CalWhite', and 'Umatilla Russet') were obtained from the University of Idaho Potato Tissue Culture Laboratory (provided by Lorie Ewing). Plantlets were cut and transferred to new media every 8 weeks and, after transfer, these plantlets were planted in soil in 2 to 8 weeks. While in vitro, the plantlets were periodically subjected to RT-PCR tests for main potato viruses to confirm their virus-free status.

Reference isolates of PVY, inoculations, phenotype screening, and laboratory testing. All isolates of PVY used in this work were from the laboratory collection at the University of Idaho and have been previously subjected to whole-genome sequencing (Table 1). PVY isolates were maintained in 'Burley' tobacco in an insect-free, climate-controlled growth chamber. Infected tobacco tissue was used as an inoculum, homogenized in phosphate buffer (50 mM sodium phosphate [pH 7.0] plus 50 mM Na DIECA) at a dilution rate of 1:10 (wt/vol) with a mortar and pestle on ice. For all cultivars, potato plants were mechanically inoculated at the 6- to 10-leaf stage and grown in growth chambers with a cycle of 16 h of light and 8 h of darkness and maintained at 20 to 22°C. Mechanical inoculations and symptom assessment were conducted as described previously (Rowley et al. 2015). Three plants of each cultivar were inoculated with each PVY isolate per experiment. The symptom assessment started 4 to 5 days after inoculation and was carried out for 6 to 8 weeks, focusing on two types of symptoms: (i) local and systemic necrotic reactions which indicated the induction of the HR and typically revealed the presence of a corresponding *N* gene in a cultivar and (ii) presence or absence of other systemic symptoms not associated with HR. Each experiment was repeated three times.

Results

Changes in PVY strain composition in Columbia Basin potato fields between 2011 and 2015. The number of mosaic samples analyzed for the presence of PVY varied during the five seasons of testing, from 222 to 431 per season in Othello seed lot trials and from 101 to 215 in Hermiston seed lot trials (Table 2). In the span of five seasons, over 1,400 mosaic samples were tested and typed in Othello

trials and over 800 samples were analyzed in Hermiston trials. PVY-positive samples represented 84 to 93% of all collected samples exhibiting mosaic in Othello and 82 to 93% of samples collected in Hermiston (Table 2). The 7 to 18% of mosaic samples testing negative for PVY may represent a margin of error characteristic of visual symptom reading or infections by other viruses; however, PVY was responsible for the majority of the mosaic symptoms identified in potato planted in the Columbia Basin between 2011 and 2015. Typing these PVY-positive samples to strain revealed dynamic changes to the composition of PVY strains present in this potato production area. Specifically, PVY^O prevalence decreased ninefold in the Othello trials, from 63 to less than 7% (Table 3), whereas, in Hermiston, this drop was less but still pronounced, from 47 to 9% (Table 4). This less dramatic drop in Hermiston trials could be due, in part, to a large proportion of unclassified samples in 2011 at this location. In both locations, the drop in the relative abundance of the PVY^O strain was accompanied by a concomitant rise in the proportion of recombinant strains (in particular, PVY^{N-Wi}) which, in the Othello trials, almost doubled from under 27 to 53% of all PVY-positive plants between 2011 and 2015 (Table 3). Similar trends were observed in Hermiston, where the relative abundance of recombinant PVY strains PVY^{N:O} and PVY^{N-Wi} together increased from 8 to 59% from 2011 to 2015 (Table 4). During the same period, the relative abundance of PVY^{NTN} increased more than threefold (from less than 8 to more than 24%) in the Othello trials (Table 3) and increased twofold (from 12 to 24%) at the Hermiston location. In the Othello trials, the share of another recombinant strain, PVY-NE11, rose from 2011 to 2013 more than 10-fold (from less than 1 to 10%) but then dropped again to less than 1% in 2015 (Table 3). The proportion of the PVY^{N:O} strain fluctuated between 2 and 3% between 2012 and 2014 but, in 2015, rose to more than 9% (Table 3). Two nonrecombinant strains, PVY^N and PVY^{O-O5}, were found only in 2011 and 2012 (Table 3). The overall strain composition of PVY isolates circulating in the Columbia Basin potato changed drastically during the observation period, from the predominantly nonrecombinant PVY^O strain (63%) in 2011 to predominantly recombinant strains represented by PVY^{N-Wi} (53%), PVY^{NTN} (24%), PVY^{N:O} (9%), and PVY-NE11 (0.5%) (Table 3). The two recombinant strains associated with PTNRD, PVY^{NTN} and PVY-NE11, combined, represented 8.7% of all PVY-positive plants in 2011 and increased to 24.6%, nearly threefold, in 2015 (Table 3). Large changes in the proportion of PVY^{N:O} strain also occurred, although its relative abundance never reached 10% of all PVY-positive plants detected during the period of observations.

