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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Authorization to Submit Thesis

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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 U.S. 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 non-recombinant PVY^{O} isolates circulating in the Columbia Basin potato dropped nine-fold during this period, from 63% of all PVY-positives 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-positives 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 ca. 24% in 2015. To further address the shift in strain abundance, screen-house 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 non-recombinant PVY^{O} strain is likely caused by the presence of the Ny_{tbr} 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.

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Chapter 1: Potato production and effects of virus diseases – brief summary

Potato (*Solanum tuberosum* subsp. *tuberosum*) is one of the staple crops grown and consumed worldwide. It is ranked fourth behind rice, wheat, and corn in terms of significance for mankind (Karasev and Gray 2013). Grown on all continents, potatoes are marketed to consumers as either fresh tubers or processed products (chips, French fries, dehydrated products, mashed potatoes, among others), or as seed to potato growers. The top four potato producing countries are China, Russia, India, and the United States (USDA-ERS 2016). The potato tuber is a good source of protein, vitamins, and is full of other nutrients making it a very important crop for people's diet in all developed and, especially, in developing countries (CIP 2016, FAO 2008). In the U.S., potato is the leading vegetable crop [over 1 million acres grown each year and $3.6 billion of potato sold (Potato Statistical Yearbook 2017)], with Idaho possessing approximately one-third of the potato acreage in the country, and producing over 30% of the harvested potato each year. Potato is a vegetatively propagated crop, planted in the form of "potato seed" or seed tubers made from cuttings of tubers, and as such is prone to accumulation of pathogens, including multiple viruses (Summuna, 2016; Karasev and Gray 2013). Management of potato diseases, and viruses among them, includes exclusion or reduction of pathogen inocula (through certification programs and quarantine), development of resistance in potato cultivars (through breeding), and management of vectors transmitting the disease.

Due to the vegetative propagation of potato, viruses represent one of the biggest threats to potato production, affecting tuber yield and also tuber quality. More than 20 viruses are considered important for potato production, with 6 to 8 routinely included in standard potato certification testing schemes in developed countries. Disease symptoms caused by

viruses can be grouped into foliar and tuber symptoms. Foliar symptoms include various types of mosaic, crinkling, mottling, leaf deformations, stunting, and local or systemic necrotic reactions, like lesions, vein burning, leaf drop, and stem streaking. Many of these foliar symptoms are non-specific to any particular virus. In tubers, symptoms of virus infections can be broadly separated into surface/external and internal symptoms. The external or surface symptoms include potato tuber necrotic ringspot disease (PTNRD) syndrome (Beczner et al. 1984; LeRomancer et al. 1994) and an external cracking syndrome (Benedict et al. 2015); both of these syndromes can be caused by certain strains of *Potato virus Y* (PVY) in susceptible potato cultivars. Internal symptoms are exhibited in the tuber flesh as net necrosis, necrotic areas, spots or arches, and affect the processing qualities of tubers. These may be caused by *Potato virus X* (PVX) transmitted mechanically or through potato seed (Nyalugwe et al. 2012), by viruses transmitted through soil, like *Potato mop-top virus* (PMTV) (Harrison and Reavy 2002) or *Tobacco rattle virus* (TRV) (Sahi et al. 2016), or by aphid-transmitted *Potato leafroll virus* (PLRV) (Jayasinghe and Salazar, 1998; Peters and Jones, 1981) and *Alfalfa mosaic virus* (AlMV) (Nie et al. 2015; Slack 1981).

Although some viruses can be transmitted mechanically, through plant wounding, the most typical transmission in the field occurs through vegetative potato seed and, hence, is a seed-borne transmission. For current season transmission, most viruses require the help of a vector to move from plant to plant. These vectors may be insects, like many different species of aphids transmitting PVY, PLRV, *Potato virus A* (PVA), *Potato virus S* (PVS), and *Potato virus M* (PVM). Two soil-transmitted viral diseases have two other types of vectors: PMTV is vectored by the fungus *Spongospora subterranea*, and TRV is vectored by stubby-root nematode species in the genera *Paratrichodorus* and *Trichodorus* (Trichodoridae).

Over the last 20 years PVY has emerged as a major problem in potato with impacts across the world (Gray et al. 2010; Karasev and Gray 2013). It is the type member of the genus *Potyvirus*, family *Potyviridae* and has a single-stranded positive-sense RNA genome. This virus has a relatively wide host range which includes Solanaceae species with many weeds growing wild around fields as well as crops like potato, tobacco, tomato, and pepper. PVY affects both yield and quality of potato tubers, and consequently has large economic impacts on the potato industry. This effect is two-fold: first, it can cause a 40-70% yield reduction; and, second, it may affect the overall tuber quality, reducing the value of the crop even further. The virus can induce PTNRD causing tubers to be unmarketable due to their appearance (raised rings that develop into necrotic sunken tissue) and tuber cracking.

PVY exists as a complex of strains (currently 10 strains have been found in the U.S.) that produce varying symptoms on a range of potato cultivars. These can be separated into foliar and tuber symptoms, and are cultivar dependent. The main tuber symptom was discussed before (PTNRD). Some foliar symptoms are mosaic, vein necrosis, necrotic lesions, leaf drop, and stunting. Each of these symptoms can be expressed in a range of severity from mild to severe. A mild mosaic can be quite faint compared to a more severe mosaic that looks more like a mottling of yellow and green on the leaves. Vein necrosis and lesions can be present on very few leaves, or be the cause of leaf drop, which happens when lower leaves have been killed off by the necrotic reaction to the virus. PVY has proven to be a very adaptable disease through its ability to generate new strains using mutation or recombination. This is seen in two ways. One is that a large number of strains present are recombinant from three parent strains that are not commonly found anymore. The other is the ability of some strains to overcome the strain-specific resistance found in some potato cultivars.

PVY strains are classified based on biological and molecular properties. Biological classification is based on reactions of a specific set of potato cultivars with defined genetic background and *Nicotiana tabacum* to PVY infection. Some strain types trigger a certain resistance gene (*Ny*, *Nc*, or *Nz*) producing the hypersensitive resistance (HR) response visible as various types of local or systemic necrosis restricting virus spread through the plant (Singh et al 2008; Karasev and Gray 2013). Molecular typing is based on differences in genome sequences between different strains of PVY, and can be done using various laboratory tests, including Enzyme-linked Immunosorbent Assay (ELISA) to differentiate between serotypes of PVY (Nikolaeva et al. 2012), reverse-transcription (RT) polymerase chain reaction (PCR) to probe natural nucleotide polymorphisms around main recombinant junctions (Lorenzen et al. 2006; Chikh-Ali et al. 2013a), or whole-genome sequencing with phylogenetic and recombination analysis (Green et al. 2017).

Chapter 2: Changes in composition of PVY Strains circulating in the Columbia Basin potato production fields[1]

Introduction

In the past 15 to 20 years, *Potato virus Y* (PVY) has been a major problem for potato in the U.S. (Gray et al., 2010; Karasev and Gray, 2013) due to the gradual spread of recombinant strains often associated with potato tuber necrotic ringspot disease (PTNRD) (Crosslin et al., 2002, 2006; Piche et al., 2004; Lorenzen et al., 2006, 2008; Karasev et al., 2008; Gray et al., 2010). 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 (Singh et al., 2008; Karasev and Gray, 2013). 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 (Hane and Hamm, 1999; Nolte et al., 2004; LeRomancer et al., 1994; C. McIntosh, unpublished).

Epidemiological data on PVY strain prevalence in potato is available for different production areas and sometimes over multiple years (Ellis et al., 1997; Kerlan et al., 1999; Baldauf et al., 2006; Crosslin et al., 2006; Lorenzen et al, 2006, 2008; Karasev et al., 2008; Djilani-Khouadja et al., 2010; Schubert et al., 2007, 2015; Gray et al., 2010; Galvino-Costa et al., 2012b; Anfoka et al., 2014; Chikh-Ali et al., 2010, 2016a,b). In Western Europe (Kerlan et al., 1999; Schubert et al., 2007), in Brazil (Galvino-Costa et al., 2012b), the Mediterranean (Djilani-Khouadja et al., 2010), the Middle East (Anfoka et al., 2014; Chikh-Ali et al., 2010,

[1]This chapter represents a fragment of the published paper by Funke et al. (2017) Strain-specific resistance to *Potato virus Y* (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. Plant Dis. 101: 20-28.

2016a), and Indonesia (Chikh-Ali et al., 2016b), there was a consistent trend over the past 30 years, where recombinant strains like PVY^{NTN} and PVY^{N-Wi} replaced non-recombinant 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 2004-2006 PVY strain survey (Gray et al., 2010). However, recombinant strains were identified in the U.S. as well (Crosslin et al., 2002, 2006; Baldauf et al., 2006; Lorenzen et al., 2006, 2008; Karasev et al., 2008; Gray et al., 2010), and there was a general concern that recombinant strains may continue to spread as they had in other countries (Gray et al., 2010).

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 the U.S. potato production (NASS, 2015). Here, I 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 U.S., particularly since the hundreds of seed lots tested were also shipped and used throughout North America.

Materials and methods

Field plot design and locations

The Washington seed lot trials were planted at the Washington State University research farm near Othello, WA. These trials were conducted from 2011 to 2015 using

certified seed lots imported into Washington from a number of U.S. states and Canadian provinces (Pavek and Holden 2016). Two hundred whole tubers per seed lot were submitted by the growers in the Columbia Basin and planted in single rows. 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 by Crosslin et al. (2006).

Plant sampling, PVY testing and serotyping

Between 2011 and 2015, each year, in early to mid-June, seed lot trials were visually assessed by a group of Pacific Northwest (PNW; Washington, Oregon, Idaho, and Montana) potato pathologists, extension specialists, and volunteers for abiotic and 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 hours 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 depended on the number of seed lot samples submitted for the testing each year, as well as the relative incidence of infection for any given season. Three leaves per flagged plant were collected and placed in a

sealable plastic bag labelled with the seed lot number. Bags were kept in coolers on ice or in a cold room at approximately 4^{o}C until processed for PVY strain typing, usually within 2-3 days after collecting. Samples were processed with a set of controls maintained by the University of Idaho to make identification of strains easier and to assure the tests are working properly. These controls have been subjected to whole genome sequencing (Table 2.1) and are grown in insect-free, climate-controlled growth chambers.

In the 2011-2015 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). 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 Scottish Agricultural Science Agency (SASA, Edinburgh, Scotland). 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.

ELISA plates were coated with G500, a capturing antibody for all strains of PVY, at 100 µl per well using a 10K dilution in 1x concentration coating buffer (20x – 15.9g Na_2CO_3, 29.3g $NaHCO_3$, dissolved into 500 mL diH_2O, pH 9.6) and incubated overnight at 4^oC. Plates were blocked with a 3% milk solution (3% dry milk dissolved in diH_2O), at 300 µl per well for a minimum of 4 hours. The plates were rinsed with diH_2O and used immediately or kept frozen in a -20^oC freezer until use. Samples of 0.5 g of tissue were ground at a 1:10 (w:v) dilution in grinding buffer (PBST, 20% polyvinylpyrrolidone, 10% of 3% milk) and loaded 100 µl into the wells. Each sample was loaded twice into four plates – one plate for each detection antibody. Plates were incubated overnight at 4^oC. Samples were washed out of the plates with six cycles of a water rinse and filling each well with 300 µl of PBST. After washing, the plates were separated into stacks for each detection antibody: Asc5, SASA-O, SASA-N, and Agdia-N. All of the detecting antibodies were mice antibodies, allowing for use of the single anti-mouse conjugate for all. A cocktail of detecting antibody and conjugated antibody was used for this step, where each antibody was used at a 10K dilution. Using ELISA buffer (PBST, 10% of volume is 3% milk), the four cocktails were made consisting of one detection antibody and the single conjugated anti-mouse antibody. These were loaded onto the plates at 100 µl per well. The plates were incubated overnight at 4^oC. After incubation, the plates were washed again 6 times. Following the final rinse, developing substrate (10% triethanolamine, 0.6 mg/mL alkaline phosphatase (pNPP), 90% diH_2O) was added to the plates at 100 µl per well. Optical density readings were taken at 4 hours after adding substrate using an ELISA plate reader set at the wavelength of 405 nm, with a maximum optical density of 4. A final reading was taken after overnight incubation at 4^oC. These readings were graphed and any signal exceeding the one of the background reading

three times or more was considered as positive. Each positive sample was subsequently tested in immunocapture-RT-PCR.

