2024 ISSUE

POTATO VIRUS INITIATIVE

DEVELOPING SOLUTIONS

BRINGING TOGETHER RESEARCHERS, EXTENSION PROFESSIONALS AND SEED CERTIFICATION PROGRAMS FROM ACROSS THE COUNTRY

www.uidaho.edu/cals/potato-virus-initiative

TO DEVELOP SOLUTIONS FOR POTATO VIRUSES

The Potato Virus Initiative: Developing Solutions is a federally funded research and Extension-based program with the mission to develop potato virus management strategies and decisions aids, specifically for potato mop top virus (PMTV) and for potato virus Y (PVY), by improving detection and strain typing methods, breeding for resistance, and developing in-season management solutions for use by the potato industry to produce a healthy and high-quality potato crop.

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OBJECTIVE 1

To improve high throughput detection of PVY and PMTV in dormant potato tubers, in their vectors and for PMTV, in soil, and to train seed certification agencies in these methods

Delivering potato virus information and optimized detection tools to the seed certification program in Michigan

JAIME WILLBUR AND MIO SATOH-CRUZ, MICHIGAN STATE UNIVERSITY

BACKGROUND

Detection of potato virus Y (PVY) in early generation potato seed lots helps prevent infected material from entering the production chain and reduces unnecessary yield and profit loss. PVY is a complex of multiple strains that cause a variety of foliar and tuber symptoms. Several current strains have potential to result in yield and quality losses dependent on variety. Understanding the local strain population is critical for detection and management. Furthermore, understanding varietal responses to PVY strains is valuable for growers, inspectors, and future breeding and selection efforts. We collaborate with Michigan Department of Agriculture and Rural Development and Michigan Seed Potato Association to optimize detection methods for the seed certification program in Michigan.

OBJECTIVES

Michigan State University conducted research to:

1) Investigate accurate, timely, and cost-effective methods for use in Michigan seed potato certification,

2) Monitor PVY strain prevalence in Michigan seed potatoes, and

3) Characterize PVY strain by chipping potato variety responses.

WHAT WE DID

Objectives 1 and 2. We evaluated direct tuber testing methods, which do not require breaking tuber dormancy to sample sprouts or plantlets. General reverse-transcriptase (RT) high-fidelity polymerase chain reaction (PCR) protocols (Mackenzie et al. 2015) were compared to existing leaflet enzyme-linked immunosorbent assays (ELISA). For each tested lot, tubers were cored for RT-PCR, treated with Rindite to break dormancy, and subsamples grown out in our greenhouse for standard leaflet ELISA. Results from summer visual inspection, direct tuber, and leaflet ELISA methods were compared. Subsets of positive samples (from research and commercial testing) were subjected to PVY strain confirmation by multiplex RT-PCR (Lorenzen et al. 2006, 2010; Chikh-Ali et al. 2013).

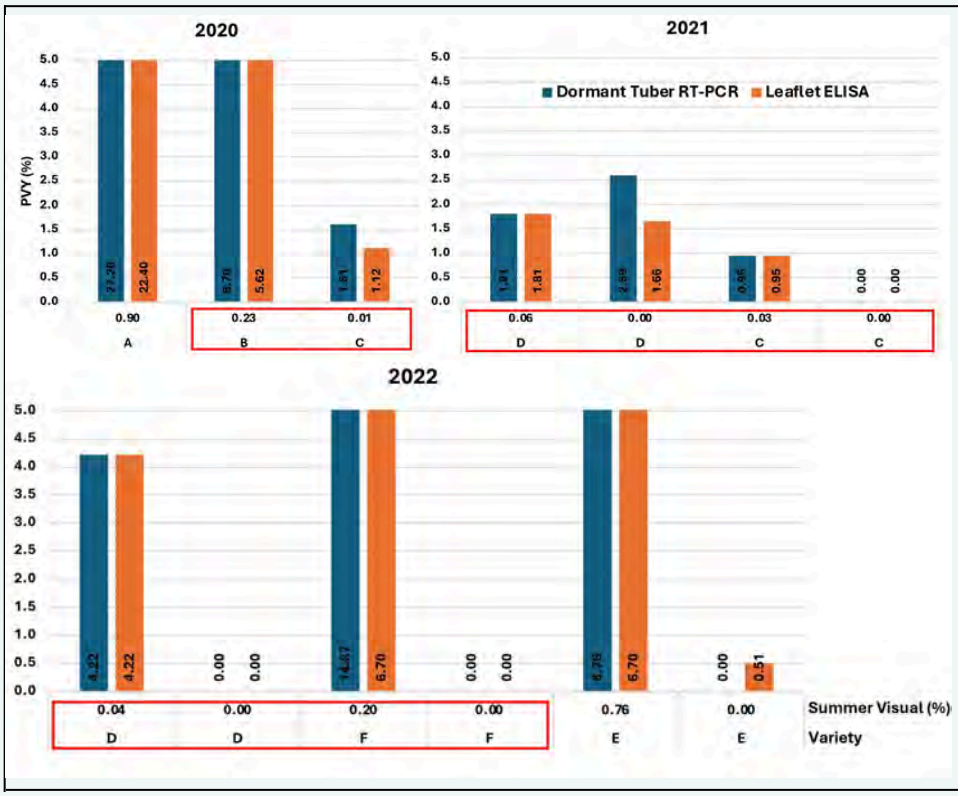


Figure 1. Comparison of direct tuber RT-PCR and ELISA testing methods. Direct tuber RT-PCR and leaflet ELISA results (from the same 10-tuber subsamples) in 13 seed lots (200- or 400-tuber samples) from 2020-2022. Red boxed varieties are less reliable visual symptoms.

WHAT WE FOUND

Objectives 1 and 2. When tested in the same 10-tuber subsamples, direct tuber RT-PCR and leaflet ELISA results were comparable across 13 seed lots from 2020-2022 (Figure 1). This tool aims to complement observations made in summer and winter field inspections and to offer a rapid option for use in seed certification testing. Direct tuber results are available 3-4 months sooner than standard winter grow out methods, supporting early decision making as needed. This method may be of particular interest when considering varieties with less reliable visual symptoms.

Over the past five years, PVYN-Wi remains most prevalent in Michigan, however, tuber necrotic strains PVYNTN and PVYE also continue to be detected (Figure 2). Strains NTN and E, as well as some N:Wi isolates, are known to cause necrotic symptoms in tubers, which causes yield and quality reductions for susceptible potato cultivars.

"Over the past 5 years, PVY N-Wi remains most prevalent in Michigan, however, tuber necrotic strains PVYNTN and PVYE also continue to be detected."

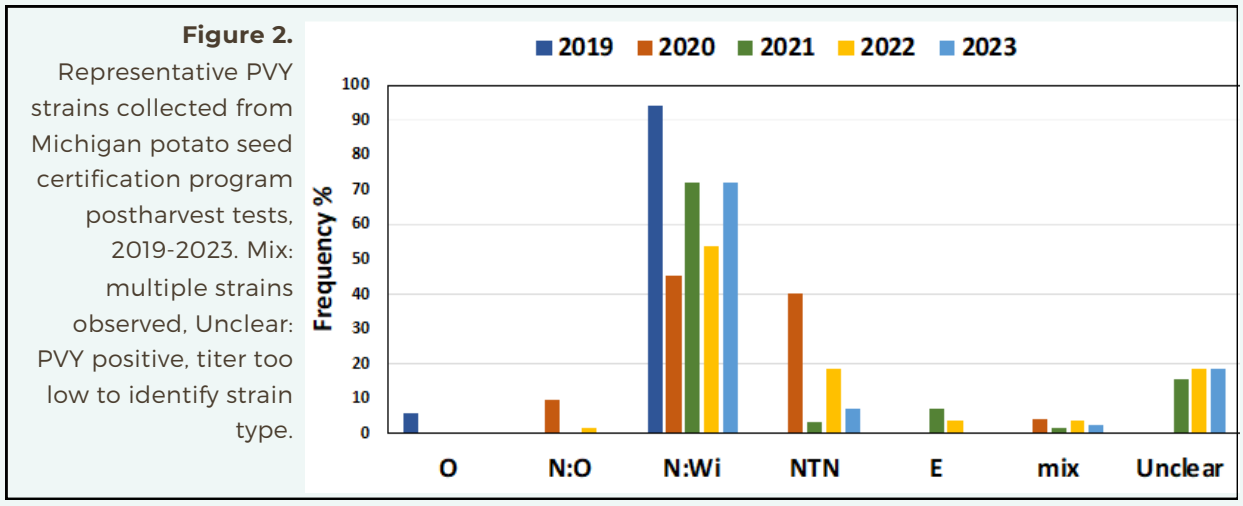


Figure 2. Representative PVY strains collected from Michigan potato seed certification program postharvest tests, 2019-2023. Mix: multiple strains observed, Unclear: PVY positive, titer too low to identify strain type.