Strain-specific resistance in potato cultivars may be driving the changes in PVY strain composition. Visual assessment of the virus infection in different strain–cultivar combinations in the screenhouse experiment indicated that PVY^O infection was often associated with systemic necrotic symptoms typical of HR in Alturas, Umatilla Russet, and Ranger Russet. These consisted of multiple green rings, necrotic lesions, and vein necrosis on infected leaves (Fig. 2A and B) and, for Ranger Russet, of necrotic leaf-drop and necrotic stem streaks (Fig. 2C). Similar systemic HR reactions against the PVY^O

strain have been reported previously for Alturas and Ranger Russet (Rowley et al. 2015) infected under greenhouse conditions. However, the response of Umatilla Russet against different PVY strains has not previously been studied.

In the screenhouse, Russet Burbank did not display any reduction in systemic movement of the three PVY strains used as a challenge, with infection incidence varying from 70% for PVY^{NTN} to 78% for

Table 2. Proportion of *Potato virus Y* (PVY)-positive samples collected exhibiting mosaic symptoms at two commercial seed lot trial locations, 2011 to 2015^a

Season	Othello, WA		Hermiston, OR	
	N	PVY+ (%)	N	PVY+ (%)
2011	246	87.7	101	82
2012	235	84.3	206	86
2013	283	88.0	114	89
2014	222	90.5	215	93
2015	431	92.6	192	88

^a N = number of mosaic samples tested and PVY+ = PVY-positive samples.

Table 3. *Potato virus Y* (PVY) strain breakdown at Othello, WA, seed lot trials, 2011 to 2015

Season	PVY strain type, percentage of all PVY-positive plants							
	O	N-Wi	N:O	NTN	NE-11	NA-N	N	O5
2011 ^b	62.6	27.4	7.8	0.9	0	0.5	0.9	3.5
2012	30.8	37.4	3.0	13.1	5.6	0	5.6	1.0
2013	17.3	46.6	2.4	20.5	10.0	0	0	0
2014	15.9	59.2	2.0	6.0	8.0	0	0	0
2015	6.8	52.9	9.3	24.1	0.5	0	0	0

^a Unclassified or inconclusive.

^b In 2011, samples from Othello were typed to strain by the methodology of Lorenzen et al. (2006b) only and, consequently, no distinction could be made between N-Wi and N:O strains or between NE-11 and NA-N strains.

Table 4. *Potato virus Y* (PVY) strain breakdown at Hermiston, OR, seed lot trials, 2011 to 2015^a

Season	PVY strain type, percentage of all PVY-positive plants						
	O	N-Wi, N:O	NTN	NE-11, NA-N	N	O5	Uncl. ^b
2011	47	8	12	0	0	0	33
2012	39	50	11	0	0	0	0
2013	26	53	14	0	0	0	7
2014	35	45	17	0	0	0	3
2015	9	59	24	0	0	0	5

^a Samples were typed to strain by the methodology of Lorenzen et al. (2006b) only and, consequently, no distinction could be made between N-Wi and N:O strains or between NE-11 and NA-N strains.

^b Unclassified or inconclusive.

