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Potato Cultivar and Seed Type Affect the Development of Systemic *Potato virus Y* (PVY^{N-Wi}) Infection

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Abstract

Potato virus Y (PVY) infection is one of the greatest challenges to seed potato production in the United States. To determine how cultivar and seed type affect the development of systemic PVY infection, Russet Burbank and Russet Norkotah Colorado 3 cultivars were grown from two types of pre-nuclear seed (i.e., plantlets and minitubers) and Generation 3 (G3) tubers and challenged with PVY strain Wilga (PVY^{N-Wi}). Systemic PVY infection was measured by assaying spread of virus from the inoculation site to upper non-inoculated leaves. The Burbank cultivar had a lower incidence of systemic PVY infection compared to the incidence of systemic PVY that developed in the Colorado 3 cultivar. Furthermore, Burbank plants grown from G3 tubers had a lower incidence of systemic PVY infection, as compared to Burbank plants grown from plantlets. Together our results indicate that both cultivar and seed type affect the development of systemic PVY^{N-Wi} infections post-inoculation.

Resumen

La infección por virus Y (PVY) es uno de los mayores retos en la producción de semilla de papa en los Estados Unidos. Para determinar cómo afecta la variedad y el tipo de semilla al desarrollo de la infección sistémica de PVY, se cultivaron las variedades Russet Burbank y Russet Norkotah Colorado 3 de dos tipos de semilla pre-nuclear (p. e., plántulas y minitubérculos) y de tubérculos de la Generación 3 (G3), y se inocularon con PVY variante Wilga (PVYN-Wi). La infección sistémica de PVY se midió mediante la detección de la dispersión del virus desde el sitio de inoculación hacia las hojas superiores no inoculadas. La variedad Burbank tuvo la incidencia más baja de infección sistémica de PVY en comparación con la incidencia de PVY sistémico que se desarrolló en la variedad Colorado 3. Aún más, las plantas de Burbank originadas de los tubérculos de G3 tuvieron la incidencia más baja de infección sistémica de PVY, en comparación con las plantas de Burbank originadas de plántulas. Juntos, nuestros resultados indican que tanto la variedad como el tipo de semilla afectan el desarrollo de infecciones sistémicas de PVYN-Wi después de la inoculación.

Keywords Russet Burbank · Russet Norkotah Colorado 3 · Potato virus Y · PVY strain Wilga

Introduction

Potatoes (*Solanum tuberosum* subsp. *tuberosum*) are the fourth most consumed food crop grown worldwide and were valued at $6 billion globally in 2005 (FAO 2008). Potatoes are vegetatively propagated and pathogens, including viruses, spread more readily to progeny compared to crops that are propagated from true seeds (Karasev and Gray 2013). Potato seed certification laboratories and growers utilize several measures including tissue culture propagation, field inspection, and diagnostic testing to ensure pathogen-free seed potato stock. Pathogen transmission and disease are reduced by extensive pathogen monitoring programs and by limiting the number of field-grown generations.

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Potato virus Y (PVY) is the most important pathogenic threat to the seed potato industry and is the primary factor limiting seed potato certification (Gray et al. 2010). PVY infects a broad range of plants in the *Solanaceae* family, including important agricultural crops such as potatoes, tobacco, tomato, pepper, and petunia (Kerlan and Moury 2006). PVY is a Potyvirus with a 9.7 kb positive sense single-stranded RNA genome and a filamentous capsid (11 nm diameter × 740 nm length) (Scholthof et al. 2011). Mutation and recombination have resulted in a great diversity of PVY strains worldwide (MacKenzie et al. 2015). The majority of currently circulating PVY strains are recombinant viruses that contain segments of both PVY^{O} and PVY^{N} genomic sequences (Karasev et al. 2011). PVY^{N-Wi} is a recombinant virus with the parent strains PVY^{O} and PVY^{N}. PVY^{O} induces a hypersensitive response (HR) in potatoes carrying the potato Ny_{tbr} gene, and mosaic symptoms in tobacco. PVY^{N} does not induce an HR response in potatoes (i.e., Ny_{tbr} or Nc_{tbr} are not induced), whereas PVY^{N} induces vein necrosis in tobacco (Cockerham 1970; Jones 1990; Karasev and Gray 2013; Rowley et al. 2015; Singh et al. 2008, and reviewed in Funke et al. 2017). Similar to PVY^{N}, PVY^{N-Wi} does not induce an HR response in potato and causes vein necrosis in tobacco (Cockerham 1970; Jones 1990; Karasev and Gray 2013; Rowley et al. 2015; Singh et al. 2008; and reviewed in Funke et al. 2017). PVY^{N-Wi} infection of potato plants may be asymptomatic or result in transient mild mosaic patterning on foliage (Karasev and Gray 2013). Interactions between specific PVY strains and potato cultivar hosts affect viral pathogenicity, potato quality and yield loss (Funke et al. 2017). In this study, we focused on the PVY^{N-Wi} strain that has increased in prevalence in Montana and the United States since its initial detection in 2002 (Karasev 2016).