RT-PCR-based PVY strain typing

In 2011, samples identified by TAS-ELISA as positive were subjected to a separate total nucleic acid extraction using our adjusted Dellaporta protocol (Dellaporta et al., 1983), and then typed to strain using the RT-PCR assays according to Lorenzen et al. (2006b). With this methodology, non-recombinant PVY^{O} and PVY^{N} strains can be distinguished from recombinant PVY^{NTN} and $PVY^{N\text{-}Wi/N:O}$ strains, however no distinction can be made between $PVY^{N:O}$ and $PVY^{N\text{-}Wi}$ strains, and no distinction is possible between $PVY^{NA\text{-}N}$ and PVY-NE11 strains (Lorenzen et al., 2006; Chikh-Ali et al., 2013a). In 2012, PVY-positive samples from Othello, WA 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 2013-2015, the samples were typed using the immuno-capture (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 non-recombinant PVY^{O}, PVY^{N}, and $PVY^{NA\text{-}N}$, and recombinant PVY^{NTN}, $PVY^{N\text{-}Wi}$, $PVY^{N:O}$, and PVY-NE11 (Chikh-Ali et al., 2013a).

Because immunocapture-RT-PCR uses the same sample extract as ELISA, we were able to load ELISA and immunocapture plates at the same time. The immunocapture plates were coated in the same way as the ELISA plates and were incubated overnight at 4^{o}C. After incubation, the plates were rinsed with distilled H_2O, emptied, and frozen at -20^{o}C until needed. The plates were loaded with 100 µl of sample extract and incubated overnight at 4^{o}C and followed the same washing as ELISA plates. After the last rinse, the plates were emptied and frozen until results from the ELISA test were finished. The samples that tested positive in ELISA were tested through RT-PCR for strain typing.

Immunocapture master mix was made using 25 µl per sample consisting of 5 µl 5x buffer with $MgCl_2$ (Promega), 1.25 µl 10mM dNTPs (Promega), 0.625 µl rRNasin (RNase Inhibitor, Promega), 2.4 µl 3µM oligo dT/Random Hexamers (54 µl 10 µM Random Hexamers (IDT), 6 µl 10 µM oligo dT (IDT), 140 µl sterile ddH_2O), 1 µl M-MLV (Promega), 13.725 µl sterile ddH_2O. The plates were ran on a thermocycler using the program: 25°C 2 min; 18 cycles of 25°C for 30 s (+1 degree every cycle); 42°C for 45 min; 19 cycles of 42°C for 2 min (+1 degree every cycle); 70°C for 10 min; and held at 4°C.

The two PCRs were performed using the same amplified cDNA from the immunocapture plates. The “Lorenzen” master mix was 25 µl per sample made up of 2.5 µl 10x buffer (Genscript), 0.13 µl 10 mM dNTPs, 0.64 µl 100 µM multiplex primer, 0.25 µl Genscript GreenTaq, 19.48 µl sterile ddH_2O, 2 µl cDNA. The samples were ran on a PCR program using: 94°C for 2 min, 12 cycles of 94°C for 20 s, 66°C for 20 s (-0.5°C every cycle), 72°C for 1 min; 23 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 1 min; 72°C for 5 min; and held at 4°C.

"Chikh-Ali" master mix was 20 µl per sample made up of 2 µl 10x buffer, 0.4 µl 10mM dNTPs, 0.6 µl 100 µM multiplex primers, 0.3 µl Genscript GreenTaq, 13.7 µl sterile ddH_2O, 3 µl cDNA. The samples were ran on a PCR program using: 94°C for 5 min; 10 cycles of 94°C for 15 s, 64°C for 30 s, 72°C for 1.5 min; 10 cycles of 94°C for 15 s, 62°C for 30 s, 72°C for 1.5 min; 10 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1.5 min; 72°C for 5 min; and held at 4°C.

The amplified PCR products were separated on a 1.2% agarose gel, stained with ethidium bromide and visualized under UV light. A 100-bp or 1-kb DNA standard (Fisher) was loaded into each row to be able to determine the approximate sizes of the bands produced.

Results

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 (Table 2.2). In the span of 5 seasons, over 1,400 mosaic samples were tested and typed. PVY positive samples represented 84 to 93% of all collected samples exhibiting mosaic in Othello (Table 2.2). The 7 to 16% of mosaic samples testing negative for PVY may represent a margin of error characteristic of visual symptoms reading or infections by other viruses, but 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^O strains present in this potato production area. Specifically, PVY^O

prevalence decreased nine-fold in the Othello trials, from 63 to less than 7% from 2011 to 2015 (Table 2.3). 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 $PVY^{N\text{-}Wi}$, which almost doubled from under 27 to 53% of all PVY-positives between 2011 and 2015 (Table 2.3).

During the same period, the relative abundance of PVY^{NTN} increased more than three-fold, from less than 8 to more than 24% (Table 2.3). In the Othello trials, the share of another recombinant strain, PVY-NE11, rose between 2011 and 2013 more than ten-fold, from less than 1% to 10%, but then dropped again to less than 1% in 2015 (Table 2.3). 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 2.3). Two non-recombinant strains, PVY^{N} and PVY^{O}-O5, were found only in 2011 and 2012 (Table 2.3).

The overall strain composition of PVY isolates circulating in the Columbia Basin potato changed drastically during the observation period, from predominantly non-recombinant PVY^{O} strain (63%) in 2011 to predominantly recombinant strains represented by $PVY^{N\text{-}Wi}$ (53%), PVY^{NTN} (24%), $PVY^{N:O}$ (9%), and PVY-NE11 (0.5%) (Table 2.3). The two recombinant strains associated with PTNRD, PVY^{NTN} and PVY-NE11, combined, represented 8.7% of all PVY-positives in 2011, and increased to 24.6%, nearly three-fold, in 2015 (Table 2.3). Large changes in the proportion of $PVY^{N:O}$ strain also occurred, although its relative abundance never reached 10% of all PVY-positives detected during the period of observations.

Discussion

Recombinant strains of PVY have spread in the past thirty years through the main potato production areas of the world, including PVY^{NTN} and PVY-NE11, which are associated with PTNRD (Kerlan et al., 1999; Piche et al., 2004; Baldauf et al., 2006; Kerlan, 2006; Blanchard et al., 2008; Singh et al., 2008; Gray et al., 2010; Galvino-Costa et al., 2012a,b; Karasev and Gray, 2013; Chikh-Ali et al., 2016a,b). This spread coincided with the virtual disappearance of other, non-recombinant 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 U.S., recombinant strains of PVY (PVY^{N-Wi} and PVY^{NTN}) were first reported on potato in the early 2000s (Crosslin et al., 2002, 2006; Piche et al., 2004; Baldauf et al., 2006; Lorenzen et al., 2006a), although in Canada they were found a few years earlier (McDonald and Kristjansson, 1993). Nevertheless, the non-recombinant strain PVY^{O} remained dominant in the U.S. up to the early 2010s (see Baldauf et al., 2006; Gray et al., 2010; Karasev et al., 2010; Table 2.3).

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 (Table 2.3), resulting in up to a 9-fold reduction of the previously dominant strain PVY^{O} 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% four years later (Table 2.3).

Table 2.1. Genotypes and phenotypic traits of the Potato Virus Y (PVY) isolates used in this study.

Isolates	Strain [a]	Genotype	Tobacco 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	$PVY^{N\text{-}Wi}$	$PVY_{N\text{-}Wi}$	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	$PVY^{NA\text{-}N}$	$PVY_{NA\text{-}N}$	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; Mos, mosaic and vein clearing

[c] Sequences deposited in GenBank

Table 2.2. Proportion of Potato Virus Y (PVY)-positive samples collected exhibiting mosaic symptoms at one commercial seed lot trial location, 2011-2015.

Season	**Othello, WA**	
	Number of visually assessed mosaic samples tested	PVY-positive, %
2011	246	87.7
2012	235	84.3
2013	283	88.0
2014	222	90.5
2015	431	92.6

Table 2.3. Potato Virus Y (PVY) strain breakdown at Othello, WA seed lot trials, 2011-2015.

Season	**PVY strain type, % of all PVY-positives**								
	O	N-Wi	N:O	NTN	NE-11	NA-N	N	O5	Uncl. [a]
2011 [b]	62.6	27.4		7.8	0.9		0	0.5	0.9
2012	30.8	37.4	3.0	13.1	5.6	0	5.6	1.0	3.5
2013	17.3	46.6	2.4	20.5	10.0	0	0	0	3.2
2014	15.9	59.2	2.0	6.0	8.0	0	0	0	9.0
2015	6.8	52.9	9.3	24.1	0.5	0	0	0	6.6

[a] Uncl. = 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, and between NE-11 and NA-N strains.

Chapter 3: Screening of five potato cultivars for resistance to PVY strains: search for new sources of resistance[1]

Introduction

Two types of genes confer resistance to viruses in potato (Gebhardt and Valkonen, 2001). *R* genes confer an extreme resistance (ER) or immunity which is very durable and is effective against a broad range of virus strains. The origin of *R* genes is in a pool of wild relatives of potato (*Solanum tuberosum*), and it takes many years to introgress these genes into commercially acceptable cultivars. *N* genes confer a hypersensitive resistance (HR) response where a small group of plant cells infected with the virus dies forming a necrotic lesion which often restricts further movement of the virus outside of this lesion. Occasionally, when the virus spread is not completely restricted, the infection may become systemic, and in this case the HR reaction becomes systemic, visible as various types of systemic necrosis, such as vein necrosis, leaf drop syndrome, and stem streaking. Unlike ER, HR is strain specific, and very sensitive to environmental factors, especially temperature – it can be broken due to changes in the temperature. And, unlike *R* genes, *N* genes are widely available in many commercial cultivars, and in theory, could be used to manage resistance against PVY in potato.

Originally, strains of PVY were classified based on the induction of HR response in a standard set of potato (*S. tuberosum*) cultivars with known genetic backgrounds (Cockerham,

[1]This chapter includes data from a published paper by Funke et al. (2017) Strain-specific resistance to *Potato virus Y* (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. Plant Dis. 101: 20-28.

1970; deBokx and Huttinga, 1981; Jones, 1990; Sigh 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_{tbr}), PVY^{C} (Nc_{tbr}), PVY^{Z} (Nz_{tbr}), and PVY^{N} (overcoming all three *N* genes without the HR response) (Cockerham, 1970; Jones, 1990; Singh et al., 2008, Chikh-Ali et al., 2014). Molecular characterization of PVY strains revealed that PVY^{O}, PVY^{N}, and PVY^{C} had non-recombinant genomes that formed three separate phylogenetic clades (Glais et al., 2002; Lorenzen et al., 2006; Singh et al., 2008; Karasev and Gray, 2013). 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 $PVY^{NTN\text{-}NW}$ recombinant based on molecular characteristics (Hu et al., 2009; Kerlan et al., 2011; Chikh-Ali et al., 2010, 2013, 2014; Karasev and Gray, 2013). Recently, PVY^{Z} was proposed to include other, non-recombinant isolates as well (Kehoe and Jones, 2016; Jones and Kehoe, 2016). There are multiple other recombinants, most often built of PVY^{O} and PVY^{N} parental sequences, named $PVY^{N\text{-}Wi}$, $PVY^{N:O}$, PVY-NE11 and others (Karasev and Gray, 2013), but these were not defined genetically and were classified only based on molecular properties.

Most of the studies of these HR-inducing *N* genes were conducted in Europe, on European cultivars which are not in use in North America, and the information on *N* genes available in potato cultivars grown in the U.S. is limited. Previuosly, eight potato cultivars grown in the U.S. were tested against five PVY strains, and the presence of Ny_{tbr} gene was demonstrated in cultivars Ranger Russet, Alturas, Western Russet, Yukon Gem, and Rio Grande Russet (Rowley et al. 2015). The Nz_{tbr} gene was found in two cultivars, Yukon Gem and Rio Grande Russet (Rowley et al. 2015). In addition to these two *N* genes, the existence

of several others were postulated in Yukon Gem, eliciting HR against multiple strains of PVY (PVY^{N}, PVY^{NA-N}, PVY^{N-Wi}, $PVY^{N:O}$, PVY-NE11). A North American cultivar Yukon Gold was demonstrated to carry the *Ny*$_{tbr}$ and *Nz*$_{tbr}$ genes eliciting HR against PVY^{O} and PVY^{NTN}, respectively (Kerlan et al. 2011). Potato cultivar Umatilla Russet was studied by us in a screen-house setting and showed HR reaction to PVY^{O} (Funke et al. 2017). To confirm the symptoms that were seen in the screen-house, we needed to perform testing under more controlled settings in the greenhouse.

Here, four popular potato cultivars were studied in the greenhouse for their ability to elicit a HR response against four of the most common strains of PVY. The cultivars Dark Red Norland, CalWhite, Chieftain, and Umatilla were tested against strains PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, and PVY^{N}. A fifth potato cultivar, Payette Russet, known to have an extreme resistance gene *Ry*$_{sto}$ (Novy et al. 2017), was challenged with six strains of PVY to confirm the presence of the *R* gene. Payette Russet has not been tested against PVY^{N}, but was tested against three additional strains; PVY^{O5}, $PVY^{N:O}$, and PVY-NE11. This study was conducted to expand our screening of North American cultivars for various *N* resistance genes.

