

POTATO AND POTATO VIRUS Y: THE EFFECT OF CULTIVAR, SEED TYPE,
AND DEFENSE-INDUCING AGENTS DIFFERENCES IN INCIDENCE

by

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ABSTRACT

Potato (*Solanum tuberosum subsp. tuberosum*) is an important crop grown worldwide. Propagated vegetatively, a sustainable potato industry relies on pathogen free, tissue culture-produced plantlets to maintain low disease incidence in seed potato stock. Potato virus Y (PVY) infection is the most significant threat to seed potato production. Therefore, determining the influence of cultivar and seed type on PVY incidence could lead to improved management practices. Data from seed potato certification inspection in Montana indicated that plants grown from seed produced in sterile conditions (plantlets and minitubers) had a greater incidence of PVY than plants grown from field-produced tubers. We hypothesized that differences in cultivar, seed type and growth conditions (i.e., sterile vs. field grown) impacted PVY incidence. Systemic acquired resistance (SAR) is a plant immune response that is induced by pathogens and resistance-inducing agents. Resistance-inducing agents are effectively used to limit fungal pathogens, and we hypothesized that they may also limit PVY infection. To test these factors, potato plants (cultivars Russet Burbank and Norkotah Colorado 3) were grown from multiple seed types (i.e., plantlet, minituber, and Generation 3 tuber (G3)) and mechanically inoculated with PVY strain Wilga in the presence and absence of Systemic Acquired Resistance (SAR) inducing agents. Percent infection (incidence) was measured with double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) at three time points post-inoculation. These studies resulted in the following conclusions: (1) incidence of PVY infection varies by cultivar, specifically, the Russet Burbank cultivar had a lower incidence of PVY than the Norkotah Colorado 3 cultivar, (2) Russet Burbank plants grown from tissue culture plantlets had a higher incidence of PVY infection than Russet Burbank plants grown from field-produced G3 tubers, and (3) phosphorous acid (Phostrol®), an inducer of Systemic Acquired Resistance (SAR) may reduce PVY infection, particularly in Russet Burbank plantlets. These findings advance our understanding of the differences in PVY incidence by cultivar and seed type, and in turn provide valuable information for maintaining pathogen free potato seed.

CHAPTER 1

INTRODUCTION

Potatoes and Seed Potato Production

Potato (*Solanum tuberosum* subsp. *tuberosum*) is a member of the *Solanaceae* family, which includes numerous agriculturally important crops such as tomato, eggplant, and tobacco. Potatoes originated in the Andes Mountains of South America. They were brought to Europe by Conquistadores in the 1500's and have subsequently been cultivated worldwide (Jong et al. 2011). Valued for their tubers, which are enlarged starch-storing stolons, potatoes are cultivated for their dense nutritional content. A 100 gram (g) tuber contains: 1.87 g protein, 20 g carbohydrates, 0.1 g fat and vitamins and minerals including potassium (379 mg) and vitamin C (13 mg) (FAO 2008). Potatoes are an important source of nutrition throughout the world, ranking fourth in human consumption after wheat, rice, and corn. In 2005, the global potato crop was valued at \$6 billion (FAO 2008). Unlike many crops, which are grown from true seeds, potatoes are vegetatively propagated. In the Montana Seed Certification Program, all seed material originates from tissue culture produced plantlets, microtubers, or greenhouse grown minitubers. This material is transplanted into the field for the first (Nuclear) generation from which tubers produced are planted and certified as seed for four additional generations designated G1-G4 (Rules and Regulations MSU Seed Potato Certification Program). This limited generation system reduces

pathogen propagation in seed stock, and is an important strategy for limiting Potato virus Y (PVY).

Potato Virus Y (PVY): Virology and Management Strategies

Potato virus Y (PVY) is the most economically damaging and challenging virus in the seed potato industry (Gray et al. 2010). Virus infection causes yield losses as well as foliar and tuber symptoms (Hane et al. 1999). In addition to reducing yield, potato lots above state-mandated PVY tolerance levels cannot be certified as seed. Losses seen in the earliest seed generations compound annually. Data from the Montana Seed Potato Certification Laboratory indicates that PVY incidence typically increases by 10-fold during the three months between summer and post-harvest testing (Zidack unpublished). Seed potato shortages due to PVY impact both seed producers and commercial growers.

PVY is a Potyvirus with a 9.7 kb positive sense single-stranded RNA genome and a filamentous capsid (11 nm diameter x 740 nm length) (Scholthof, K. B. et al. 2011). Recombination of viral genomes has resulted in a diversity of PVY strains. The most prevalent PVY strains, including PVY^{N-Wi} (Wilga), are recombinants of PVY^N (necrotic) and PVY^O (ordinary) (Chikh Ali et al. 2010). Strain PVY^{N-Wi} prevalence in the United States has increased since the original detection in 2002 (Karasev 2016). PVY is transmitted by propagation of infected seed, aphid vectors, and mechanical damage (Fageria et al. 2014). Aphid (*Myzus persicae*)-mediated transmission occurs in a nonpersistent manner. Virus

is transferred by aphid stylets between plants, and do not replicate in the aphid (Uzest et al. 2007). Likewise, mechanical transmission occurs when infectious sap is transferred from infected to healthy plants in both the field and laboratory settings.

To reduce virus infection in the potato crop, seed certification programs include both visual inspection and laboratory testing for PVY. In Montana, fields are typically inspected three times per growing season and leaf samples are tested for PVY using DAS-ELISA. All Nuclear and G1 plants are indexed and tested for PVY, which allows growers to eliminate (rogue) infected plants from their planting stock. Currently, seed certification programs are the primary control method for PVY. A better understanding of the influence of potato cultivar and seed type (sterile vs. field grown) on PVY incidence will impact seed potato production.

Plant Immune Systems

Plants have innate immune systems to defend against pathogens including viruses, bacteria, fungi, and nematodes. Plant immune responses include RNA interference (RNAi), microbe associated molecular pattern (MAMP) triggered immunity (MTI), effector triggered immunity (ETI), and systemic acquired resistance (SAR) (Durrant et al. 2004).

RNAi, an anti-viral immune response, is a post-transcriptional gene silencing mechanism that is sequence-specific (Durrant et al. 2004, Jones et al.

2006, Muthamilarasan et al. 2013). MTI and ETI are triggered through membrane-associated pathogen recognition receptors. Pathogen recognition receptors (PRRs) bind conserved pathogen-associated molecular patterns (PAMPs) such as chitinase and flagellar proteins (Dodds et al. 2010). Pathogen recognition in ETI is mediated by plant resistance proteins (R proteins) that bind pathogen-excreted effectors. Both MTI and ETI activate defense pathways that trigger the mitogen-activated protein kinase (MAPK) cascade. WRKY transcription factors are activated leading to increased salicylic acid (SA) production and expression of proteins involved in defense mechanisms including Systemic Acquired Resistance (SAR) (Muthamilarasan et al. 2013). Salicylic acid plays a role in SAR and the hypersensitive response (HR). The hypersensitive response results in programmed cell death and induction of defense genes (Jones et al. 2006). Salicylic acid travels from infected tissue throughout the plant triggering the expression of host defense genes, thus resulting in pathogen resistance throughout the entire plant. The SAR pathway is also induced by applications of resistance-inducing agents (Durrant et al. 2004).

Systemic Acquired Resistance-Inducing Agents

Systemic Acquired Resistance (SAR) is induced by biotic and abiotic factors including water, heat, and pH stress, pathogens, synthetic chemicals, and biological control agents (Durrant et al. 2004). Potato plants grown in the field are exposed to conditions that potentially induce SAR and therefore limit PVY

infection. The SAR pathway can be triggered in the absence of pathogens. Applications that use the activation of plant SAR for pathogen control include compost tea (Scheuerell et al. 2002, Zhang et al. 1998), *Bacillus mycoides* isolate J (BmJ) (Bargabus et al. 2002), acibenzolar-S-methyl (Actigard ®) (Walters et al. 2005) and phosphorous acid (Phostrol ®) (Guest et al. 1990) (Johnson et al. 2004, Miller et al. 2011).

Compost tea refers to a fermentation of compost in continually aerated water. Efficacy for pathogen control is lost when the microbial component is inactivated (Scheuerell et al. 2002). Aerated compost tea limits disease caused by *Sphaerotheca pannosa* on rose (Scheuerell et al. 2002), *Colletotrichum orbiculare* on cucumber, and *Pseudomonas syringae* on arabidopsis (Zhang et al. 1998), and was shown to induce SAR in cucumber and arabidopsis (Zhang 1998).

Bacillus mycoides isolate J (BmJ) is a gram-positive fermicute isolated from sugarbeet and developed as a biological control agent. Application of BmJ induced systemic acquired resistance in sugarbeet and limited infection on plants challenged with *Cercospora beticola*, the causative agent of *Cercospora* Leaf Spot (Bargabus et al. 2002).

Acibenzolar-S-methyl (Actigard ®) is a synthetic chemical labeled for use against bacterial, fungal, and viral (Iris yellow spot virus) pathogens of many crops (mostly fruits and vegetables). Acibenzolar-S-methyl triggers plant SAR (Walters et al. 2005).

Phosphorous acid (Phostrol ®) (H_2PO_3), treatment protects against diseases caused by oomycetes (Johnson et al. 2004), and is efficacious on potato against late blight (*Phytophthora infestans*) and pink rot (*Phytophthora erythroseptica*) when applied to foliage during the growing season or to tubers prior to storage (Gachango et al. 2012, Miller et al. 2015). Application of phosphorous acid in high concentrations inhibits fungal growth (Fenn et al. 1984), and at lower concentrations, triggers SAR (Jackson et al. 2000, Shanghyun 2012). Resistance-inducing agents are efficacious against bacterial and fungal diseases; however, their effect on PVY infection in potato has not been reported.

Age-Related Resistance

Age-related resistance is observed when developmentally younger plants are more susceptible to a pathogen than older plants (Develey-Riviere et al. 2007). Age-related resistance has been observed for PVY infection of potato (Gibson 1991, Sigvald 1985) and late blight, *Phytophthora infestans* infection of potato which has been known to cause serious losses in potato crops worldwide (Fry et al. 1986). As potato plants aged during the growing season, they were less susceptible to pathogens. Additionally, disease resistance occurs at developmental stages such as flowering. Potato plants inoculated with PVY pre-flowering are more susceptible than plants inoculated post flowering (Shrestha et al. 2014). Pathways for age-related resistance and SAR overlap, for instance,

salicylic acid (SA) is involved in flowering and the SAR pathway (Raskin et al. 1987, Yalpani et al. 1993). While plants grown from different seed types are the same age in days, they exhibit a response similar to age-related resistance and we speculated that plantlets may be developmentally younger than G3 tubers.

Objectives and Experimental Approach

The first objective of this research was to test the effect of potato cultivar and seed type on PVY incidence. Specifically, is there a difference in PVY incidence between potato cultivars Russet Burbank and Norkotah Colorado 3. Additionally, do plants grown from plantlets have a higher incidence of PVY infection than plants grown from G3 tubers? The second objective was to evaluate whether systemic acquired resistance (SAR) inducing agents could effect PVY incidence. Do potato plants treated with agents known to induce SAR have lower percent PVY infections compared to non-induced controls?

To conduct experiments with PVY, a standardized inoculum procedure was developed. To quantify viral inoculum two SYBR Green quantitative polymerase chain reaction (qPCR) assays were developed. Preliminary experiments determined that viral inoculum was too heterogeneous for accurate quantification with qPCR. To improve viral inoculum preparation, additional centrifugation, filtration, and dilution steps were incorporated. Mechanical inoculation experiments were carried out with potato cultivars Russet Burbank (accounts for most acreage planted) and Norkotah Colorado 3 (also widely

planted) (Potato Association of America 2015) grown from three seed types (plantlets, minitubers and G3 tubers). Plants were mechanically inoculated with PVY^{N-Wi} (Wilga) and percent infection (incidence) was measured using DAS-ELISA at two, three, and four weeks post inoculation. We determined that the cultivar Norkotah Colorado 3 had a higher incidence of PVY^{N-Wi} infection than the cultivar Russet Burbank in mechanically inoculated plants. In addition, Russet Burbank plants grown from plantlets had a higher incidence of PVY^{N-Wi} infection than Russet Burbank plants grown from G3 tubers. Experiments with the same cultivars and seed types were conducted to compare the effects of a panel of resistance-inducing agents (compost tea, BmJ, phosphorous acid, and acibenzolar-S-methyl) on PVY infection. Russet Burbank plantlets treated with phosphorous acid had a lower percent infection when compared to non-induced controls in all four experiments conducted. These findings provide insight into factors that affect PVY infection of early generation seed potatoes, and have implications for seed potato management.

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CHAPTER TWO

POTATO CULTIVAR AND SEED TYPE AFFECT INCIDENCE OF POTATO VIRUS Y (PVY^{N-WI}) INFECTION

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CHAPTER 2

POTATO CULTIVAR AND SEED TYPE AFFECT INCIDENCE OF POTATO
VIRUS Y (PVY^{N-Wi}) INFECTION

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Abstract

Potato virus Y (PVY) is one of the greatest challenges to seed potato (*Solanum tuberosum* subsp. *tuberosum*) production in the United States. To determine how potato cultivar and seed type affect incidence of PVY strain Wilga (PVY^{N-Wi}) infection, cultivars Russet Burbank and Norkotah Colorado 3 were grown from multiple seed types (plantlets, minitubers, and G3 tubers) and challenged with PVY^{N-Wi}. The standard PVY inoculum preparation method was improved, and two quantitative-PCR (qPCR) assays were developed. By evaluating the percentage of infected plants (incidence) at two, three, and four weeks post-inoculation it was established that the cultivar Russet Burbank had a lower incidence of PVY^{N-Wi} infection than the cultivar Norkotah Colorado 3. Furthermore, Russet Burbank plants grown from plantlets had a higher incidence of PVY^{N-Wi} infection than Russet Burbank plants grown from field-propagated G3 tubers.

Introduction

Potato (*Solanum tuberosum* subsp. *tuberosum*) is valued at \$6 billion globally in 2005 and is the fourth most consumed food crop grown worldwide (FAO 2008). Potatoes are vegetatively propagated and pathogens, including viruses, are more readily spread to progeny compared to crops that are propagated from true seeds (Karasev et al. 2013). Several measures are taken to ensure pathogen-free seed potato stock, including tissue culture propagation, field inspection, and diagnostic testing. Pathogen transmission and disease are reduced by extensive pathogen monitoring programs and by limiting the number of field-grown generations. Numerous states within the US have set maximum thresholds such as 0.5% in Generation 4 seed in Montana for disease incidence within seed potato lots.

Potato virus Y (PVY) is the most important pathogenic threat to the seed potato industry, and is the primary factor limiting certification (Gray et al. 2010). PVY infects a broad range of plants in the *Solanaceae* family, many of which are important agricultural crops such as potatoes, tobacco, tomato, pepper, and petunia (Kerlan 2006). PVY is a Potyvirus with a 9.7 kb positive sense single-stranded RNA genome and a filamentous capsid (11 nm diameter x 740 nm length) (Scholthof et al. 2011). Mutation and recombination have resulted in a great diversity of PVY strains (MacKenzie et al. 2015). The majority of currently predominant PVY strains, including PVY^{N-Wi}, are recombinant viruses that contain varying proportions of PVY^O (ordinary) and PVY^N (necrotic) genomic

sequences (Chikh Ali et al. 2010, Karasev et al. 2013). PVY^O causes mosaic patterning and vein clearing on susceptible potato leaves but does not cause Potato Tuber Necrotic Ringspot Disease (PTNRD). PVY^N causes foliar vein necrosis on potato but not PTNRD (Karasev et al. 2013). PVY^{N-Wi}, a recombinant of PVY^O and PVY^N, causes foliar vein necrosis, but not PTNRD (Karasev et al. 2013). Interactions between PVY strain and potato cultivar affect pathogenicity, yield loss, and quality (Nie et al. 2012). The PVY^{N-Wi} strain has been increasing in prevalence, in Montana and the US, since its initial detection in 2002 (Karasev 2016).

In the United States, seed potatoes are produced using a limited generation system where the first planting is initiated from tissue culture stock produced in sterile conditions, or minitubers produced in clean greenhouses. In Montana, the nuclear generation is the first planting, which is followed by four more generations (Generation 1 (G1)- Generation 4 (G4)) can be certified as seed. In Montana, 100% of Nuclear and G1 are tested for PVY using DAS-ELISA, and G2 is randomly tested. Testing for G3-G4 is optional. Montana has a maximum *Potato virus Y* (PVY) infection incidence of 0.1% for Generation 2, 0.2% for Generation 3 and 0.5% for Generation 4 during summer field testing; below this threshold potato stock may be planted to produce the next generation of seed. In addition, certification of seed potatoes in Montana is limited to five generations (Nuclear and Generations 1-4) (Rules and Regulations MSU Seed Potato Certification Program). Cumulative data from potato field inspections

indicate that PVY incidence varies by cultivar and seed type. Specifically, the cultivar Norkotah Colorado 3 has a higher incidence of PVY than the cultivar Russet Burbank and the nuclear generation has greater current season PVY infections when compared to later generation plantings grown from field produced tubers (i.e., G1, G2 and G3 seed potato stock). Tissue culture propagated plantlets and microtubers are the first seed materials and are produced in a sterile environment. Plantlets are grown in pasteurized soils in sanitary greenhouse conditions to produce minitubers. Plants and tubers grown in these artificial environments have reduced pathogen pressure and in turn unchallenged immune systems.