Russet Burbank (Burbank) was initially released as "Netted Gem" in 1902 and is the most widely grown cultivar in the United States (Bethke et al. 2014; Potato Association of America 2017). It has oblong russeted tubers with excellent baking and processing quality. It is tolerant to common scab, but susceptible to Fusarium and Verticillium wilts, leafroll, and Potato virus Y (Potato Association of America 2017). Russet Norkotah is a long, smooth, shallow-eyed russet-skinned cultivar, which was released from the North Dakota potato breeding program in 1985. Originally thought to be tolerant to PVY, it was later shown to be highly susceptible to PVY, though it is resistant to the expression of PVY-associated symptoms (Draper et al. 2002). Russet Norkotah Colorado Selection 3 (referred to as Russet Norkotah Colorado 3 and Colorado 3) was selected from Russet Norkotah stock by researchers at Colorado State University in 1991 (Certification Seed Potato 2017).

In the United States, seed potatoes are produced using a limited generation system where the first planting is initiated from pre-nuclear stock, which includes tissue culture plantlets produced in sterile conditions or minitubers produced in sanitized greenhouses in pathogen-free growth media. In Montana, the nuclear generation is the first field planting and may originate from in-vitro produced plantlets and microtubers or greenhouse produced minitubers. The nuclear generation is followed by four more field plantings, Generation 1 (G1)- Generation 4 (G4), which can be certified as seed (MSU Seed Potato Certification Program Rules 2016). In this study, we use "seed type" to refer to the pre-nuclear plantlets produced in-vitro, pre-nuclear mini-tubers produced in a sanitized greenhouse, and field grown G3 tubers, which were the seed type sources for the plants used in this study.

In Montana, PVY testing using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is performed on 100% of nuclear and G1 plants and on 200 plants per acre for G2 plants; PVY testing of G3-G4 plants is optional. Montana regulations allow a maximum level of 0.5% PVY incidence for seed potato recertification. In addition, visual inspections are performed during the potato seed certification process to evaluate the incidence of mosaic symptoms, which may be indicative of either tuber born or current season PVY infection. Montana has a maximum allowed mosaic incidence of 0.1% for Generation 2, 0.2% for Generation 3, and 0.5% for Generation 4.

Cumulative data from potato field inspections and laboratory diagnostic tests of leaf samples, performed as part of the Montana State University Seed Potato Certification Program, indicated that PVY incidence varied by cultivar and seed type. Data from the Montana Seed Certification Program from 2011 to 2015 indicated that the cultivar Colorado 3 had a higher incidence of PVY than the cultivar Burbank (Fig. 1) and that nuclear generation plants had a greater number of current season PVY infections when compared to plants grown from field produced tubers (i.e., G1, G2, and G3 seed potato stock). Aseptically produced plantlets or microtubers are initially grown in pasteurized soils in sanitary greenhouse conditions and then transplanted to the field as the nuclear generation (Field Year 1). Plants and tubers grown in these artificial environments have reduced pathogen pressure, as well as reduced exposure to soil microbes, and in turn it is likely that their immune systems have not developed the capability to generate an appropriate response to biotic challenges as compared to that of field propagated plants (Pospóšilová et al. 1999; Jones and Dangl 2006). Mature plant resistance and developmental age of the plant is also a factor in virus susceptibility, as older potato plants or plants that are in later developmental stages are less likely to become infected by virus (Sigvald 1985). The goal of this study was to further examine the influence of cultivar and seed type on the development of PVY infections. Greater PVY prevalence in nuclear plantings could jeopardize successive generations of seed potatoes.

We determined that the percentage of systemic PVY^{N-Wi} infections was lower in the Burbank cultivar compared to the Colorado 3 cultivar. In addition, we determined that Russet Burbank plants grown from field produced G3 tubers were less likely to develop systemic PVY infections compared to plants

grown from plantlets. Together, these results indicate that both cultivar and seed type affect the percentage of potato plants that develop systemic PVY^{N-Wi} infection post-inoculation.