Table 1. Genotypes and phenotypic traits of the *Potato virus Y* (PVY) isolates used in this study

Isolates	Strain ^a	Genotype	Bioassay ^b	Serotype	Genome sequence ^c
Tb60	PVY ^O	PVY ^O	Mos	O	O-type sequence EF026074
ID269	PVY ^O	PVY ^{O-O5}	Mos	O5	O5-type sequence FJ643477
N1	PVY ^{N-Wi}	PVY ^{N-Wi}	VN	O	Recombinant HQ912863
Alt	PVY ^{N:O}	PVY ^{N:O}	VN	O	Recombinant AY884985
Mont	PVY ^N	PVY ^N	VN	N	N-type sequence AY884983
HR1	PVY ^Z	PVY ^{NTN} (PVY ^Z -NTN)	VN	N	Recombinant FJ204166
L26	PVY ^Z	PVY ^{NTN} (PVY ^Z -NTN)	Mos	N	Recombinant FJ204165
RRA-1	PVY ^{NA-N}	PVY ^{NA-N}	VN	N	NA-N-type sequence AY884984
ID20	NE-11	NE-11	VN	N	Recombinant HQ912867

^a Strains listed according to Karasev and Gray (2013).

^b Tobacco symptoms: VN = vein necrosis and Mos = mosaic and vein clearing.

^c Sequences deposited in GenBank.

PVY^{N-Wi} (Fig. 3). The three other cultivars exhibited reduced systemic movement for PVY^O, with PVY^O infection incidence ranging from 55% for Alturas to 40% for Umatilla Russet and less than 28% for Ranger Russet (Fig. 3). Under these same screenhouse conditions, infection incidence of PVY^{N-Wi} was significantly higher in these three cultivars than in Russet Burbank: 85% in Ranger Russet, 90% in Umatilla Russet, and 92% in Alturas. Infection incidences for PVY^{NTN} were also significantly higher in all of the three cultivars relative to Russet Burbank (Fig. 3).

Examination of the number of plants infected systemically in each cultivar–strain combination provided evidence that the strain-

specific resistance exhibited by Alturas, Umatilla Russet, and Ranger Russet in the screenhouse resulted in a significant difference in strain prevalence between the expected infection from the inoculation and observed infection at the end of the experiment (Table 5). Although each of the three PVY strains was initially inoculated into 33.3% of the plants, by 5 weeks after inoculation, the proportions of PVY strains significantly changed for at least two cultivars (Umatilla Russet and Ranger Russet) (Table 5), with Alturas exhibiting a similar trend. At the end of the 5-week experiment, the proportions among all PVY-positive plants in the screenhouse were 22% PVY^O, 40% PVY^{N-Wi}, and 38% PVY^{NTN} (Table 5).

Fig. 2. Symptoms observed in the screenhouse experiment. **A**, Symptoms of green, water-soaking rings and necrotic lesions; and **B**, vein necrosis induced by *Potato virus Y* (PVY) isolate Tb60 (PVY^O) in Umatilla Russet as observed at 5 weeks postinoculation (wpi). **C**, Severe systemic leaf-drop and necrotic stem streaks induced by PVY isolate Tb60 (PVY^O) in Ranger Russet at 5 wpi. All plants were tested at 5 wpi by triple-antibody sandwich enzyme-linked immunosorbent assay and reverse-transcription polymerase chain reaction to confirm systemic infection with the isolate or strain indicated.

Fig. 3. Infection incidences in the screenhouse experiment determined by triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) and reverse-transcription polymerase chain reaction (RT-PCR) 5 weeks postinoculation. Forty individual plants per cultivar–strain combination were inoculated with isolates Tb60 (PVY^O), N1 (PVY^{N-Wi}), and HR1 (PVY^{NTN}) in this experiment. The X-axis represents the proportion of the 40 plants found infected with the corresponding PVY strain for each of the four cultivars tested: Russet Burbank (RB), Alturas, Umatilla Russet, and Ranger Russet; vertical bars represent standard error. Each sample was tested for systemic infection using TAS-ELISA, and each positive sample was typed to strain by RT-PCR.