To evaluate potential differences in PVY^{N-Wi} incidence, the Russet Burbank cultivar, which is the most widely planted cultivar in the United States, and Norkotah Colorado 3, also widely planted, were grown from plantlets, minitubers, and G3 tubers and exposed to PVY via mechanical inoculation (Potato Association of America 2015). To conduct these experiments, two quantitative polymerase chain reaction (qPCR) assays and a standardized virus inoculation method were developed. Percent infection (incidence) using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was measured at 2, 3- and 4-week post-inoculation (wpi). The incidence of PVY^{N-Wi} infection was lower in the Russet Burbank cultivar, compared to the Norkotah Colorado 3 cultivar. In addition, we determined that PVY^{N-Wi} incidence was less in Russet Burbank plants grown from field produced G3 tubers, compared to the

incidence in plants grown from plantlets. Together, these results indicate that PVY^{N-Wi} incidence is dependent on potato cultivar and seed type.

Methods

Experimental Design

Greenhouse experiments were arranged in a two (cultivars) by three (seed types) factorial, completely randomized block design with 10 blocks. Each treatment group was composed of ten experimental units (potato plants). The percent PVY infection was measured using DAS-ELISA by sampling every plant (each experimental unit) at two, three, and four weeks post inoculation. The experiment was replicated three times.

Plant Production

Potato cultivars 'Russet Burbank' and 'Norkotah Colorado 3' were propagated in three different ways. Tissue culture plantlets were propagated using 4.44 g/L Murashige and Skoog medium with Gamborg's vitamins (PhytoTechnology Laboratories, Shawnee Mission, KS). Minitubers were grown in the greenhouse in Sunshine Mix 1 (Sungro Horticulture, Vancouver, Canada), and G3 tubers were field grown and certified by Montana Seed Potato Certification. Tubers were treated with Rindite (7 parts ethylene chlorohydrin, 3 parts ethylene dichloride, and 1 part carbon tetrachloride) to break dormancy (Bryan, J.E. 1989). Plantlets, minitubers and G3 tubers were planted into 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 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 and Inoculation of Potato Plants with PVY

Tobacco plants (*Nicotiana tabacum* 'Samsun'), used to maintain PVY^{N-Wi} virus stocks, were cultivated from seed with a 24 h photoperiod at 22°C in 10" pots. Strain PVY^{N-Wi} was isolated in Montana and strain verified (Chikh Ali 2009). Improving the preparation of this inoculum became necessary after pilot studies indicated unequal quantification results between aliquots from the same preparation. To improve consistency while quantifying viral inoculum, additional centrifugation, filtration, and dilution steps were incorporated. PVY-infected tobacco tissue (collected 4-5 wpi) was homogenized in 0.05 M sodium sulfite (Na₂SO₃) buffer, pH 9.7 (1:10 g tissue: mL buffer) followed by two rounds of centrifugation (4,600 x g at 4° C for 10 min) to remove leaf debris (Rupar, M. 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 to estimate relative PVY RNA equivalences (methods below). Mechanical inoculation of plants was performed by abrading a 2 cm² 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 suspension onto the leaf and spreading it across the abraded surface with a pipet tip. The estimated viral RNA equivalences delivered per inoculation for each experimental replicate (rep) were as follows: rep 1: 5.1×10^5 , rep 2: 4.7×10^7 , and rep 3: 2.0×10^7 .

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 were performed using 2 µg of total RNA in a 25 µl incubated with Maloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI), and random hexamer primers (5 µg) (IDT, Coralville, IA) for 1 hour 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 viral RNA equivalences, which includes both genomic RNA and transcriptional products, based on the relative abundance to a standard curve. We developed two qPCR assays. To detect the majority of PVY strains, we utilized universal primers that target a highly conserved capsid

protein-encoding gene of PVY; PVY^{Univ}-F9144, 5' CCA ATC GTT GAG AAT GCA AAA (M., Fageria et al. 2013) and PVY^{Univ}-R9295 5' CGC GCT AAA CCC ATA TCC CGC AGA (Supplemental Table 2.1). For strain-specific detection, we developed a primer set that targets the variable P1 region specific to PVY^{N-Wi} (Chikh Ali, M. et al. 2010); PVY^{N-Wi}-F535 5' TCA TCC ACA CAA CTC CAA GG (Supplemental Table S2.1) and PVY^{N-Wi}-R742 5' GTC CAC TCT CTT TCG TAA ACC TC (Chikh Ali, M. et al. 2010) (Supplemental Table 2.1).

Quantitative PCR was performed in triplicate using a CFX Connect Real Time machine (BioRad, Hercules, CA). Each 20 μ L reaction contained 2 μ L cDNA, 1X ChoiceTaq Mastermix (Denville, Holliston, MA), 0.4 μ M of each forward and reverse primer, 1X SYBR Green (Life Technologies, Carlsbad, CA), and 3 mM MgCl₂. To estimate the relative PVY abundance based on a standard curve, the corresponding segments of PVY were cloned into plasmids. Plasmid standards containing from 10³ to 10⁹ copies were used as templates for qPCR to generate standard curves. The detection limit was 10³ copies per reaction for both PVY^{Univ} and PVY^{N-Wi} primer sets. The linear standard equations for each primer set, generated by plotting the crossing point (Cp) versus the log₁₀ of the initial plasmid copy number were as follows: PVY^{Univ} Cp = -3.78x + 43.66, R₂ = 0.98 and PVY^{N-Wi} Cp = -3.20x + 44.54, R₂ = 0.99 (Supplemental Figure S2.4). No template containing qPCR reactions served as negative controls. Melt point analyses and gel electrophoresis were used to verify qPCR specificity (Ginzinger 2002)

Potato Leaf Tissue Collection and DAS-ELISA

PVY infection was measured using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) at two, three and four weeks post-inoculation. The compound leaf two nodes above the inoculated leaf was collected two weeks post inoculation. At three and four weeks post inoculation, the compound leaf three and four nodes above the inoculation site respectively were collected and tested. Leaf tissue samples were weighed, placed in 12 cm by 12 cm universal extraction bags (Bioreba, Reinach, Switzerland) and homogenized in a 1:10 dilution of Blotto (5.84 M NaCl, 0.003 M KH_2PO_4 , 0.002M Na_2HPO_4 , 0.14 M KCl, 0.05% Tween-20) using a tissue homogenizer (Bioreba, Reinach, Switzerland) mounted on a drill press (Craftsman, Hoffman Estates, IL). DAS-ELISA (Clark et al. 1976) was performed with 200 μL of plant filtrate per reaction using PVY-specific IgG polyclonal antibody developed by MSU's Seed Potato Certification laboratory and validated by the laboratory of Dr. Stewart Grey, Cornell University. Optical densities were measured at 405 nm on an Epoch Microplate Spectrophotometer (Biotek, Winooski, VT). Negative controls of uninfected potato tissue and positive controls of infected tobacco tissue were included on each plate.

Statistical Analysis

Percent infection data was analyzed using the Restricted Maximum Likelihood Method (REML). Variance-covariance was tested, and compound

symmetry (CS) was determined to be the most appropriate using Akaike Information Criterion (AIC), Bayesian Criterion (BIC), and Hurvich and Tsui (AICC). 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)

Results

Tissue from greenhouse mechanical inoculation studies was analyzed for presence or absence of PVY infection using DAS-ELISA. Across all cultivars and seed types there was a significant increase in percent PVY infection detected between the first (2 wpi) and last (4 wpi) time points. The number of infections detected peaked between the 2 wpi and 3 wpi time points (P-value <0.0001), and the upward trend continued between 3 wpi and 4 wpi (P-value 0.13) (Supplemental Figure 2.5).

Potato cultivars differed in incidence of PVY infection after mechanical inoculation. The cultivar Russet Burbank had a lower incidence of PVY^{N-Wi} infection (mean of 41%) than the cultivar Norkotah Colorado 3 (mean of 63%) when data from plantlets, minitubers, and G3 tubers was analyzed together (P-value 0.0069). PVY incidence varied by seed type for Russet Burbank but not for Norkotah Colorado 3. Russet Burbank plants had a lower incidence of PVY^{N-Wi} infection than Norkotah Colorado 3 plants grown from both minitubers (P-value 0.04), and G3 tubers (P-value 0.01). However, plants grown from Russet Burbank

and Norkotah Colorado 3 plantlets did not significantly differ in incidence (P-value 0.82) (Figure 2.1).

Potato seed type affects incidence of PVY^{N-Wi} infection in Russet Burbank. Plants grown from Russet Burbank plantlets had a significantly higher incidence of PVY^{N-Wi} infection than plants grown from Russet Burbank G3 tubers (P-value 0.03). However, plants grown from Norkotah Colorado 3 plantlets did not have significantly higher incidence of PVY^{N-Wi} infection than plants grown from Norkotah Colorado 3 G3 tubers (P-value 0.82) (Figure 2.2).

Discussion

Producing pathogen-free potato seed relies on a limited generation system starting with tissue culture produced plantlets and microtubers, or greenhouse grown minitubers. It can be speculated that plants not challenged by organisms or stressors in the environment may have deficient immune responses to pathogens such as *Potato virus Y* (PVY). Understanding differences in incidence of PVY infection by potato cultivar and seed type has implications for management of the earliest generation of seed produced in the field.

These studies demonstrate that across all seed types (plantlet, minituber and G3 tuber) the cultivar Norkotah Colorado 3 has a higher incidence of PVY^{N-Wi} infection than the cultivar Russet Burbank. Russet Burbank plants grown from tissue culture plantlets and mechanically inoculated with PVY had a higher incidence of PVY^{N-Wi} infection than Russet Burbank plants grown from G3 tubers.

This implies that there may be a defense response that develops in the cultivar Russet Burbank when it is grown in the field, and exposed to environmental factors including pathogens. It is also possible that the Norkotah Colorado 3 cultivar may be a superior host to the virus potentially due to virus transmission efficiency or movement within the plant.

Russet Burbank plants grown from tissue culture produced plantlets had a higher incidence of PVY^{N-Wi} infection than Russet Burbank plants grown from G3 tubers when mechanically inoculated in the greenhouse. Field exposure to microbes and environmental stress as well as, differential immune responses and developmental maturity of seed may play a role in reducing percent PVY infection in plants grown from G3 tubers (Raskin et al. 1987, Yalpani et al. 1993).

For Russet Burbank, there was a distinct difference in incidence of PVY infection between plants grown from tissue culture plantlets and plants grown from G3 tubers. This demonstrates that an increased level of vigilance is required to protect this valuable first field generation from infection. For Norkotah Colorado 3, which has higher incidence of PVY infection, there was no difference in virus incidence between seed types. The differential response of these two cultivars indicates that there could be differences in development of resistance in other cultivars.

To achieve consistent inoculation in these experiments, a standardized PVY inoculum preparation and quantification method was needed. To our knowledge, previous studies had not used qPCR to quantify inoculum. We

developed two qPCR assays and used them to quantify relative viral RNA equivalences in viral inoculum. Standard mechanical inoculation with PVY involves rubbing target potato leaf surfaces with the abrasive agent carborundum and sap mixture in buffer (Shrestha et al. 2014). Preliminary experiments indicated that this method of PVY^{N-Wi} inoculum preparation affected accurate relative quantification using qPCR. Heterogeneity in the preparation may be due to viral aggregates (Luciano et al. 1991) which could lead to variation in quantification results. The preparation was improved by adding centrifugation, filtration, and dilution steps, however additional steps to disperse PVY aggregates should be explored. In addition to inoculum preparation, inoculum concentration is important for standardizing experimental replicates. For studies where 100% infection is not desirable, targeting certain relative viral RNA equivalences is valuable. Preliminary inoculation experiments demonstrated that inoculum concentration may affect overall infection rate, which was also observed in the experimental replicates of this study. Preliminary studies indicated that if inoculum is too concentrated, infections as high as 100% are observed regardless of treatment. Future work to assess inoculum concentrations and the resulting infection incidence is needed. Standardized inoculum and a method to predict a range of percent infections would improve uniformity between experimental replicates. Inoculum concentrations used for experiments were similar enough to compare differences in PVY infection by Potato cultivar and seed type.

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Author Contributions

Conceived and designed experiments: N.Z., M.F., and E.B. Primer design and inoculum standardization: M.F., N.Z., and E.B. Greenhouse experiments: N.Z., E.B., and E.C. Analyzed the data: J.R., N.Z., E.B. Wrote the paper: E.B., N.Z., and M.F.

Conflicts of Interest

The authors declare no conflict of interest.

Figures

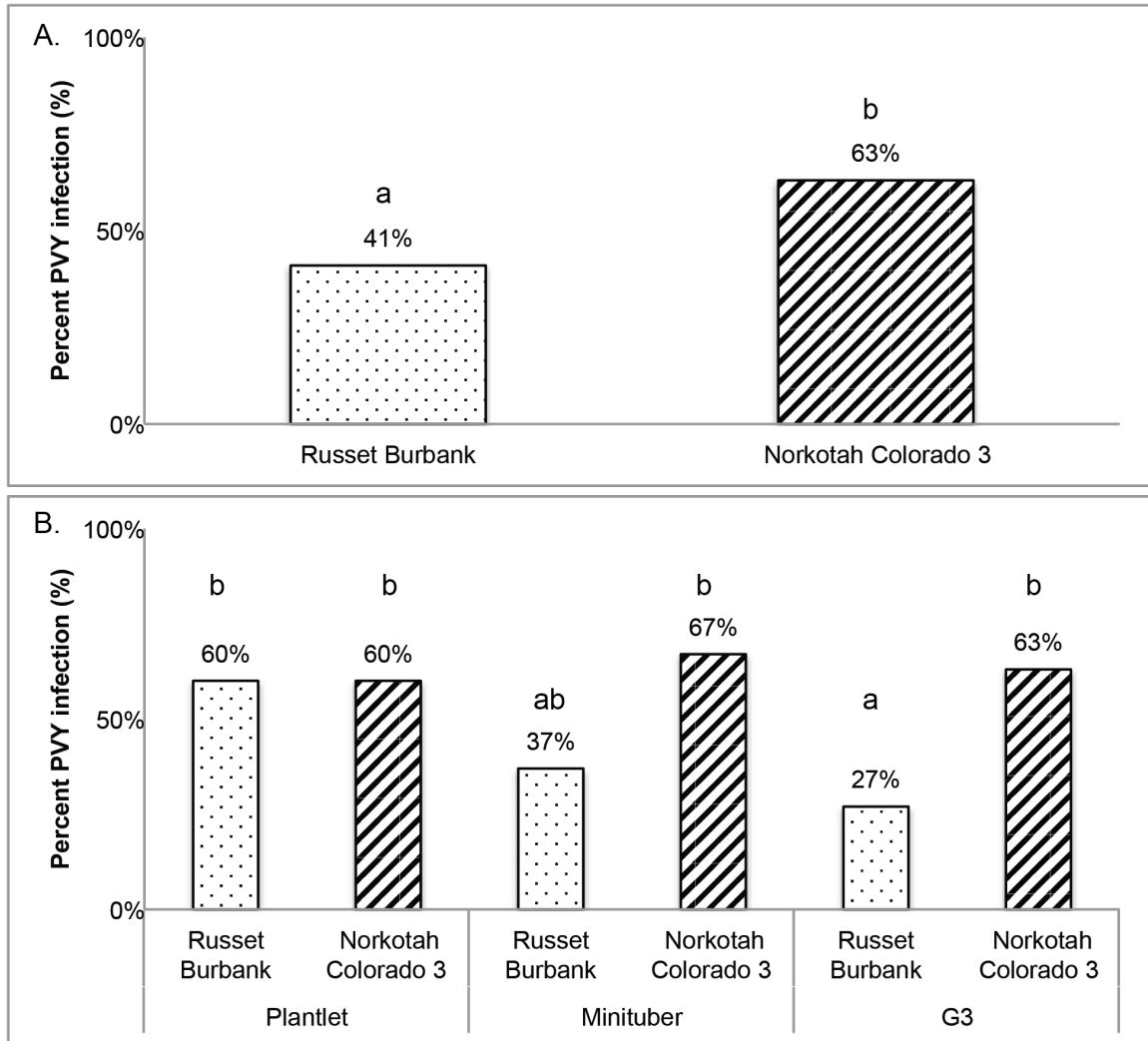


Figure 2.1. Potato Cultivars Russet Burbank and Norkotah Colorado 3 Differ in Incidence of PVY^{N-Wi} Infection. Average percent PVY^{N-Wi} infection over three experiments of Russet Burbank and Norkotah Colorado 3 potato cultivars grown from plantlets, minitubers, and G3 tubers inoculated with PVY^{N-Wi} measured by DAS-ELISA 4-weeks post inoculation. (A.) All seed types averaged for both Russet Burbank and Norkotah Colorado 3 cultivars. (B.) Average percent infection in Russet Burbank and Colorado 3 plants grown from three seed types. Treatments with statistically different means are indicated by different letters (P-value < 0.05).