WHAT WE DID

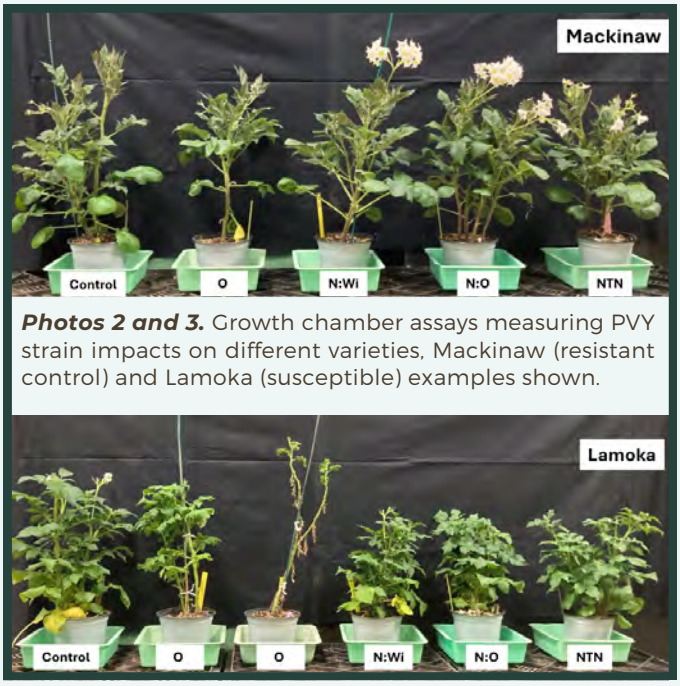
Objective 3. We conducted growth chamber assays using characterized PVY strains (provided by Karasev, University of Idaho) and inoculating elite potato germplasm using previously reported methods by Gundersen et al. (2019). Based on our local PVY strains and varieties of interest, we selected four characterized strains (N:Wi, NTN, N:O, and O) and six varieties (Snowden, Lamoka, Mackinaw, Lady Liberty, Petoskey and MSZ242-13 (Dundee)). In 2023, three varieties were added (MSW474-1, NY163, and Manistee) and screened in a greenhouse using three PVY isolates from Michigan (N:Wi, NTN, N:O). These entries represent current chip varieties used in Michigan and elite experimental varieties originating from the MSU Potato Breeding and Genetics program. We planted clean tubers and mechanically inoculated mother plants with PVY strains; infected daughter tubers were then harvested and re-planted to monitor symptom expression and yield impacts caused by seedborne PVY.

WHAT WE FOUND

Objective 3. We observed low to mild to severe foliar symptoms in susceptible varieties depending on strain. Across varieties, reductions in total tuber weight relative to the mock-inoculated control were observed with strain N:Wi reaching 23% and 38% in growth chamber and greenhouse assays, respectively. Direct tuber tests of daughter tubers showed an average 94% (range of 83 to 100%) of PVY transmission from leaves to tubers in susceptible mother plants. In resistant varieties, Mackinaw and Lady Liberty, no detectable levels of virus were observed in daughter tubers. Variety responses to seedborne infection were greatly strain-dependent. Repeated experiments are currently in progress and will validate PVY responses to multiple strains, further informing variety selection and breeding efforts.

TAKE-HOME HIGHLIGHTS

- In three years of testing, results from direct tuber and standard grow out methods were comparable.
- As in other U.S. potato-growing regions, PVY strains N:Wi and NTN are most prevalent in Michigan seed potato lots. Of note, mild to severe tuber necrosis has been observed in diagnostic samples positive with local N:Wi strains.
- In strain x variety response assays, results indicate some notable impacts on second-generation biomass and tuber yield in susceptible varieties (Photo 2 and 3).
- These results have been shared with Michigan potato commercial and seed industry members in annual reports and seed potato association meetings and in new interactive demonstration formats at our Winter Potato Conference (Photo 1).



Photos 2 and 3. Growth chamber assays measuring PVY strain impacts on different varieties, Mackinaw (resistant control) and Lamoka (susceptible) examples shown.

NEXT STEPS

We continue to compare RT-PCR and IC-RT-PCR tools to traditional growout methods to identify cost-effective options for Michigan seed certification, and to characterize potato varietal responses to regionally-prevalent PVY strains to inform certification and breeding efforts. Validated results will be shared to our stakeholders through local outlets as well as at regional and national meetings and in scientific journals.

Photo 1. Stakeholder participants interact with Michigan State University Potato and Sugar Beet Pathology Drs. Satoh-Cruz and Willbur during the annual Winter Potato Conference's interactive "Potato University" tour of our research facilities on January 31st, 2024. Small-group demonstrations of potato virus observations and variety responses to PVYN:Wi were presented.

Accelerating dormancy break to aid the seed certification community

NATHAN GELLES(GRADUATE STUDENT),
RABECKA HENDRICKS, AND NORA OLSEN, UNIVERSITY OF IDAHO

BACKGROUND

Seed certification programs seek quick, reliable methods to detect Potato Virus Y (PVY) in seed potatoes. PVY levels within a given seed lot could make it ineligible for recertification or impact productivity if planted in the field. Having fast, yet reliable results in the hands of growers soon after harvest will help make seed purchasing and planting decisions.

Direct tuber testing (DTT) is a laboratory test used by certification programs to test for PVY. Research is needed to determine the reliability of detecting PVY with this method compared to standard techniques such as the winter grow out (WGO). Previous research on DTT indicated more reliable analysis when tubers were sprouting; therefore, some DTT protocols require tubers to have broken dormancy and have visual sprout development. The inherent dormancy period of potatoes can be difficult to break in some varieties.

OBJECTIVES

The University of Idaho conducted research trials to
1) identify ways to initiate sprouting soon after harvest to enable direct tuber testing.
2) compare direct tuber testing to leaf testing results obtained from the winter grow out and a spring grow out.

INVESTIGATING METHODS TO PROMOTE SPROUTING

Methods to break dormancy for PVY detection were tested on Ranger Russet, Clearwater Russet, and Umatilla Russet over two years. Treatments included an untreated control placed at 65˚F and 95% RH, a cold shock (40˚F for 2 weeks), alternating temperatures (40˚F for 4 days 65˚F for 5 days and 40˚F for 4 days), a gibberellic acid dip (GA; 20 ppm for 15 min), three rates of cold aerosol smoke, and a combination of smoke plus a dip in GA. Treatments were applied to separate batches of tubers at 1 month, 2 months, and 3 months after harvest to determine if efficacy improved as time after harvest increased. Tubers were periodically evaluated for sprout development and sprout length and weight.

WHAT WE FOUND

"The use of smoke alone or in combination with GA would decrease the time after harvest to have acceptable sprout development."

Sprout development increased significantly with a smoke application in all three varieties and monthly timings (See Table 1 for the October results). However, smoke combined with GA produced a synergistic effect with greater sprout development and larger sprouts compared to the other treatments in all three varieties and monthly timings. Tubers treated one month after harvest and treated with smoke + GA resulted in dormancy break within 22 days after treatment (DAT) in Clearwater Russet and Umatilla Russet, and 44 DAT in Russet Burbank, while the untreated controls did not break dormancy break for another 20-40 days after that. Temperature fluctuation and cold shock treatments produced equal or less sprout development than the untreated control in all varieties and treatment timings and are not a viable option to break dormancy soon after harvest. GA alone produced more sprouts and broke dormancy sooner than the untreated control in all varieties and application timings. Overall, the use of smoke alone or in combination with GA would decrease the time after harvest to have acceptable sprout development for efficient virus detection via various laboratory direct tuber testing detection methods.

Table 1. Average sprout length (mm/tuber) at the final evaluation after the October treatments.

	Russet Burbank	Clearwater Russet	Umatilla Russet
Treatment	Avg sprout length (mm)		
UTC	1.0 a	1.5 ab	6.0 b
Cold-stratification	2.0 ab	0.3 a	1.6 a
Temp. fluctuation	1.2 a	0.4 ab	2.6 a
GA dip	3.5 abc	6.2 d	12.6 d
Smoke 1h 20h	4.8 bc	6.8 d	8.9 c
Combination	14.9 d	16.2 e	13.3 d
Smoke 2h 4h	6.1 c	4.6 cd	8.1 bc
Smoke 1h 4h	3.2 abc	3.1 bc	7.6 bc

Values followed by the same letter are not significantly different (α=0.05) within each column.
UTC: 65F for 14 day (d); Cold-stratification: 40F for 14 d; Temp. fluctuation: 40F for 5 d -> 65F for 4 d -> 40F for 5 d; GA dip: Gibberellic acid (GA) at 20 ppm; Smoke 1 h 20 h: smoke injected 1 hour (h), recirculated 20 h; Combination: 1 h 20 h + GA Dip; Smoke 2 h 4 h: smoke injected 2 h, recirculated 4 h; Smoke 1 h 4 h: smoke injected 1 h, recirculated 4 h. All tubers stored at 65F post-treatment.