Materials and Methods

Postharvest Evaluation All G1 seed potato lots were sampled at 400 tubers per lot after harvest, collected by the Montana Seed Potato Certification Laboratory, and grown in Oahu, HI. Plots were planted in mid-November and visual evaluations and leaf sampling were initiated in late December. For each plot, one leaflet was picked per plant. All samples were transported within 2 days to the Montana State University Seed Potato Laboratory and processed using 10 leaflet subsamples for DAS-ELISA detection of PVY. PVY incidence data from 2011 to 2015 was aggregated for each cultivar (i.e., Colorado 3 and Burbank) (Fig. 1). From 2011 to 2015, there was an average of four seed lots per year of Colorado 3 and an average of 19 seed lots per year of Burbank from all Montana growers. The PVY incidence for each seed lot was classified as one of three categories: 1–0% PVY; 2–0-0.5% PVY; 3 – >0.5% PVY.

PVY Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) A PVY-specific polyclonal IgG antibody stock was developed by MSU's Seed Potato Certification laboratory, screened against several strains of PVY by the laboratory of Dr. Stewart Gray at Cornell University, and utilized to detect PVY in the leaf homogenates described herein. In brief, PVY-specific polyclonal antibodies were produced by inoculating rabbits with a PVY^{O} positive virus preparation from Agdia, Inc.; PVY^{O} strain determination was based on monoclonal antibody testing and PCR (Chikh Ali et al. 2010). The PVY detection capacity of the resulting polyclonal antibody serum was evaluated by testing its ability to detect 23 different PVY strains, including O, N:O, NTN, as well as some mixed and unclassified strains (data not included, Dr. Stewart Gray, USDA-ARS, Cornell, 2007); the polyclonal antibody detected all of these strains, and further testing confirmed that this polyclonal antibody detected PVY strain Wilga (PVY^{N-Wi}), including the strain obtained from potato tubers grown near Townsend, Montana, USA, which was utilized for the experiments described herein. ELISA plates were prepared by incubating 200 μl of anti-PVY IgG (1 μg/mL) in coating buffer (14.2 mM sodium carbonate, 35 mM sodium bicarbonate, 3.1 mM sodium azide) per well of an Immulon-1B flat bottom 96-well microtiter plate (ThermoScientific) overnight at 4 °C, followed by washing three times with PBS-Tween buffer, blocking overnight at 4 °C in buffer (5.84 M NaCl, 3 mM KH_2PO_4, 2 mM Na_2HPO_4, 140 mM KCl, 0.05% Tween-20, 0.2% nonfat dry milk), and lastly removing the buffer and storing at −20 °C until use (Clark and Adams 1976).

To assay for PVY infection, leaf tissue samples (200 mg) were placed in 12 cm by 12 cm universal extraction bags (Bioreba, Reinach, Switzerland) and homogenized in buffer (5.84 M NaCl, 3 mM KH_2PO_4, 2 mM Na_2HPO_4, 140 mM KCl, 0.005% Tween-20, 0.02% nonfat dry milk) using a tissue homogenizer (Bioreba, Reinach, Switzerland) mounted on a drill press (Craftsman, Hoffman Estates, IL). Each PVY-DAS-ELISA was performed with 200 μL of plant filtrate per sample and an overnight incubation at 4 °C, followed by washing and incubation with the PVY-specific poly-clonal antibody (developed by MSU's Seed Potato Certification Laboratory), followed by washing, incubation with an alkaline phosphatase conjugated anti-rabbit IgG secondary antibody, and developed using 4-nitrophenyl phosphate (Gold Biotechnology). Results were obtained as optical density (OD) measurements at 405 nm on an Epoch Microplate Spectrophotometer (Biotek, Winooski, VT), with a positive threshold of 0.2 OD. Negative controls of uninfected potato tissue and positive controls of infected tobacco tissue were included on each plate.