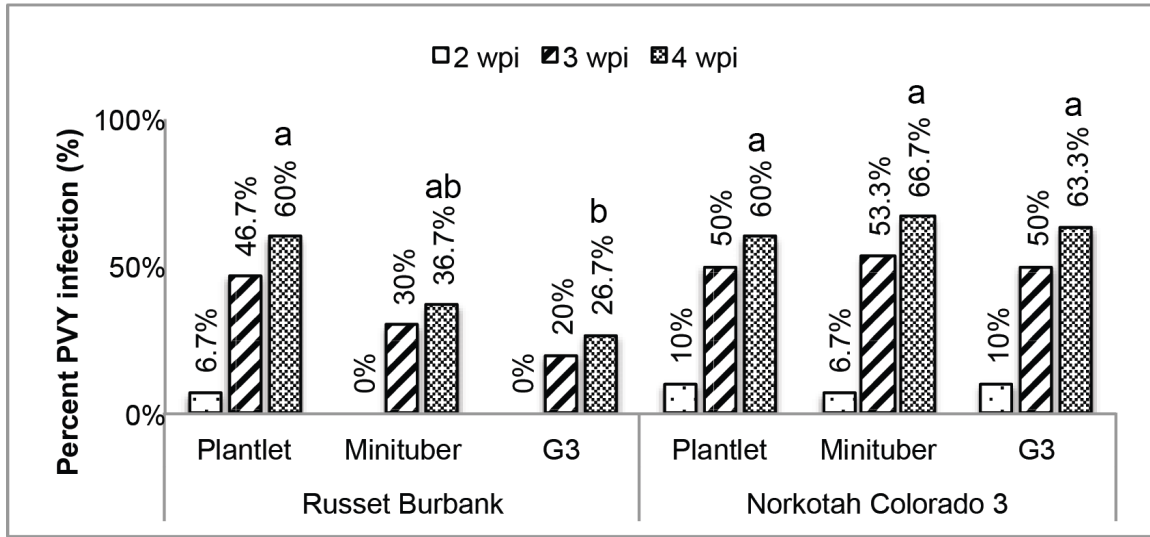
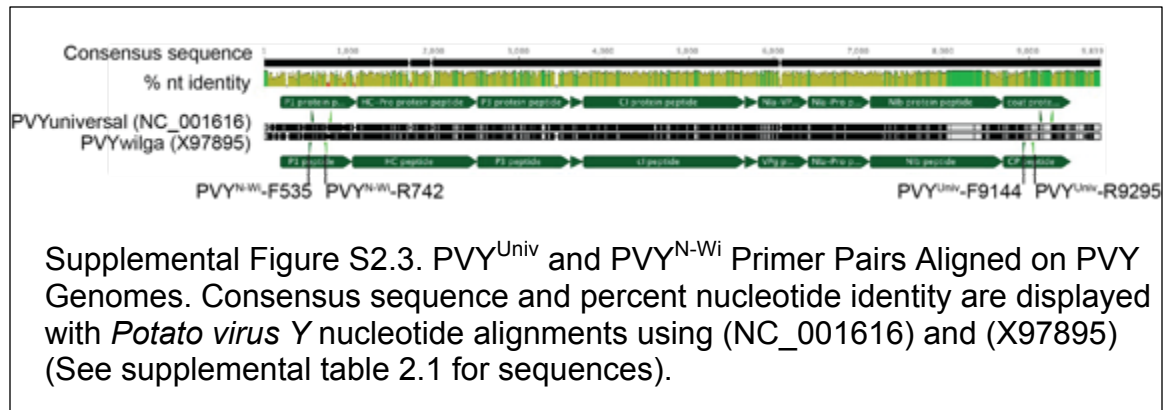
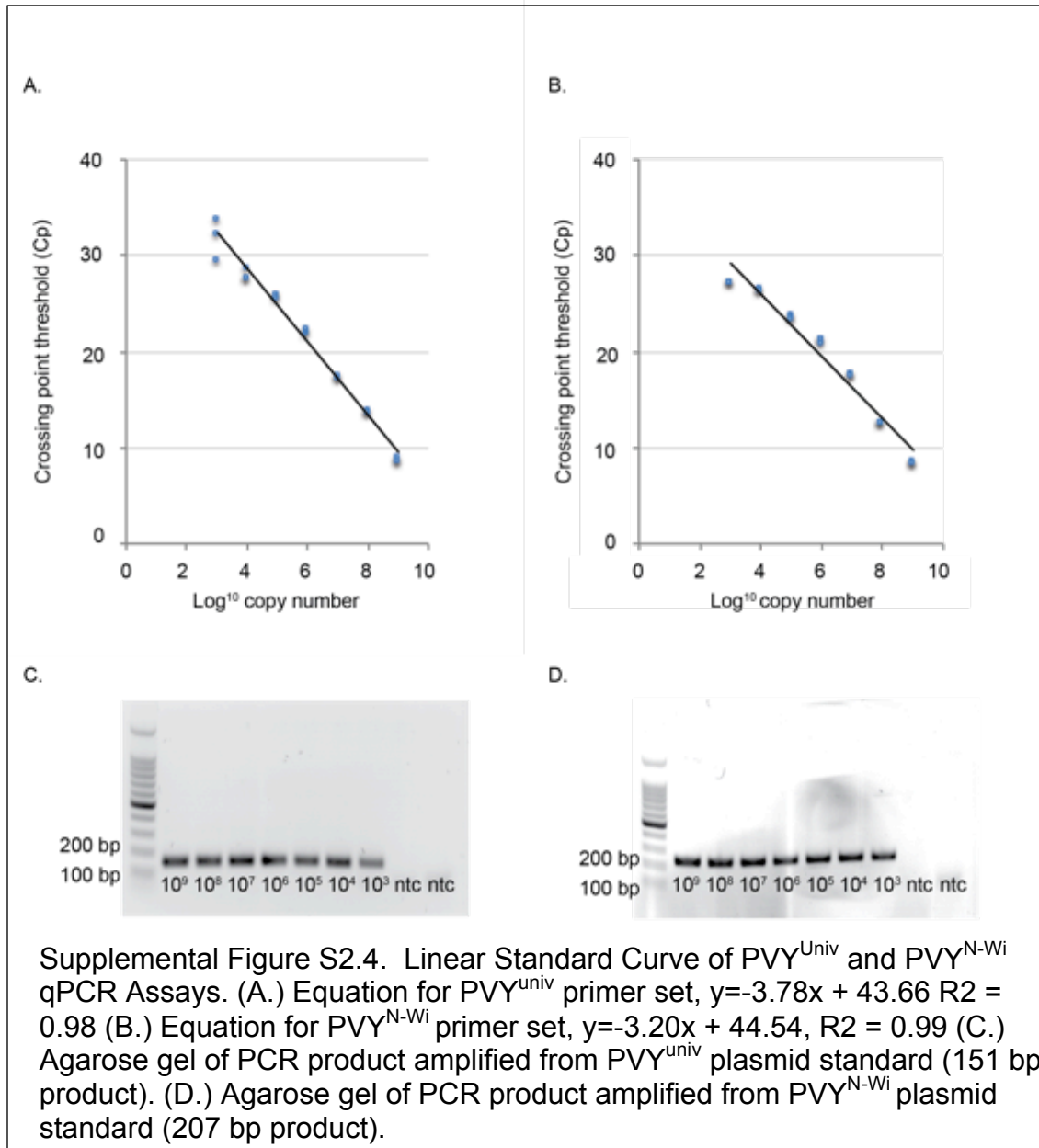


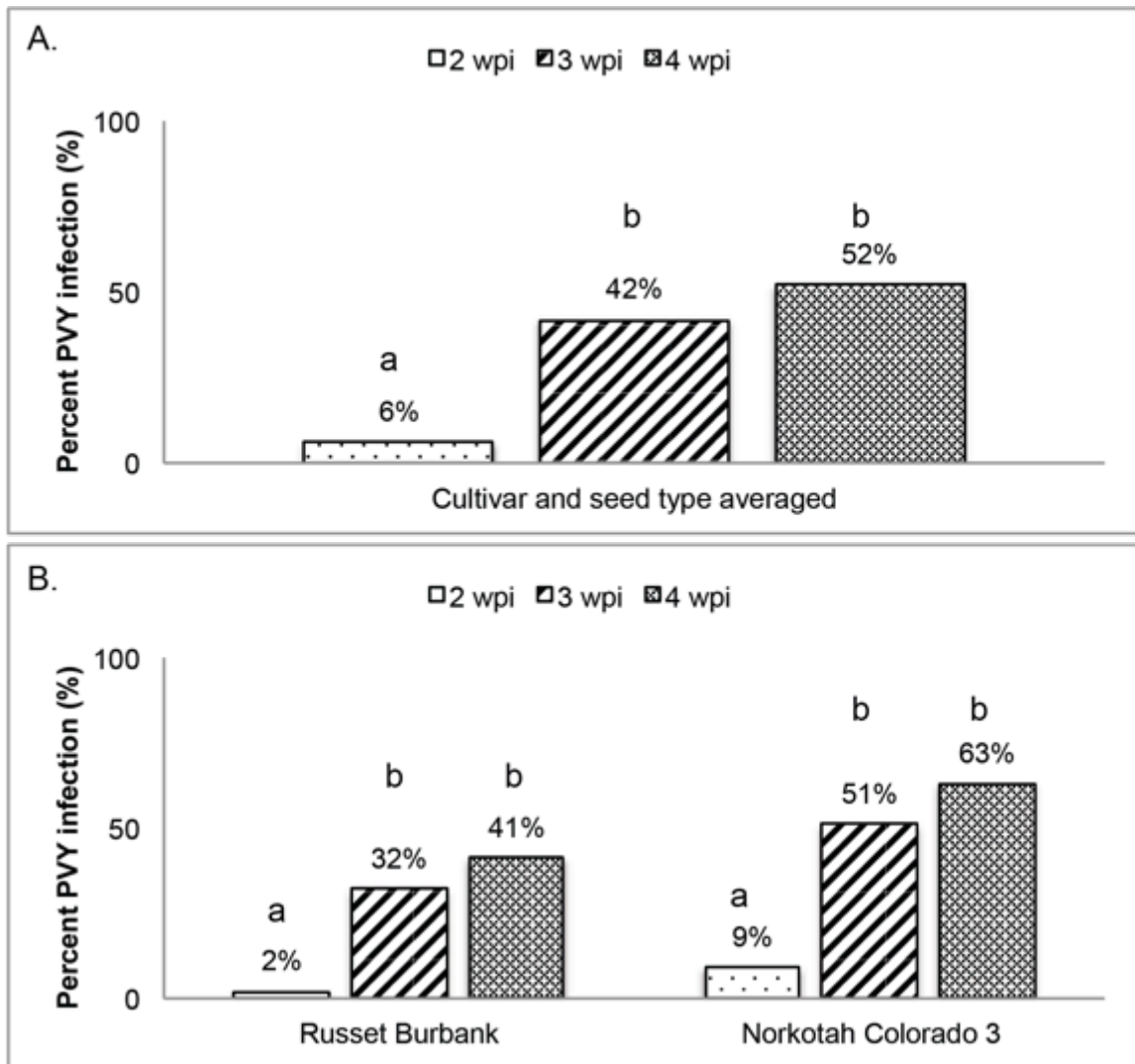
Figure 2.2. Potato Seed Type Affects Incidence of PVY^{N-Wi} infection in Russet Burbank. Percent PVY^{N-Wi} infection (y-axis) was measured at 2, 3- and 4- weeks post inoculation (wpi) in Russet Burbank and Norkotah Colorado 3 potato cultivars grown from plantlets, minitubers, and G3 tubers (x-axis). Percent 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).

Supplemental Table 2.1. Primer Sequences used for qPCR.

Genome / Gene Name	NCBI # GI #	Primer Name	Sequence (5'-3')	Product Size (bp)	Reference
<i>Potato virus Y</i> , genes encoding viral polyprotein	X97895.1 GI:1430929	PVY ^{N-Wi} -F535	TCATCCACACAA CTCCAAGG	207	Boyd et al 2016 in preparation / submitted
		PVY ^{N-Wi} -R742	GTCCACTCTCTTT CGTAAACCTC		Chikh Ali (2009) Journal of Virological Methods
<i>Potato virus Y</i> , complete genome	NC_001616.1 GI:9627728	PVY ^{Univ} -F9144	CCAATCGTTGAG AATGCAAAA	151	Fageria (2013) Plant Disease Crosslin unpublished
		PVY ^{Univ} -R9295	CGCGCTAAACCC ATATCCCGCAGA		







Supplemental Figure S2.5. Detection of Percent PVY^{N-Wi} Infection of Potatoes Increases Over Time. Average percent PVY^{N-Wi} infection measured by DAS-ELISA 2, 3- and 4-weeks post inoculation (wpi) from three experiments with Russet Burbank and Norkotah Colorado 3 potato cultivars grown from plantlets, minitubers, and G3 tubers inoculated with PVY^{N-Wi}. (A.) Average percent infection of all plants by post inoculation time point. (B.) Average percent infection of all plants within each cultivar by post inoculation time point. Treatments with statistically different means are indicated by different letters (P-value <0.05).

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CHAPTER 3

EXAMINATION OF RESISTANCE-INDUCING COMPOUNDS TO LIMIT
POTATO VIRUS Y (PVY^{N-WI}) INFECTION IN POTATOES

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Abstract

Potato virus Y (PVY) is one of the greatest disease challenges to seed potato (*Solanum tuberosum* subsp. *tuberosum*) production in the United States. All seed potatoes originate from plantlets produced in sterile tissue culture, which have a higher incidence of PVY infection than plants grown from field produced seed. Plant systemic acquired resistance (SAR) can be induced in the absence of pathogens by resistance-inducing agents. To determine if SAR-inducing agents could lower the risk of infection by PVY, Russet Burbank and Norkotah Colorado 3 plants were grown from three seed types in the presence or absence of SAR-inducing compounds. Plants were inoculated with PVY^{N-Wi} (Wilga) and the percentage of plants infected (incidence) with PVY^{N-Wi} was measured at 2, 3, and 4 weeks post-inoculation. Preliminary data suggests that phosphorous acid treatment may limit PVY infection in Russet Burbank plantlets. To our knowledge

this is the first examination of the effect of phosphorous acid application on PVY infection.

Introduction

Potato (*Solanum tuberosum* subsp. *tuberosum*) production depends on the availability of pathogen-free seed. Production of *Potato virus Y* (PVY)-free seed relies on tissue culture propagation, extensive field inspections, and diagnostic testing for PVY, Potato virus X, Potato virus A and other pathogens (MSU Seed Potato Certification Program). All seed potato stock originates from pathogen-free tissue culture produced plantlets. Limiting the number of field-grown generations and continually obtaining pathogen-free seed stock reduces pathogen prevalence and diseases in the field (MSU Seed Potato Certification Program).

In the Montana Seed Certification Program, all seed material originates from Pre-Nuclear planting stock, which consists of plantlets, microtubers, and minitubers (greenhouse grown from plantlets). This material is transplanted into the field for the first (Nuclear) generation from which tubers produced are planted and certified as seed for four additional generations (G1-G4) (Rules and Regulations MSU Seed Potato Certification Program). Pre-Nuclear stock is not exposed to pathogens and in turn, has unchallenged immune systems. Cumulative data from potato field inspections and greenhouse studies demonstrate that cultivar Norkotah Colorado 3 has a higher incidence of PVY^{N-Wi}

infection than cultivar Russet Burbank. Additionally, Russet Burbank plants grown from plantlets have a higher incidence of PVY^{N-Wi} infection than Russet Burbank plants grown from G3 tubers after mechanical inoculation (Boyd et al. in preparation 2016).

PVY is a filamentous virus 11 nm in diameter x 740 nm in length, with a 9.7 kb positive sense, single-stranded RNA genome. Viral genome mutation and recombination results in a diversity of PVY strains which cause a range of foliar and tuber symptoms (MacKenzie et al. 2015). The majority of currently predominant PVY strains, including PVY^{N-Wi} (Wilga), are recombinant viruses that contain varying proportions of PVY^O (ordinary) and PVY^N (necrotic) (Chikh Ali et al. 2010, Karasev et al. 2013). In this study, strain PVY^{N-Wi} was used due to its prevalence in the US. Detection of PVY^{N-Wi} strains has increased in Montana since its initial detection in 2002 (Karasev 2016).

Plant innate immune systems respond to pathogens such as bacteria, fungi, nematodes, and viruses. Defense mechanisms include microbial associated molecular pattern (MAMP) triggered immunity (MTI), effector triggered immunity (ETI), RNA interference (RNAi), and systemic acquired resistance (SAR) (Muthamilarasan et al. 2013). Systemic acquired resistance (SAR) is triggered by local pathogen challenge and results in long-lasting resistance throughout the entire plant. Salicylic acid is produced at the infection site and travels throughout the plant where it induces defense genes (Jones et al.