Materials and Methods

Potato cultivar sources and plant maintenance

The cultivar Maris Bard was originally received from the National Potato Germplasm Collection in Sturgeon Bay, WI, as tissue culture plantlets. The other cultivars Desiree, CalWhite, Dark Red Norland, Payette Russet, Chieftain, and Umatilla Russet, were obtained from the University of Idaho Nuclear Seed Potato Program (provided by Lorie Ewing).

Plantlets were cut and transferred to new media every 8 weeks and after transfer, these plantlets were transplanted in soil in 2-8 weeks. While *in vitro*, the plantlets were periodically subjected to RT-PCR tests for main potato viruses to confirm their virus-free status.

Maris Bard and Desiree were used as control cultivars in each experiment, with three plants inoculated per strain. This was done to help determine correct infection with each strain as well as infectivity of inoculum. Their symptoms have been well documented for the strains we used in these tests.

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 2.1). PVY isolates were maintained in tobacco cv. Burley in an insect-free, climate-controlled growth chamber. Infected tobacco tissue was used as an inoculum source for the potato plants. Tobacco leaves were homogenized in a phosphate inoculation buffer (6.7g Na_2HPO_4 $7H_2O$, 3.3g KH_2PO_4, dissolved in 500 mL diH_2O, pH 7.0, autoclaved) at a dilution rate of 1:10 (w:v) with a mortar and pestle on ice. For all cultivars, potato plants were mechanically inoculated at the six- to ten-leaf stage, and grown in climate-controlled growth chambers, with a 16h light/8h dark cycle and maintained at 20-22°C.

Mechanical inoculations were performed using carborundum (silicon carbide) and the homogenized leaf tissue (inoculum). Carborundum was dusted onto the potato leaves that were chosen for inoculation, and rubbed with a cotton tipped swab dipped in inoculum. The carborundum was used to wound the leaves so virus can enter the cells. Each inoculated leaf

was punched to mark it and allow for symptom tracking. After the plants were inoculated they were rinsed to remove excess inoculum. They were then placed in the growth chamber (same environmental conditions as described above) according to virus strain to help eliminate cross-contamination (Rowley et al., 2015). Three plants of each cultivar were inoculated with each PVY isolate per experiment, and three plants of each cultivar were left as healthy controls.

The symptom assessment started 4-5 days after inoculation and was carried out for 6-8 weeks, focusing on two types of symptoms: a) local and systemic necrotic reactions which indicated the induction of the HR and typically reveal the presence of a corresponding *N* gene in a cultivar, and b) presence or absence of other systemic symptoms, not associated with HR. Each experiment was repeated two times.

Results

Umatilla and CalWhite

To study the response of Umatilla Russet to four different strains of PVY (PVY^{O}, PVY^{N}, PVY^{NTN}, and $PVY^{N\text{-}Wi}$), a growth room experiment was conducted under controlled temperature and lighting conditions as well as side-by-side analysis with CalWhite and control cultivars Maris Bard and Desiree. Both PVY^{O} and PVY^{NTN} induced a typical HR response (Fig. 3.1) similar to previously described, in the control cv. 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 post-inoculation (dpi). Within 10-14 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 (Fig. 3.2). Another control, cv. Desiree, produced a very similar HR upon infection with PVY^{O}, whereas PVY^{NTN} induced mild systemic mosaic on upper non-inoculated leaves 14-21 dpi.

PVY^{N} and $PVY^{N\text{-}Wi}$ 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 non-inoculated leaves in Maris Bard infected with PVY^{N} and $PVY^{N\text{-}Wi}$ (Table 3.1). Systemic infections of all four isolates representing four strains of PVY were easily confirmed 21-28 dpi using ELISA (Fig. 3.3) 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. 3.3) due to the presence of Ny_{tbr} gene (Desiree) or Ny_{tbr} and Nz_{tbr} genes (Maris Bard) (Cockerham, 1970; Jones, 1990; Singh et al., 2008; Chikh-Ali et al., 2014).

$PVY^{N\text{-}Wi}$ and PVY^{NTN} induced very mild mosaic in systemically infected, non-inoculated upper leaves of cv. CalWhite, clearly visible by about 14-21 dpi, but no other local or systemic symptoms (Table 3.1). PVY^{N} and PVY^{O} induced no local or systemic symptoms in CalWhite (Table 3.1) although virus was easily detected 18-21 dpi by ELISA (Fig. 3.3) 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 four strains. PVY^{O} induced a distinct local and systemic HR reaction in Umatilla Russet (Fig. 3.1; Table 3.1). This included formation of local lesions that later expanded and led to completely necrotized inoculated leaves by 12-14 dpi. This local HR was followed by a clear systemic HR, which displayed a typical leaf drop syndrome.

Some of the Umatilla plants inoculated with Tb60 (PVY^{O}) failed to establish systemic infection, suggesting possible presence of the *Ny_{tbr}* 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 cv. CalWhite in the same experiment (Fig. 3.3). $PVY^{N\text{-}Wi}$ and PVY^{NTN} induced mosaic and crinkling in Umatilla Russet (Fig. 3.1), and led to visible stunting of the infected plants. $PVY^{N\text{-}Wi}$ induced characteristic chlorotic spots in upper, non-inoculated leaves of Umatilla (Fig. 3.1). In the case of PVY^{NTN}, typical local HR was visible at 14 dpi (Fig. 3.1, A), but only mild systemic vein necrosis became visible late in infection, by the end of the experiment, at 50 dpi (Fig. 3.1, B). PVY^{N} induced only mild to very mild mosaic in Umatilla Russet (Table 3.1). All plants infected with PVY^{NTN}, $PVY^{N\text{-}Wi}$, and PVY^{N} strains established systemic infection easily confirmed at 21-28 dpi by ELISA (Fig. 3.3) and RT-PCR (not shown).

Dark Red Norland and Chieftain

Cultivars Dark Red Norland and Chieftain were tested against the four isolates representing the same strains as above – Tb60 (PVY^{O}), Mont (PVY^{N}), HR1 (PVY^{NTN}), and N1 ($PVY^{N\text{-}Wi}$). PVY^{O} (Tb60) induced local lesions on inoculated leaves of both cultivars, which appeared at 6-11 dpi in Dark Red Norland, and at 18 dpi in Chieftain (Fig. 3.4). These lesions expanded and resulted in pronounced vein necrosis of the inoculated leaves at 18 dpi for Dark Red Norland, and at 11-13 dpi for Chieftain. At 27-31 dpi both cultivars developed systemic vein necrosis and at 44 dpi they developed necrotic lesions on upper, non-inoculated leaves (Table 3.1). Leaf drop was observed by 37 dpi for Dark Red Norland and 44 dpi for

Chieftain, which also developed mottling at 13-24 dpi (Table 3.1). Three of the six Dark Red Norland plants inoculated with PVY^{O} were dead after 50 dpi.

Mont (PVY^{N}) infection stayed largely asymptomatic in both Dark Red Norland and Chieftain inducing only systemic necrotic reactions, and only very late into the infection in Chieftain. Dark Red Norland only developed leaf drop by 44 dpi. Chieftain developed vein necrosis in upper, non-inoculated leaves at 27-37 dpi and systemic mosaic at 35 dpi.

L26 (PVY^{NTN}) induced vein necrosis on inoculated leaves in Dark Red Norland at 18 dpi, with symptoms of mosaic in upper non-inoculated leaves (13 dpi), crinkling (13 dpi), and leaf drop (44 dpi) developing over the course of the testing period. Following PVY^{NTN} inoculation, Chieftain developed mosaic in inoculated leaves at 13 dpi and vein necrosis by 18 dpi. Systemic mosaic symptoms in upper, non-inoculated leaves developed into systemic mottle at 27-31 dpi, and at the same time vein necrosis was appearing systemically. Leaf drop was observed at 44 dpi.

$PVY^{N\text{-}Wi}$ induced mosaic but no necrotic lesions on inoculated leaves for both cultivars, Dark Red Norland and Chieftain. Chieftain developed systemic mosaic in upper, non-inoculated leaves at 13 dpi which turned into mottle at 27 dpi. Dark Red Norland showed systemic mosaic in upper, non-inoculated leaves at 13 dpi, and very mild systemic vein necrosis was observed at 31 dpi on one plant.

Our control cultivars Desiree and Maris Bard were tested along to confirm the presence of known *N* genes. Both test cultivars showed a necrotic HR response to PVY^{O} in inoculated leaves, exhibiting vein necrosis and necrotic lesions (Table 3.1). The local necrotic reactions continued to spread through the growth period, becoming systemic symptoms. Leaf

drop, vein necrosis and necrotic lesions were all observed as necrotic reactions. Non-necrotic symptoms of mosaic and crinkling were observed as well (Table 3.1). In the same experiments, Desiree exhibited the HR reaction to PVY^{O} (Tb60) but not to PVY^{NTN} (L26), while Maris Bard exhibited HR to both PVY^{O} (Tb60) and PVY^{NTN} (L26) (Table 3.1). These responses of the cultivars under testing (Dark Red Norland and Chieftain) and the control cultivars (Desiree and Maris Bard) indicated the possible presence of Ny_{tbr} and Nz_{tbr} genes in both Dark Red Norland and Chieftain. The plants were tested at 5 weeks post inoculation and all plants were systemically infected with PVY^{N}, PVY^{NTN}, and $PVY^{N\text{-}Wi}$ (Fig. 3.5) despite presence of the HR response locally or systemically.

Payette Russet

Plants were mechanically inoculated as above and checked for symptoms weekly starting at 6 dpi. PVY^{O} and PVY^{O5} elicited no symptoms from Payette for the duration of the test period. Desiree began showing symptoms for both strains 14-16 dpi with vein necrosis and necrotic lesions forming on inoculated leaves. PVY^{O5} caused water soaked rings to appear at 16-21 dpi, which progressed into necrotic lesions. The vein necrosis and lesion symptoms then spread and became systemic 21 dpi. Mosaic symptoms developed between 21-40 dpi and leaf drop was observed at 28-43 dpi. The control cultivar Maris Bard began developing symptoms at 9 dpi showing necrotic lesions on inoculated leaves (Table 3.2). Vein necrosis developed 9-16 dpi and spread systemically at 14-21 dpi. Severe mosaic appeared by 14 dpi and at 21 dpi leaf drop developed. All plants were tested at 28 dpi (Fig. 3.6) and 3/6 plants for both strains in controls Maris Bard and Desiree were found

systemically infected. This 50% infection rate was expected due to the HR response from both cultivars, showing that they were able to restrict virus movement in both cultivars carrying the *Ny$_{tbr}$* gene.

Payette was inoculated with PVY^{NTN} and PVY-NE11 but displayed no symptoms in inoculated or upper, non-inoculated leaves (Table 3.2) during the entire observation period, and all plants tested at the end were found negative for systemic infection (Fig. 3.6). In our controls PVY^{NTN} and PVY-NE11 produced vein necrosis 9-16 dpi on inoculated leaves in Desiree and Maris Bard. Necrotic lesions began forming by 9-16 dpi on Maris Bard for both strains, but none on Desiree. However, both cultivars showed systemic symptoms of both necrotic types after 21 dpi. Desiree showed vein necrosis with both strains 21-28 dpi, and necrotic lesions and leaf drop with PVY-NE11 28 dpi. Maris Bard began showing systemic vein necrosis and necrotic lesions 21-28 dpi, and leaf drop at 28-35 dpi. Both cultivars showed systemic mosaic (Table 3.2) for both PVY-NE11 and PVY^{NTN} at 14-21 dpi. When tested at 28 dpi, all plants inoculated with PVY^{NTN} were infected, but 5/6 Desiree and 4/6 Maris Bard were infected with PVY-NE11.

Payette was also challenged with PVY$^{N\text{-}Wi}$ and PVY$^{N:O}$ but again showed no symptoms, and all plants tested at 35 dpi were negative. Maris Bard and Desiree inoculated with PVY$^{N\text{-}Wi}$ didn't show any local symptoms of infection on inoculated leaves. Systemic symptoms began developing around 14 dpi for Maris Bard and 21 dpi for Desiree when mosaic/mottling began to show. These symptoms continued to be expressed on any new leaves formed for the rest of the testing period (Fig. 3.7), and gradually became more pronounced. The plants inoculated with PVY$^{N:O}$ showed only systemic symptoms. Maris Bard plants again showed mosaic (Table 3.2) around 14 dpi which eventually became more

severe and turned into mottling. Desiree plants were slower to show symptoms, with the earliest sign of mosaic at 21 dpi. Five out of six Desiree plants were infected with $PVY^{N\text{-}Wi}$ when tested at 28 dpi (Fig. 3.6) and 6/6 plants infected with $PVY^{N:O}$. All 12 Maris Bard plants inoculated with both $PVY^{N\text{-}Wi}$ and $PVY^{N:O}$ were infected.