Plant Production Potato cultivars 'Russet Burbank' and 'Russet Norkotah Colorado 3' were propagated as follows: pre-nuclear tissue culture plantlets were propagated using 4.44 g/L Murashige and Skoog medium with Gamborg's vitamins (PhytoTechnology Laboratories, Shawnee Mission, KS), pre-nuclear minitubers were grown in the greenhouse in Sunshine Mix 1 (Sungro Horticulture, Vancouver, Canada), and G3 tubers were field grown and certified by the Montana Seed Potato Certification Program. Tubers were treated with Rindite (7 parts ethylene chlorohydrin, 3 parts ethylene dichloride, and 1 part carbon tetrachloride) to break dormancy (Bryan, 1989). Plantlets, minitubers, and G3 tubers were planted in 7″ pots containing Sunshine Mix 1 (Sungro Horticulture, Vancouver, Canada), and grown in the greenhouse under a 16:8 day:night photoperiod at 22 °C. Watering was performed as needed and 200 ppm 20-20-20 fertilizer was applied weekly (JR Peter's Inc., Allentown, PA). Tubers from G3 planting stock had no detectable PVY in postharvest testing conducted by Montana Seed Potato Certification Laboratory and were confirmed to be negative for PVY by DAS-ELISA in leaf tissue prior to inoculation. Plants were grown for four weeks prior to mechanical inoculation.

PVY Inoculum Preparation PYV strain Wilga (PVY^{N-Wi}) was obtained from potato tubers grown near Townsend, Montana, USA. Sequence analysis, using the primers listed in Supplemental Table S1, was used to verify strain designation. Strain validation including assessment by multiplex PCR, which confirmed the presence of PVY^{N-Wi} and the absence of other strains, as described by Chikh Ali et al. 2010 (Supplemental Fig. S1 and Supplemental Table S1) and sequence analysis, which is a more precise characterization of the PVY^{N-Wi} isolate

used in these studies (Supplemental Fig. S2). Sequencing of the PVY virus genome from two preparations of inoculum confirmed that a PVY^{N-Wi} strain was utilized for these experiments. Specifically the PVY^{N-Wi} isolate described herein share 99.6% nucleotide identity (i.e., 3912 of 3927 nucleotides sequenced) with PVY^{N-Wi} (Accession HQ912863) (Supplemental Figs. S2, S3, and Supplemental Table S1).

PVY^{N-Wi} stock was maintained in tobacco plants (*Nicotiana tabacum* 'Samsun'), which were cultivated from seed with a 24 h photoperiod at 22 °C in 10″ pots. Tobacco plants were grown for approximately four weeks prior to virus inoculation. Tobacco tissue that exhibited mosaic symptoms four to five weeks post-inoculation and was PVY-positive by DAS-ELISA was used to prepare inoculum. Virus inoculum was prepared as described by Rupar et al. 2013 with the addition of modifications that improved consistency in viral inoculum quantification including additional centrifugation, filtration, and dilution steps. In brief, PVY-infected tobacco tissue (collected 4–5 wpi) was homogenized in 0.05 M sodium sulfite (Na_2SO_3) buffer, pH 9.7 (1 g tissue: 10 mL buffer) followed by two rounds of centrifugation (4600 x g at 4 °C for 10 min) to remove leaf debris (Rupar et al. 2013). The virus-containing supernatant was filtered through a 0.45 μm filter (Fisher Scientific, Hampton, NH) to remove debris and a 0.22 μm filter (Fisher Scientific, Hampton, NH) to remove bacterial and fungal contaminants. To facilitate accurate quantification by qPCR, the supernatant was diluted (1:1) in sodium sulfite buffer before RNA extraction, reverse transcription, and quantitative PCR. The PVY RNA (including genome and transcript copies) abundance in virus preparations was determined by qPCR using strain-specific qPCR primers as described below (Supplemental Table S1 and Supplemental Figs. S2 - S3).

RNA Extraction and cDNA Synthesis RNA was extracted from PVY inoculum samples using TRIzol reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Reverse transcription reactions (25 μl) were performed using 2 μg of total RNA and random hexamer primers (500 ng) (IDT, Coralville, IA) incubated with Maloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI) for 1 h at 37 °C, according to the manufacturer's instructions.