2006). In the absence of pathogens, resistance-inducing agents can also trigger SAR in plants (Durrant et al. 2004).

Resistance-inducing agents tested in this study were compost tea (Kimm Seed Potatoes, Manhattant, MT), *Bacillus mycoides* isolate J (BmJ, Montana State University, Bozeman, MT), acibenzolar-S-methyl (Actigard ® Syngenta, Basel, Switzerland), and phosphorous acid (Phostrol ® Nufarm, Melbourne, Australia). Compost that has been fermented in continually aerated water is known as compost tea. Compost tea limited infection in *Colletotrichum orbiculare* on cucumber and *Pseudomonas syringae* on *Arabidopsis* by inducing SAR (Zhang et al. 1998). *Bacillus mycoides* isolate J (BmJ) induced SAR in sugarbeet which was then challenged (and limited infection) with *Cercospora beticola* the pathogen responsible for Cercospora leaf spot (Bargabus et al. 2002).

Acibenzolar-S-methyl (Actigard ®) is labeled as a fungicide and triggers SAR in many crops including tobacco (Friedrich et al. 1996) and cantaloupe (Smith-Becker et al. 2003, Walters et al. 2005). Phosphorous acid (H₂PO₃) products are used for both foliar and postharvest application and protect against some diseases caused by oomycetes (Johnson et al. 2004). It is efficacious against late blight (*Phytophthora infestans*) and pink rot (*Phytophthora erythroseptica*) when applied to foliage during the growing season or to tubers prior to storage (Gachango et al. 2012, Miller et al. 2015). Fungal growth is inhibited by high concentrations of phosphorous acid (Fenn et al. 1984), and at lower concentrations, it induces SAR (Jackson et al. 2000, Shanghyun 2012). The

efficacy of this resistance-inducing compound to limit PVY infection of potato has not been examined.

To examine the effect of resistance-inducing agents on PVY infection, plants from cultivars Russet Burbank and Norkotah Colorado 3 were grown from seed types; plantlets, minitubers, and G3 tubers in the presence or absence of resistance-inducing agents and mechanically inoculated with PVY^{N-W}. Percent PVY infection (incidence) was measured using PVY-specific double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) at 2, 3- and 4-weeks post inoculation. Although differences were statistically non-significant at four weeks post inoculation, phosphorous acid treated Russet Burbank plantlets had a lower percent infection than, non-induced control plants in each of four experiments conducted. Trends to date indicate further studies are warranted.

Methods

Experimental Design

Experiments 1 and 2 (Table 3.2) were set up in a randomized complete block design with ten experimental units and blocks for experiment 1 and 12 experimental units and blocks for experiment 2. Experiments 3 and 4 (Table 3.2) were set up in a two (cultivars) by three (seed types) factorial randomized complete block design. Ten experimental units (potato plants) made up each treatment. For all experiments, percent infection was measured 2, 3, and 4 weeks post inoculation (wpi).

Plant Production

Potato cultivars 'Russet Burbank' and 'Norkotah Colorado 3' were propagated in three different ways. Tissue culture plantlets were propagated using 4.44 g/L Murashige and Skoog medium with Gamborg's (PhytoTechnology Shawnee Mission, KS). Minitubers were grown in the greenhouse in Sunshine Mix 1 (Sungro Horticulture, Vancouver, Canada), and G3 tubers were field grown and certified by Montana Seed Potato Certification. Tubers were treated with Rindite (7 parts ethylene chlorohydrin, 3 parts ethylene dichloride, and 1 part carbon tetrachloride) to break dormancy (Bryan, J.E. 1989). Plantlets, minitubers and G3 tubers were planted into 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 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.

Treatment Application

All resistance-inducing compounds were applied to foliage until runoff. An aerosol sprayer (Crown Spra-Tool, Philadelphia, PA) was used for application of treatments for inducer panel experiments, and an Ace Home and Garden pump up sprayer model 7437668 (Ace Hardware, Oak Brook, IL) was used for

phosphorous acid application in generational/phosphorous acid experiments. Rates for each compound were: undiluted compost tea made from forest humus, hay silage, and cow manure (Kimm Seed Potatoes, Manhattan, MT), 1 g/L *Bacillus mycoides* Isolate J (BmJ) (Jacobsen Lab MSU, Bozeman, MT), 4 ml/L phosphorous acid (Phostrol® Nufarm, Melbourne, Australia), and 0.1 g/L acibenzolar-S-methyl (Actigard® Syngenta, Basel, Switzerland). Products were applied five days prior to PVY^{N-Wi} inoculation to allow resistance response development.

PVY Inoculum Preparation and Inoculation of Potato Plants with PVY

Tobacco plants (*Nicotiana tabacum* 'Samsun'), used to maintain PVY^{N-Wi} virus stocks, were cultivated from seed with a 24 h photoperiod at 22°C in 10" pots. Strain PVY^{N-Wi} was isolated in Montana and strain verified (Chikh Ali 2009). PVY-infected tobacco tissue (collected 4-5 wpi) was homogenized in 0.05 M sodium sulfite (Na₂SO₃) buffer, pH 9.7 (1:10 g tissue: mL buffer) followed by two rounds of centrifugation (4,600 x g at 4° C for 10 min) to remove leaf debris (Rupar, M. 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 to estimate relative PVY RNA equivalences (methods

below). Mechanical inoculation of plants was performed by abrading a 2 cm² 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 suspension onto the leaf and spreading it across the abraded surface with a pipet tip. The estimated viral RNA equivalences delivered per inoculation for each experimental replicate (rep) were as follows: rep 1: 5.1x10⁵, rep 2: 4.7x10⁷, and rep 3: 2.0x10⁷.

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 were performed using 2 µg of total RNA in a 25 µl incubated with Maloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI), and random hexamer primers (5 µg) (IDT, Coralville, IA) for 1 hour at 37° C, according to the manufacturer's instructions.

Quantitative PCR (qPCR)

In order to compare and standardize PVY inoculum across experimental replicates, we utilized qPCR to estimate the viral RNA equivalences, which includes both genomic RNA and transcriptional products, based on the relative abundance to a standard curve. For strain specific detection, a primer set that targets the variable P1 region specific to PVY^{N-Wi} (Chikh Ali, M. et al. 2010); PVY^{N-Wi}-F535 5' TCA TCC ACA CAA CTC CAA GG (Supplemental Table S2.1)

and PVY^{N-Wi}-R742 5' GTC CAC TCT CTT TCG TAA ACC TC (Chikh Ali, M. et al. 2010)(Supplemental Table 2.1).

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 MgCl₂. To estimate the relative PVY abundance based on a standard curve, the corresponding segment of PVY was cloned into plasmids. Plasmid standards containing from 10³ to 10⁹ copies were used as templates for qPCR to generate the standard curve. The detection limit was 10³ copies for the PVY^{N-Wi} primer set. The linear standard equations generated by plotting the crossing point (Cp) versus the log₁₀ of the initial plasmid copy number was as follows: PVY^{N-Wi} Cp = -3.20x + 44.54, R₂ = 0.99. Wells containing no template were run as negative controls. Melt point analysis and gel electrophoresis were used to verify qPCR specificity (Ginzinger 2002)

Potato Leaf Tissue Collection and DAS-ELISA

PVY infection was measured using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) at two, three and four weeks post-inoculation. The compound leaf two nodes above the inoculated leaf was collected two weeks post inoculation. At three and four weeks post inoculation, the compound leaf three and four nodes above the inoculation site respectively

were collected and tested. Leaf tissue samples were weighed, placed in 12 cm by 12 cm universal extraction bags (Bioreba, Reinach, Switzerland) and homogenized in a 1:10 dilution of Blotto (5.84 M NaCl, 0.003 M KH_2PO_4 , 0.002M Na_2HPO_4 , 0.14 M KCl, 0.05% Tween-20) using a tissue homogenizer (Bioreba, Reinach, Switzerland) mounted on a drill press (Craftsman, Hoffman Estates, IL). DAS-ELISA (Clark et al. 1976) was performed with 200 μL of plant filtrate per reaction using PVY-specific IgG polyclonal antibody developed by MSU's Seed Potato Certification laboratory and validated by the laboratory of Dr. Stewart Grey, Cornell University. Optical densities were measured at 405 nm on an Epoch Microplate Spectrophotometer (Biotek, Winooski, VT). Negative controls of uninfected potato tissue and positive controls of infected tobacco tissue were included on each plate.

Statistical Analysis

Percent infection data was analyzed using the Restricted Maximum Likelihood Method (REML). Variance-covariance was tested, and compound symmetry (CS) was determined to be the most appropriate using Akaike Information Criterion (AIC), Bayesian Criterion (BIC), and Hurvich and Tsui (AICC). Once determined, CS was used in a repeated measures analysis using Tukey's adjustment to make inferences using a mixed model in SAS 9.4.

Results

To examine the effect of systemic acquired resistance (SAR) inducing agents on PVY infection, mechanical inoculation studies were conducted with plants treated with inducing agents prior to inoculation. Experiments 1 and 2 (Table 3.2) were conducted with plantlets grown from cultivars Russet Burbank and Norkotah Colorado 3. Plants were treated with the SAR-inducing agents compost tea, BmJ, phosphorous acid, and acibenzolar-S-methyl prior to inoculation with PVY^{N-Wi}. At 2, 3, and 4 weeks post inoculation, infection was evaluated by DAS-ELISA. Experiments 3 and 4 (Table 3.2) were conducted with Russet Burbank and Norkotah Colorado 3 plants grown from plantlets, minitubers and G3 tubers, non-induced or treated with phosphorous acid, and inoculated with PVY^{N-Wi}.

All results were statistically non-significant, however interesting trends were observed. Russet Burbank plantlets treated with phosphorous acid had lower percent infections at four weeks post inoculation compared with the non-induced control in experiments 1, 2, 3, and 4. Specifically, Russet Burbank plantlets at four weeks post inoculation, treated with phosphorous acid resulted in 66.7% lower infection in experiment 1 (Figure 3.1), a 77.3% lower infection in experiment 2 (Figure 3.2), a 30% lower infection in experiment 3 (Figure 3.3), and a 75% lower infection in experiment 4 compared to the non-induced controls (Figure 3.4). The cultivar Norkotah Colorado 3 has a higher incidence of infection than Russet Burbank (Boyd, E. et al. in preparation 2016). This trend was observed in experiments 1 and 2. In experiment 1, Russet Burbank non-

induced controls had 30% infection and the Norkotah Colorado 3 non-induced controls had 60% infection at four weeks post inoculation (Figures 3.1). In experiment 2, the Russet Burbank non-induced controls had 75% infection and the Norkotah Colorado 3 non-induced controls had 100% infection (Figure 3.2).

Together, the preliminary studies performed to date suggest that treatment of Russet Burbank plantlets with phosphorous acid reduced PVY infections, however, due to an insufficient number of replications the results are statistically non-significant and thus additional experiments are required to validate these initial findings. We expect that additional replications of these experiments will result in significant findings.

Discussion

Identifying a resistance-inducing agent to limit PVY infection could aid in protecting the most susceptible seed generation. We tested the ability of a panel of systemic acquired resistance-inducing agents to limit percent PVY infection. Russet Burbank and Norkotah Colorado 3 plants were grown from plantlets, minitubers, and G3 tubers, in the presence or absence of inducing agents, and inoculated with PVY^{N-Wi}.

Though treatment differences were statistically non-significant at α 0.05, the trend at four weeks post inoculation that Russet Burbank plantlets treated with phosphorous acid had a lower percent infection compared with the non-induced control (between 30% and 77.3% lower) indicates that further

experiments are warranted. Plants grown from Russet Burbank plantlets have a higher incidence of PVY^{N-Wi} infection than plants grown from Russet Burbank G3 tubers (Boyd et al. in preparation 2016). Since Russet Burbank plantlets have a higher incidence of PVY infection than their field grown counterparts, phosphorous acid treatment could be a beneficial management tool. Percent PVY infection in phosphorous acid treated Norkotah Colorado 3 plantlets was only lower in experiment 1. Norkotah Colorado 3 plants have a higher incidence of PVY^{N-Wi} infection than Russet Burbank plants, and cultivar differences in incidence may play a role in differential responses to phosphorous acid application (Boyd et al. in preparation 2016).

The PVY inoculation for Experiment 5 (1.6×10^7 viral RNA equivalences) resulted in final percent infections at, or near 100% and negated our ability to compare treatment groups. Inoculum concentration was comparable to experiments 1 and 2 (1.4×10^7 and 1.7×10^7 viral RNA equivalences). High percent infections across all treatments in experiment 5 may have resulted from greenhouse conditions at the time of inoculation or a higher percentage of infectious PVY particles in the virus prep used.

Phosphorous acid may activate SAR in plantlets, limiting PVY infection. The PR-1 (NCBI Accession number XM_006343477) gene could be examined with qPCR to confirm induction of SAR. To our knowledge there are currently no primers targeting PR-1 in potato but could be designed using Primer 3 Plus (Rozen et al. 2000). Phosphorous acid is currently used to manage diseases

such as late blight (*Phytophthora infestans*), and pink rot (*Phytophthora erythroseptica*) (Miller et al. 2015, Miller et al. 2011). If phosphorous acid did limit PVY infection, it would be a useful tool in integrated pest management programs. Studies should be replicated to determine if there are statistical differences between treatments. Including additional cultivars in future studies would add valuable information about differential responses to application of this product. If phosphorous acid limits PVY infection in potatoes of many cultivars, it would be a valuable management tool in the seed potato industry.

Acknowledgments

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Author Contributions

Conceived and designed experiments: N.Z., M.F., and E.B. Primer design and inoculum standardization: M.F., N.Z., and E.B. Greenhouse experiments: N.Z., E.B., and E.C. Analyzed the data: J.R., N.Z., E.B. Wrote the paper: E.B., N.Z., and M.F.

Conflicts of Interest

The authors declare no conflict of interest.

Figures

Table 3.1. Primer Sequences used for qPCR.

Genome / Gene Names	NCBI # GI #	Primer Name	Sequence (5'-3')	Product Size (bp)	Reference
<i>Potato virus Y</i> , genes encoding viral polyprotein	X97895.1 GI:1430929	PVY ^{N-Wi} -F535	TCATCCACACAAC TCCAAGG	207	Boyd et al 2016 in preparation / submitted Chikh Ali (2009) Journal of Virological Methods
		PVY ^{N-Wi} -R742	GTCCACTCTCTTT CGTAAACCTC		

Table 3.2. Resistance-Inducing Agent Experiments. Experiments presented in Chapter 3 with their relative inoculum concentration, cultivars used, seed types used, treatments applied, and how statistical analysis was conducted.

experiment number	relative PVY RNA abundance per inoculation	cultivar	seed type	treatments	statistical analysis
1	1.4×10^7	Russet Burbank Norkotah Colorado 3	plantlet	mock inoculated compost tea BmJ phosphorous acid acibenzolar-S-methyl non-induced control	analyzed individually
2	1.7×10^7	Russet Burbank Norkotah Colorado 3	plantlet	mock inoculated compost tea BmJ phosphorous acid acibenzolar-S-methyl non-induced control	analyzed individually
3	4.7×10^7	Russet Burbank Norkotah Colorado 3	plantlet minituber G3 tuber	non-induced control phosphorous acid	analyzed with experiment 4
4	2.0×10^7	Russet Burbank Norkotah Colorado 3	plantlet minituber G3 tuber	non-induced control phosphorous acid	analyzed with experiment 3
5	1.6×10^7	Russet Burbank	plantlet	non-induced control phosphorous acid	not statistically analyzed