Resistance in Umatilla Russet against PVY^O is expressed as HR. To further study the response of Umatilla Russet to four different strains of PVY (PVY^O, PVY^N, PVY^{NTN}, and PVY^{N-Wi}), a growth-room experiment was conducted under controlled temperature and lighting conditions and side by side with control cultivars Maris Bard, Desiree, and CalWhite. Both PVY^O and PVY^{NTN} induced a typical HR response (Fig. 4), similar to previously described, in Maris Bard following mechanical inoculation (Rowley et al. 2015). The HR started as necrotic rings developing into local lesions on inoculated leaves at about 7 days postinoculation (dpi). Within 10 to 14 dpi, inoculated leaves were fully necrotized. By 21 dpi, systemic necrosis and leaf drop were observed, as well as severe mosaic and crinkling of the top, young leaves. Another control, Desiree, produced a very similar HR upon infection with PVY^O, whereas PVY^{NTN} induced mild systemic mosaic on upper noninoculated leaves 14 to 21 dpi. PVY^N and PVY^{N-Wi} infection resulted in mild to very mild systemic mosaic with no local lesions observed on the inoculated leaves in either Desiree or Maris Bard. Slight crinkling was visible in upper noninoculated leaves in Maris Bard infected with PVY^N and PVY^{N-Wi} (Table 6). Systemic infections of all four isolates representing four strains of PVY were easily confirmed 21 to 28 dpi using ELISA (Fig. 5) and RT-PCR (not shown). However, not all of the Desiree and Maris Bard plants inoculated with Tb60 (PVY^O) and not all of the Maris Bard plants inoculated with L26 (PVY^{NTN}) established systemic infection (Fig. 5) due to the presence of the *Ny_{ibr}* gene (Desiree) or *Ny_{ibr}* and *Nz_{ibr}* genes (Maris Bard)

(Chikh-Ali et al. 2014; Cockerham 1970; Jones 1990; Singh et al. 2008).

PVY^{N-Wi} and PVY^{NTN} induced very mild mosaic in systemically infected, noninoculated upper leaves of CalWhite, clearly visible by about 14 to 21 dpi, but no other local or systemic symptoms (Table 6). PVY^N and PVY^O induced no local or systemic symptoms in CalWhite (Table 6), although virus was easily detected 18 to 21 dpi by ELISA (Fig. 5) and RT-PCR (not shown). Lack of resistance to any of the four PVY strains tested combined with the lack of symptoms exhibited by CalWhite demonstrated that this cultivar is susceptible to these three strains.

PVY^O induced a distinct local and systemic HR reaction in Umatilla Russet (Fig. 4; Table 6). This included formation of local lesions that later expanded and led to completely necrotized inoculated leaves by 12 to 14 dpi. This local HR was followed by a clear systemic HR, which displayed a typical leaf-drop syndrome. Some of the Umatilla Russet plants inoculated with Tb60 (PVY^O) failed to establish systemic infection, suggesting possible presence of the *Ny_{ibr}* gene conferring strain-specific HR against PVY^O. This was not due to the loss of infectivity of the Tb60 (PVY^O) inoculum; the infectivity of the same inoculum was confirmed in CalWhite in the same experiment (Fig. 5). PVY^{N-Wi} and PVY^{NTN} induced mosaic and crinkling in Umatilla Russet (Fig. 4) and led to visible stunting of the infected plants. PVY^{N-Wi} induced characteristic chlorotic spots in upper, noninoculated leaves of Umatilla Russet (Fig. 4). In the case of PVY^{NTN}, typical local HR was visible at 14 dpi (Fig. 4A) but only mild

Table 5. Infection incidences in four potato cultivars in the screenhouse after mechanical inoculation with three different strains of *Potato virus Y* (PVY)^a

Strain	Russet Burbank			Alturas			Umatilla Russet			Ranger Russet			All combined		
	Exp	Obs	Tot (%)	Exp	Obs	Tot (%)	Exp	Obs	Tot (%)	Exp	Obs	Tot (%)	Exp	Obs	Tot (%)
O	40	29	33.0 (±9.8)	40	22	23.4 (±8.6)	40	16	19.1 (±8.4)	40	11	13.3 (±7.3)	160	78	22.3 (±5.0)
N-Wi	40	31	35.2 (±10.0)	40	37	39.4 (±9.9)	40	36	42.9 (±10.6)	40	34	41.0 (±10.5)	160	138	39.5 (±5.3)
NTN	40	28	31.8 (±9.7)	40	35	37.2 (±9.8)	40	32	38.1 (±10.4)	40	38	45.8 (±10.7)	160	133	38.1 (±5.2)
Total	120	88	...	120	94	...	120	84	...	120	83	...	480	349	...