DIRECT TUBER TESTING VERSUS LEAF SAMPLING

Three 400 tuber samples from each cultivar were treated with: 1) untreated control (UTC), 2) application of cold aerosol smoke, or 3) application of Rindite. Samples were held at 65˚F and sprout development monitored weekly. Treatments were direct tuber tested for PVY via ELISA (by Idaho Crop Improvement) when one treatment of that cultivar achieved three sprouts at ½ inch long. A fourth 400 tuber sample was collected, treated with Rindite, and shipped to be included in the Idaho winter grow out plots in Waialua, Hawaii and leaves were sampled and evaluated for PVY using ELISA. Laboratory tested seed was stored and planted in a spring grow out (Kimberly, Idaho) and leaf samples analyzed for PVY by ELISA.

WHAT WE FOUND

The application of Rindite produced greater sprout development compared to the smoke treatment and untreated control. Final PVY results from DTT were produced by the middle to last week of November (42 to 70 days after harvest) while the traditional WGO samples were not processed until the middle of January: ~45 day difference. In addition, DTT samples had comparable PVY detection as samples sent to the WGO: 15% PVY detected via DTT versus 14% PVY in the WGO (Table 2). Tuber samples could have been tested even sooner. A spring grow-out of the tubers used for direct tuber testing further confirmed the accuracy of ELISA at detecting PVY in non-dormant, sprouted tubers. Overall, results from this study indicate that DTT via ELISA in tubers that have broken dormancy, or at least a few weeks from harvest, provide comparable results to the WGO and the level of PVY a grower would expect to see in the field the following spring.

Table 2. Quick snapshot on how the direct tuber testing compared to the WGO and the spring grow out. Data is combined over cultivars and years. The percent of PVY varied from 0.3% in Clearwater Russet to 35% in Ranger Russet.

Treatment	WGO	Direct tuber testing	Spring grow out
	% PVY		
Untreated	--	13.5	16.7
Smoke	--	14.7	17.2
Rindite	13.9	16.9	17.5
Average	*13.9*	*15.0*	*17.1*

TAKE-HOME HIGHLIGHTS

Methods such as a smoke application and/or GA dip have the ability to break dormancy soon after harvest and can be used to help facilitate accurate direct tuber testing of seed for PVY. Direct tuber testing PVY results were comparable to the WGO and field plantings.

NEXT STEPS

To continue evaluating means of using cold smoke applications to break dormancy and encourage sprout development for the use in direct tuber testing and as a Rindite replacement for seed planted in the WGO.

"Results from this study indicate DTT via ELISA provide comparable results to the WGO and the level of PVY a grower would expect to see in the field the following spring."

Impact of seedborne PVY infection on yield – Larger plot results

NATHAN GELLES (GRADUATE STUDENT), RABECKA HENDRICKS, AND NORA OLSEN, UNIVERSITY OF IDAHO

BACKGROUND

Seedborne PVY can express a wide variation of symptoms and depending upon the cultivar and strain may result in yield loss and quality defects. Previous studies have focused on the impact of PVYO on yield since it was the predominant PVY observed in commercial fields. But due to the complexity of PVY and the capability of producing several recombinant strains, PVY has shifted in the Pacific Northwest in the last several years to PVYNTN and PVYN-Wi, which produce more mild symptoms compared to PVYO.

Research with current strains is needed to determine the impact commercial potato growers may experience if planting seed with seedborne PVY infection. Previous studies have also focused on the effects of seedborne PVY on yield compared to individual plants or very small plots. Although a yield decrease due to PVY infection is anticipated, commercial growers question the overall impact seedborne PVY has on a farm level. Research evaluating the effects of seedborne PVY on yield needs to be conducted on a larger scale to provide relatable impacts of seedborne PVY on potato production.

WHAT WE DID

Multiple seed lots of three cultivars were sampled from two commercial seed growers' storages and stored at University of Idaho Kimberly Research and Extension Center until planting in April 2022. Russet Norkotah, Russet Burbank, and Ranger Russet were collected based upon post-harvest PVY test results. Seed within a cultivar was collected from the same seed grower except the Ranger Russet lots which were from two growers. Russet Norkotah treatments will be referred to as low (2%) and high (11%). Russet Burbank treatments will be referred to as low (0%), medium (5%), and high (8%). Ranger Russet treatments will be referred to as low (3%) and high (34%) for ease of discussion.

OBJECTIVE

The study's objective was to determine the effects of seedborne PVY on yield, focusing on commercial production conditions.

Cut seed was planted in a randomized block design with five replicates. When plants reached approximately 30 cm tall (June 27, 2022; 67 DAP) visual evaluations were conducted based upon PVY symptoms to determine the level of seedborne PVY in each seed lot. Plants in question were tested using Agdia Immunostrips. In addition, leaf tissue samples were collected from plants expressing PVY symptoms and sent to University of Idaho Virology Lab (Karasev) for PVY strain identification. Vines were mechanically flailed 14 days prior to harvest. Plots were harvested September 20, 2022 (152 DAP). Total yield and grade of each plot was collected. Potatoes were produced and harvested in a manner that mimicked commercial production practices.

WHAT WE FOUND

Plots in this study contained the two most prevalent strains of PVY (NTN and N-Wi) in the Pacific Northwest. Russet Burbank plots were planted from 0% (low), 5% (medium), and 8% (high) seedborne PVY seed lots. Total yield of Russet Burbank seed lots ranged from 495 (high PVY) to 571 (low PVY) cwt/A (Table 1). Total yield of the low and medium PVY infected seed lots were similar, but the high infected lot resulted in a 14% yield reduction compared to the low treatment. Significant differences in yield were observed between PVY seed lot treatments in all size profile categories indicating not only an effect on yield, but also size profile (data not shown).

Contrary to Russet Burbank, significant total yield differences were not observed in Ranger Russet. Ranger Russet plots were planted from seed lots with 3% (low) and 34% (high) PVY infection, although the seed lots were produced by two different seed growers in different regions. An argument could be made that physiological age was different between the two seed lots and contributed to the lack of yield differences, although this would indicate age of the seed can be a greater influence on seed performance than the level of PVY.

Russet Norkotah yield losses were comparable to yield loss observed between high and low PVY infection in Russet Burbank. Russet Norkotah had a 13% yield decrease between the seed lot with low (2%) and the high (11%) seedborne PVY incidence.

Table 1. Total harvested tuber yield (cwt/A) of multiple seed lots with seedborne PVY infection in Russet Burbank, Ranger Russet, and Russet Norkotah in 2022.

	Total Yield (cwt/A) [1]		
Seedborne PVY level	Russet Burbank[2]	Ranger Russet[3]	Russet Norkotah[4]
Low	571 b	522 a	593 b
Medium	569 b	--	--
High	495 a	486 a	517 a
Standard error	22	14	16

1Values followed by the same letter are not significantly different (α=0.05) within each column.
2Low = 0%, Medium = 5%, and High = 8% seedborne PVY infection based upon post-harvest test.
3 Low = 3% and High = 34% seedborne PVY seed lots based upon post-harvest test.
4 Low = 2% and High = 11% seedborne PVY seed lots based upon post-harvest test.

TAKE-HOME HIGHLIGHTS

Findings of this research showed PVY infection impacts potato yield although yield reductions were not always observed. PVY infection and yield loss may not be a linear function in some cultivars. It was concluded that PVY impact on total yield was highly dependent on cultivar and level of seedborne PVY in the seed lot. The current study contributed relevant information for commercial producers to make seed purchasing decisions and anticipate losses associated with PVY on a farm level.

"Findings of this research showed PVY infection impacts potato yield although yield reductions were not always observed."

NEXT STEPS

Further evaluation on the impact that PVY has on a commercial production scale needs to continue to adequately assess the risk of seedborne PVY to the potato industry. The effect of PVY was observed, however differences in physiological age of the seed lots may have influenced the overall outcome on production.