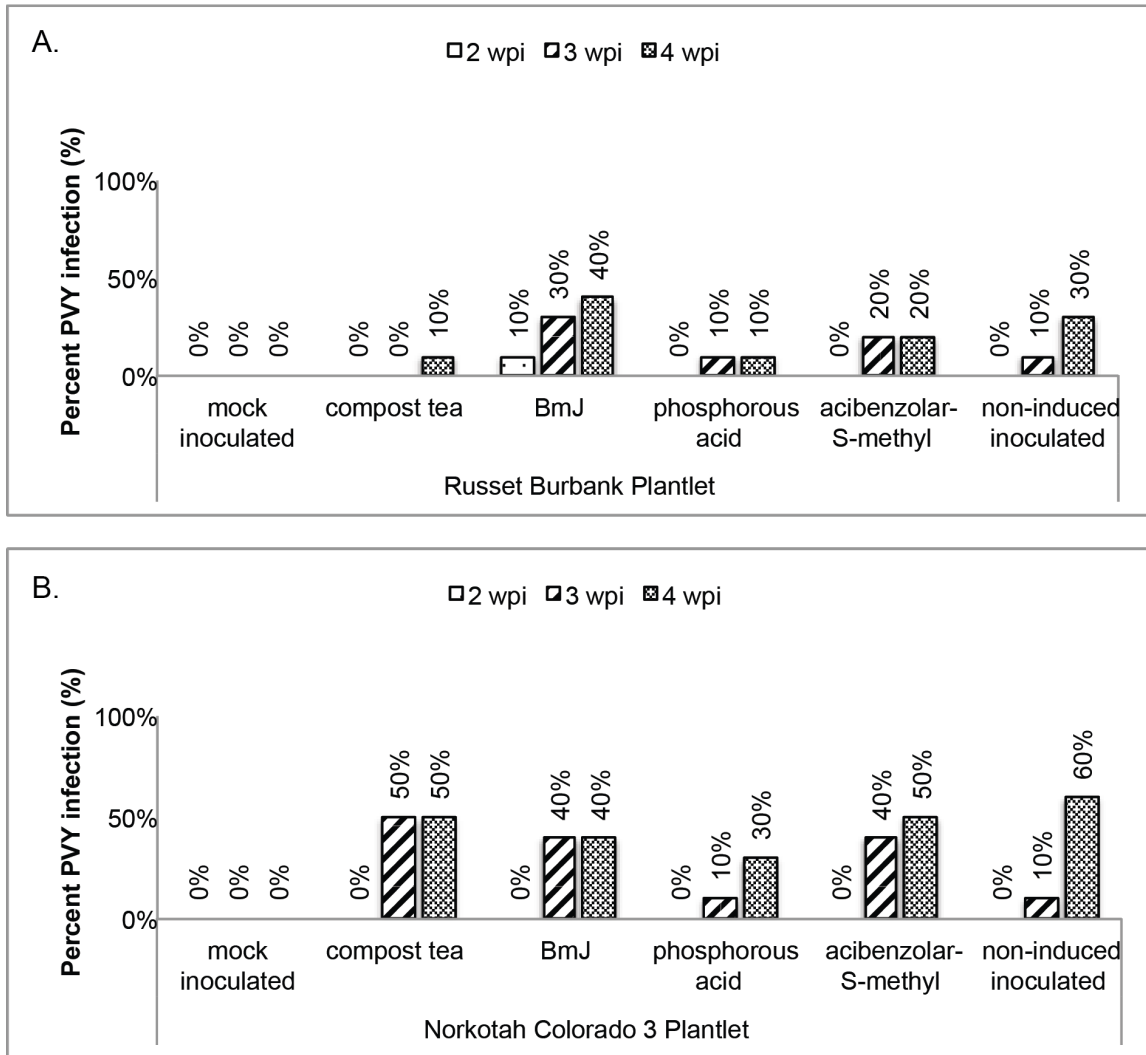


Figure 3.1. Experiment 1: Effect of Resistance-Inducing Agents on Percent PVY^{N-Wi} Infection of Potato. Russet Burbank and Norkotah Colorado 3 potato cultivars grown from plantlets, treated with inducers, and inoculated with 1.4×10^7 PVY^{N-Wi} genome copies. All mock inoculated controls remained negative. Percent PVY^{N-Wi} infection measured 2, 3- and 4-weeks post inoculation (wpi) in plants grown from (A.) Russet Burbank plantlets and (B.) Norkotah Colorado 3 plantlets.

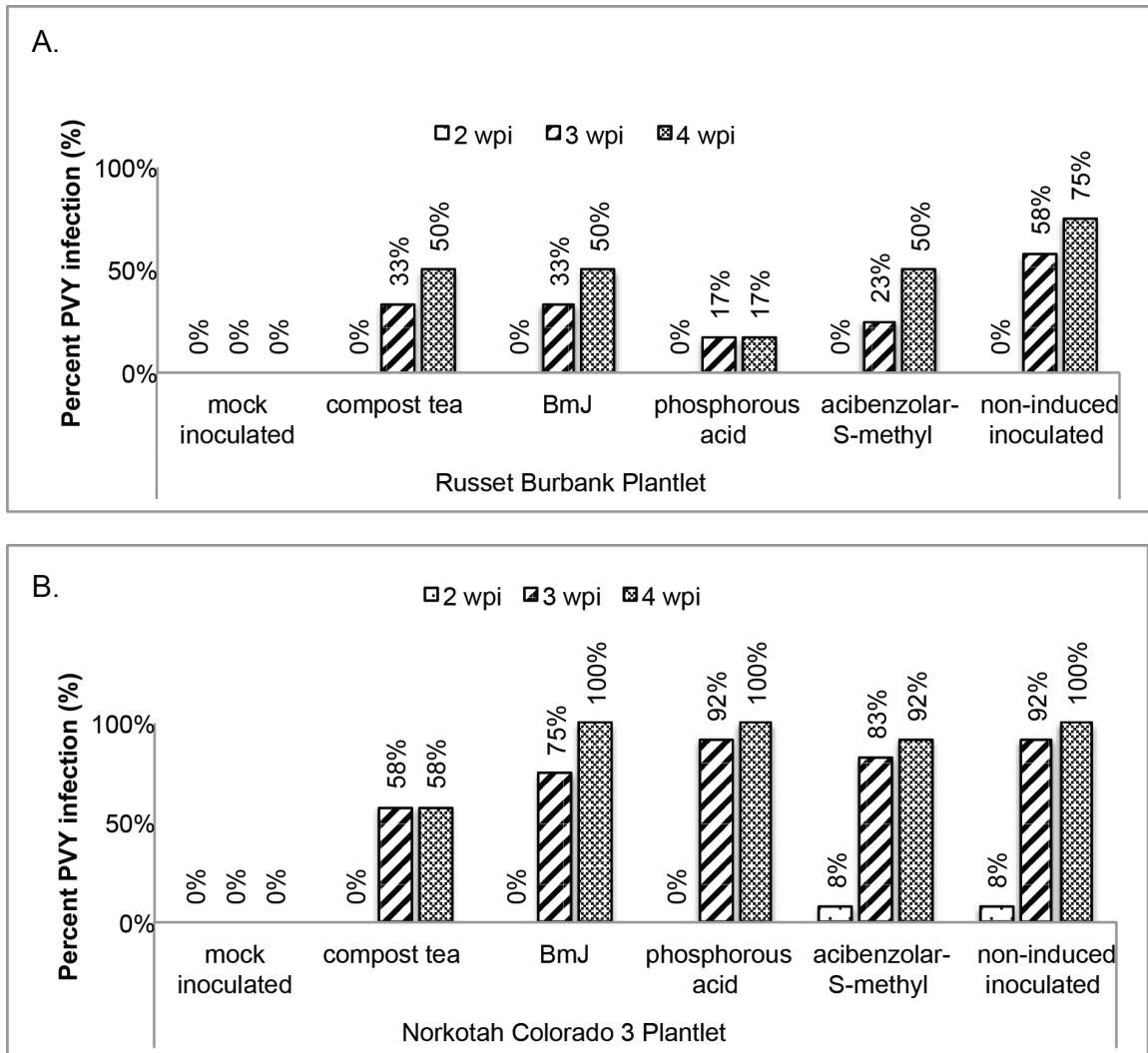


Figure 3.2. Experiment 2: Effect of Resistance-Inducing Agents on Percent PVY^{N-Wi} Infection of Potato. Russet Burbank and Norkotah Colorado 3 potato cultivars grown from plantlets, treated with inducers, and inoculated with 1.7×10^7 PVY^{N-Wi} genome copies. All mock inoculated controls remained negative. Percent PVY^{N-Wi} infection measured 2, 3- and 4-weeks post inoculation (wpi) in plants grown from (A.) Russet Burbank plantlets and (B.) Norkotah Colorado 3 plantlets.

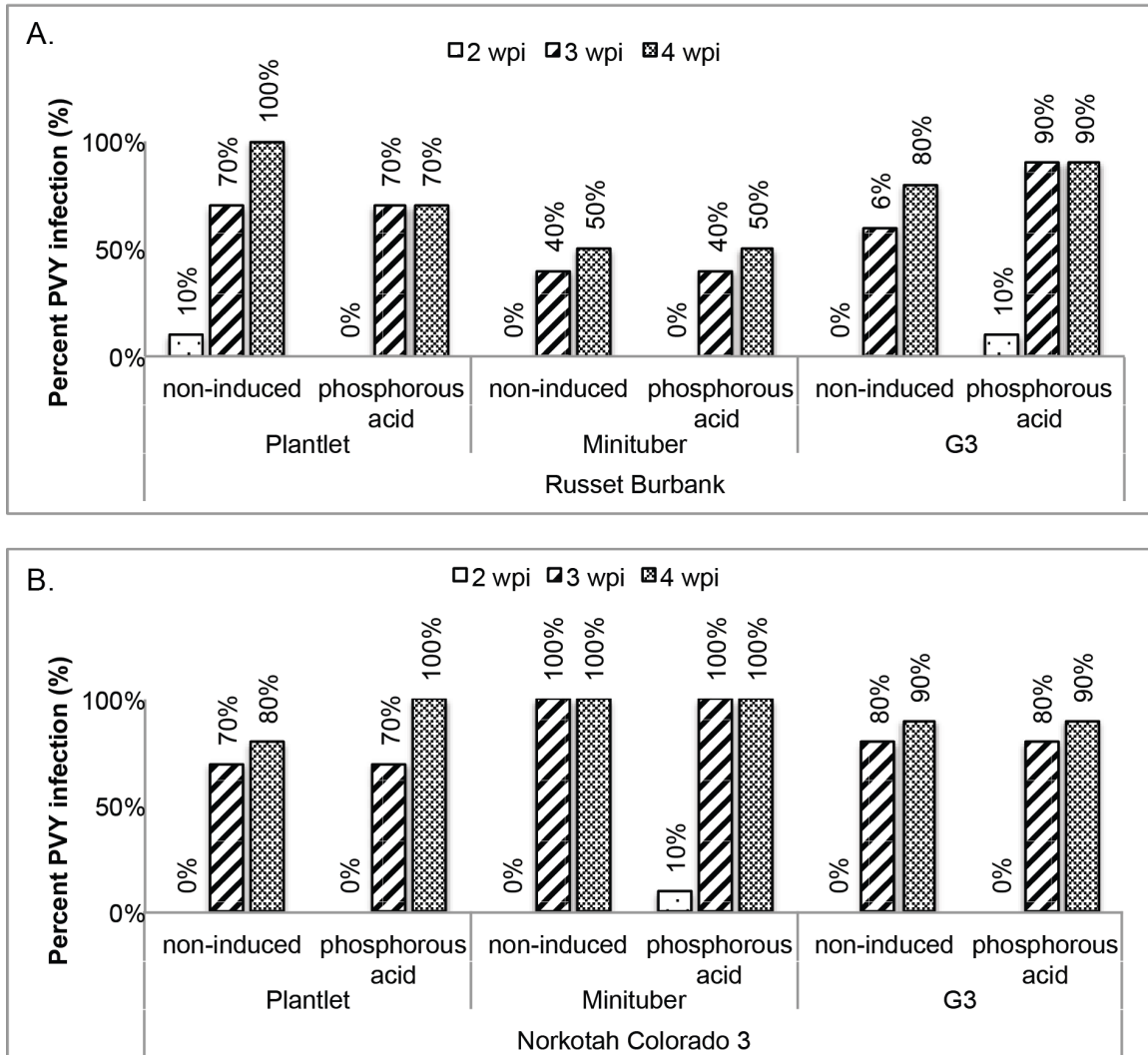


Figure 3.3. Experiment 3: Effect of Phosphorous Acid Application on Two Potato Cultivars and Three Seed Types Inoculated with PVY^{N-Wi}. First experimental replicate of potato plants treated with phosphorous acid or non-induced and inoculated with 4.7×10^7 PVY^{N-Wi} genome copies. All mock inoculated controls remained negative. Percent PVY^{N-Wi} infection measured 2, 3- and 4-weeks post inoculation (wpi) in plants grown from (A) Russet Burbank plantlets, minitubers and G3 tubers and (B) Norkotah Colorado 3 plants grown from plantlets, minitubers, and G3 tubers.

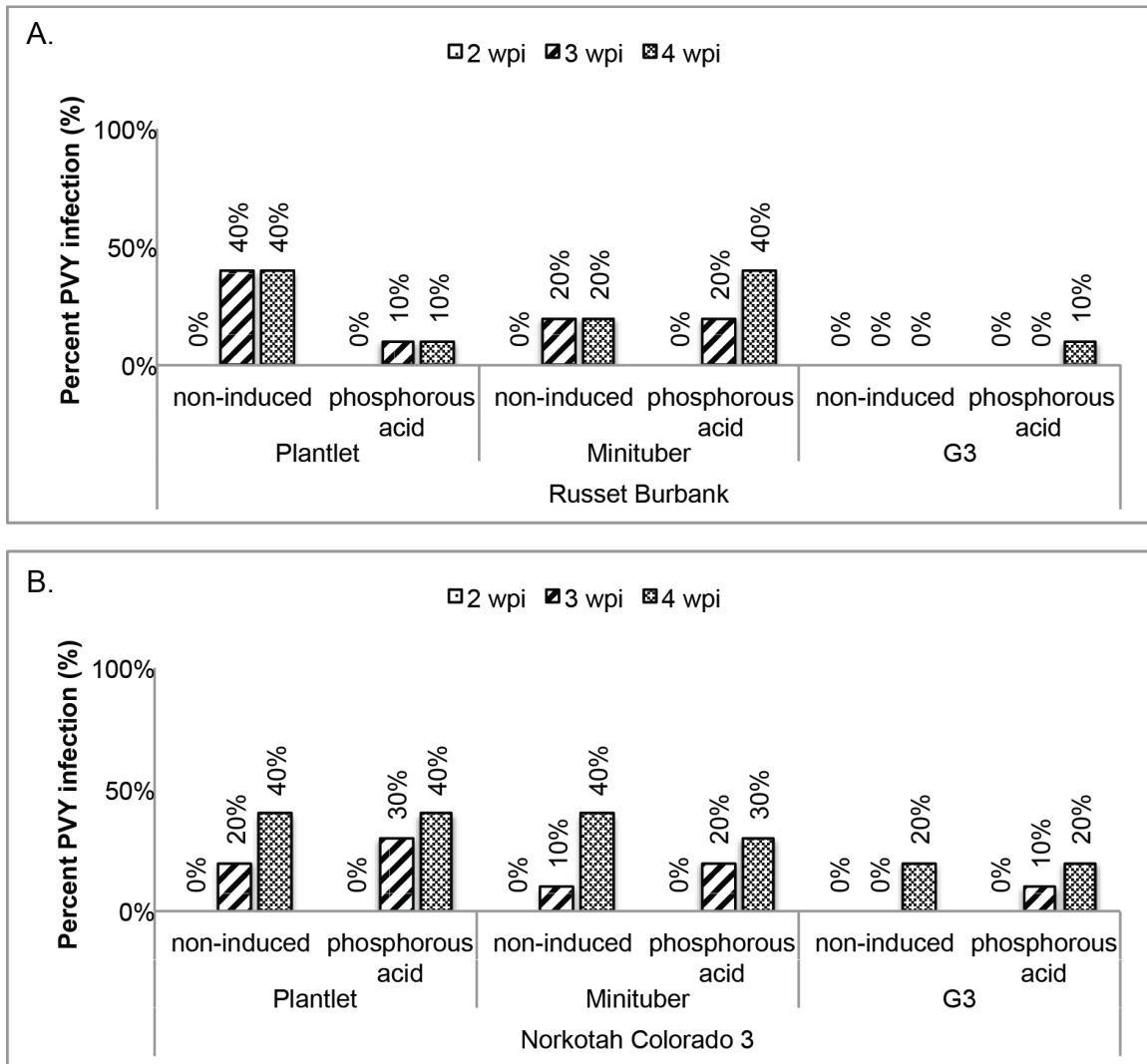


Figure 3.4. Experiment 4: Effect of Phosphorous Acid Application on Two Potato Cultivars and Three Seed Types Inoculated with PVY^{N-Wi}. Second experimental replicate of potato plants treated with phosphorous acid or non-induced and inoculated with 2.0×10^7 PVY^{N-Wi} genome copies. All mock inoculated controls remained negative. Percent PVY^{N-Wi} infection measured 2, 3- and 4-weeks post inoculation (wpi) in plants grown from (A.) Russet Burbank plantlets, minitubers and G3 tubers and (B.) Norkotah Colorado 3 plants grown from plantlets, minitubers, and G3 tubers.

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CHAPTER 4

CONCLUSION

Information derived from potato cultivar and seed type studies validate cumulative field inspection data showing cultivar and generational differences in incidence of *Potato virus Y* (PVY), and have implications for PVY management in seed potato production. Improved inoculum preparation and qPCR quantification were used to conduct greenhouse mechanical inoculation studies where PVY incidence in two potato cultivars and three seed types was compared. These studies determined that the cultivar Russet Burbank had a significantly lower incidence of PVY^{N-Wi} infection than the cultivar Norkotah Colorado 3. Additionally, consistent with field inspection data, plants grown from Russet Burbank plantlets had a higher incidence of PVY^{N-Wi} infection than plants grown from Russet Burbank G3 tubers.

To compare differences in PVY incidence in plants non-induced or induced with systemic acquired resistance (SAR) inducing agents, the same cultivars and seed types were treated and challenged with PVY. Trends for SAR-inducing experiments indicate further research is warranted. While results were statistically non-significant, in experiments 1-4 the percentage of PVY infection in Russet Burbank plantlets treated with phosphorous acid was lower compared to controls. In contrast, this trend was not observed in Norkotah Colorado 3

plantlets, nor were differences in PVY incidence observed for phosphorous acid treated plants grown from minitubers or G3 tubers from either cultivar.