Quantitative PCR (qPCR) In order to compare and standardize PVY inoculum across experiments, we used qPCR to estimate the relative abundance of viral RNA, including both genomic RNA and transcriptional products. As described above and assessed by multiplex PCR and sequence analysis, only PVY^{N-Wi} was detected in the inoculum produced for the experiments described herein. To determine the abundance of PVY^{N-Wi} RNA, we utilized a qPCR primer set that targets the variable P1 region (i.e., P1–1-F 5' TCA TCC ACA CAA CTC CAA GG and n787-R 5' GTC CAC TCT CTT TCG TAA ACC TC (Chikh Ali et al. 2010), Supplemental Table S1). Quantitative PCR was performed in triplicate using a CFX Connect Real Time machine (BioRad, Hercules, CA). Each 20 μl reaction contained 2 μL cDNA, 1X ChoiceTaq Mastermix (Denville, Holliston, MA), 0.4 μM of each forward and reverse primer, 1X SYBR Green (Life Technologies, Carlsbad, CA), and 3 mM $MgCl_2$. To estimate the relative PVY abundance based on a standard curve, the corresponding segments of PVY were cloned into plasmids. Plasmid standards containing from 10^3 to 10^9 copies per reaction were used as templates for qPCR to generate standard curves. The accurate detection limit was 10^3 copies per reaction for the PVY^{N-Wi} qPCR primer set. The linear standard equations for the PVY^{N-Wi} qPCR primer set, generated by plotting the crossing point (Cp) versus the $\log_{10}$ of the initial plasmid copy number was as follows: PVY^{N-Wi} $Cp = -3.20x + 44.54$, $R^2 = 0.99$ (Supplemental Fig. S4). Quantitative-PCR reactions containing no template served as negative controls. Melt point analyses, agarose gel electrophoresis, and sequencing of initial qPCR products were used to verify qPCR specificity (Ginzinger 2002).

Experimental Design To assess differences in systemic spread of PVY in two potato cultivars from three seed types (i.e., pre-nuclear plantlet, pre-nuclear mini-tuber, and Generation 3) we performed mechanical inoculation of potato plants with PVY^{N-Wi} in a greenhouse setting. Greenhouse experiments were arranged in a two cultivars by three seed type factorial completely randomized block design with 10 blocks. Treatment groups included inoculated and un-inoculated plants (Burbank and Colorado 3) grown from three seed types (plantlets, minitubers, and G3 tubers). Each treatment had 10 replicates and the experiment was repeated three times. To ensure uniform inoculation we mechanically inoculated potato plants at the five to six leaf stage with fresh, quantified preparations of PVY^{N-Wi}. Mechanical inoculation was performed by abrading a 2 cm^2 area on the fourth leaf from the apical meristem with carborundum (320 grit, Van Waters and Rogers Inc., Radnor, PA) and then pipetting a 45 μl PVY^{N-Wi} suspension onto the leaf and spreading it across the abraded surface with a pipet tip (Fageria et al. 2014). The estimated viral RNA equivalences delivered per inoculation for each experimental replicate (rep) were as follows: experimental replicate 1, 5.1×10^5; experimental replicate 2, 4.7×10^7; and experimental replicate 3, 2.0×10^7. In pilot studies, we determined that mechanical inoculation of ~ 1.5×10^4 to 4.7×10^7 RNA equivalents resulted in between 40 and 100% systemic PVY^{N-Wi} infection by four weeks post-inoculation (wpi). In order to quantifiably assay differences in virus spread by ELISA by four wpi, we optimized the amount of virus inoculum to achieve less than 100% infection, though 100% infection was achieved with higher concentration of PVY in the inoculum. The percentage of productive PVY^{N-Wi} infection was determined by evaluating the number of plants, out of the total number of plants per treatment ($n = 10$ per biological

replicate) that exhibited systemic spread of PVY^{N-Wi}, which was assayed using the DAS-ELISA described above. At two, three, and four wpi, the compound leaf two, three, and four nodes above the inoculated leaf, respectively, was collected for immediate PVY-testing.

Statistical Analysis Percent systemic PVY infection data was analyzed using the Restricted Maximum Likelihood Method. Variance-covariance was tested, and compound symmetry (CS) was determined to be the most appropriate using Akaike Information Criterion, Bayesian Criterion, and Hurvich and Tsui. Once determined, CS was used in a repeated measures analysis to make inferences using a mixed model in SAS 9.4 (SAS Software, Cary, NC). The fixed effects were variety, generation, and time, and the random effect was experimental replicate. P-values were adjusted using the Tukey method of multiple comparisons and differences and least square means were determined using a two-tailed t-test critical value of +/− 2.032. Differences with a P-value <0.05 were considered significant.

Results

The Montana Seed Certification Program postharvest test results over a five-year period indicate that a higher percentage of Colorado 3 cultivar seed potato lots exceed the maximum certifiable level of 0.5% PVY incidence as compared to Burbank seed lots (Fig. 1). In addition, farms growing both Burbank and Colorado 3 had higher PVY incidence in Burbank cultivar seed lots (48%) as compared to Burbank G1 seed lots aggregated across all farms (24%, which included both 0%–0.5% and >0.5%) (Fig. 1).