Discussion

The HR reaction conferred by *N* genes specific to individual strains of PVY is considered an indication of a host defense response in potato (Cockerham, 1970; de Bokx and Huttinga, 1981; Jones, 1990; Singh et al., 2008; Chikh-Ali et al., 2014). The strain-specific genes Ny_{tbr} and Nc_{tbr} 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; Lorenzen et al., 2006a; Singh et al., 2008; Hu et al., 2009a; Karasev and Gray, 2013). Recently, an additional Nz_{tbr} gene was identified in potato conferring resistance to the PVY^{NTN} recombinant, defining the PVY^{Z}-NTN strain of PVY (Jones, 1990; Barker et al., 2009; Kerlan et al., 2011; Chikh-Ali et al., 2014). Ny_{tbr}, Nz_{tbr}, and possibly other strain-specific resistance genes were identified in multiple commercial potato cultivars grown in the U.S. (Kerlan et al., 2011; Rowley et al., 2015), including Alturas and Ranger Russet, commonly grown in the Columbia Basin (Rowley et al., 2015).

Here, the reactions of the cultivar CalWhite to PVY infection suggested the absence of any resistance genes conferring HR to the four tested strains of PVY; PVY^{O}, PVY^{N}, PVY^{NTN},

and $PVY^{N\text{-}Wi}$ (Table 3.1). The lack of any HR response to the infection was consistent with the systemic infection established by all tested strains in all inoculated CalWhite plants. The reaction of three other cultivars, Umatilla Russet, Chieftain, and Dark Red Norland to infection with four strains of PVY, demonstrated HR response to the PVY^{O} and PVY^{NTN} infection which indicated presence of the Ny_{tbr} and Nz_{tbr} genes in their genetic backgrounds (Figs 3.1 and 3.3; Table 3.1).

No symptoms were observed in Payette Russet following inoculation of all six tested strains, indicating that the plant did not produce a HR reaction to any of the strains and neither were any of these six strains able to systemically infect Payette Russet. The control cultivars Maris Bard and Desiree both showed appropriate symptoms for each strain used (Table 3.2). Both maintained symptom expression as described in previous experiments (Kerlan et al. 2011; Rowley et al. 2015; Funke et al. 2017) showing local HR in all strains except $PVY^{N:O}$ and $PVY^{N\text{-}Wi}$. Desiree also did not react locally to PVY^{NTN}. The systemic necrotic response across the tested strains was able to restrain systemic infection in some plants. The only two strains to give no necrotic reaction either locally or systemically in Maris Bard was $PVY^{N:O}$ and $PVY^{N\text{-}Wi}$ (Table 3.2).

Systemic testing occurred at 28 dpi to check for infection. All Payette Russet plants tested negative showing it was able to prevent the infection from spreading systemically. The control cultivars Maris Bard and Desiree allowed us to confirm infective inoculum was used and was able to spread systemically (Fig. 3.6). We used another control to ensure the inoculum was good by infecting *Nicotiana benthamiana*. This tobacco plant is susceptible to all our strains of PVY, and will give a positive result when tested at the same time as the

potatoes. All Desiree and Maris Bard plants infected with PVY^{NTN} and $PVY^{N:O}$ were systemically infected, same with all Maris Bard plants infected with $PVY^{N\text{-}Wi}$ (Fig. 3.6). All the other strains were only able to establish systemic infection in some of the plants tested.

Table 3.1. Summary of symptoms expressed by different potato cultivars upon mechanical inoculation with four isolates of PVY representing four PVY strains.

Cultivar	**Isolate (PVY strain)**			
	N1 (PVY^{N-Wi})	L26 (PVY^{NTN})	Tb60 (PVY^{O})	Mont (PVY^{N})
Maris Bard	M, Cr, St [a]	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
Chieftain	M, LL	M, SN, LD	M, LL, SN, LD	M
Dark Red Norland	M, LL, SN	M, Cr, SN, LD	LL, SN, LD	NS

[a] Symptom abbreviations: M, Mosaic; Cr, crinkling; St, stunting; LL, local lesions; SN, systemic necrosis; LD, leaf drop; MM, mild mosaic. "NS" designates no symptoms, however systemic virus infection was confirmed by ELISA and RT-PCR.

Table 3.2. Summary of symptoms expressed by Payette Russet and control cultivars Desiree and Maris Bard when tested against six strains of PVY.

Isolate (PVY strain)	Payette Russet		Desiree		Maris Bard	
	Local	Systemic	Local	Systemic	Local	Systemic
Tb60 (PVY^{O})	NI [a)]	NI	VN, LL	M, LL, SN, Cr, LD	VN, LL	M, Cr, LD
N1 (PVY^{N-Wi})	NI	NI	NS	M, Cr	NS	M, Cr, St
HR1 (PVY^{NTN})	NI	NI	NS	M, St	VN, LL	M, SN, LL, LD
ID20 (PVY^{NE-11})	NI	NI	VN	M, SN, LL	VN, LL	M, Cr, SN, LL, LD
ID269 (PVY^{O5})	NI	NI	VN, LL	M, SN, LL, LD, WSR	VN, LL	M, SN, LD
Alt ($PVY^{N:O}$)	NI	NI	NS	M	NS	M

[a] Symptom abbreviations: M, Mosaic; Cr, crinkling; St, stunting; LL, local lesions; SN, systemic necrosis; LD, leaf drop; MM, mild mosaic; WSR, water soaked rings. "NS" designates no symptoms, but the systemic virus infection was confirmed by ELISA and RT-PCR. "NI" designates no symptoms and no infection confirmed by ELISA and RT-PCR in any of the plants tested.

Fig. 3.1

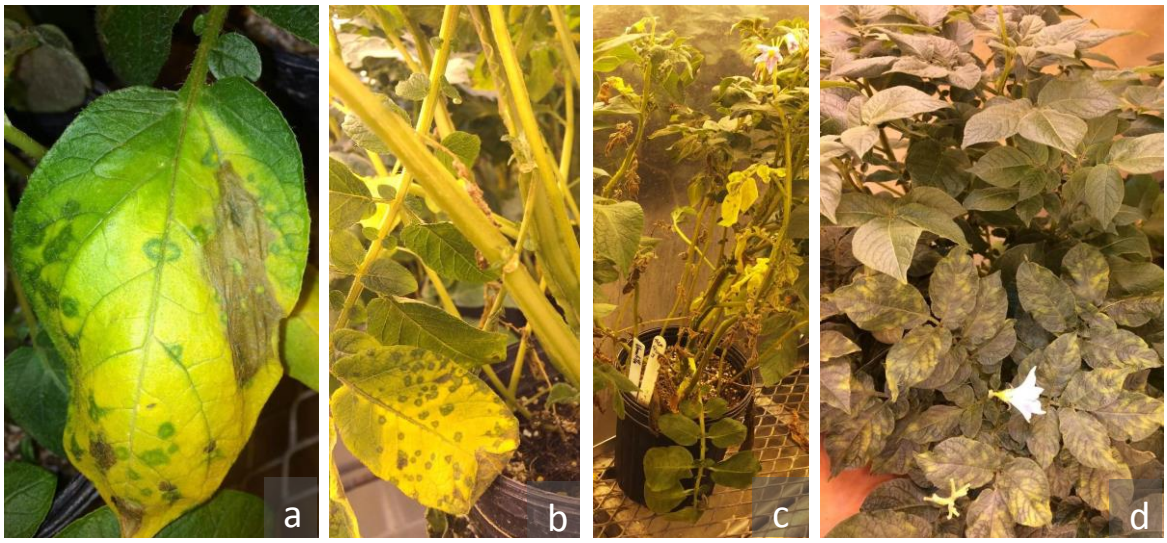

Fig. 3.1. Symptoms observed in the climate-controlled growth room experiment. (a) Inoculated leaf of cv. Umatilla Russet, 2 weeks after inoculation with PVY isolate L26 (PVY^{NTN}): symptoms of green water soaking rings, necrotic lesions, and vein necrosis; (b) Mild systemic necrosis, leaf-drop and necrotic stem streaks induced by PVY isolate L26 (PVY^{NTN}) in cv. Umatilla Russet at 7 weeks post-inoculation (p.i.). (c) Severe leaf-drop and necrotic stem streaks induced by PVY isolate Tb60 (PVY^{O}) in cv. Umatilla Russet at 7 weeks p.i. (d) Mosaic, mottling, and chlorotic spots induced by PVY isolate N1 ($PVY^{N\text{-}Wi}$) in upper, non-inoculated leaves of cv. Umatilla Russet at 7 weeks p.i.; background – a healthy Umatilla Russet plant. All plants were tested at 4 weeks p.i. by TAS-ELISA and RT-PCR to confirm systemic infection with the isolate/strain indicated.

Fig. 3.2

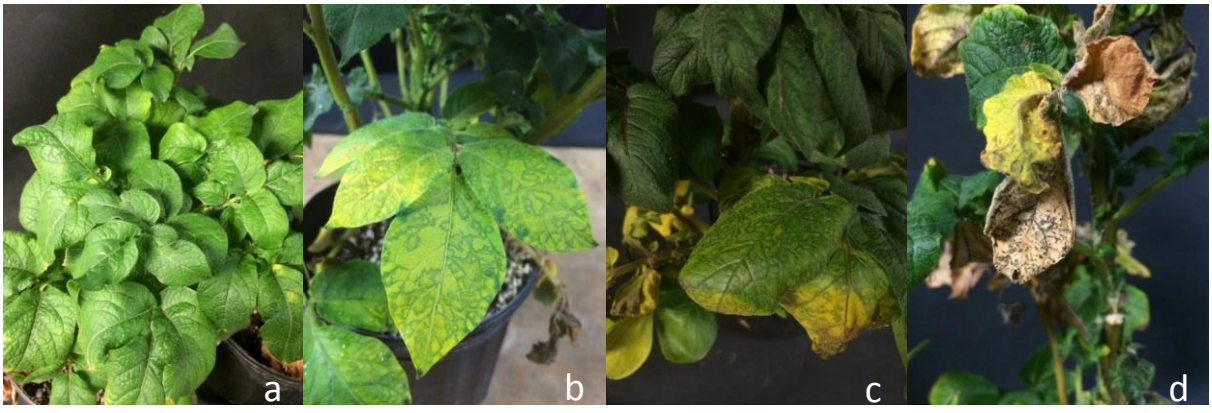

Fig. 3.2. Symptoms in upper, non-inoculated leaves of four cultivars inoculated with isolate L26 (PVY^{NTN}): (a) mild mosaic in CalWhite at 53 days post-inoculation (dpi); (b) green necrotic rings in Umatilla Russet at 24 dpi; (c) mosaic and green rings in Desiree at 24 dpi; (d) systemic necrosis and leaf drop in Maris Bard at 53 dpi. Experiment was performed in climate-controlled rooms.