Greenhouse studies and data from field studies highlight the importance of limiting PVY infection in the earliest generations of seed. If further studies indicate phosphorous acid is an efficacious chemical application to limit PVY infection, it would be a useful management tool. Differential incidence to PVY across cultivars and seed types indicates that viral immune responses may vary. Transcriptome level analysis could be performed using high throughput sequencing (RNAseq) to examine differentially expressed genes. Further testing would allow us to hypothesize about the mechanisms potentially involved in differential antiviral responses in potato challenged with PVY.

APPENDICES

APPENDIX A

TABLE OF EXPERIMENTS REPRESENTED IN THIS THESIS

Appendix A Table 1. Each experiment represented in this thesis described by a unique identifying number (used for the rest of appendices) and corresponding information. Experiment number is the chapter number.experiment number (2.1 is equivalent to chapter 2, experiment 1). Date represents the date inoculated.

Unique ID #	Experiment Number	relative PVY RNA abundance	cultivar	seed type	treatments	name in lab notebook, notebook number, page number of inoculation	Date
1	2.1	5.1×10^5	Russet Burbank Norkotah Colorado 3 Ranger Russet Alturas Western	Plantlet Minituber G3 tuber	mock inoc. virus inoculated	Mechanical Inoculation Experiment 1 Notebook 1 page 48	Jan. 16, 2015
2	2.2	4.7×10^7	Russet Burbank Norkotah Colorado 3	Plantlet Minituber G3 tuber	non-induced control phosphorous acid	Mechanical Inoculation Experiment 5 Notebook 1 page 143	Dec. 17, 2015
3	2.3	2.0×10^7	Russet Burbank Norkotah Colorado 3	Plantlet Minituber G3 tuber	non-induced control phosphorous acid	Mechanical Inoculation Experiment 6 Notebook 2 page 11	Mar. 4, 2016

Appendix A Table 1 Continued

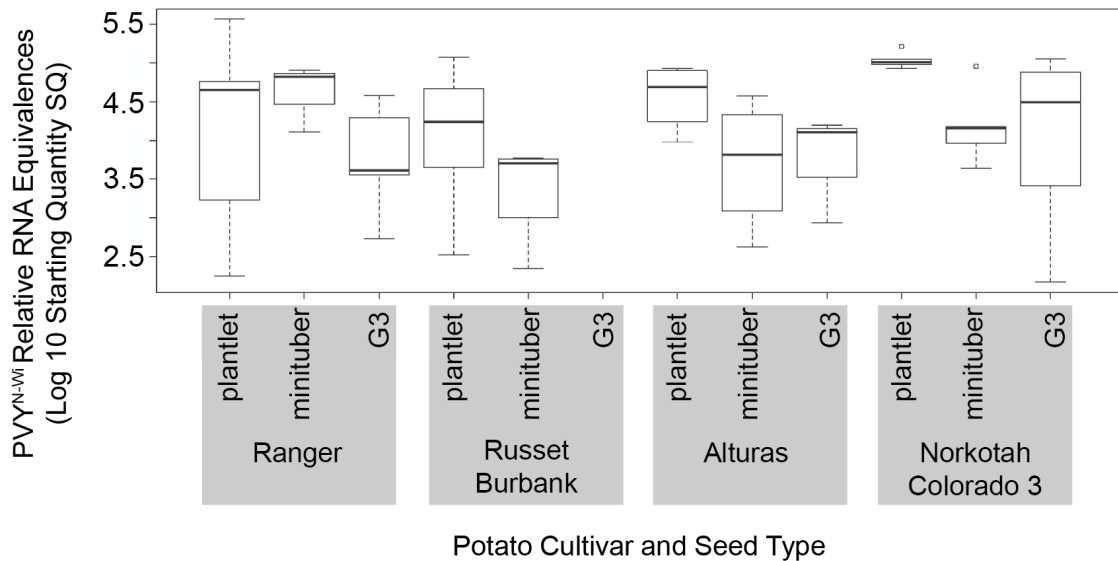
4	3.1	1.4×10^7	Russet Burbank Norkotah Colorado 3	plantlet	mock inoc. compost tea BmJ phosphorous acid acibenzolar-S-methyl non-induced control	Biocontrol 1 Notebook 1 page 117	Sept. 2, 2015
5	3.2	1.7×10^7	Russet Burbank Norkotah Colorado 3	plantlet	mock inoc. compost tea BmJ phosphorous acid acibenzolar-S-methyl non-induced control	Biocontrol 3 Notebook 2 page 6	Feb. 3, 2016
2	3.3	4.7×10^7	Russet Burbank Norkotah Colorado 3	Plantlet Minituber G3 tuber	non-induced control phosphorous acid	Mechanical Inoculation Experiment 5 Notebook 1 page 143	Dec. 17, 2015
3	3.4	2.0×10^7	Russet Burbank Norkotah Colorado 3	Plantlet Minituber G3 tuber	non-induced control phosphorous acid	Mechanical Inoculation Experiment 6 Notebook 2 page 11	Mar. 4, 2016
6	3.5	1.6×10^7	Russet Burbank	plantlet	non-induced control phosphorous acid	Phostrol Experiment 1 Notebook 2 page 12	Mar. 14, 2016

APPENDIX B

TRANSCRIPTOME LEVEL ANALYSIS OF POTATO VIRUS Y (PVY)
INFECTION OF POTATO

Differential PVY^{N-Wi} incidence between potato cultivars and seed types indicate that viral immune responses may vary. Transcriptome level analysis can allow us to examine differentially expressed genes between potato cultivars and seed types to hypothesize about mechanisms involved in plant antiviral responses. We will perform high throughput sequencing (RNAseq) on a subset of select samples from Experiment 2.1 (unique ID# 1) (Appendix Table 1). As described in Chapters 2 and 3, two potato cultivars (Russet Burbank and Norkotah Colorado 3) were grown from three distinct seed types (i.e., plantlets, minitubers, and G3 tubers) and challenged with Potato virus Y^{N-Wi} (Wilga). Leaf tissue was collected 2, 3, and 4 weeks post inoculation (wpi) and virus infection status was measured using DAS-ELISA. As presented in Chapter 2, the potato cultivar Norkotah Colorado 3 had a higher incidence of PVY^{N-Wi} infection than the cultivar Russet Burbank. Additionally, plants grown from Russet Burbank plantlets had a higher incidence of PVY^{N-Wi} infection than plants grown from Russet Burbank G3 tubers. Relative quantification of PVY in samples at four weeks post inoculation is necessary. Samples were measured with DAS-ELISA and qPCR quantified at three weeks post inoculation (Appendix Figure 1). Quantification results were obtained from RNA which was TRIzol extracted from leaf tissue following the manufacturer's instructions. Typical RNA yields ranged between 20,000 and 40,000 ng total RNA extracted from 200 mg of leaf tissue. To extend upon these results presented in Chapter 2, future studies will include

qPCR quantification of virus abundance for samples to be sequenced and transcriptome level analysis of differential potato gene expression.



Appendix B Figure 1. PVY^{N-Wi} relative RNA equivalences measured with qPCR by potato cultivar and seed type for experiment 1, 3 weeks post inoculation.

The most representative experiment from which to obtain samples for transcriptome analysis is Experiment 2.1 (Unique ID# 1) (Appendix A Table 1). Experiment 2.1 was performed in February 2015, with the final tissue collection on 2/13/15 (lab notebook 1, pages 48-57).

Experiment 2.1, was the first replicate of a set of experiments aimed at determining the influence of potato cultivar and seed type on PVY incidence.

In Experiment 2.1, Russet Burbank and Norkotah Colorado 3 plants were grown from plantlets, minitubers, and G3 tubers and mechanically inoculated with PVY^{N-Wi} (1.4×10^7 relative PVY RNA equivalences, Appendix Table 1). Infection levels

ranged from 0% to 80% (Appendix C Table 1), representing mid-range levels of infection. Importantly, the infection percentages obtained in experiment 2.1 are in line with the results from the statistical analysis of three experimental replicates (experiments 2.1, 2.2, and 2.3 (Appendix A Table 1).

Transcriptome level analyses will examine differentially expressed genes between potato cultivars and seed types challenged with PVY. Questions of interest are presented with corresponding samples listed for each. A key for sample identification numbers is presented below.

Pot Tag / Tube Label Key

Example: 2-1-10 (cultivar - seed type - pot # ie. rep)

Cultivar: 2 = Russet Burbank (RB), 4 = Norkotah Colorado 3 (CO3)

Seed type: 1 = plantlet, 2 = minituber, 3 = G3 tuber

pot numbers: virus inoculated plants were paired with a mock inoculated plant (example 10= inoculated, 5 = mock inoculated)

Q1. What genes are differentially expressed during PVY infection?

To identify genes potentially involved in PVY antiviral immune responses in the most susceptible generation at peak infection 4 wpi, we will sequence infected Russet Burbank (RB) plantlets and infected Norkotah Colorado 3 (CO3) plantlets.

1. Infected RB plantlet (2-1-10, 2-1-8, 2-1-17) / mock inoculated RB plantlet (2-1-5, 2-1-3, 2-1-12) (Experiment Unique ID# 1, Appendix A Table 1)

2. Infected CO3 plantlet (4-1-10, 4-1-9, 4-1-16) / mock CO3 plantlet (4-1-5, 4-1-4, 4-1-11) (Experiment Unique ID# 1, Appendix A Table 1)

Q2. What genes are differentially expressed between potato cultivars?

To identify the genes that may play a role in reducing the number of PVY infections in RB plants as compared to CO3 plants we will examine transcriptional differences between RB and CO3 plants grown from plantlets and G3 tubers. The differentially expressed genes in these comparisons may be involved in immune responses, though bioinformatics analyses and additional experiential studies would be required. Between different potato cultivars, we will sequence less susceptible RB plants and more susceptible CO3 plants at peak infection 4 wpi, grown from both plantlets as well as G3 tubers.

3. infected RB plantlet (2-1-10, 2-1-8, 2-1-17) / mock inoculated RB plantlet (2-1-5, 2-1-3, 2-1-12) (Experiment Unique ID# 1, Appendix A Table 1)
4. infected CO3 plantlet (4-1-10, 4-1-9, 4-1-16) / mock CO3 plantlet (4-1-5, 4-1-4, 4-1-11) (Experiment Unique ID# 1, Appendix A Table 1)
5. inoculated/uninfected RB G3 (2-3-10, 2-3-9, 2-3-16) / mock inoculated RB G3 (2-3-5, 2-3-4, 2-3-11) (Experiment Unique ID# 1, Appendix A Table 1)

6. infected CO3 G3 (4-3-10, 4-3-9, 4-3-8)/ mock inoculated CO3 G3 (4-3-5, 4-3-4, 4-3-3) (Experiment Unique ID# 1, Appendix A Table 1)

Q3. What genes are differentially expressed between plants that were inoculated and became infected and plants that were inoculated and did not become infected (“fought off” PVY challenge)?

To understand genes that may play a role in limiting PVY infection we will sequence and compare samples from RB and CO3 plants that were inoculated and became infected with ones that were inoculated but did not become infected at peak infection 4wpi.

7. infected RB plantlet 2-1-10, 2-1-8, 2-1-17) / inoculated-uninfected RB plantlet (2-1-9, 2-1-16, 2-1-7) (Experiment Unique ID# 1, Appendix A Table 1)
8. infected CO3 plantlet (4-1-10, 4-1-9, 4-1-16) / inoculated-uninfected CO3 plantlet (4-1-8, 4-1-17, 4-1-18) (Experiment Unique ID# 1, Appendix A Table 1)

Q4. What genes are differentially expressed over the time course?

To understand differential gene expression as it changes through time, we will sequence the same plants sampled at 2, 3, and 4 weeks post inoculation (wpi).

1. RB infected plantlet 2-1-10, 2-1-8, 2-1-17) 2,3,4 wpi (Experiment Unique ID# 1, Appendix A Table 1)

2. RB inoculated-uninfected plantlet (2-1-9, 2-1-16, 2-1-7) 2,3,4 wpi
(Experiment Unique ID# 1, Appendix A Table 1)
3. RB mock inoculated plantlet (2-1-5, 2-1-3, 2-1-12) 2,3,4wpi
(Experiment Unique ID# 1, Appendix A Table 1)
4. CO3 infected plantlet (4-1-10, 4-1-9, 4-1-16) 2,3,4 wpi (Experiment
Unique ID# 1, Appendix A Table 1)
5. CO3 inoculated-uninfected plantlet (4-1-8, 4-1-17, 4-1-18) 2,3,4 wpi
(Experiment Unique ID# 1, Appendix A Table 1)
6. CO3 mock inoculated plantlet (4-1-5, 4-1-4, 4-1-11) 2,3,4wpi
(Experiment Unique ID# 1, Appendix A Table 1)

Appendix B Table 1. Samples for sequencing. 57 unique samples are needed to address questions 1-4. If Question 4 (time course) is left out, only 27 unique samples require sequencing.

question number	sample	cultivar	seed type	block number	time point	PVY infection status
1	2-1-10	Russet Burbank	plantlet	5	4 wpi	positive
1	2-1-8	Russet Burbank	plantlet	3	4 wpi	positive
1	2-1-17	Russet Burbank	plantlet	7	4 wpi	positive
1	2-1-5	Russet Burbank	plantlet	5	4 wpi	negative
1	2-1-3	Russet Burbank	plantlet	3	4 wpi	negative
1	2-1-12	Russet Burbank	plantlet	7	4 wpi	negative
1	4-1-10	Colorado 3	plantlet	5	4 wpi	positive
1	4-1-9	Colorado 3	plantlet	4	4 wpi	positive
1	4-1-16	Colorado 3	plantlet	6	4 wpi	positive
1	4-1-5	Colorado 3	plantlet	5	4 wpi	negative
1	4-1-4	Colorado 3	plantlet	4	4 wpi	negative
1	4-1-11	Colorado 3	plantlet	6	4 wpi	negative
2	2-1-10	Russet Burbank	plantlet	5	4 wpi	positive
2	2-1-5	Russet Burbank	plantlet	5	4 wpi	negative
2	4-1-10	Colorado 3	plantlet	5	4 wpi	positive
2	4-1-9	Colorado 3	plantlet	4	4 wpi	positive
2	4-1-16	Colorado 3	plantlet	6	4 wpi	positive
2	4-1-5	Colorado 3	plantlet	5	4 wpi	negative
2	4-1-4	Colorado 3	plantlet	4	4 wpi	negative
2	4-1-11	Colorado 3	plantlet	6	4 wpi	negative
2	2-3-10	Russet Burbank	G3	5	4 wpi	inoculated/ uninfected
2	2-3-9	Russet Burbank	G3	4	4 wpi	inoculated/ uninfected
2	2-3-16	Russet Burbank	G3	6	4 wpi	inoculated/ uninfected

Appendix B Table 1 Continued

2	2-3-5	Russet Burbank	G3	5	4 wpi	negative
2	2-3-4	Russet Burbank	G3	4	4 wpi	negative
2	2-3-11	Russet Burbank	G3	6	4 wpi	negative
2	4-3-10	Colorado 3	G3	5	4 wpi	positive
2	4-3-9	Colorado 3	G3	4	4 wpi	positive
2	4-3-8	Colorado 3	G3	3	4 wpi	positive
2	4-3-5	Colorado 3	G3	5	4 wpi	negative
2	4-3-4	Colorado 3	G3	4	4 wpi	negative
2	4-3-3	Colorado 3	G3	3	4 wpi	negative
3	2-1-10	Russet Burbank	plantlet	5	4 wpi	positive
3	2-1-8	Russet Burbank	plantlet	3	4 wpi	positive
3	2-1-17	Russet Burbank	plantlet	7	4 wpi	positive
3	2-1-9	Russet Burbank	plantlet	4	4 wpi	inoculated/ uninfected
3	2-1-16	Russet Burbank	plantlet	6	4 wpi	inoculated/ uninfected
3	2-1-7	Russet Burbank	plantlet	2	4 wpi	inoculated/ uninfected
3	4-1-10	Colorado 3	plantlet	5	4 wpi	positive
3	4-1-9	Colorado 3	plantlet	4	4 wpi	positive
3	4-1-16	Colorado 3	plantlet	6	4 wpi	positive
3	4-1-8	Colorado 3	plantlet	3	4 wpi	inoculated/ uninfected
3	4-1-17	Colorado 3	plantlet	7	4 wpi	inoculated/ uninfected
3	4-1-18	Colorado 3	plantlet	8	4 wpi	inoculated/ uninfected
4	2-1-10	Russet Burbank	plantlet	5	2 wpi	positive
4	2-1-8	Russet Burbank	plantlet	3	2 wpi	positive
4	2-1-17	Russet Burbank	plantlet	7	2 wpi	positive