To further evaluate potential differences between two potato cultivars (i.e., Burbank and Colorado 3) and three seed generations (i.e., plantlets, minitubers, and G3 tubers) to support systemic PVY^{N-Wi} infection, potato plants were grown in greenhouse conditions and exposed to PVY strain Wilga (PVY^{N-Wi}) via mechanical inoculation (Supplemental Figs. 1–4). Foliar potato symptoms were undetectable, but as expected, the percentage of PVY infected plants increased with the weeks post-inoculation (Supplemental Fig. S5). Specifically, at four weeks post-inoculation there was a significantly higher percentage of systemic PVY infections detected (mean of 52%) compared to the mean infection percentage detected at two weeks post-inoculation (mean of 6%) (P-value <0.0001). Likewise, the number of new infections detected at three weeks post-inoculation (mean of 42%) compared to two weeks post-inoculation was significantly greater (P-value <0.0001). In contrast, the number of new infections detected at four weeks post-inoculation was not statistically different than at three weeks post-inoculation (P-value = 0.1289) (Supplemental Fig. 5) indicating that the majority of systemic PVY infections would be detected by 4 weeks post-inoculation.

Potato cultivars differed in the percent systemic PVY infection detected after mechanical inoculation. The cultivar Burbank exhibited a lower incidence of systemic PVY infections (mean of 41%), as compared to the cultivar Colorado 3 (mean of 63%), when data from plantlets, minitubers, and G3 tubers were analyzed together (P-value = 0.0069) (Fig. 2a). Burbank plants had a lower percent systemic PVY infection than Colorado 3 plants grown from both minitubers (Burbank 37%, Colorado 3 67%) (P-value = 0.0446), and G3 tubers (Burbank - 27%, Colorado 3–63%) (P-value = 0.0117) (Fig. 2b). However, plants grown from Burbank (mean of 60%) and Colorado 3 (mean of 60%) plantlets did not significantly differ in percent systemic PVY (P-value = 0.8182) (Fig. 2b).

Interestingly, the potato seed type affected the incidence of systemic PVY in the Burbank cultivar, but not the Colorado 3

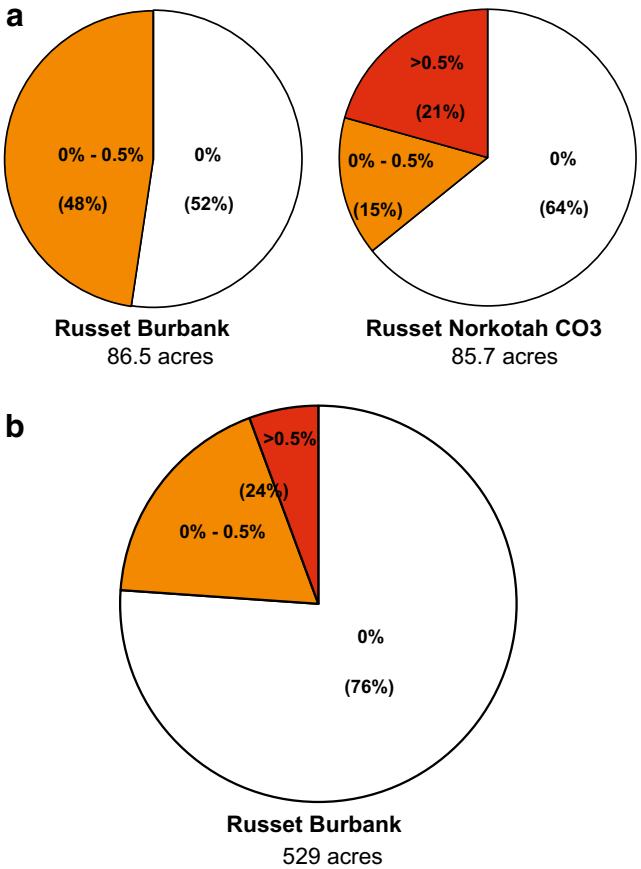

Fig. 1 PVY Incidence in Postharvest Tests from 2011 to 2015. Percentage of Russet Burbank and Russet Norkotah Colorado 3 G1 acres that had 0% (white), 0–0.5% (orange) or >0.5% (red) PVY detected in postharvest tests and measured by DAS-ELISA. Numbers in parenthesis indicate the percent acreage each PVY incidence category. From 2011 to 2015 there were an average of four seed lots per year of Russet Norkotah Colorado 3 and an average of 19 seed lots per year of Russet Burbank across all Montana Growers. **a** PVY incidence data from growers that had seed lots of both cultivars; 86.5 acres of Russet Burbank and 85.7 acres of Norkotah CO3. **b** PVY incidence data from all 529 acres of Russet Burbank G1 seed lots