Post-harvest potato mop-top virus detection

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BACKGROUND

Potato mop top virus (PMTV), vectored by the plasmodiophorid pathogen Spongospora *subterranea*, induces tuber necrosis. The necrotic arcs and rings induced by PMTV are similar to those associated with tobacco rattle virus, alfalfa mosaic virus, and potato virus Y (Fig. 1). PMTV is seed-borne and seed-transmitted, making effective and efficient seed testing paramount to disease management and limiting pathogen spread. Effective PMTV detection assays are available; however, our understanding of the relative effectiveness of PMTV detection over time is limited. Additionally, the cost and time involved in laboratory methods to test tubers for PMTV are significant. Increasing our understanding of optimal detection timing will allow seed testing agencies, breeding programs, and other stakeholders to more efficiently deploy resources to maximize testing effectiveness. Despite PMTV infection inducing tuber necrosis, tubers can be infected asymptomatically. Symptom expression is known to vary across cultivars and time in storage, but our knowledge of PMTV infection vs. symptom expression is limited. The lack of symptoms or signs in infected tubers makes molecular tools like PCR indispensable to test potato seed for PMTV

OBJECTIVES

North Dakota State University conducted trials to determine the
1) optimal timing for PMTV detection post-harvest;
2) susceptibility of 11 cultivars to PMTV.

WHAT WE DID

Seed-tubers from eleven potato cultivars were planted at a field site with known S. *subterranea* and PMTV disease pressure in 2020 and 2021. Following harvest, tubers were stored at 4˚C until processing. Beginning one month after harvest, and continuing monthly for 5 months, ten tubers were arbitrarily selected to be tested for PMTV following previously established methods, with minor modification. A 4mm disposable biopsy punch was used to excise a single tuber core, approximately 250 mg, from the stem and bud end of the tuber. Total RNA was extracted from each tuber core sample, and PMTV presence was assessed via RT-qPCR.

Figure 1. Potato mop-top virus symptoms in potato tubers.

"Effective PMTV detection assays are available; however, our understanding of the relative effectiveness of PMTV detection over time is limited."

WHAT WE FOUND

Differences were observed across time the testing was conducted and across cultivars. While some inconsistencies were observed between years, in both years, the highest detection frequencies were observed three-months post-harvest, significantly so when compared to one-month (Fig. 2). In 2020, testing at months two through six did not differ significantly, while in 2021 PMTV detection frequency at three-months was significantly higher than all other months. This finding will make seed testing more efficient, potentially decreasing false negative results. Additionally, this information could aid breeding programs in making selections more efficiently about advanced breeding lines resistant to PMTV. PMTV infection frequency ranged from 3% to 58% in 2020 and 9% to 51% in 2021 across cultivars (Fig. 3). PMTV was detected in Bannock Russet least frequently (<10%) in both 2020 and 2021, while Modoc and Atlantic were most frequently infected with PMTV. These results show cultivars vary significantly in PMTV infection rate, suggesting cultivar choice could be a potential management strategy for fields with known high disease pressure. Additionally, these results support the continued efforts in breeding for resistance to PMTV.

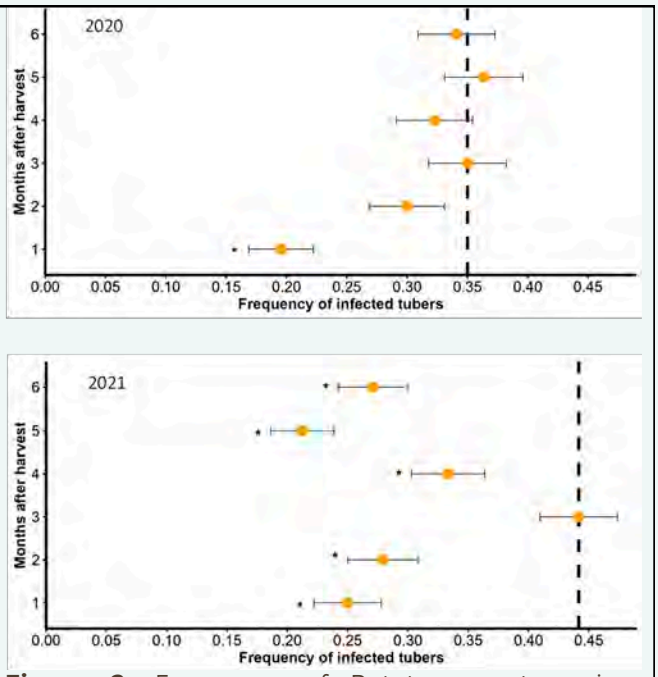

Figure 2. Frequency of Potato mop-top virus detection in potato tubers of 11 cultivars from one to six-months post-harvest in 2020 (top) and 2021 (bottom). Error bars based on standard variance of the mean. Values designated with an asterisk* are significantly different from three-months post-harvest. The dotted line represents the mean frequency 3 months post-harvest.

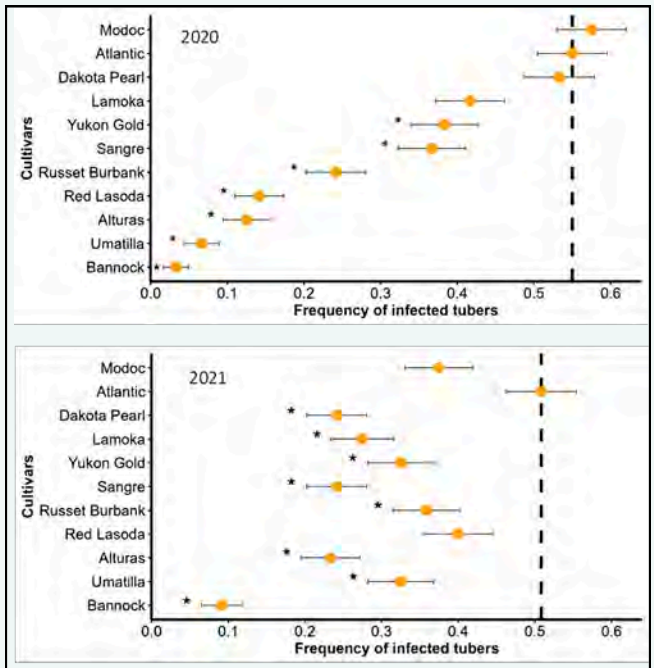

Figure 3. Frequency of Potato mop-top virus detection in potato tubers from one to six-months post-harvest in 2020 (top) and 2021 (bottom) across 11 cultivars. Error bars based on standard variance of the mean. Values designated with an asterisk* are significantly different from three-months post-harvest. The dotted line represents the mean frequency of cultivar Atlantic.

TAKE-HOME HIGHLIGHTS

Infection by PMTV can vary across trials, highlighting the need for consistent testing procedures conducted across several years. It is recommended to wait at least 3 months post-harvest to test for PMTV in tubers. Bannock was infected at very low levels (<10%) both years the trial was conducted.

NEXT STEPS

Screening potato cultivars and breeding material for resistance to PMTV will continue in order to accelerate the availability of PMTV resistance. Furthermore, we will investigate diversity in the PMTV vector, Spongospora *subterranea*, to aid in our understanding of resistance to it, and PMTV.

Does size of the mother seed tuber influence PVY infection level in the field?

NATHAN GELLES (GRADUATE STUDENT), RABECKA HENDRICKS, AND NORA OLSEN, UNIVERSITY OF IDAHO

BACKGROUND

The seed potato certification process has multiple steps to ensure quality seed is maintained throughout the system. Most commonly, there are two in-season field inspections, a storage inspection, a shipping point inspection for grade, and a post-harvest test. Currently most seed producing states in the US use the winter grow out (WGO) method to conduct post-harvest testing soon after harvest while others may use greenhouse grow outs or rely solely on in-season field inspections. The WGO is a process where whole- single drop tubers (1.5 to 4 oz) are collected at the time of harvest for each seed lot. The size of tubers in the seed lot may be larger or more variable in size, but the smaller sized tubers are desired for the WGO process for several reasons. The planting equipment cannot plant seed potatoes larger than approximately 4 oz without being cut or causing issues to the planting of the crop. Cut seed can lead to potential cross contamination and spread of disease through mechanical inoculation and the logistics involved with cutting seed would add an extra step and complexity to the WGO system, which could cause delays in the process. Although logistically easier to plant, the smaller sized tubers planted in the WGO may not represent the size range of tubers within a given seed lot.

Due to seed certification requiring single drop tubers to be submitted for WGO post-harvest testing, it is valuable to know if the size of the tuber dictates a preference for PVY accumulation. If so, this could cause an under or over- estimation of PVY in a seed lot. Although there is considerable research on PVY distribution within a plant, there is a gap in research determining if PVY preferentially accumulates in tubers based upon final size of the tuber.

OBJECTIVE

The objective of this study was to determine if seedborne PVY incidence in a seed lot is impacted by the size of the mother tuber used at planting. This would help clarify if seed certification agencies are inadvertently selecting potatoes with higher or lower levels of PVY due to tuber size restrictions during WGO sampling.