Fig. 3.3

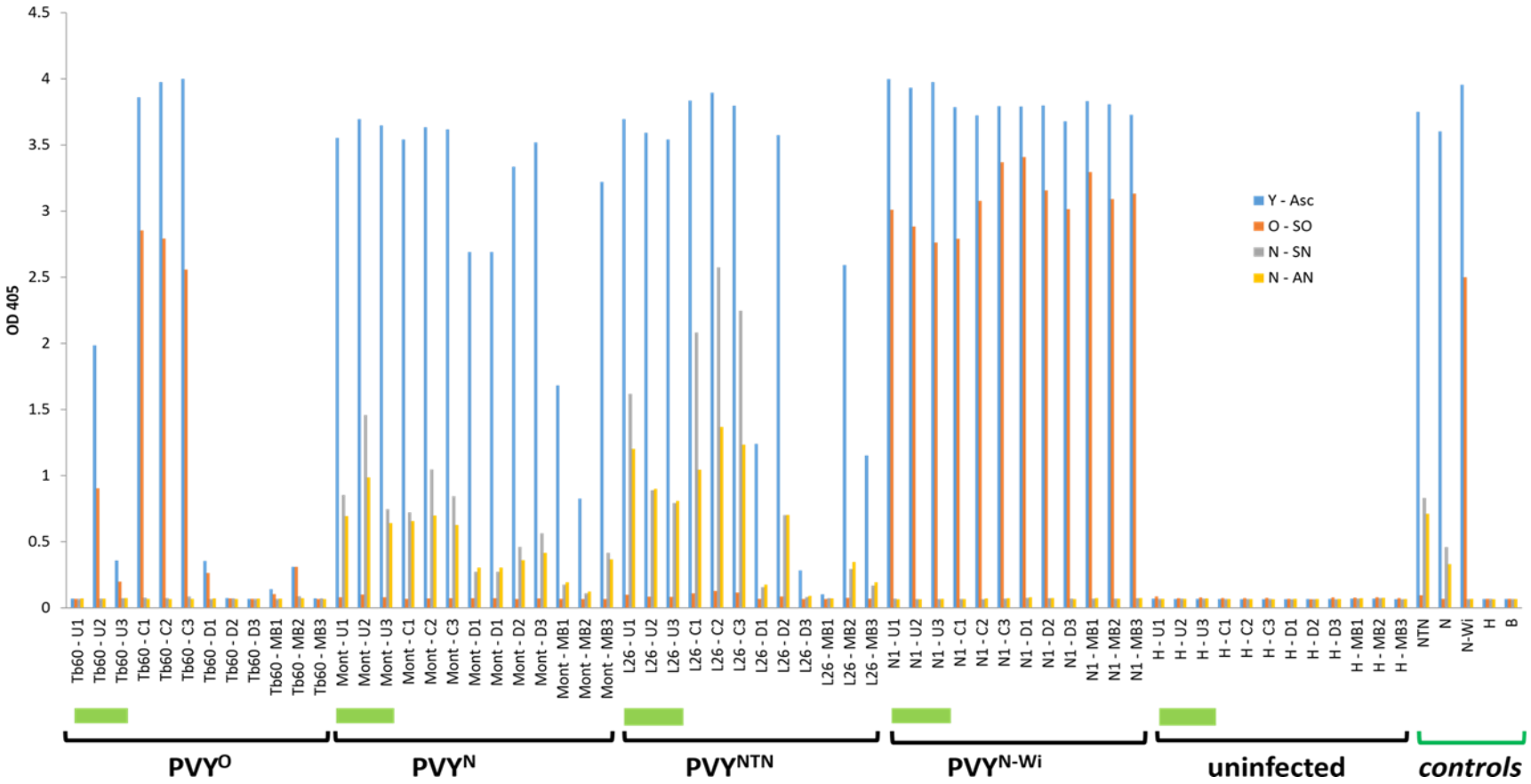

Fig. 3.3. An example of the triple-antibody sandwich (TAS)-ELISA assessment of PVY infection in four potato cultivars, Umatilla Russet (U), CalWhite (C), Desiree (D), and Maris Bard (MB), 4 weeks post-inoculation. Three individual plants per cultivar were infected with each isolate, Tb60 (PVY^{O}), Mont (PVY^{N}), L26 (PVY^{NTN}), and N1 ($PVY^{N\text{-}Wi}$), and three plants were left uninfected. Umatilla Russet plants are labelled with green rectangles. Controls represent tobacco samples infected with corresponding PVY strains. OD_{405} signal reflects the concentration of the respective PVY strain in each individual plant sampled. Bars with different colors represent signals generated with polyclonal (PVY-specific, blue) or three different monoclonal antibodies, SASA-O (O-specific, orange), SASA-N (N-specific, gray), and Agdia-N (1F5, N-specific, yellow). TAS-ELISA plates were read 16 hrs after the developing solution was added to the wells, and samples were considered positive if the OD_{405} signal for the infected plant exceeded the one for an uninfected plant 3-fold.

Fig. 3.4

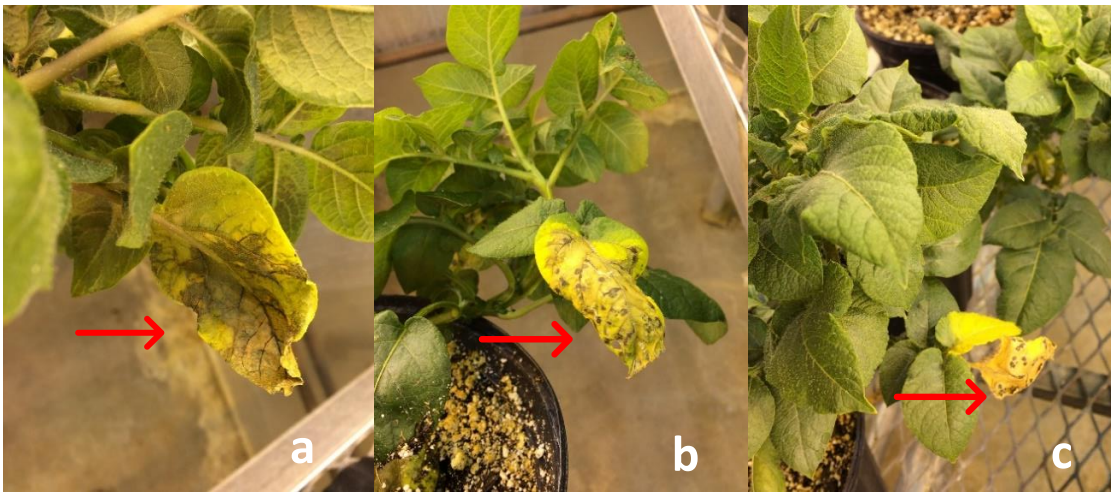

Fig. 3.4. Symptoms of local lesions and vein necrosis expressed on inoculated leaves, 18 days post-inoculation with isolate Tb60 (PVY^{O}): (a) cv. Chieftain; (b) cv. Dark Red Norland; and (c) cv. Maris Bard.

Fig. 3.5

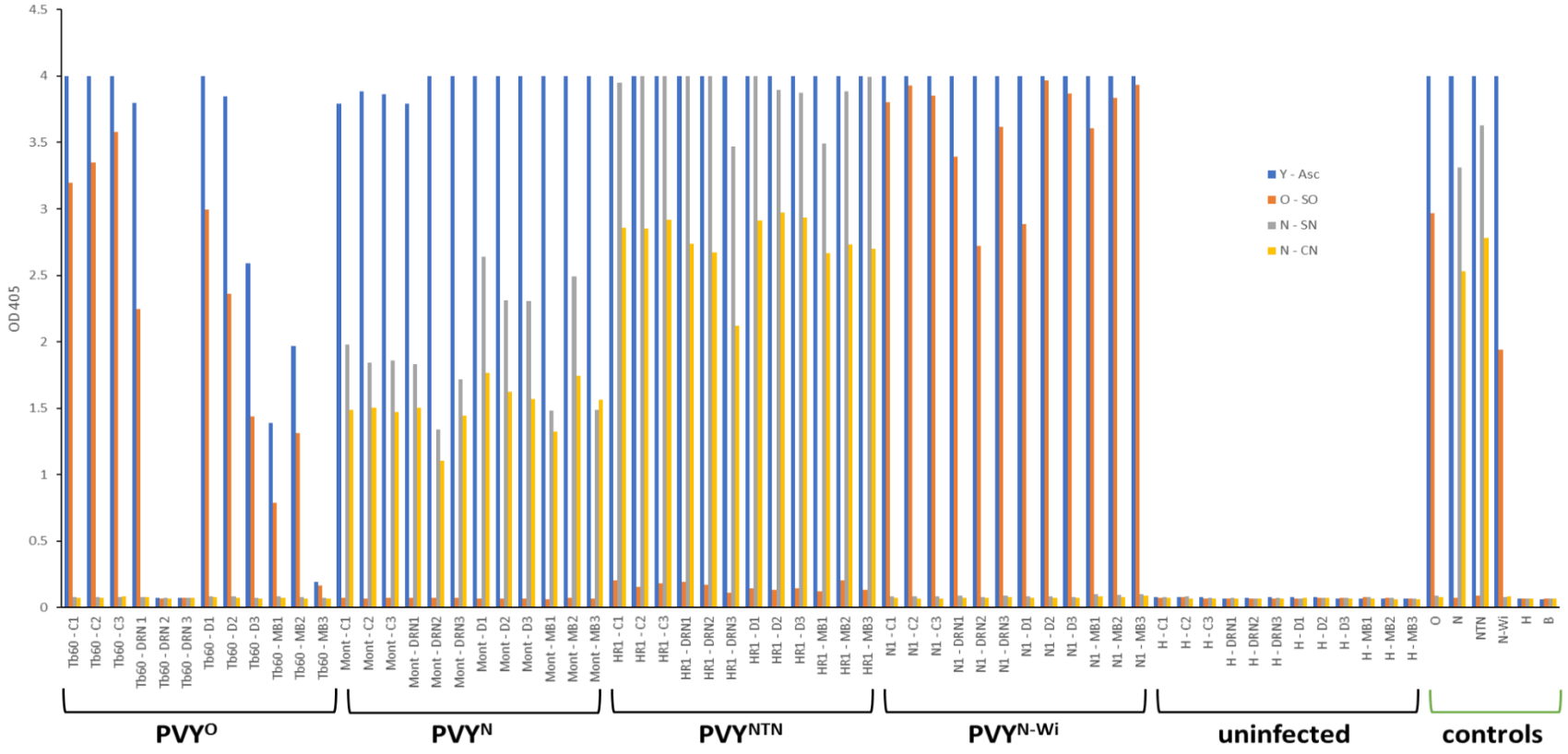

Fig. 3.5. TAS-ELISA results of PVY infection in potato cultivars Chieftain (C), Dark Red Norland (DRN), Desiree (D), and Maris Bard (MB) 4 weeks post-inoculation. Inoculated three individual plants of each cultivar with the isolates, Tb60 (PVY^{O}), Mont (PVY^{N}), HR1 (PVY^{NTN}), and N1 (PVY^{N-Wi}). Three plants were left uninfected for healthy controls. Selection of controls came from the tobacco collection, and correspond to isolates used to perform inoculations. OD_{405} signal represents the concentration of the PVY strain in each individual plant. Different colored bars represent signals from a polyclonal antibody (PVY-specific, blue) and three different monoclonals (SASA-O, orange; SASA-N, gray; Agdia-N, yellow). Plates were read after 16 hours with developing solution, and samples were considered positive if the signal for infected plants was three times higher than for an uninfected plant

Fig. 3.6

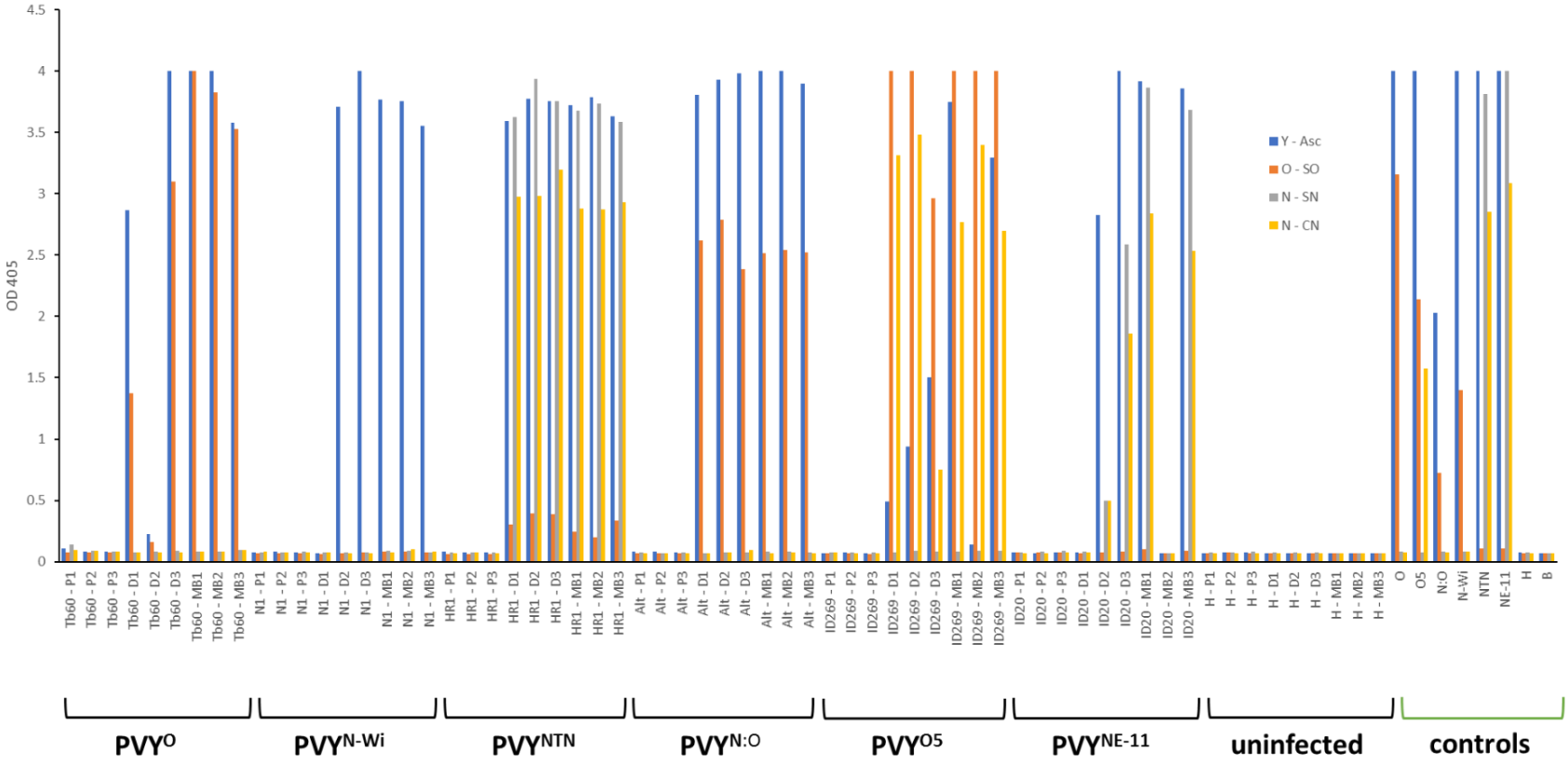

Fig. 3.6. TAS-ELISA test on three potato cultivars, Payette Russet (P), Desiree (D), and Maris Bard (MB) 4 weeks post inoculation. Three plants per cultivar were inoculated with each isolate; Tb60 (PVY^{O}), N1 ($PVY^{N\text{-}Wi}$), HR1 (PVY^{NTN}), Alt ($PVY^{N:O}$), ID269 (PVY^{O5}), ID20 ($PVY^{NE\text{-}11}$), and three plants left as uninoculated controls. The controls represent tobacco samples taken from the maintained collection, and correspond to the strains used to inoculate test plants. OD_{405} signal reflects the concentration of PVY in each individual sample tested. Different colored bars represent signals from the polyclonal (PVY-specific, blue) or three monoclonal antibodies, SASA-O, orange; SASA-N, gray; and Agdia-N, yellow. The plates were read 16 hours after developing substrate was added, and samples were considered positive if the OD_{405} signal was three-times that of the signal for an uninfected plant.