Appendix B Table 1 Continued

4	2-1-10	Russet Burbank	plantlet	5	3 wpi	positive
4	2-1-8	Russet Burbank	plantlet	3	3 wpi	positive
4	2-1-17	Russet Burbank	plantlet	7	3 wpi	positive
4	2-1-10	Russet Burbank	plantlet	5	4 wpi	positive
4	2-1-8	Russet Burbank	plantlet	3	4 wpi	positive
4	2-1-17	Russet Burbank	plantlet	7	4 wpi	positive
4	2-1-9	Russet Burbank	plantlet	4	2 wpi	inoculated/ uninfected
4	2-1-16	Russet Burbank	plantlet	6	2 wpi	inoculated/ uninfected
4	2-1-7	Russet Burbank	plantlet	2	2 wpi	inoculated/ uninfected
4	2-1-9	Russet Burbank	plantlet	4	3 wpi	inoculated/ uninfected
4	2-1-16	Russet Burbank	plantlet	6	3 wpi	inoculated/ uninfected
4	2-1-7	Russet Burbank	plantlet	2	3 wpi	inoculated/ uninfected
4	2-1-9	Russet Burbank	plantlet	4	4 wpi	inoculated/ uninfected
4	2-1-16	Russet Burbank	plantlet	6	4 wpi	inoculated/ uninfected
4	2-1-7	Russet Burbank	plantlet	2	4 wpi	inoculated/ uninfected
4	4-1-10	Colorado 3	plantlet	5	2 wpi	positive
4	4-1-9	Colorado 3	plantlet	4	2 wpi	positive
4	4-1-16	Colorado 3	plantlet	6	2 wpi	positive
4	4-1-10	Colorado 3	plantlet	5	3 wpi	positive
4	4-1-9	Colorado 3	plantlet	4	3 wpi	positive
4	4-1-16	Colorado 3	plantlet	6	3 wpi	positive
4	4-1-10	Colorado 3	plantlet	5	4 wpi	positive
4	4-1-9	Colorado 3	plantlet	4	4 wpi	positive
4	4-1-16	Colorado 3	plantlet	6	4 wpi	positive

Appendix B Table 1 Continued

4	4-1-8	Colorado 3	plantlet	3	2 wpi	inoculated/ uninfected
4	4-1-17	Colorado 3	plantlet	7	2 wpi	inoculated/ uninfected
4	4-1-18	Colorado 3	plantlet	8	2 wpi	inoculated/ uninfected
4	4-1-8	Colorado 3	plantlet	3	3 wpi	inoculated/ uninfected
4	4-1-17	Colorado 3	plantlet	7	3 wpi	inoculated/ uninfected
4	4-1-18	Colorado 3	plantlet	8	3 wpi	inoculated/ uninfected
4	4-1-8	Colorado 3	plantlet	3	4 wpi	inoculated/ uninfected
4	4-1-17	Colorado 3	plantlet	7	4 wpi	inoculated/ uninfected
4	4-1-18	Colorado 3	plantlet	8	4 wpi	inoculated/ uninfected
4	4-1-5	Colorado 3	plantlet	5	2 wpi	negative
4	4-1-4	Colorado 3	plantlet	4	2 wpi	negative
4	4-1-11	Colorado 3	plantlet	6	2 wpi	negative
4	4-1-5	Colorado 3	plantlet	5	3 wpi	negative
4	4-1-4	Colorado 3	plantlet	4	3 wpi	negative
4	4-1-11	Colorado 3	plantlet	6	3 wpi	negative
4	4-1-5	Colorado 3	plantlet	5	4 wpi	negative
4	4-1-4	Colorado 3	plantlet	4	4 wpi	negative
4	4-1-11	Colorado 3	plantlet	6	4 wpi	negative

Transcriptome Sequencing Details

One of the most important considerations for transcriptome level sequencing experiments is ensuring adequate sequence coverage of expressed genes. While it is cost prohibitive to sequence each sample at sufficient levels to obtain quantitative data for every expressed transcript, there are guidelines that help ensure sequencing will provide an adequate data set. The potato genome

consists of 840 million bp encoding 39,000 genes (Potato Genome Sequencing Consortium et al. 2011). Coverage is determined by the number of reads, multiplied by the average read length, divided by the length of the genome (ENCODE Consortium 2011). Some coverage standards for potato transcriptional studies are 5X (Zhang, N. et al. 2014) and 7X (Goyer, A. et al. 2015). An example of 10X and 5X coverage are outlined below. Budget estimates were calculated based on the University of Illinois Sequencing Facility's pricing. Cost is dependent on the number of libraries prepared and the number of lanes used on the HiSeq. Since sequencing is costly, processing all 57 unique samples with enough coverage is not feasible. If samples addressing question 4 (time course) were omitted there would be 27 unique samples to sequence. See details below for costs pertaining to the sequencing of 27 unique samples.

Sequencing Coverage for 27 samples

Coverage = number reads X average read length (200bp) / length of
genome (840 million bp for potato) (Potato Genome
Sequencing Consortium et al. 2011)

Coverage standards: 5X (Zhang, N. et al. 2014)

7X (Goyer, A. et al. 2015)

Coverage example with 10X coverage:

10X coverage = number reads X 200bp / 840,000,000 bp potato genome

10X coverage requires 42 million reads per sample

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OR

5X coverage = number reads X 200bp / 840,000,000 bp potato genome

5X coverage requires 21 million reads per sample

Illumina HiSeq has 400 million reads/lane

42mil reads/sample = 9.5 samples/lane = 9 samples/lane

22 samples / 9 samples per lane = 3 (2.4) lanes on HiSeq needed

OR

21mil reads/sample = 19 samples/lane

27 samples / 19 samples per lane = 1.4 lanes -> 2 lanes on HiSeq needed

Sequencing Budget Estimate (University of Illinois Sequencing Facility 2016)

Library prep (eukaryotic RNA) costs \$275 per sample

1. 25.4% overhead=\$333.85 per sample
2. \$9,013.95 for library preparation of 27 samples

Each HiSeq lane costs \$2,600

3. 25.4% overhead=\$3260.40
4. 4 lanes needed (10X coverage) = \$13,041.60
5. 2 lanes needed (5X coverage) = \$6,520.80

Cost (library prep and lanes)

1. 10X coverage: \$22,055.55
2. 5X coverage: \$15,534.75

Data processing will follow a transcriptome analysis pipeline as outlined (Trapnell, C. et al. 2014). FastQC is used to assess information in raw fastq files

such as number of sequences, poor quality sequences, sequence lengths, etc. Trimmomatic is used to trim Illumina adaptor sequences from reads. Bowtie 2 Build writes a bowtie-indexed FASTA file from the genome of choice to be used in Tophat. Tophat aligns reads to the bowtie indexed file, Cufflinks assembles transcripts, Cuffmerge finalizes the transcriptome assembly, Cuffdiff outputs differential expression results, and CummeRbund produces expression plots of differentially expressed genes (Cufflinks version 2.2.0). Differentially expressed genes can be explored for function by using gene ontology tools.

Appendix B. Table 2

Gene	NCBI Accession Number	Immune Response	Organism	Reference
Ry _{sto}	EF205363	“extreme resistance”	potato	(Valkonen et al. 2008)
Ry _{adg}		“extreme resistance”	potato	(Andrade et al. 2009)
N _y -1	EF555209	Hypersensitive Response	potato	(Szajko et al. 2008)
PR-1	XM_006343477	SAR	potato	NCBI prediction

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APPENDIX C

CHAPTER 2 RAW DATA

Percent *Potato virus Y* (PVY) infection data from Experiment 2.1, Experiment 2.2, and Experiment 2.3 (Appendix A Table 1). Variety RB is equivalent to Russet Burbank, and variety CO3 is equivalent to Norkotah Colorado 3. Tissue was sampled at three time points: 2, 3, and 4 weeks post inoculation (wpi). Number infected represents number of plants detected as positive out of ten inoculated when measured with DAS-ELISA. Mean is mean percent infection for each treatment.

Appendix C Table 1. Percent *Potato virus Y* Infection Data from Experiments 2.1, 2.2, and 2.3. Number of plants infected out of ten potato plants inoculated measured with DAS-ELISA two, three, and four weeks post inoculation (wpi). Means are calculated for each treatment group across three experimental replicates (Experiment 2.1, Experiment 2.2, and Experiment 2.3).

experimental replicate	cultivar	seed type	Percent infection 2 wpi	Percent infection 3 wpi	Percent infection 4 wpi
1	Russet Burbank	Plantlet	1	3	4
2	Russet Burbank	Plantlet	1	7	10
3	Russet Burbank	Plantlet	0	4	4
mean			0.7	4.7	6.0
1	Russet Burbank	Minituber	0	3	4
2	Russet Burbank	Minituber	0	4	5
3	Russet Burbank	Minituber	0	2	2
mean			0.0	3.0	3.7

Appendix C Table 1 Continued

experimental replicate	cultivar	seed type	2 wpi	3 wpi	4 wpi
1	Russet Burbank	G3	0	0	0
2	Russet Burbank	G3	0	6	8
3	Russet Burbank	G3	0	0	0
mean			0.0	2.0	2.7
1	Colorado 3	Plantlet	3	6	6
2	Colorado 3	Plantlet	0	7	8
3	Colorado 3	Plantlet	0	2	4
mean			1.0	5.0	6.0
1	Colorado 3	Minituber	2	5	6
2	Colorado 3	Minituber	0	10	10
3	Colorado 3	Minituber	0	1	3
mean			0.7	5.3	6.3
1	Colorado 3	G3	3	7	8
2	Colorado 3	G3	0	8	9
3	Colorado 3	G3	0	0	2
mean			1.0	5.0	6.3

APPENDIX D

STATISTICAL ANALYSIS OF CHAPTER 2, DATA FORMATTED FOR SAS AND
CODE USED FOR ANALYSIS

Statistical analysis for chapter 2 where PVY incidence for two potato cultivars and three seed types was analyzed using SAS 9.4. A mixed effects model is ideal for situations where repeated measurements are taken on the same statistical units (2wpi, 3wpi, and 4wpi tissue collection on the same plants). The fixed effects were variety, generation, and time, and the random effect was rep (rep of experiment). Initially the model was run with each of six covariance structures since the ideal criteria were not met. In order to satisfy ideal criteria, variety and generation would have had to be assigned at random to each plant, which is not feasible. The Compound Symmetry covariance structure had the lowest fit statistic, meaning it explained the variation in our data the best (observations are correlated in time and the correlation is the same across time). The model was run again using just the compound symmetry model to generate the output used. Below is the code used for analysis.

```
ods rtf;
data Generational;
input variety $ generation $ time $ rep $ infected;
datalines;
```

RB	plantlet	2wpi	1	1
RB	minituber	2wpi	1	0
RB	G3	2wpi	1	0
CO3	plantlet	2wpi	1	3
CO3	minituber	2wpi	1	2
CO3	G3	2wpi	1	3
RB	plantlet	3wpi	1	3
RB	minituber	3wpi	1	3
RB	G3	3wpi	1	0
CO3	plantlet	3wpi	1	6

CO3	minituber	3wpi	1	5
CO3	G3	3wpi	1	7
RB	plantlet	4wpi	1	4
RB	minituber	4wpi	1	4
RB	G3	4wpi	1	0
CO3	plantlet	4wpi	1	6
CO3	minituber	4wpi	1	6
CO3	G3	4wpi	1	8
RB	plantlet	2wpi	2	1
RB	minituber	2wpi	2	0
RB	G3	2wpi	2	0
CO3	plantlet	2wpi	2	0
CO3	minituber	2wpi	2	0
CO3	G3	2wpi	2	0
RB	plantlet	3wpi	2	7
RB	minituber	3wpi	2	4
RB	G3	3wpi	2	6
CO3	plantlet	3wpi	2	7
CO3	minituber	3wpi	2	10
CO3	G3	3wpi	2	8
RB	plantlet	4wpi	2	10
RB	minituber	4wpi	2	5
RB	G3	4wpi	2	8
CO3	plantlet	4wpi	2	8
CO3	minituber	4wpi	2	10
CO3	G3	4wpi	2	9
RB	plantlet	2wpi	3	0
RB	minituber	2wpi	3	0
RB	G3	2wpi	3	0
CO3	plantlet	2wpi	3	0
CO3	minituber	2wpi	3	0
CO3	G3	2wpi	3	0
RB	plantlet	3wpi	3	4
RB	minituber	3wpi	3	2
RB	G3	3wpi	3	0
CO3	plantlet	3wpi	3	2
CO3	minituber	3wpi	3	1
CO3	G3	3wpi	3	0
RB	plantlet	4wpi	3	4
RB	minituber	4wpi	3	2

RB	G3	4wpi	3	0
CO3	plantlet	4wpi	3	4
CO3	minituber	4wpi	3	4
CO3	G3	4wpi	3	2

```

;
run;
proc print data = Generational;
title 'Full data set of generation';
run;

ODS RTF;
PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=CS SUBJECT=generation R;
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT1;
TITLE2 'Repeated Measures Analysis - Assuming Compound Symmetry';
RUN;
DATA FIT1; SET FIT1; TYPE='CS          ';
RUN;

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=HF SUBJECT=generation R;
TITLE2 'Repeated Measures Analysis - Assuming H-F conditions';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT2;
RUN;
DATA FIT2; SET FIT2; TYPE='HF          ';
RUN;

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=UN SUBJECT=generation R;
TITLE2 'Repeated Measures Analysis - No Assumptions on the Covariance
Matrix';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT3;
RUN;
DATA FIT3; SET FIT3; TYPE='UN          '; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=AR(1) SUBJECT=generation R;
TITLE2 'Repeated Measures Analysis - Assuming Autogressive Errors';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT4;
RUN;
DATA FIT4; SET FIT4; TYPE='AR(1)'; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=CSH SUBJECT=generation R;
TITLE2 'Repeated Measures Analysis - Assuming Heterogenous Compound Symmetry';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT5;
RUN;
DATA FIT5; SET FIT5; TYPE='CSH'; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=ARH(1) SUBJECT=generation R;
TITLE2 'Repeated Measures Analysis - Assuming Assuming Heterogenous AR(1)';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT6;
RUN;
DATA FIT6; SET FIT6; TYPE='ARH(1)'; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=CS SUBJECT=generation R;
GROUP=variety;
TITLE2 'Repeated Measures Analysis - Assuming Compound Symmetry';
TITLE3 'Covariance Matrix Allowed to Change for Each Variety';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT7;
RUN;

```

```

DATA FIT7; SET FIT7; TYPE='CS, HETEROGENOUS GROUPS'; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=HF SUBJECT=generation R;
GROUP=variety;
TITLE2 'Repeated Measures Analysis - Assuming H-F conditions';
TITLE3 'Covariance Matrix Allowed to Change for Each Variety';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT8;
RUN;
DATA FIT8; SET FIT8; TYPE='HF, HETEROGENOUS GROUPS'; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=UN SUBJECT=generation R;
GROUP=variety;
TITLE2 'Repeated Measures Analysis - No Assumptions on the Covariance
Matrix';
TITLE3 'Covariance Matrix Allowed to Change for Each Variety';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT9;
RUN;
DATA FIT9; SET FIT9; TYPE='UN, HETEROGENOUS GROUPS'; RUN;

```

```

PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=AR(1) SUBJECT=generation R;
GROUP=variety;
TITLE2 'Repeated Measures Analysis - Assuming Autogressive Errors';
TITLE3 'Covariance Matrix Allowed to Change for Each Variety';
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT10;
RUN;
DATA FIT10; SET FIT10; TYPE='AR(1), HETEROGENOUS GROUPS'; RUN;

```

```

DATA FITSTATS; SET FIT1 FIT2 FIT3 FIT4 FIT5
FIT6 FIT7 FIT8 FIT9 FIT10;
CRITERION=DESCR;
FITSTAT=VALUE;
DROP DESCR VALUE;

```



```
PROC PRINT DATA = FITSTATS; WHERE CRITERION = '-2 Res Log
Likelihood';
TITLE 'FIT STATISTICS';
RUN;
```

```
PROC PRINT DATA = FITSTATS; WHERE CRITERION = 'AIC (smaller is
better)';
TITLE 'FIT STATISTICS';
RUN;
```

```
PROC PRINT DATA = FITSTATS; WHERE CRITERION = 'AICC (smaller is
better)';
TITLE 'FIT STATISTICS';
RUN;
```

```
PROC PRINT DATA = FITSTATS; WHERE CRITERION = 'BIC (smaller is
better)';
TITLE 'FIT STATISTICS';
RUN;
```

Working code identifies CS:

```
ODS RTF;
PROC MIXED DATA=Generational;
CLASS variety generation time rep;
MODEL infected =variety generation variety*generation;
REPEATED TIME/TYPE=CS SUBJECT=generation R;
ODS RTF SELECT R;
ODS OUTPUT FITSTATISTICS=FIT1;
TITLE2 'Repeated Measures Analysis - Assuming Compound Symmetry';
LSMEANS variety generation variety*generation/PDIFF adjust=TUKEY;
LSMEANS variety*generation/PDIFF SLICE=variety;
LSMEANS variety*generation/PDIFF SLICE=generation;
ODS OUTPUT DIFF=DIFFS;
RUN;
```

```
proc print data=DIFFS;
where effect = 'variety*generation' and variety_variety;
title 'time differences by level of variety';
run;
```

```
proc print data=DIFFS;  
where effect = 'variety*generation' and time_time;  
title 'variety differences by level of time';  
run;  
ODS RTF CLOSE;  
  
PROC MIXED DATA=Generational;  
CLASS variety generation time rep;  
MODEL infected=variety generation variety*generation;  
Repeated time/type=CS;  
Lsmeans variety generation variety*generation;  
  
RUN
```

APPENDIX E

CHAPTER 3 RAW DATA

Systemic Acquired Resistance-Inducing Agents Panel Experiments

Percent *Potato virus Y* (PVY) infection measured with DAS-ELISA data from Experiment 3.1 and 3.2 (Appendix A Table 1). Variety RB is equivalent to Russet Burbank, and variety CO3 is equivalent to Norkotah Colorado 3. All plants were grown from plantlets. Tissue was analyzed at three time points: 2, 3, and 4 weeks post inoculation (wpi). Entries of 0 represent plants negative for PVY and 1s represent plants positive for PVY. Plant rep is the individual plant replicate (10 per treatment in experiment 1 and 12 per treatment in experiment 2). Percent infection is the number of positive plants (1s) out the number of plant reps.