WHAT WE DID

Distribution of PVY within a seed potato lot was evaluated over two years, 2021 (year one) and 2022 (year two). Year one included two cultivars, Russet Norkotah (Selection 3) and Umatilla Russet. Russet Norkotah was sampled from a commercial grower storage and had an estimated 60% seedborne PVY infection based upon post-harvest testing. Umatilla Russet had an estimated 26% seedborne PVY infection. Year two included one cultivar, Russet Norkotah, which was sampled from a commercial seed grower storage and had an estimated 11% seedborne PVY based upon post-harvest testing. All samples were stored at 40 F and 95% RH at the University of Idaho Kimberly Research and Extension Center (KREC), Kimberly, ID. Prior to planting, tubers were classified into mother tuber size categories of a) single drop: 1.5 to 4 oz, b) small: 4 to 6 oz, c) medium: 6 to 8 oz, d) large: 10 to 12 oz, and e) mixed: a mixed sample (year one only).

Prior to planting, all tubers were cut into 2 to 3 oz seed pieces except for the single drop sample. The single drop category was left uncut to simulate the current WGO system. In year one, all seed pieces within size range from a cut mother tuber were used. Year two, only one bud or one stem seed piece (in a 1:1 ratio) was used from each cut mother tuber. Plots were planted in a randomized block design with five replicates and were grown at KREC fields according to University of Idaho management guidelines.

When plants reached approximately 30 cm tall (59 DAP year one and 68 DAP year two) visual evaluations were conducted to determine the incidence of seedborne PVY in each plot. Plants with questionable symptoms were tested using Agdia Immunostrip test kits. In addition, to confirm the accuracy of visual evaluation, each plant in the third replicate plot of Russet Norkotah was tested using Agdia Immunostrips. Leaf tissue samples were also collected from plants expressing PVY symptoms and sent to University of Idaho Virology Lab (Karasev) for PVY strain identification each year (~50% PVYntn and 50% PVYN-Wi).

Vines were mechanically flailed eight (year 1) and 14 (year 2) days prior to harvest. Each plot was mechanically harvested to determine total yield and grade on September 13, 2021 (145 DAP) and September 20, 2022 (152 DAP).

WHAT WE FOUND

Umatilla Russet plots were planted from an estimated 26% seedborne PVY seed lot. Visual in-season PVY incidence ranged from 38% to 43% with a mean of 40% and no significant differences in visual foliar symptoms between mother tuber size treatments were observed (Table 1). Russet Norkotah (selection 3) plots were planted from a seed lot with an estimated 60% seedborne PVY and in-season visual PVY incidence ranged from 68 to 73% with a mean of 70%. No significant differences in visual PVY incidence were observed between mother tuber size treatments (Table 1). The following year, Russet Norkotah plots were planted from a seed lot with an estimated 11% seedborne PVY infection. In-season visual PVY incidence ranged from 5 to 6% with a mean of 5.8% and no significant differences in PVY levels between mother tuber size treatments were observed (Table 1). Agdia Immunostrip test kit results confirmed the accuracy of the visual evaluations of one replicate and showed 71% PVY compared to 72% visual PVY and 7% compared to 6% visual in 2022.

Total yield, tuber number, harvested tuber size distribution, and USDA grade were impacted by the mother tuber treatment (Table 2). Typically, the single drop mother tuber treatment had higher total yield and tuber numbers than the large mother tuber treatment. Due to PVY levels being similar in each of the treatments, yield and grade differences were attributed to size of mother tuber rather than virus infection.

"PVY accumulated equally within tubers of previously infected mother plants without regards to final daughter tuber size."

TAKE-HOME HIGHLIGHTS

This study examined the potential of seed certification agencies selecting for higher or lower levels of PVY based upon tuber size restrictions for post-harvest WGO testing. In this study, several mother tuber size categories were sorted from Umatilla Russet and Russet Norkotah seed lots with one of the treatments simulating a sample collected for the WGO. There were no significant differences between observable PVY incidence for any of the mother tuber size categories regardless of cultivar, seed lot, seedborne PVY level, or year. These results indicated PVY accumulated equally within tubers of previously infected mother plants without regards to final daughter tuber size, which supports the claim that seed certification agencies are not preferentially selecting for higher or lower levels of PVY based upon tuber size restrictions.

Although not the focus of this study, insights were gained on the influence of cut compared to single drop seed and size of mother tuber that seed is taken from.

NEXT STEPS

Findings from this study could be used to instigate research studies on the agronomics and economics of using various sizes of mother seed tubers. Also, investigation into cut seed compared to whole seed tubers could be beneficial to the potato industry.

Table 1. Percent of plants showing visual seedborne foliar PVY symptoms for each mother tuber size treatment in Umatilla Russet and Russet Norkotah. All treatments were cut to 2 to 3 oz seed pieces except single drop was left uncut.

	Umatilla Russet	Russet Norkotah	
	Visual PVY infection (%)[1]		
Mother tuber size [2]	**2021**	**2021**	**2022**
Single drop	40 a	69 a	6 a
Small	43 a	73 a	5 a
Medium	40 a	68 a	6 a
Large	39 a	68 a	6 a
Mixed	38 a	70 a	N/A
Standard error	3	2	1

1Values followed by the same letter are not significantly different (α=0.05) within each column.
2Single Drop = 2 to 4 oz, Small = 4 to 6 oz, Medium = 6 to 10 oz, Large = 10 to 12 oz, Mixed = equal amounts of each mother tuber category. N/A = category not planted in 2022.

Table 2. Total harvested tuber yield (cwt/A) of five mother tuber size treatments of Umatilla Russet and Russet Norkotah. All treatments were cut to 2 to 3 oz seed pieces except single drop was left uncut.

	Total Yield (cwt/A) [1]		
Mother tuber size[2]	**Umatilla Russet 2021**	**Russet Norkotah 2021**	**Russet Norkotah 2022**
Single drop	642 c	545 b	566 a
Small	560 a	500 ab	543 a
Medium	592 abc	501 ab	536 a
Large	568 ab	473 a	523 a
Mixed	636 bc	486 a	NA
Standard error	25	18	13

1Values followed by the same letter are not significantly different (α=0.05) within each column.
2Single Drop = 2 to 4 oz, Small = 4 to 6 oz, Medium = 6 to 10 oz, Large = 10 to 12 oz, Mixed = equal amounts of each mother tuber category. N/A = category not planted in 2022.

OBJECTIVE 2

To improve virus-vector management through development of epidemiological models and through research-based recommendations for potato production

Mineral crop oils for PVY management under overhead irrigation

KELIE YOHO AND ERIK WENNINGER, UNIVERSITY OF IDAHO

BACKGROUND

Potato virus Y (PVY) management is challenging in part because the virus is transmitted non-persistently by many different aphid vectors that may transiently visit potato fields and infect plants within seconds to minutes. Many insecticides work too slowly to prevent PVY transmission by aphids to healthy plants. One potential option for managing PVY is the incorporation of mineral crop oil sprays into existing integrated pest management (IPM) programs. A good IPM program manages target pests while minimizing other off-target effects. Mineral crop oils have low environmental persistence, limited effects on nontarget organisms, and have been shown to reduce the transmission of non-persistent viruses when used consistently, making them a great candidate for IPM programs. However, nearly all the potato growing acreage in Idaho relies on overhead irrigation, and the efficacy of these oils under such conditions has yet to be evaluated. This knowledge gap has contributed to a slow adoption of crop oils into PVY-focused IPM programs in the region.

OBJECTIVES

Field trials were conducted to:

1) Evaluate the efficacy of mineral crop oils at reducing in-season spread of PVY under overhead irrigation

2) Determine how various PVY-focused IPM spray regimes impact beneficial arthropod communities in potato

WHAT WE DID

To assess the efficacy of the crop oils, 'Russet Burbank' potatoes grown under typical field conditions with hand-line irrigation were subjected to one of four IPM regimens, each lasting from emergence until vine kill: 1) an untreated check (UTC); 2) crop oil only – a weekly application of a 0.75% mineral crop oil (JMS Stylet Oil) solution; 3) insecticide only – an in-furrow application of imidacloprid (Admire Pro) followed by fortnightly applications of one of four rotating insecticides – pymetrozine (Fulfill), spirotetramat (Movento), flonicamid (Beleaf), and lambda-cyhalothrin (Lamcap II); 4) crop oil + insecticide – a combination of treatments 2 and 3. PVY infection was dependent on natural aphid and virus pressure in the field. Tuber samples were collected after harvest, grown in the greenhouse, and the resulting plants were tested for PVY infection using enzyme-linked immunosorbent assays (ELISAs). To evaluate responses of beneficial insects, vacuum samples were taken fortnightly throughout the growing season (Fig. 1). Captured insects were identified and counted to compare taxonomic richness and abundance among treatments.

Figure 1. Vacuum sampling was used to sample beneficial arthropods from the foliage of potato plots.