Fig. 3.7

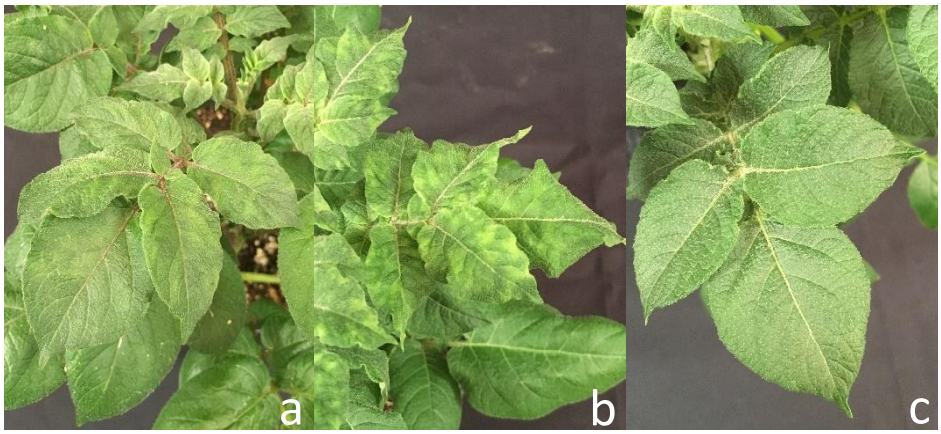

Fig. 3.7. Symptoms in upper, non-inoculated leaves of three cultivars inoculated with isolate N1 ($PVY^{N\text{-}Wi}$), 29 days post-inoculation: (a) cv. Desiree and (b) cv. Maris Bard showing mosaic and mottling; and (c) asymptomatic leaves of cv. Payette Russet.

Chapter 4: Strain specific resistance to the current-season infection by three PVY strains studied for four potato cultivars in a screen-house[1]

Introduction

Various explanations for the observed transition from non-recombinant 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 have been suggested (Karasev and Gray, 2013). Experimental evidence specifically for any of these factors being involved in the observed shift to the recombinant strains of PVY in potato production was limiting and not convincing. Partly, the problem was due to the shortage of studies on the (strain-specific) HR induction in the U.S. potato cultivars, and partly due to the lack of consistent epidemiological surveys addressing PVY strain composition in an area representing a substantial segment of the potato production in the U.S. Nevertheless, PVY isolates from strains PVY^O and PVY^Z were studied for their ability to elicit HR in several potato cultivars harboring Ny_{tbr} and Nz_{tbr} genes, respectively, and grown in the U.S. (Karasev et al., 2011; Kerlan et al., 2011; Chikh-Ali et al., 2014; Rowley et al., 2015; Kehoe and Jones, 2016). The virus determinant involved in interaction with the Ny_{tbr} 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).

[1]This chapter represents a fragment of the published paper by Funke et al. (2017) Strain-specific resistance to *Potato virus Y* (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. Plant Disease **101**: 20-28.

One of the possible explanations of the PVY strain composition changes may be a steady change in the potato cultivars grown in the Columbia Basin region of Washington and Oregon, where in the past 30 years the acreage of once dominant Russet Burbank was on a gradual decline. New potato cultivars have emerged with similar processing and storage qualities that have started to take over parts of the Russet Burbank production total. Though Russet Burbank is still considered the best option, this allows for a variety of cultivars to be grown without sacrificing the end consumer's expectations. To address these recently released cultivars as possible drivers of the PVY strain composition change, a model experiment was conducted in a screen-house with several potato cultivars widely grown in the Columbia Basin, compared to Russet Burbank as a control.

These cultivars have been selected by the industry and growers as the preferred potatoes for the Columbia Basin. For this study, we used Alturas, Umatilla Russet, Ranger Russet, and Russet Burbank. After emergence, equal quantities of three strains of PVY was introduced and we documented how the infection grew and changed throughout the growing seasons. The three strains were PVY^{O}, PVY^{NTN}, and $PVY^{N\text{-}Wi}$ which represent the bulk of the strains of the virus circulating in the Columbia Basin. We were able to observe a similar strain composition shift as we had observed previously in Othello over five years, this time 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 Russet Ranger.

Materials and Methods

Layout of the screen-house experiment

In Spring and Summer 2015 and 2016, a screen-house experiment was conducted at the Hermiston Agricultural Research and Extension Center (HAREC), Oregon State University, OR, to assess the infection rate of three strains of PVY in the four most common potato varieties grown in the Columbia Basin. The screen-house was used both to mimic the field conditions for the potato production, and to minimize any current season spread of the virus by excluding aphids, by thorough insecticide application, and use of mechanical inoculation. One hundred-sixty plants for each of the cultivars Russet Burbank, Alturas, Umatilla Russet, and Ranger Russet were divided into four groups, infected with PVY^{O} (isolate Tb60), $PVY^{N\text{-}Wi}$ (N1), PVY^{NTN} (HR1), or left uninfected. On April 16, 2015 (April 19, 2016), nuclear seed mini-tubers of the four potato varieties were planted into a 72 x 35 feet screen house in a randomized complete block design (RCBD) in Hermiston, OR (Figs 4.1 and 4.2). The experiment had 16 treatments [4 varieties x 3 strains of PVY, plus 1 untreated check (UTC) x 10 plants x 4 replications = 640 plants]. Hence, each of the four potato varieties were 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 and bulked (10 plants per group) and assayed for PVY using RT-PCR (Cating et al., 2015). No PVY-positives were detected prior to inoculation (data not shown). Potato plants were then mechanically inoculated with the PVY^{O} (isolate Tb60), PVY^{NTN} (HR1), and $PVY^{N\text{-}Wi}$ (N1) strains of PVY on May 27, 2015 and May 26, 2016, when plants were approximately 20-76 cm in height (Fig. 4.3).

The plants were treated as though grown in fields in the surrounding area. They received similar watering, pesticide and fertilization schedules to that of the fields, though not applied by tractor due to space constraints. Although the plants were in a screen-house, they were also exposed to all the same weather events that occurred during each of the growing seasons.

Preparation of inoculum and inoculation

All preparations of inoculum were done in the lab immediately before use where the inoculation buffer was stored at 4ºC. To prepare inoculum 50 g of confirmed infected potato leaf tissue were sampled for each of the three strains. These strain samples were individually homogenized with a blender using a 1:10 (w:v) dilution. The inoculation buffer was added in increments during blending to ensure the leaf tissue was not floating above the blades. After homogenization, the inoculum was strained through cheesecloth to remove leaf tissue, leaving behind only liquid to take to the screenhouse. This liquid was poured into 50 mL tubes containing 2-3 g of carborundum powder (silicon carbide) and mixed well. Carborundum was used to create wounds on the leaves and allow the virus to enter the cells. The tubes were inverted multiple times during inoculation to prevent the carborundum from settling to the bottom. After straining, the blender was washed using dish soap and bleach to prevent cross-contamination of the strains.

Inoculations were performed mechanically using cotton swabs and nitrile gloves to prevent contamination from our hands. Three leaflets per plant (each on a separate stem) were inoculated. Each virus strain was prepared and inoculated one at a time to avoid potential cross-

contamination. After inoculation of each strain, the plants were rinsed with water to remove excess inoculum from the leaves. Healthy plants were left as non-inoculated controls.

Sampling and Testing

At the sampling time points five weeks and three months (2015) or four weeks and eight weeks (2016) mentioned below, (plant status examples Fig. 4.3) each plant was individually sampled. Walking down the rows, 3 leaflets were taken from the upper area of the plant, specifically non-inoculated leaves. These were placed into previously numbered plastic bags (2015) or paper envelopes (2016). Samples were taken back to the lab in a cooler on ice and stored at 4°C in a cold room until testing was completed. Each sample was subjected to TAS-ELISA testing and serotyping, and all positives were subsequently tested using IC-RT-PCR as described previously (see Chapter 2).

In the first season, five weeks after inoculation (July 1, 2015), individual plants were sampled, and upper, uninoculated leaves were assayed for PVY. Each plant was tested by ELISA as discussed previously. All PVY-positive samples were typed to strain using Immunocapture-RT-PCR according to the previously published methodology (Nikolaeva et al., 2012; Chikh-Ali et al, 2013a) confirming only those strains that we inoculated were present. A second sampling occurred three months post inoculation (August 26, 2015) that included only the living plants. At this time, many plants had died due to age and presence of virus. All of these plants were tested the same way as the first set.

In the second season of testing, four weeks after inoculation (June 21, 2016) individual plants were sampled and tested, in the same manner as the previous year. A second sampling

occurred in 2016 (July 20, 8 weeks post-inoculation) but only included the healthy plants and the blocks inoculated with PYV^{O}.

Results

ELISA, IC-RT-PCR testing

After testing the plants with ELISA and doing strain confirmation through IC-RT-PCR it was confirmed that no cross-infection or movement of the virus had occurred. There were also no new strains introduced into the screen-house. The healthy controls were all negative after one and three months' post inoculation testing.

Again, as expected, the only strains that were found were the three inoculated into the screen-house and there was no movement of the virus out of the designated blocks. In 2015, after the first round of testing we had almost 100% infection in both PVY^{NTN} and $PVY^{N\text{-}Wi}$, so additional testing was not needed. However, during the first round only a 16.9% infection of PVY^{O} plants was detected. We needed to confirm that rate of infection in those blocks, and make sure healthy plants maintained their virus-free status. After the second round of testing we received a 51.9% infection rate of PVY^{O}.

Strain-specific resistance in potato cultivars may be driving the changes in PVY strain composition

During each sampling season, plants were observed for any foliar systemic symptoms that might be present. PVY^{O} was associated with some of the clearest symptoms across cultivars

and seasons (Fig. 4.4). PVY^{O} exhibited systemic necrotic symptoms, most notably in Umatilla Russet, all of which were present during the first sampling and were only more pronounced during the second round. These necrotic symptoms were classic representations of HR reactions, and included necrotic lesions (some of which began as green rings), vein necrosis, leaf drop, and necrotic stem streaking. Symptoms for PVY^{NTN} were most easily noticed in the 2016 season. These symptoms included necrotic lesions and vein necrosis by the first round of sampling (Fig. 4.5). There were also many plants with a variety of mosaic, from very mild to yellow blotches. In 2016, $PVY^{N\text{-}Wi}$ showed very similar symptoms to PVY^{NTN}, such as clearly visible mosaic/mottle appeared by four weeks post inoculation (Fig. 4.6). This only became more apparent at eight weeks post inoculation when sampling PVY^{O} the second time.

Visual assessment of the virus infection in different strain-cultivar combinations in the screen-house 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. 4.7, A, B), and, for Ranger Russet, of necrotic leaf-drop and necrotic stem streaks (Fig. 4.7, C). Similar systemic HR reactions against the PVY^{O} strain have been reported previously for potato cultivars Alturas and Ranger Russet (Rowley et al., 2015) infected under greenhouse conditions.

In the 2015 season, 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 $PVY^{N\text{-}Wi}$ (Fig. 4.8). 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 58% for Ranger Russet (Fig. 4.8). Under these same screen-house

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. 4.8).

These trends were repeated in the 2016 season of testing. Russet Burbank again exhibited similar infection rates to all three strains of PVY, ranging from 100% infection with PVY^{O} down to 95% for PVY^{NTN}. The other three cultivars tested showed dramatically lower incidence for PVY^{O} when compared to Russet Burbank, but also exhibited a reduction from the previous year as well. Alturas showed 50% infection, followed by Ranger Russet at 30% and Umatilla Russet at 27%. These same cultivars showed no less than 89% incidence of infection for the two remaining strains, PVY^{NTN} and PVY^{N-Wi}.