^a Forty potato plants per PVY strain were inoculated in a screenhouse with isolates Tb60 (PVY^O), N1 (PVY^{N-Wi}), and HR1 (PVY^{NTN}) at the same time, in a complete randomized block design. All plants were tested for systemic infection 5 weeks later using triple-antibody sandwich enzyme-linked immunosorbent assay and typed to strain using immunocapture reverse-transcription polymerase chain reaction. Exp = expected number of infected plants, if the incidence is assumed at 100% and Obs = actual, observed number of infected plants. Tot (%) = percentage of plants of a cultivar infected with this particular strain out of the total number of plants of this cultivar infected with all strains tested. A χ^2 test for homogeneity was done for each variety assuming a null hypothesis of equal distribution among viral types, and the 95% statistical confidence intervals are listed in parentheses.

Fig. 4. Symptoms observed in the climate-controlled growth room experiment. **A**, Inoculated leaf of Umatilla Russet, 2 weeks postinoculation (wpi) with *Potato virus Y* (PVY) isolate L26 (PVY^{NTN}): symptoms of green, water-soaking rings, necrotic lesions, and vein necrosis. **B**, Mild systemic necrosis, leaf-drop, and necrotic stem streaks induced by PVY isolate L26 (PVY^{NTN}) in Umatilla Russet at 7 wpi. **C**, Severe leaf-drop and necrotic stem streaks induced by PVY isolate Tb60 (PVY^O) in Umatilla Russet at 7 wpi. **D**, Mosaic, mottling, and chlorotic spots induced by PVY isolate N1 (PVY^{N-Wi}) in upper, noninoculated leaves of Umatilla Russet at 7 wpi. Background: a healthy Umatilla Russet plant. All plants were tested at 4 wpi by triple-antibody sandwich enzyme-linked immunosorbent assay and reverse-transcription polymerase chain reaction to confirm systemic infection with the isolate or strain indicated.

systemic vein necrosis became visible late in infection, by the end of the experiment, at 50 dpi (Fig. 4B). PVY^N induced only mild to very mild mosaic in Umatilla Russet (Table 6). All plants infected with PVY^{NTN}, PVY^{N-Wi}, and PVY^N strains established systemic infection easily confirmed at 21 to 28 dpi by ELISA (Fig. 5) and RT-PCR (not shown).

Discussion

Recombinant strains of PVY have spread in the past 30 years through the main potato production areas of the world, including strains PVY^{NTN} and PVY-NE11, which are associated with PTNRD (Baldauf et al. 2006; Blanchard et al. 2008; Chikh-Ali et al. 2016a,b; Galvino-Costa et al. 2012a,b; Gray et al. 2010; Karasev and Gray 2013; Kerlan 2006; Kerlan et al. 1999; Piche et al. 2004; Singh et al. 2008). This spread coincided with the virtual disappearance of other, nonrecombinant strains such as PVY^N and PVY^O from

potato fields in most of the production areas (Blanchard et al. 2008; Karasev and Gray 2013). In the United States, recombinant strains of PVY (PVY^{N-Wi} and PVY^{NTN}) were first reported on potato in the early 2000s (Baldauf et al. 2006; Crosslin et al. 2002, 2006; Lorenzen et al. 2006a; Piche et al. 2004) although, in Canada, they were found a few years earlier (McDonald and Kristjansson 1993). Nevertheless, the nonrecombinant strain PVY^O remained dominant in the United States up to the early 2010s (Baldauf et al. 2006; Gray et al. 2010; Karasev et al. 2010) (Tables 3 and 4).