WHAT WE FOUND

The incorporation of mineral crop oils into IPM programs reduced the in-season spread of PVY in all trial years when compared to the UTC. Both the mineral oil-only treatment and the combination oil + insecticide treatment were successful in this regard, though the magnitude of the reduction varied by year and disease pressure. In years of relatively low PVY infection (UTC infection less than 12%), the oil-only and insecticide + oil combination treatments performed similarly, with infection rates 3-6% lower than the UTC. In years of high disease pressure (UTC infection at 44% and 69%), however, the combination treatment far outperformed the oil only treatment, with infection rates 21 and 23% lower than the UTC, respectively, compared to the oil only treatment's 14 and 8% lower rates (Table 1). No clear and consistent pattern in PVY infection was observed for the insecticide only IPM program; in some years the insecticide only treatment reduced PVY infection compared to the UTC, but in other years the infection rates between these two groups were comparable. Considering the nature of transmission of the virus, this is not an altogether unexpected outcome for this treatment. Thus far, no significant differences among the four treatments have been detected with respect to beneficial arthropod communities, an ideal outcome for an IPM program. One of the goals of IPM is environmental sustainability, or minimal impact on natural ecosystems. This indicates both that the mineral crop oil used has limited impact on nontarget arthropods, but also that the insecticides chosen for were sufficiently specific to aphids to limit negative effects on beneficials. Identification efforts are ongoing, however, and results of these analyses may differ when repeated with higher taxonomic resolution.

"Mineral crop oils can help reduce the in-season spread of PVY under overhead irrigation conditions."

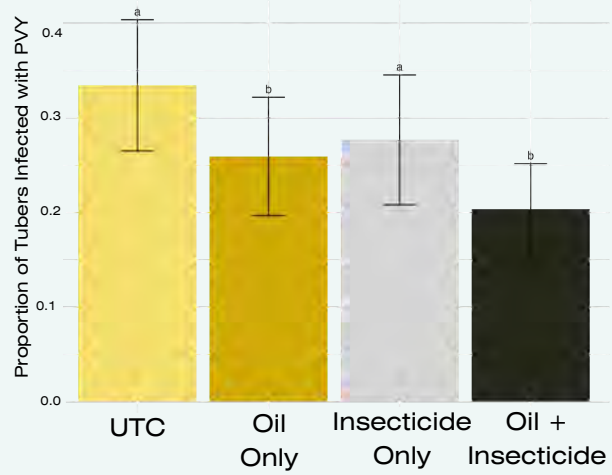

Figure 2. Mean proportion of infected tubers (± SEM) compared among treatments. Treatments marked by the same letter are not significantly different from each other (α = 0.05). The oil only and insecticide + oil treatments both significantly reduced the average rate of PVY infection compared to the UTC when all years were considered together.

TAKE-HOME HIGHLIGHTS

Mineral crop oils can help reduce the in-season spread of PVY under overhead irrigation conditions. This reduction in transmission is observed when crop oils are used alone but is even more evident when oil is used in combination with insecticides. The evidence thus far suggests that mineral crop oils do not significantly impact the species richness and abundances of predators and parasitoids, and as such should be conducive to preserving arthropod natural enemies that may further contribute to pest management.

NEXT STEPS

Continued efforts to fully identify the collected beneficial arthropods to species are in progress, and greater taxonomic resolution will allow for more robust evaluation of any nontarget effects of the treatment programs. Other ongoing work is focused on developing recommendations for potato growers regarding the timing of mineral crop oil applications. Relevant work includes identifying the timing of onset of a potato plant's innate resistance to PVY infection and development of predictive models for aphid migrations. These studies will provide insight into the necessary duration of oil sprays throughout the season as well as provide growers with resources to aid in making decisions regarding the need for mineral crop oil sprays.

Characterizing the microbiome of soil that suppress powdery scab

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HANNAH RIVEDAL AND TODD TEMPLE, USDA-ARS

BACKGROUND

Powdery scab of potato (Solanum tuberosum), is caused by infection of Spongospora *subterranea* (Sss) resulting in blemishes on the tuber, root and stolon hyperplasia, and can directly reduce tuber quality and fresh market value. Sss is the vector of PMTV, a virus that causes tuber necrosis which leads to buyer rejection of potatoes used for processing, seed production, and consumption. Although a high proportion of seed tubers and fields are infested with Sss, field-grown potatoes do not universally exhibit symptoms caused by Sss infection. This may be due, in part, to disease suppressive soils. A soil is regarded as disease suppressive if a pathogen cannot establish or persist, if the severity of disease declines over multiple plantings of a susceptible crop, or if no disease develops in the presence of a pathogen population. Over the past six decades, scientists have identified suppressive soils that possess a natural ability to suppress diseases. Recent research in New Zealand found that soil microorganisms played a primary role in disease suppression of powdery scab. This conclusion was supported by their observation that heating of suppressive soils to disrupt the native microbial community led to greater incidence and severity of powdery scab. The main goal of our project is to gain a deeper insight into the biotic and abiotic factors in soil that contribute to powdery scab incidence and severity, and identify microbial taxa and agricultural practices that contribute to disease suppression.

OBJECTIVE

Characterize soil microbial taxa linked to powdery scab suppressive activity and identify indicator species associated with disease suppression.

WHAT WE DID

We set up a greenhouse pot experiment to assay powdery scab suppressive activity of field soils from 10 locations. Soils were from potato and non-potato cropping systems in Oregon and included soils with suspected suppressive activity. Approximately, 12 gallons of each field soil was obtained from each location and chemical and physical properties were determined (e.g., Figure 1). Three different potting mixtures were created with each field soil to determine the relative importance of soil edaphic and soil biological factors leading to powdery scab suppressive activity (Figure 2). Eight replicate six-inch pots were prepared with each potting mixture and one seed tuber of cultivar 'Shepody' was planted in each of the pots. Emergence data were recorded and three pots of each treatment were destructively sampled and rated for powdery scab, assess for S. *subterranea* infection, and bulk and rhizosphere soils were sampled. The greenhouse experiment was repeated in 2023 with soils from six locations. Four replicate 3-gallon pots were prepared with each potting mixture and one minituber seed piece of cultivar 'Kennebec' was planted in each of the pots in the 2023 experiment. Soils were inoculated with S. *subterranea* and emergence was assessed. Again, the plants were destructively sampled, powdery scab galls were counted, root infection was estimated by PCR, plant and tuber biomass was estimated, and bulk and rhizosphere soils were sampled. DNA was extracted from bulk and rhizosphere soils for microbiome analysis, which is currently underway.

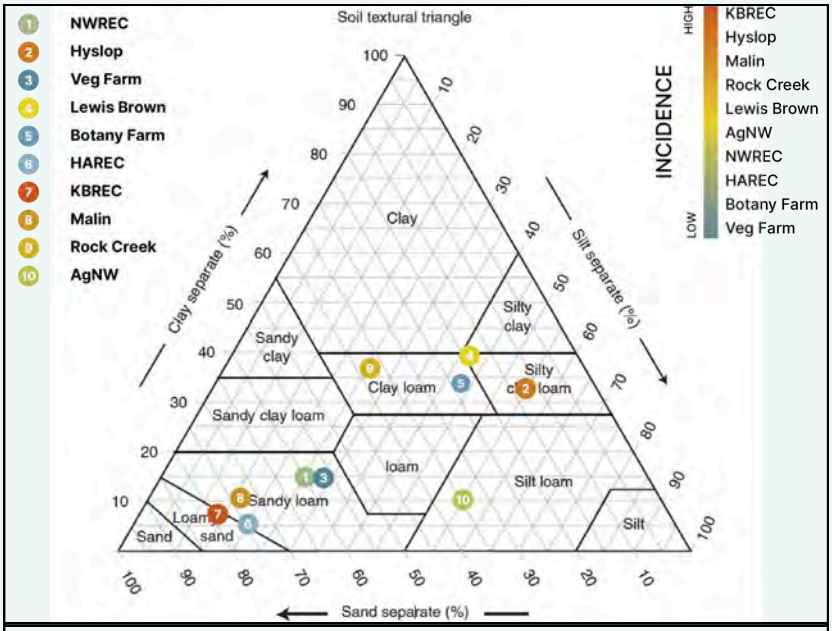

Figure 1. Soil texture triangle describing the diversity of soil textures used in the greenhouse experiment.