Phosphorous Acid Experiments with Three Seed Types

Percent *Potato virus Y* (PVY) infection measured with DAS-ELISA data from Mechanical Inoculation Experiment 3.3 (Appendix A Table 1), and Mechanical Inoculation Experiment 3.4 (Appendix A Table 1). Variety RB is equivalent to Russet Burbank, and variety CO3 is equivalent to Norkotah Colorado 3. Tissue was analyzed at three time points: 2, 3, and 4 weeks post inoculation (wpi). Number infected represents number of plants detected as positive out of ten when measured with DAS-ELISA. Mean percent is the mean of the two experimental replicates for each treatment.

Experiment 3.1

cultivar	treatment	plant rep	2 wpi	3 wpi	4 wpi
RB	mock	1	0	0	0
RB	mock	2	0	0	0
RB	mock	3	0	0	0
RB	mock	4	0	0	0
RB	mock	5	0	0	0
RB	mock	6	0	0	0
RB	mock	7	0	0	0
RB	mock	8	0	0	0
RB	mock	9	0	0	0
RB	mock	10	0	0	0
percent infection			0%	0%	0%

cultivar	treatment	plant rep	2 wpi	3 wpi	4 wpi
RB	compost tea	1	0	0	1
RB	compost tea	2	0	0	0
RB	compost tea	3	0	0	0
RB	compost tea	4	0	0	0
RB	compost tea	5	0	0	0
RB	compost tea	6	0	0	0
RB	compost tea	7	0	0	0
RB	compost tea	8	0	0	0
RB	compost tea	9	0	0	0
RB	compost tea	10	0	0	0
percent infection			0%	0%	10%

RB	BmJ	1	1	1	1
RB	BmJ	2	0	1	1
RB	BmJ	3	0	1	1
RB	BmJ	4	0	0	1
RB	BmJ	5	0	0	0
RB	BmJ	6	0	0	0
RB	BmJ	7	0	0	0
RB	BmJ	8	0	0	0
RB	BmJ	9	0	0	0
RB	BmJ	10	0	0	0
percent infection			10%	30%	40%

Appendix E Table 1 Continued

RB	phosphorous acid	1	0	1	1
RB	phosphorous acid	2	0	0	0
RB	phosphorous acid	3	0	0	0
RB	phosphorous acid	4	0	0	0
RB	phosphorous acid	5	0	0	0
RB	phosphorous acid	6	0	0	0
RB	phosphorous acid	7	0	0	0
RB	phosphorous acid	8	0	0	0
RB	phosphorous acid	9	0	0	0
RB	phosphorous acid	10	0	0	0
percent infection			0%	10%	10%

cultivar	treatment	plant rep	2 wpi	3 wpi	4 wpi
RB	acibenzolar-S-methyl	1	0	1	1
RB	acibenzolar-S-methyl	2	0	1	1
RB	acibenzolar-S-methyl	3	0	0	0
RB	acibenzolar-S-methyl	4	0	0	0
RB	acibenzolar-S-methyl	5	0	0	0
RB	acibenzolar-S-methyl	6	0	0	0
RB	acibenzolar-S-methyl	7	0	0	0
RB	acibenzolar-S-methyl	8	0	0	0
RB	acibenzolar-S-methyl	9	0	0	0
RB	acibenzolar-S-methyl	10	0	0	0
percent infection			0%	20%	20%
RB	non-induced control	1	0	1	1
RB	non-induced control	2	0	0	1
RB	non-induced control	3	0	0	1
RB	non-induced control	4	0	0	0
RB	non-induced control	5	0	0	0
RB	non-induced control	6	0	0	0
RB	non-induced control	7	0	0	0
RB	non-induced control	8	0	0	0
RB	non-induced control	9	0	0	0
RB	non-induced control	10	0	0	0
percent infection			0%	10%	30%

Appendix E Table 1 Continued

CO3	mock	1	0	0	0
CO3	mock	2	0	0	0
CO3	mock	3	0	0	0
CO3	mock	4	0	0	0
CO3	mock	5	0	0	0
CO3	mock	6	0	0	0
CO3	mock	7	0	0	0
CO3	mock	8	0	0	0
CO3	mock	9	0	0	0
CO3	mock	10	0	0	0
percent infection			0%	0%	0%

cultivar	treatment	plant rep	2 wpi	3 wpi	4 wpi
CO3	compost tea	1	0	1	1
CO3	compost tea	2	0	1	1
CO3	compost tea	3	0	1	1
CO3	compost tea	4	0	1	1
CO3	compost tea	5	0	1	1
CO3	compost tea	6	0	0	0
CO3	compost tea	7	0	0	0
CO3	compost tea	8	0	0	0
CO3	compost tea	9	0	0	0
CO3	compost tea	10	0	0	0
percent infection			0%	50%	50%

CO3	BmJ	1	0	1	1
CO3	BmJ	2	0	1	1
CO3	BmJ	3	0	1	1
CO3	BmJ	4	0	1	1
CO3	BmJ	5	0	0	0
CO3	BmJ	6	0	0	0
CO3	BmJ	7	0	0	0
CO3	BmJ	8	0	0	0
CO3	BmJ	9	0	0	0
CO3	BmJ	10	0	0	0
percent infection			0%	40%	40%

Appendix E Table 1 Continued

CO3	phosphorous acid	1	0	1	1
CO3	phosphorous acid	2	0	0	1
CO3	phosphorous acid	3	0	0	1
CO3	phosphorous acid	4	0	0	0
CO3	phosphorous acid	5	0	0	0
CO3	phosphorous acid	6	0	0	0
CO3	phosphorous acid	7	0	0	0
CO3	phosphorous acid	8	0	0	0
CO3	phosphorous acid	9	0	0	0
CO3	phosphorous acid	10	0	0	0
percent infection			0%	10%	30%

cultivar	treatment	plant rep	2 wpi	3 wpi	4 wpi
CO3	acibenzolar-S-methyl	1	0	1	1
CO3	acibenzolar-S-methyl	2	0	1	1
CO3	acibenzolar-S-methyl	3	0	1	1
CO3	acibenzolar-S-methyl	4	0	1	1
CO3	acibenzolar-S-methyl	5	0	0	1
CO3	acibenzolar-S-methyl	6	0	0	0
CO3	acibenzolar-S-methyl	7	0	0	0
CO3	acibenzolar-S-methyl	8	0	0	0
CO3	acibenzolar-S-methyl	9	0	0	0
CO3	acibenzolar-S-methyl	10	0	0	0
percent infection			0%	40%	50%

CO3	non-induced control	1	0	1	1
CO3	non-induced control	2	0	0	1
CO3	non-induced control	3	0	0	1
CO3	non-induced control	4	0	0	1
CO3	non-induced control	5	0	0	1
CO3	non-induced control	6	0	0	1
CO3	non-induced control	7	0	0	0
CO3	non-induced control	8	0	0	0
CO3	non-induced control	9	0	0	0
CO3	non-induced control	10	0	0	0
percent infection			0%	10%	60%

Experiment 3.2

cultivar	treatment	plant rep	2 wpi	3 wpi	4 wpi
RB	mock	1	0	0	0
RB	mock	2	0	0	0
RB	mock	3	0	0	0
RB	mock	4	0	0	0
RB	mock	5	0	0	0
RB	mock	6	0	0	0
RB	mock	7	0	0	0
RB	mock	8	0	0	0
RB	mock	9	0	0	0
RB	mock	10	0	0	0
RB	mock	11	0	0	0
RB	mock	12	0	0	0
percent infection			0%	0%	0%
RB	compost tea	1	0	1	1
RB	compost tea	2	0	1	1
RB	compost tea	3	0	1	1
RB	compost tea	4	0	1	1
RB	compost tea	5	0	0	1
RB	compost tea	6	0	0	1
RB	compost tea	7	0	0	0
RB	compost tea	8	0	0	0
RB	compost tea	9	0	0	0
RB	compost tea	10	0	0	0
RB	compost tea	11	0	0	0
RB	compost tea	12	0	0	0
percent infection			0%	33%	50%
RB	BmJ	1	0	1	1
RB	BmJ	2	0	1	1
RB	BmJ	3	0	1	1
RB	BmJ	4	0	1	1
RB	BmJ	5	0	0	1
RB	BmJ	6	0	0	1
RB	BmJ	7	0	0	0
RB	BmJ	8	0	0	0
RB	BmJ	9	0	0	0
RB	BmJ	10	0	0	0
RB	BmJ	11	0	0	0
RB	BmJ	12	0	0	0
percent infection			0%	33%	50%

Appendix E Table 1 Continued

RB	phosphorous acid	1	0	1	1
RB	phosphorous acid	2	0	1	1
RB	phosphorous acid	3	0	0	0
RB	phosphorous acid	4	0	0	0
RB	phosphorous acid	5	0	0	0
RB	phosphorous acid	6	0	0	0
RB	phosphorous acid	7	0	0	0
RB	phosphorous acid	8	0	0	0
RB	phosphorous acid	9	0	0	0
RB	phosphorous acid	10	0	0	0
RB	phosphorous acid	11	0	0	0
RB	phosphorous acid	12	0	0	0
percent infection			0%	17%	17%
RB	acibenzolar-S-methyl	1	0	1	1
RB	acibenzolar-S-methyl	2	0	1	1
RB	acibenzolar-S-methyl	3	0	1	1
RB	acibenzolar-S-methyl	4	0	0	1
RB	acibenzolar-S-methyl	5	0	0	1
RB	acibenzolar-S-methyl	6	0	0	1
RB	acibenzolar-S-methyl	7	0	0	0
RB	acibenzolar-S-methyl	8	0	0	0
RB	acibenzolar-S-methyl	9	0	0	0
RB	acibenzolar-S-methyl	10	0	0	0
RB	acibenzolar-S-methyl	11	0	0	0
RB	acibenzolar-S-methyl	12	0	0	0
percent infection			0%	25%	50%
RB	non-induced control	1	0	1	1
RB	non-induced control	2	0	1	1
RB	non-induced control	3	0	1	1
RB	non-induced control	4	0	1	1
RB	non-induced control	5	0	1	1
RB	non-induced control	6	0	1	1
RB	non-induced control	7	0	1	1
RB	non-induced control	8	0	0	1
RB	non-induced control	9	0	0	1
RB	non-induced control	10	0	0	0
RB	non-induced control	11	0	0	0
RB	non-induced control	12	0	0	0
percent infection			0%	58%	75%

Appendix E Table 1 Continued

CO3	mock	1	0	0	0
CO3	mock	2	0	0	0
CO3	mock	3	0	0	0
CO3	mock	4	0	0	0
CO3	mock	5	0	0	0
CO3	mock	6	0	0	0
CO3	mock	7	0	0	0
CO3	mock	8	0	0	0
CO3	mock	9	0	0	0
CO3	mock	10	0	0	0
CO3	mock	11	0	0	0
CO3	mock	12	0	0	0
percent infection			0%	0%	0%
CO3	compost tea	1	0	1	1
CO3	compost tea	2	0	1	1
CO3	compost tea	3	0	1	1
CO3	compost tea	4	0	1	1
CO3	compost tea	5	0	1	1
CO3	compost tea	6	0	1	1
CO3	compost tea	7	0	1	1
CO3	compost tea	8	0	0	0
CO3	compost tea	9	0	0	0
CO3	compost tea	10	0	0	0
CO3	compost tea	11	0	0	0
CO3	compost tea	12	0	0	0
percent infection			0%	58%	58%
CO3	BmJ	1	0	1	1
CO3	BmJ	2	0	1	1
CO3	BmJ	3	0	1	1
CO3	BmJ	4	0	1	1
CO3	BmJ	5	0	1	1
CO3	BmJ	6	0	1	1
CO3	BmJ	7	0	1	1
CO3	BmJ	8	0	1	1
CO3	BmJ	9	0	1	1
CO3	BmJ	10	0	0	1
CO3	BmJ	11	0	0	1
CO3	BmJ	12	0	0	1
percent infection			0%	75%	100%

Appendix E Table 1 Continued

CO3	phosphorous acid	1	0	1	1
CO3	phosphorous acid	2	0	1	1
CO3	phosphorous acid	3	0	1	1
CO3	phosphorous acid	4	0	1	1
CO3	phosphorous acid	5	0	1	1
CO3	phosphorous acid	6	0	1	1
CO3	phosphorous acid	7	0	1	1
CO3	phosphorous acid	8	0	1	1
CO3	phosphorous acid	9	0	1	1
CO3	phosphorous acid	10	0	1	1
CO3	phosphorous acid	11	0	1	1
CO3	phosphorous acid	12	0	0	1
percent infection			0%	92%	100%
CO3	acibenzolar-S-methyl	1	1	1	1
CO3	acibenzolar-S-methyl	2	0	1	1
CO3	acibenzolar-S-methyl	3	0	1	1
CO3	acibenzolar-S-methyl	4	0	1	1
CO3	acibenzolar-S-methyl	5	0	1	1
CO3	acibenzolar-S-methyl	6	0	1	1
CO3	acibenzolar-S-methyl	7	0	1	1
CO3	acibenzolar-S-methyl	8	0	1	1
CO3	acibenzolar-S-methyl	9	0	1	1
CO3	acibenzolar-S-methyl	10	0	1	1
CO3	acibenzolar-S-methyl	11	0	0	1
CO3	acibenzolar-S-methyl	12	0	0	0
percent infection			8%	83%	92%
CO3	non-induced control	1	1	1	1
CO3	non-induced control	2	0	1	1
CO3	non-induced control	3	0	1	1
CO3	non-induced control	4	0	1	1
CO3	non-induced control	5	0	1	1
CO3	non-induced control	6	0	1	1
CO3	non-induced control	7	0	1	1
CO3	non-induced control	8	0	1	1
CO3	non-induced control	9	0	1	1
CO3	non-induced control	10	0	1	1
CO3	non-induced control	11	0	1	1
CO3	non-induced control	12	0	0	1
percent infection			8%	92%	100%

Appendix E Table 2. Percent *Potato virus Y* (PVY^{N-Wi}) Infection Data from Experiments 3.3, and 3.4. Number of plants infected out of ten plants inoculated measured with DAS-ELISA two, three, and four weeks post inoculation. Means are calculated for each treatment group across two experimental replicates (Experiment 2, and Experiment 3).

experimental rep	cultivar	seed type	treatment	2 wpi	3 wpi	4 wpi
1	RB	plantlet	non-induced	1	7	10
2	RB	plantlet	non-induced	0	4	4
mean percent				5%	55%	70%
1	RB	plantlet	phosphorous acid	0	7	7
2	RB	plantlet	phosphorous acid	0	1	1
mean percent				0%	40%	40%
1	RB	minituber	non-induced	0	4	5
2	RB	minituber	non-induced	0	2	2
mean percent				0%	30%	35%
1	RB	minituber	phosphorous acid	0	4	5
2	RB	minituber	phosphorous acid	0	2	4
mean percent				0%	30%	45%
1	RB	G3	non-induced	0	6	8
2	RB	G3	non-induced	0	0	0
mean percent				0%	30%	40%
1	RB	G3	phosphorous acid	1	9	9
2	RB	G3	phosphorous acid	0	0	1
mean percent				5%	45%	50%

Appendix E Table 2 Continued

experimental rep	cultivar	seed type	treatment	2 wpi	3 wpi	4 wpi
1	CO3	plantlet	non-induced	0	7	8
2	CO3	plantlet	non-induced	0	2	4
mean percent				0%	45%	60%
1	CO3	plantlet	phosphorous acid	0	7	10
2	CO3	plantlet	phosphorous acid	0	3	4
mean percent				0%	50%	70%
1	CO3	minituber	non-induced	0	10	10
2	CO3	minituber	non-induced	0	1	4
mean percent				0%	55%	70%
1	CO3	minituber	phosphorous acid	1	10	10
2	CO3	minituber	phosphorous acid	0	2	3
mean percent				5%	60%	65%
1	CO3	G3	non-induced	0	8	9
2	CO3	G3	non-induced	0	0	2
mean percent				0%	40%	55%
1	CO3	G3	phosphorous acid	0	8	9
2	CO3	G3	phosphorous acid	0	1	2
mean percent				0%	45%	55%

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