The data collected in the Pacific Northwest seed lot trials from 2011 to 2015 documented a PVY strain composition shift during this period that was not only dramatic but also very rapid (Tables 3 and 4), resulting in an up to ninefold reduction of the previously dominant strain PVY^O share to an insignificant minority. At the same time, the relative abundance of the recombinant strains increased greatly from a significant 36% in 2011 to an overwhelming 93% 4 years later (Table 3). The underlying question that we attempted to answer in this work was: what were the reasons for these dramatic changes in the relative abundance of PVY strains over a period of only 5 years? Between 2011 and 2015, no new strains or variants of PVY were identified in the Othello and Hermiston trials, and the set of strains detected in the samples collected during these 5 years stayed stable (Tables 3 and 4), although improved RT-PCR typing methodology allowed differentiation of up to 14 recombinant genomes known for PVY (Chikh-Ali et al. 2013a). No new aphid species capable of vectoring PVY were reported in the Columbia Basin during this period, making the insect vector an unlikely factor contributing to the observed changes in the relative abundance of strains. Hence, neither changes in the virus nor changes in the vector composition can explain the observed shift in the PVY strain prevalence.

In the past, rapid and significant shifts of the PVY strain profile were hypothesized to occur due to differences in virus–host or virus–vector interactions for recombinant strains of PVY as compared with the nonrecombinant strains (Cockerham 1970; de Bokx

Table 6. Summary of symptoms expressed by different potato cultivars upon mechanical inoculation with four isolates of *Potato virus Y* (PVY) representing four PVY strains

Cultivar	Isolate or strain of PVY ^a			
	N1 (PVY ^{N-Wi})	L26 (PVY ^{NTN})	Tb60 (PVY ^O)	Mont (PVY ^N)
Maris Bard	M, Cr, St	M, Cr, LL, SN	M, Cr, LL, SN	M, Cr
Desiree	MM	M	M, Cr, LL, SN	MM
CalWhite	MM	MM	NS	NS
Umatilla Russet	M, Cr, St	MM, LL, SN, St, LD	M, Cr, LL, LD, SN	M

^a Symptom abbreviations: M = mosaic, Cr = crinkling, St = stunting, LL = local lesions, SN = systemic necrosis, LD = leaf drop, and MM = mild mosaic. NS indicates no symptoms but the systemic virus infection was confirmed by enzyme-linked immunosorbent assay and reverse-transcription polymerase chain reaction.

Fig. 5. Example of the triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) assessment of *Potato virus Y* (PVY) infection in four potato cultivars—Umatilla Russet (U), CalWhite (C), Desiree (D), and Maris Bard (MB)—4 weeks postinoculation. Three individual plants per cultivar were infected with each isolate—Tb60 (PVY^O), Mont (PVY^N), L26 (PVY^{NTN}), and N1 (PVY^{N-Wi})—and three plants were left uninfected. Umatilla Russet plants are labeled with green rectangles. Controls represent tobacco samples infected with corresponding PVY strains. Optical density at 405 nm (OD₄₀₅) signal reflects the concentration of the respective PVY strain in each individual plant sampled. Bars with different shades represent signals generated with polyclonal (PVY-specific, medium-dark gray) or three different monoclonal antibodies: SASA-O (O-specific, dark gray), SASA-N (N-specific, medium-light gray), and Agdia-N (1F5, N-specific, light gray). TAS-ELISA plates were read 16 h after the developing solution was added to the wells, and samples were considered positive if the OD₄₀₅ signal for the infected plant exceeded the one for an uninfected plant threefold.

and Huttinga 1981; Jones 1990; Karasev and Gray 2013; Singh et al. 2008). Translocation efficiency of the virus moving from the infected foliage into the tuber was studied for different PVY strains in several potato cultivars (Basky and Almasi 2005; Beemster 1976; Draper et al. 2002). Changes in the efficiency of virus translocation during the development of the plant have long been known to result in the “mature resistance” phenomenon (Sigvald 1985). PVY^N was found to translocate into tubers more efficiently than PVY^O (Basky and Almasi 2005; Beemster 1976); however, the studies included multiple other variables, hampering the interpretation of the data. Aphid transmission efficiencies in potato were found to differ about twofold between PVY^O and PVY^{NTN} for at least one colonizing aphid (Carroll et al. 2016), although other tests on a broader range of PVY strains and colonizing and noncolonizing aphids revealed similar transmission efficiencies across most of the strain–species combinations; in particular, no difference was found between PVY^O and PVY^{N-Wi} transmission (Mello et al. 2011; Verbeek et al. 2010). One of the factors contributing to the transmission efficiencies was found to be the cultivar reaction to the PVY^O infection. In a cultivar exhibiting HR against PVY^O, aphid transmission was significantly reduced (Carroll et al. 2016).