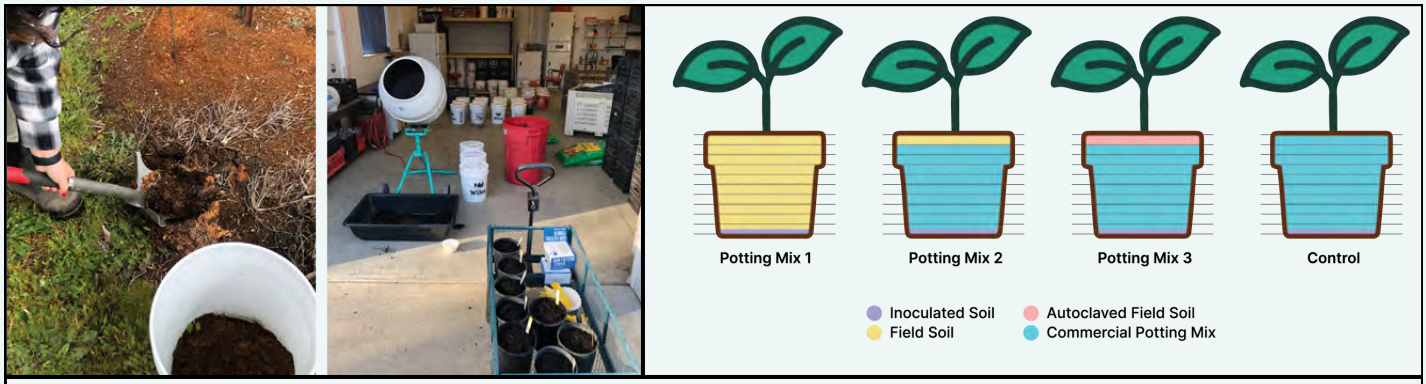

Figure 2. Sampling and mixing soils for the greenhouse suppressive soil assay. Three potting mixes were created using different amounts of field soil, soil medium, and inoculant medium.

WHAT WE FOUND

In our 2022 greenhouse experiment, differences in emergence occurred among the different soils and different potting mix treatments (data not shown). However, no visible disease symptoms (i.e., root galls) developed on any of the plant roots. We believe the summer greenhouse temperatures were too high for disease symptoms to occur. Based on PCR testing of the roots for the presence of S. *subterranea*, 2/3 of the potato plants growing in the control treatment were infected by the pathogen. Potato plants growing in other soil treatments were infected by S. *subterranea*, to varying degrees and it was difficult to determine if any of the soils had disease suppressive activity. In our 2023 greenhouse experiment, we did not observe clear differences in emergence among different soils and soil treatments (data not shown). Unlike in 2022, plants developed excellent root gall symptoms (Figure 3; root galls) and based on PCR testing, nearly all of the roots were infected with S. *subterranea* (data not shown). Disease incidence and severity varied by potting mix treatment and field soil. Both physical and biological components of the soil environment seemed to be playing a roll in root gall development. Additionally, there was a clear difference between disease development in live versus autoclaved treatments of some field soils (e.g., Fields G & I). In each of these soils, autoclaving the field soil resulted in greater root galling. This observation is consistent with disease suppression that is biological in nature.

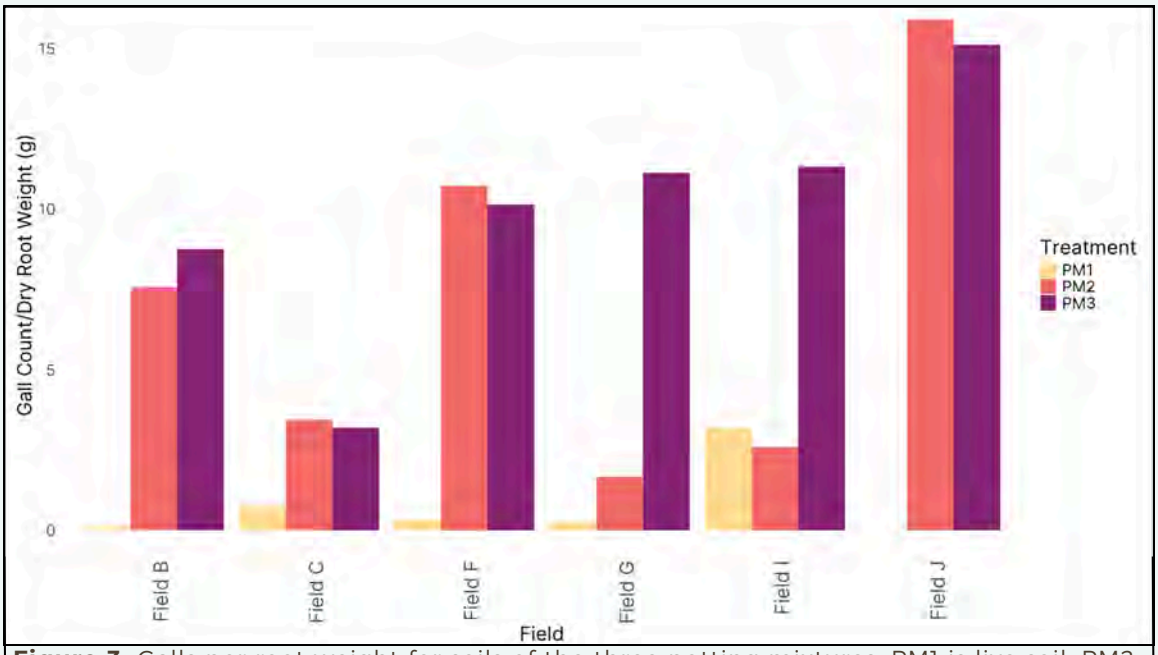

Figure 3. Galls per root weight for soils of the three potting mixtures, PM1 is live soil, PM2 is diluted live soil, and PM3 is diluted autoclaved soil. Differences in disease between PM2 and PM3 for Fields G & I are consistent with biological disease suppressive activity.

TAKE-HOME HIGHLIGHTS

We have developed greenhouse assays that help to identify soils with PS suppressive activity. We have identified a few soils with PS suppressive activity and are currently analyzing the bulk and rhizosphere soil microbiomes associated with PS suppressive and conducive soils.

What time during the growing season is most important for PVY in-season transmission?

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BACKGROUND

Potato virus Y (PVY) continues to pose challenges for seed potato growers. In Idaho, a potato seed lot is ineligible for re-certification (that is, seed to be used for further increase) if it has more than 1% incidence of PVY based on in-field visual evaluations and post-harvest laboratory testing. PVY is vectored by numerous species of aphids in a non-circulative, non-persistent manner. Mineral oil applications to potato foliage as means to reduce PVY in daughter tubers has been touted for decades, but this approach requires a substantial time commitment. Research suggests that mineral oil applications must occur at least weekly, beginning early in the growing season and continuing until vines are killed. Research from the University of Idaho has demonstrated the phenomenon of age-related resistance in a greenhouse experiment, where efficiency of mechanically transmitted PVY in 'Yukon Gold' potatoes dropped markedly 5-8 weeks after transplanting plantlets. We wondered if this same phenomenon might occur in the field, and if so, would it reduce the requirement of a season-long mineral oil regimen? To provide insight on when PVY spread is most important in Idaho (and thus, when mineral oil applications may need to be initiated or terminated), and whether harnessing age-related resistance under field conditions is feasible, we established an experiment in southern Idaho in 2021 and 2022, using potato variety 'Russet Burbank' and using the approach of physically excluding aphids at different times throughout the growing season.

Figure 1. Aphid-proof mesh was used to exclude aphids at different times during the growing season to identify when PVY is transmitted during the growing season in Idaho. This information may help determine when to initiate and terminate mineral oil applications. (Photo: E.J. Wenninger)

OBJECTIVE

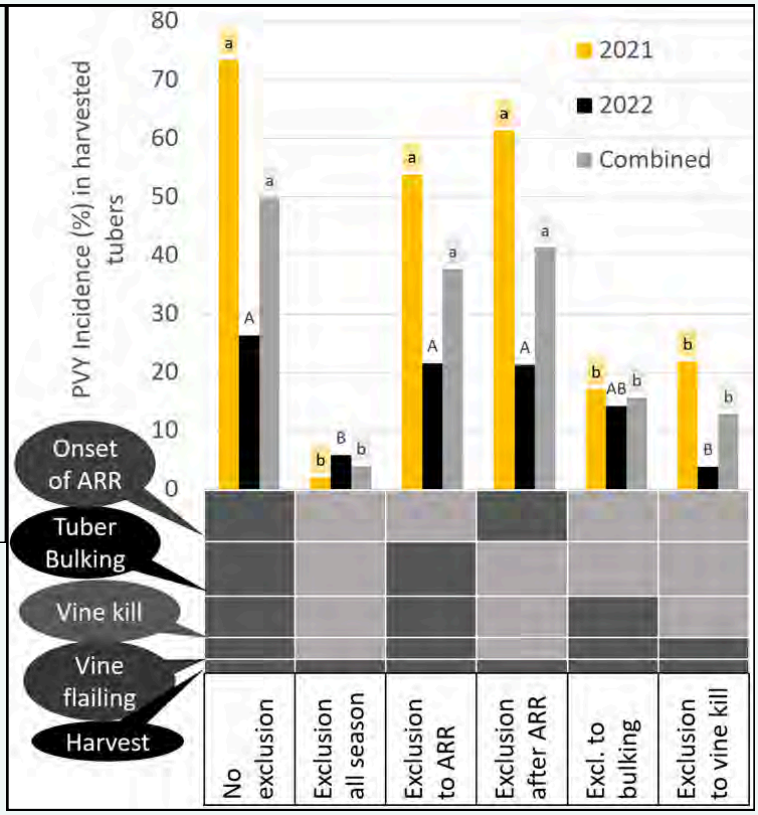
To determine if PVY incidence in daughter tubers from potato plants differs based on timing of exposure to aphids, and if protection is required after estimated onset of age-related resistance