Examination of the number of plants infected systemically in each cultivar/strain combination provided evidence that these strain-specific resistances exhibited by Alturas, Umatilla Russet, and Ranger Russet in the screen-house resulted in a significant difference of the strain prevalence, between the expected infection from inoculation and the observed infection at the end of the experiment (Table 4.1). Although each of the three PVY strains were initially inoculated into 33.3% of the plants, five weeks after inoculation the proportions of PVY strains significantly changed for at least two cultivars, Umatilla Russet and Ranger Russet (Table 4.1), with Alturas exhibiting a similar trend. At the end of the 5-week experiment, the proportions among all PVY-positive plants in the screen-house for both seasons were, on average, 22% PVY^{O}, 40% PVY^{N-Wi}, and 38% PVY^{NTN} (Table 4.1).

In 2016, we saw similar trends in infection rates across all four cultivars which supports the conclusions made from the first year. Despite Russet Burbank exhibiting less of an overall resistance to PVY, our trends are similar between the years. In 2015, there was an overall infection of PVY^{N-Wi} and PVY^{NTN} above 80% (Fig. 4.8), this was reproduced in 2016. The incidence of PVY^{O} in 2015 was reproduced in 2016, with Alturas, Ranger Russet and Umatilla Russet, showing resistance to that strain when compared to the other two strains tested. Indeed, 2016 showed a more defined difference between the strains (Fig. 4.8).

Discussion

In the screen-house study, the reduction in the relative abundance of PVY^{O} was very rapid; detectable within five weeks post-inoculation (Fig. 4.8). This significant, one-third reduction in the PVY^{O} strain share observed in the screen-house experiment after a single round of infection appeared to be the direct result of strain-specific resistance to PVY^{O} infection exhibited by three potato cultivars: Alturas, Ranger Russet, and, in particular, Umatilla Russet (Fig. 4.8). Russet Burbank had no strain-specific response against any of the three strains tested (Fig. 4.8), although this cultivar might have exhibited a weak non-specific resistance against all three PVY strains, reducing slightly the overall number of PVY-infected plants (Table 4.1) in 2015.

A factor to consider while looking at the data from both seasons is the genes of resistance that are being looked at are *N* genes. These genes give hypersensitive resistance under specific weather and temperature conditions. Taking this into consideration the results become even more interesting where they are representing not only the rate of infection across two years

but also the efficacy of resistance genes. The beginning of the season in 2015 was considerably warmer than the season in 2016. This difference in temperature could account for the slight difference in percent of plants infected between the years. It could be speculated that some *N* genes worked better under a cooler summer in 2016 than a hotter one in 2015. Russet Burbank might have lost its overall weak non-specific resistance once the temperatures dropped while all the other cultivars were able to enhance their levels of partial resistance to PVY^{O}.

The changes in temperature in 2016 did not have an effect on the resistance of these cultivars to PVY^{O}, as the infection rates decreased with a lower temperature. In contrast to the rates of infection in the other two strains, the plants were able to display the restriction of systemic spread of PVY^{O}. It was observed that they managed to restrict the virus even better than in 2015 for the three cultivars Alturas, Ranger Russet, and Umatilla Russet (Fig. 4.8).

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, currently representing approximately 30% of all potatoes grown in Washington, and approximately 18% of all potatoes grown in Oregon (NASS, 2015). If the screen-house 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, Russet Ranger, Umatilla Russet, and Alturas the rapid, within one month, PVY strain composition change observed in the screen-house following inoculations with three strains of PVY (Table 4.1, Fig. 4.8) would reasonably mimic the changes observed between individual growing seasons in the field (see Table 2.3).

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. 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 screen-house 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 non-mainstream cultivars made up the remaining 22% to 25% of all seed lots (Pavek and Holden 2016).

Table 4.1. Infection incidences in four potato cultivars in the screen-house after mechanical inoculation with three different strains of PVY. Forty potato plants per PVY strain were inoculated in a screen-house with isolates Tb60 (PVY^{O}), N1 ($PVY^{N\text{-}Wi}$), and HR1 (PVY^{NTN}) at the same time, in a complete randomized block design (see Figs. 4.1, 4.2). All plants were tested for systemic infection five weeks later, using TAS-ELISA, and typed to strain using IC-RT-PCR.

Strain of PVY	Russet Burbank					Alturas					Umatilla Russet					Ranger Russet					All Combined				
	Exp[a]	Obsrvd[a]		% of total[b]		Exp	Obsrvd		% of total		Exp	Obsrvd		% of total		Exp	Obsrvd		% of total		Exp	Obsrvd		% of total	
		2015	2016	2015	2016		2015	2016	2015	2016		2015	2016	2015	2016		2015	2016	2015	2016		2015	2016	2015	2016
O	40	29	40	33.0 (±9.8)	34.2 (±8.6)	40	22	20	23.4 (±8.6)	21.1 (±8.2)	40	16	11	19.1 (±8.4)	13.6 (±7.5)	40	23	12	13.3 (±7.3)	13.3 (±7.0)	160	90	83	22.3 (±5.0)	21.7 (±4.1)
N-Wi	40	31	39	35.2 (±10.0)	33.3 (±8.6)	40	37	38	39.4 (±9.9)	40.0 (±9.9)	40	36	38	42.9 (±10.6)	44.4 (±10.3)	40	34	40	41.0 (±10.5)	46.9 (±10.9)	160	138	155	39.5 (±5.3)	40.5 (±4.9)
NTN	40	28	38	31.8 (±9.7)	32.5 (±8.5)	40	35	37	37.2 (±9.8)	39.0 (±9.8)	40	32	32	38.1 (±10.4)	39.5 (±10.6)	40	38	38	45.8 (±10.7)	42.2 (±10.2)	160	133	145	38.1 (±5.2)	37.9 (±4.9)
Total:	120	88	117			120	94	95			120	84	81			120	95	90			480	361	383		

[a] Exp = expected number of infected plants, if the incidence is assumed at 100%; Obsrvd = actual, observed number of infected plants.

[b] Percent 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 chi-square 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 bracket.

Fig. 4.1

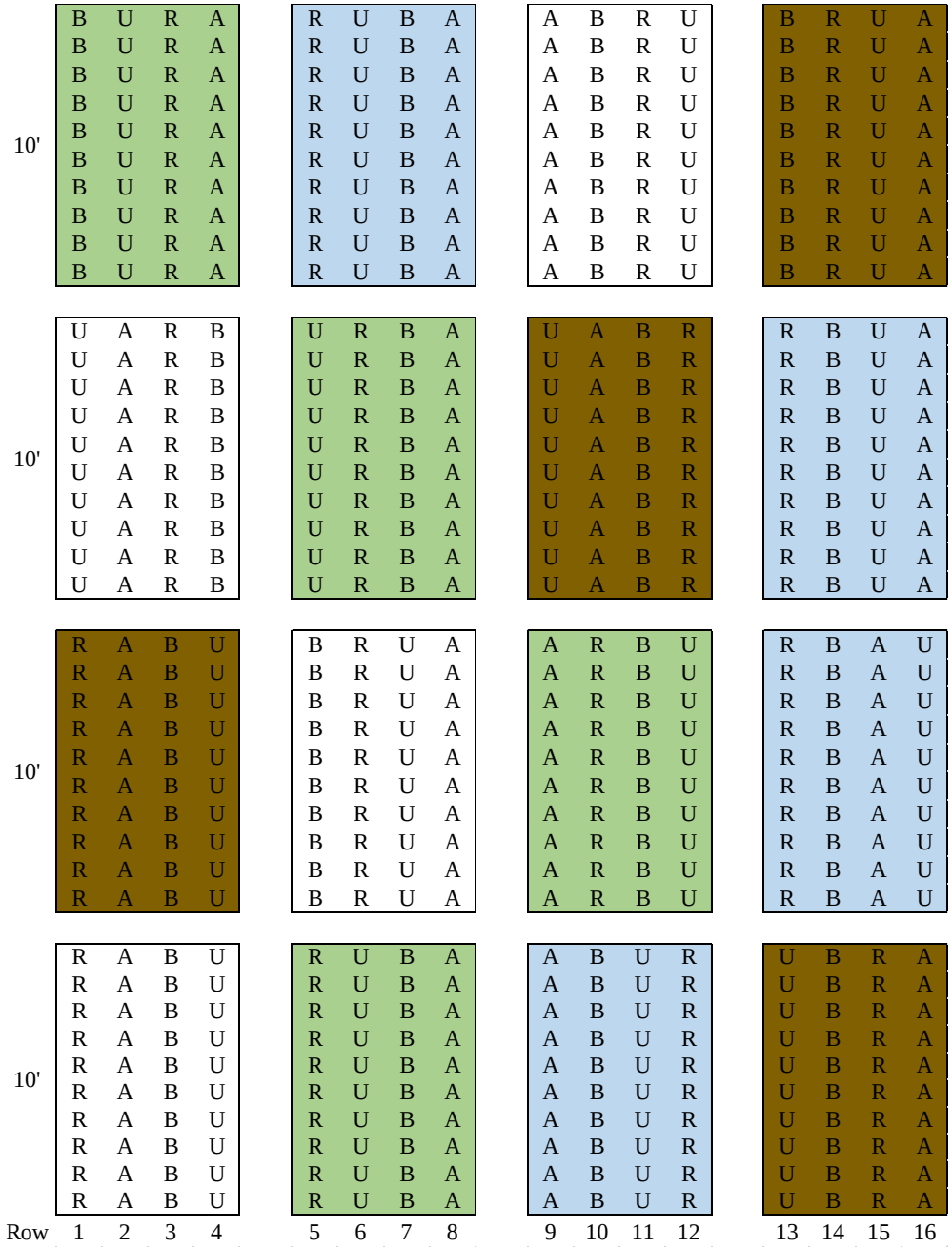

Fig. 4.1. Screen-house layout in 2015, showing experiment layout, dimensions and randomized block design. Letters indicate individual potato plants. B = Russet Burbank, U = Umatilla Russet, R = Ranger Russet, A = Alturas. Shading color designates the *Potato virus Y* (PVY) strain used for inoculation: green means PVY^{O} (isolate Tb60), blue means PVY^{NTN} (isolate HR1), brown means $PVY^{N\text{-}Wi}$ (isolate N1), and no shading means non-inoculated.

Fig. 4.2

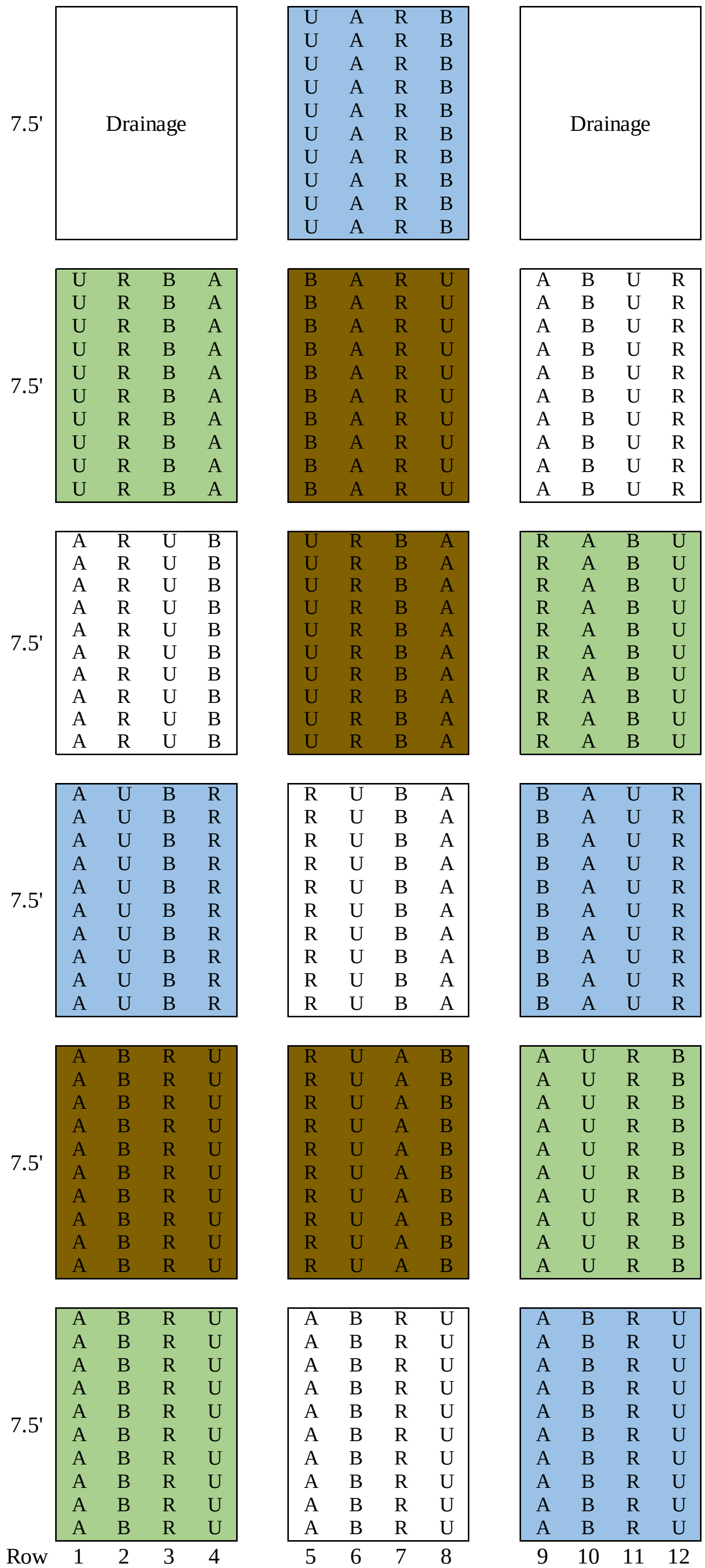

Fig. 4.2. Screen-house layout in 2016. Letters and colors are represented identically from 2015. The layout changed to accommodate better drainage and reduce compaction and plant competition.