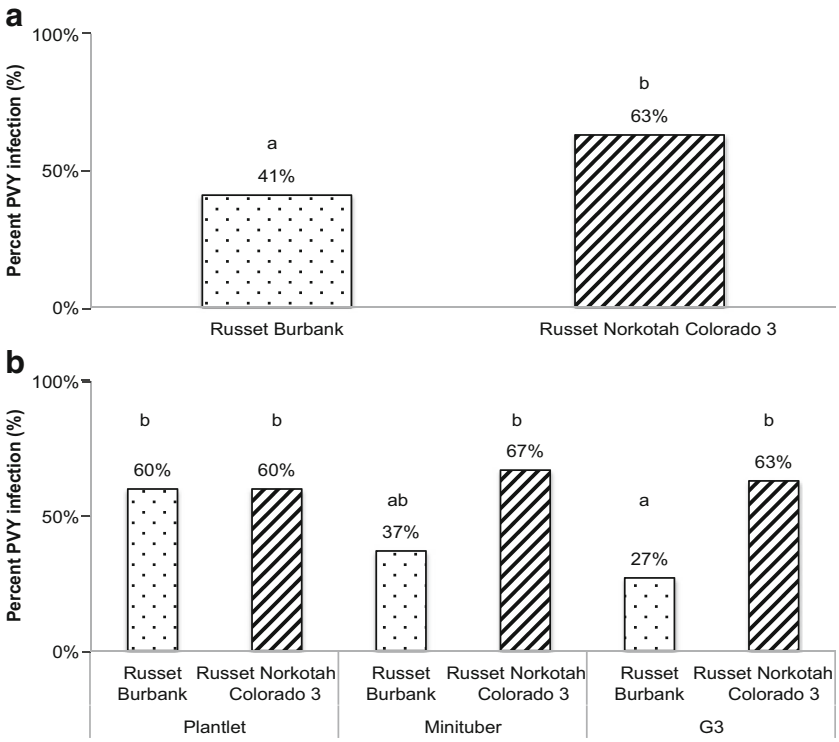

Fig. 2 Potato cultivars Russet Burbank and Russet Norkotah Colorado 3 differ in percent systemic PVY$^{N\text{-}Wi}$ infection. Average percent systemic PVY$^{N\text{-}Wi}$ over three experiments of Russet Burbank and Russet Norkotah Colorado 3 potato cultivars grown from different seed types including pre-nuclear plantlets and minitubers, and G3 tubers inoculated with PVY$^{N\text{-}Wi}$ measured by DAS-ELISA 4-weeks post inoculation. **a** All seed types averaged for both Russet Burbank and Russet Norkotah Colorado 3 cultivars (P-value = 0.007). **b** Average percent systemic PVY$^{N\text{-}Wi}$ in Russet Burbank and Colorado 3 plants grown from three seed types. Cultivar difference for plants grown from minitubers (P-value = 0.04) and G3 tubers (P-value = 0.01). Treatments with statistically different means are indicated by different letters (P-value <0.05)

cultivar (Fig. 3). Plants grown from Burbank plantlets had a significantly higher percentage of systemic PVY infections (mean of 60%) than plants grown from G3 tubers, whereas Colorado 3 plantlets did not exhibit a significantly higher percent systemic PVY infection than plants grown from G3 tubers (P-value = 0.82).

Discussion

Montana Seed Potato Certification data collected as part of regular postharvest testing demonstrated that Colorado 3 G1 seed lots are more likely to exceed maximum seed certifiable PVY levels than Burbank (Fig. 1), and has also been observed