The HR reaction conferred by the *N* genes specific to individual strains of PVY is considered an indication of a host defense response in potato (Chikh-Ali et al. 2014; Cockerham 1970; de Bokx and Huttinga 1981; Jones 1990; Singh et al. 2008). The strain-specific genes *Ny_{ibr}* and *Nc_{ibr}* conferring resistance to PVY^O and PVY^C were found to be triggered by genetic determinants of the virus located in the HC-Pro cistron (Moury et al. 2011; Tian and Valkonen 2013, 2015), which may explain the selection of the PVY recombinants carrying the HC-Pro cistron from the PVY^N parent unable to trigger these *N* genes (Glais et al. 2002; Hu et al. 2009a,b; Karasev and Gray 2013; Lorenzen et al. 2006a; Singh et al. 2008). Recently, an additional *Nz_{ibr}* gene was identified in potato conferring resistance to the PVY^{NTN} recombinant, defining the PVY^{Z-NTN} strain of PVY (Barker et al. 2009; Chikh-Ali et al. 2014; Jones 1990; Kerlan et al. 2011). *Ny_{ibr}*, *Nz_{ibr}*, and possibly other strain-specific resistance genes were identified in multiple commercial potato cultivars grown in the United States (Kerlan et al. 2011; Rowley et al. 2015), including Alturas and Ranger Russet, commonly grown in the Columbia Basin (Rowley et al. 2015). The reaction of Umatilla Russet to infection with four strains of PVY and demonstrated HR response to the PVY^O infection in this cultivar indicated the presence of the *Ny_{ibr}* gene (Figs. 4 and 5; Table 6).

This study documents the unequal impacts that the predominant cultivars grown in the Columbia Basin have on the different recombinant and nonrecombinant PVY strains. These studies provided the first direct evidence that a strain-specific resistance exhibited by the most popular potato varieties grown in this area could result in a significant drop of the PVY^O strain share of infected plants in a screenhouse experiment, where current-season spread was excluded (Fig. 3). In the screenhouse study, the reduction in the relative abundance of PVY^O was very rapid, detectable within 5 weeks postinoculation (Fig. 3). This significant, one-third reduction in the PVY^O strain share observed in the screenhouse experiment after a single round of infection appeared to be the result of strain-specific resistance to PVY^O infection exhibited by three potato cultivars: Alturas, Umatilla Russet, and, in particular, Ranger Russet (Table 5). Russet Burbank had no strain-specific response against any of the three strains tested (Fig. 3), although this cultivar might have exhibited a weak, nonspecific resistance against all three PVY strains, reducing slightly the overall number of PVY-infected plants (Table 5).

The acreage of the Russet Burbank grown in the states of Washington and Oregon was on a steady, albeit slow decline from 2002 to 2015, representing now approximately 30% of all potato grown in Washington and approximately 18% of all potato grown in Oregon (NASS 2015). If the screenhouse were to approximate the various seed potato production areas supplying potato seed to the Columbia Basin, and representing 25% shares for each of Russet Burbank, Ranger Russet, Umatilla Russet, and Alturas (Fig. 1), the rapid (within 1 month) PVY strain composition change observed in the

screenhouse following inoculations with three strains of PVY (Table 5; Fig. 3) would reasonably mimic the changes observed between individual growing seasons in the field (Tables 3 and 4). However, in the Columbia Basin production area, many more potato cultivars may contribute to the observed strain-specific resistance expression, and other additional factors may contribute to the observed strain composition changes. Indeed, between 2011 and 2015, Russet Burbank made up 18 to 26% of the seed lots entered into the Washington seed lot trials (Pavek and Holden 2016), which is close to the Russet Burbank share in our screenhouse experiments. Collectively, 26 to 38% of all seed lots during this period were Alturas, Ranger Russet, and Umatilla Russet; Russet Norkotah contributed between 15 and 17% of the total; and more than 35 newer or nonmainstream cultivars made up the remaining 22 to 25% of all seed lots (Pavek and Holden 2016).

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