Fig. 4.3

Fig. 4.3. Screen-house – plant size during inoculation, and status of plants during second sampling in 2015.

Fig. 4.4

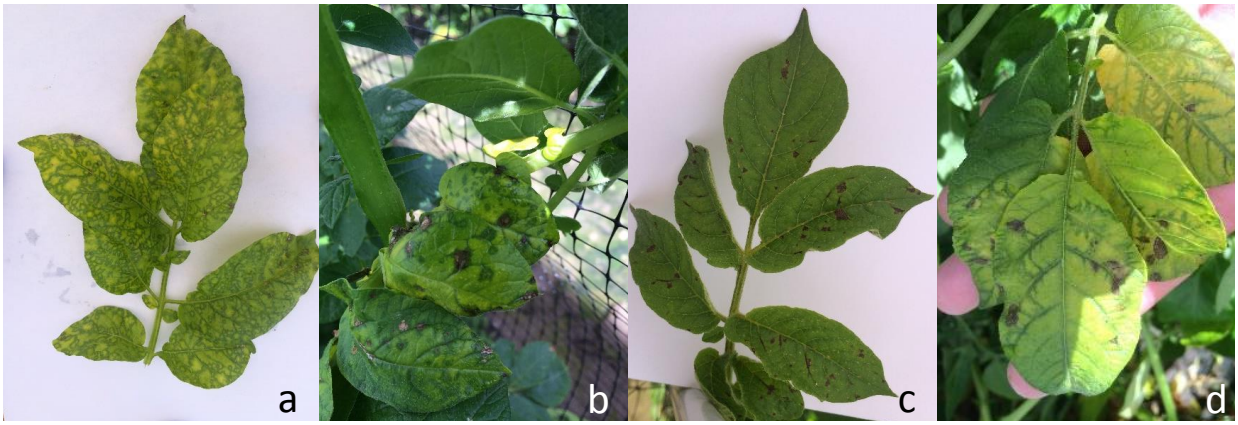

Fig. 4.4. Symptoms in upper, non-inoculated leaves expressed in different cultivars infected with isolate Tb60 (PVY^{O}) at 5 weeks p.i. in 2015 and 8 weeks p.i. in 2016. (a, d) cv. Russet Burbank; (b) cv. Umatilla Russet; (c) cv. Alturas. The experiment was conducted in the screen-house in 2015 (b) or in 2016 (a, c, and d).

Fig. 4.5

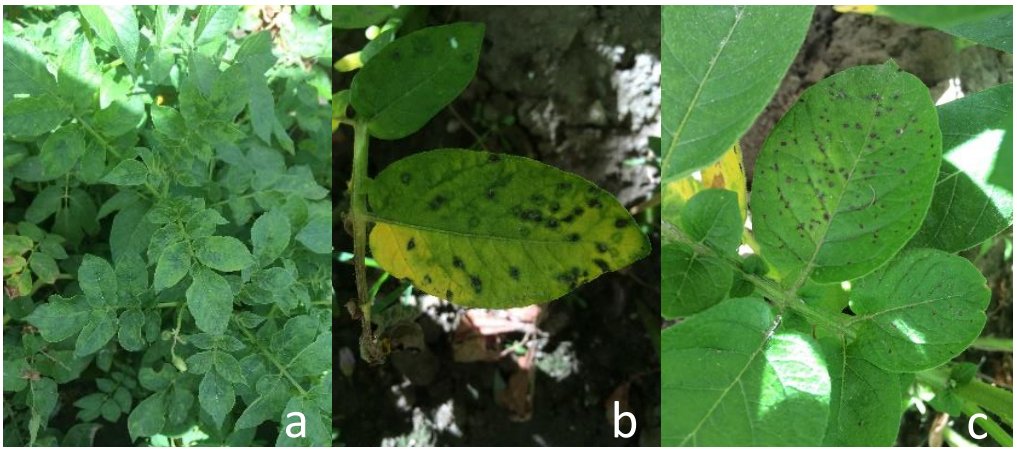

Fig. 4.5. Symptoms in upper, non-inoculated leaves expressed in different cultivars infected with isolate HR1 (PVY^{NTN}): (a) cv. Alturas 8 weeks p.i.; and (b,c) cv. Ranger Russet 4 weeks p.i.. The experiment was conducted in the screen-house in 2016.

Fig. 4.6

Fig. 4.6. Symptoms in upper, non-inoculated leaves expressed in two cultivars infected with isolate N1 (PVY^{N-Wi}) 8 weeks p.i.: (a) mosaic and mottling in cv. Alturas; and (b) mild mosaic in cv. Ranger Russet. The experiment was conducted in the screen-house in 2016.

Fig. 4.7

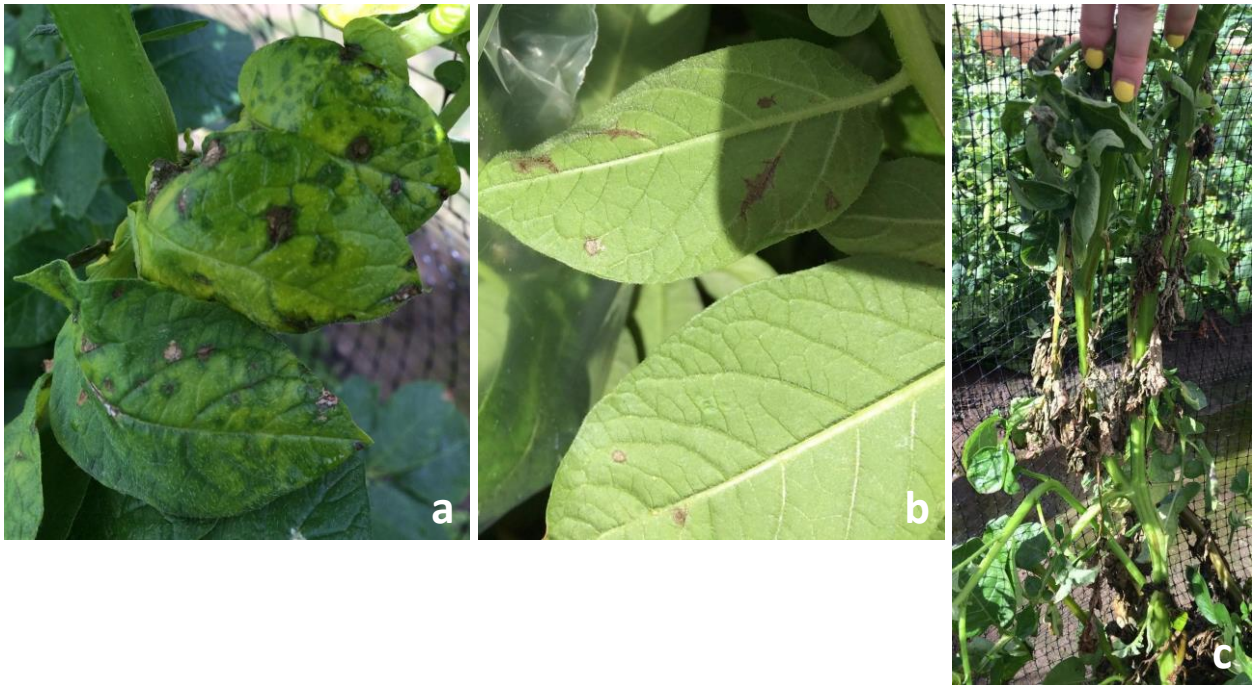

Fig. 4.7. Symptoms observed in the screen-house experiment. (a) Symptoms of green water soaking rings, necrotic lesions, and (b) vein necrosis induced by PVY isolate Tb60 (PVY^{O}) in cv. Umatilla Russet as observed at 5 weeks post-inoculation (p.i.). (c) Severe systemic leaf-drop and necrotic stem streaks induced by PVY isolate Tb60 (PVY^{O}) in cv. Ranger Russet at 5 weeks p.i. All plants were tested at 5 weeks p.i. by TAS-ELISA and RT-PCR to confirm systemic infection with the isolate/strain indicated.

Fig. 4.8

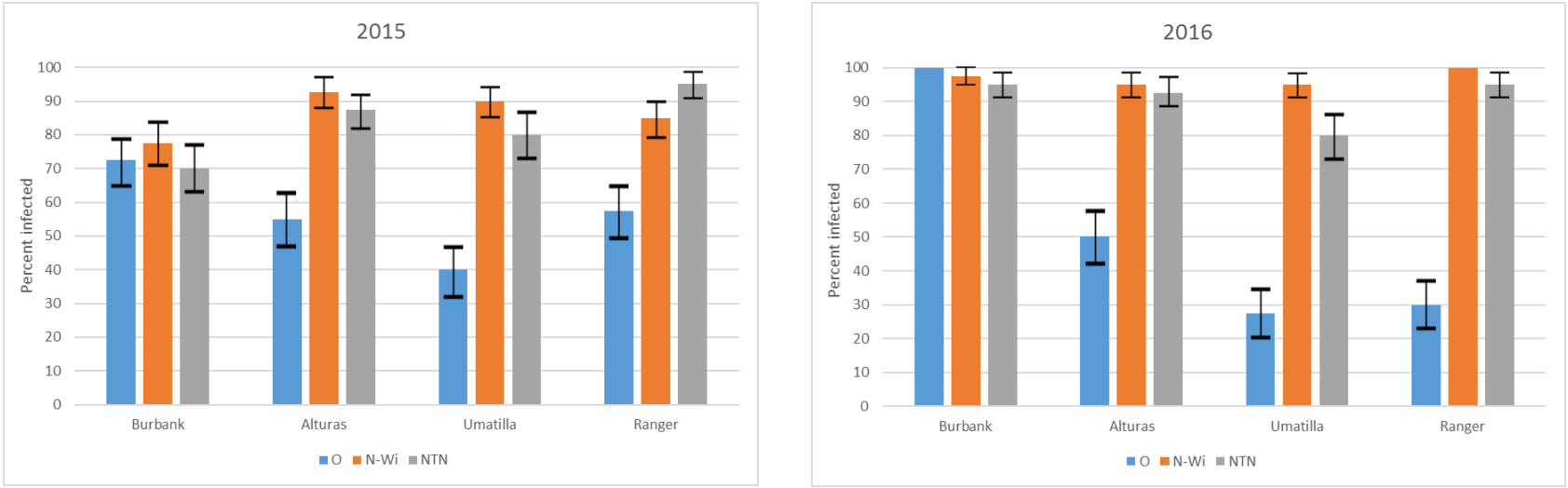

Fig. 4.8. Graphs showing incidence of infection in the screen-house across two years; determined by triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) and reverse-transcription polymerase chain reaction (RT-PCR) 5 weeks post-inoculation. Forty individual plants per cultivar-strain combination were inoculated with isolates Tb60 (PVY^{O}), N1 ($PVY^{N\text{-}Wi}$), 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 (Burbank), 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.

Chapter 5: Conclusions

The underlying question we attempted to answer in these studies was what may be the reasons for these dramatic changes in the relative abundance of PVY in the Columbia Basin strains over recent years? Between 2011 and 2015, no new strains or variants of PVY were identified in the Othello seed lot trial, and the set of strains detected in the samples collected during these five years remained stable (Table 2.3), 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 to the non-recombinant strains (Cockerham, 1970; de Bokx and Huttinga, 1981; Jones, 1990; Singh et al., 2008; Karasev and Gray, 2013). Translocation efficiency of the virus moving from the infected foliage into the tuber was studied for different PVY strains in several potato cultivars (Beemster, 1976; Draper et al., 2002; Basky and Almasi, 2005). 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 (Beemster, 1976; Basky and Almasi, 2005), however the studies included multiple other variables hampering the interpretation of the data. Aphid transmission efficiencies in potato were found to differ

about two-fold 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 non-colonizing aphids revealed similar transmission efficiencies across most of the strain/species combinations; in particular, no difference was found between PVY^{O} and $PVY^{N\text{-}Wi}$ 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).

This study documents the unequal impacts that the predominant cultivars grown in the Columbia Basin have on the different recombinant and non-recombinant 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 screen-house experiment, where current season spread was excluded (Fig. 4.8).

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