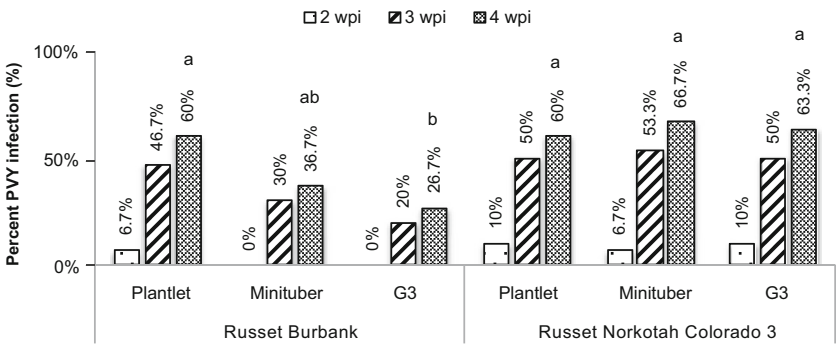

Fig. 3 Potato seed type affects percent systemic PVY$^{N\text{-}Wi}$ infection in Russet Burbank. Percent systemic PVY$^{N\text{-}Wi}$ infection (y-axis) was measured at 2-, 3- and 4- weeks post inoculation (wpi) in Russet Burbank and Russet Norkotah Colorado 3 potato cultivars grown from different seed types including pre-nuclear plantlets and minitubers, and G3 tubers (x-axis). Percent systemic PVY$^{N\text{-}Wi}$ infection data is an average of three experimental replicates. Treatments at 4 wpi with statistically different means are indicated by different letters (P-value <0.05). Difference in percent systemic PVY$^{N\text{-}Wi}$ infection between plants grown from plantlets versus G3 tubers (P-value = 0.03)

in summer field inspections (data not shown). This is consistent with observations that both Colorado 3 and its parent cultivar, Russet Norkotah, had higher rates of current season PVY infection than Burbank (Hamm et al. 2010; Whitworth et al. 2010).

To confirm the influence of potato cultivar and investigate the influence of seed type on the development of systemic PVY infections we performed greenhouse-based studies and utilized well-controlled mechanical inoculation to ensure uniform PVY exposure in three biological replicate experiments. These studies demonstrate that for all seed types (plantlet, minituber, and G3 tuber) the cultivar Burbank had a lower percentage of systemic PVY infection post-inoculation as compared to the cultivar Colorado 3 (Fig. 2). Investigation of the influence of seed type on the development of systemic PVY infections determined that Burbank plants grown from G3 tubers exhibited a lower percentage of systemic PVY infections than plants grown from tissue culture produced plantlets (Fig. 3). In contrast, we did not observe an effect of seed type on the percentage of systemic PVY infections that developed post-inoculation of the Colorado 3 cultivar.

Differences in immune responses, field exposure to microbes, environmental stress, and developmental maturity of seed, likely influence PVY- potato plant host interactions and the development of systemic PVY infections developed post-inoculation in the greenhouse (Raskin et al. 1987; Yalpani et al. 1993). We evaluated the influence of cultivar and seed type on the percentage of systemic PVY that developed post-mechanical inoculation in the greenhouse setting in order to minimize confounding variables inherently associated with aphid-mediated inoculation (e.g., delivery of non-standardized doses of virus) and field-based studies (e.g., environmental factors including nutrient availability and weather events). Measuring relatively subtle differences in the percentages of systemic PVY infection required the standardization of inoculum concentration using qPCR and mechanical inoculation, which ensured that all plants within each experimental replicate were exposed to a standardized PVY dose.

The data presented herein provide valuable information regarding the impact of cultivar and seed type on PVY infection and support field observations obtained by the Montana State University Seed Potato Certification Program from 2011 to 2015. Specifically, we determined that the Burbank cultivar developed a lower percentage of systemic PVY infections post-inoculation, compared to the Colorado 3 cultivar. In addition, our data indicate that for the Burbank cultivar, seed type impacted the percentage of systemic PVY infections observed post-inoculation, whereas no effect of seed type was detected for the Colorado 3 cultivar. Statistically, Burbank plants grown from G3 tuber seed stock had a lower percentage of systemic PVY infections than plants grown from plantlets. Plants that are grown in tissue culture and in sterile soil within a greenhouse setting are exposed to a reduced number of microbes, viruses, and environmental stressors, as compared to plants grown in the field, and therefore may have insufficiently developed (or primed) immune responses against pathogens including PVY. Likewise, due to genetic predispositions, potato cultivars may vary in their ability to respond to pathogens. Overall, our data indicate that the development of systemic PVY infections post-mechanical exposure is affected by cultivar and seed generation for the Burbank cultivar. Additional studies are required to validate the field-relevance of these observations and further investigate the mechanism(s) involved, particularly in the context of aphid-mediated transmission, though PVY can be mechanically transmitted in field settings (Fageria et al. 2015). Future studies aimed at further assessing the mechanism(s) responsible for limiting the development of systemic PVY infections in potato plants may lead to the identification of genes, treatments, and management strategies that restrict virus infection and enhance seed certification.

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