WHAT WE DID

In 2021 and 2022, we planted Russet Burbank in one-row plots (50 feet long, 50 plants per plot) in Kimberly, Idaho. Plots were covered with an aphid-proof mesh at different times during the growing season. The mesh, shown in Figure 1, physically prevented aphids from landing on potato plants and potentially transmitting PVY. This experiment relied on natural inoculum of PVY. The six treatments (shown schematically in Figure 2) were as follows:

1. A non-treated positive control: no exclusion of aphids (plots were exposed all season)
2. A negative control: aphids excluded with aphid-proof mesh from before emergence until mechanical vine flailing
3. Aphids excluded from before emergence until estimated onset of age-related-resistance (4 weeks after full emergence)
4. Aphids excluded only from estimated onset of age-related resistance until mechanical vine flailing
5. Aphids excluded from before emergence until tuber bulking (about 12-13 weeks after planting)
6. Aphids excluded from before emergence until chemical vine desiccation

Figure 2. Influence of exposing potato plants to aphids at different times in the growing season on incidence of Potato virus Y in daughter tubers in 2021, 2022, and both years combined. Bars of the same color with the same letter do not significantly differ based on statistical tests (pairwise comparisons, alpha = 0.05). "Exclusion all season" refers to aphids excluded with an aphid-proof barrier until vines were mechanically flailed; "ARR" refers to age-related resistance (estimated to be 4 weeks after full emergence for Russet Burbank); "vine kill" refers to chemical desiccation with Reglone plus an adjuvant at the labeled rate.



At harvest, 50 tubers from each plot were arbitrarily sampled and subjected to a winter grow-out test in Hawaii. A leaf was picked from each plant that emerged and sent to our lab in Idaho Falls, where we tested leaves individually for presence of PVY using double-antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA).

WHAT WE FOUND

Post-harvest incidence of PVY ranged from 2.0 to 73.4% and 3.8 to 36.0% in 2021 and 2022, respectively. In each year, post-harvest incidence of PVY was highest for the treatment in which aphids were not excluded (Figure 2).

"These results support recommendations for PVY mitigation efforts to extend at least to onset of tuber bulking."

In addition, PVY incidence for the treatments where aphids were excluded from before emergence to age-related resistance or from age-related resistance to mechanical vine-flailing did not differ from the no-exclusion treatment or each other. Conversely, treatments where aphids were excluded for most of the growing season (from before emergence to mechanical vine flailing, tuber bulking, or chemical vine desiccation) experienced the lowest post-harvest incidence of PVY in both years, and incidences for these treatments did not differ from each other.

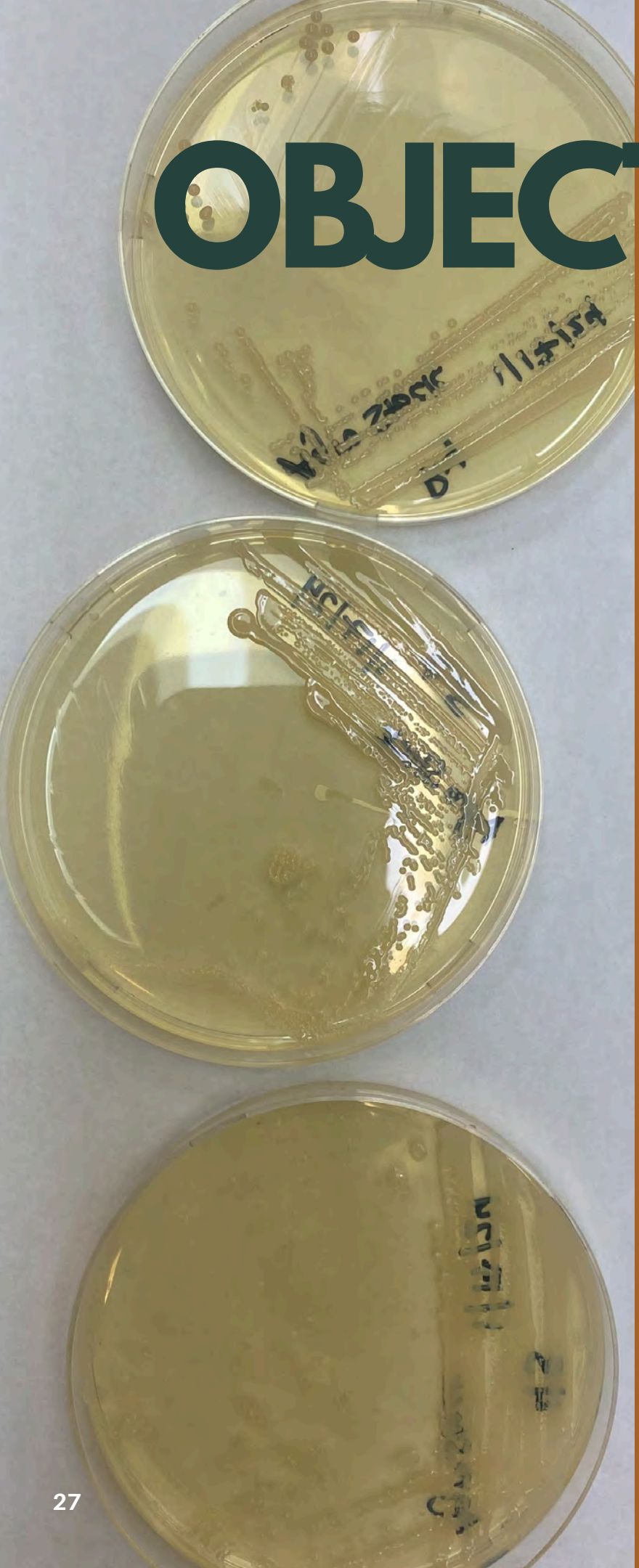
Mean PVY incidence was higher in 2021 compared to 2022 (45.6% vs. 20.9%, respectively), and this could be explained by greater disease pressure in 2021. As in individual years, PVY incidence for treatments encompassing age-related resistance did not differ from each other or from the no-exclusion treatment. Treatments that were covered for most of the growing season did not differ from each other and had significantly lower PVY incidence compared to no-exclusion and the two age-related resistance treatments.

TAKE-HOME HIGHLIGHTS

These results support recommendations for PVY mitigation efforts to extend at least to onset of tuber bulking. Furthermore, our data suggest that onset of age-related resistance may not contribute substantively to PVY management in a field setting until later in the season.

NEXT STEPS

Deeper insight into timing of aphid transmission of PVY during the growing season is needed, particularly for later times during the growing season. We will continue research in Aberdeen where we will focus on the impact of exposing plants to aphids at different time periods later in the season.



OBJECTIVE 3

To develop molecular markers for resistance genes against PVY and PMTV and Ss; to clone at least one PVY resistance gene; and to understand virus impacts on the physiology of stored potatoes

POTATO VIRUS INITIATIVE
DEVELOPING SOLUTIONS

Genetic analysis of resistance to recombinant PVY isolate ID-20 in multiple tetraploid potato populations

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BACKGROUND

Potato cultivars have been characterized with two different types of resistance to PVY conveyed by Ny genes and Ry genes. Ny genes tend to be PVY strain specific (typically non-recombinant PVY strains), however this type of response is widely scattered among existing cultivars. Ny gene-like response was identified for recombinant PVY strain NE-11 (isolate ID-20), in a cross of Yukon Gem x Russet Norkotah, however a small number of progeny exhibited resistance to viral spread (systemic resistance, assessed using ELISA and RT-PCR). Developing a better understanding of the genetics of this resistance may make it more assessable to growers.

OBJECTIVES

1) After the first cross, Yukon Gem x Russet Norkotah, generate additional populations for genetic evaluation of resistance.
2) Evaluate local and systemic responses and score progeny challenged with isolate ID-20.

WHAT WE DID

All hybridizations were conducted in a greenhouse environment in the early Fall or early Spring. Initially resistant F1 progeny were backcrossed to Russet Norkotah, generating two populations, YGRN147 and YGRN154. Selected F1 and BC1 resistant progeny were then intercrossed among individuals to generate additional populations.

All local and systemic responses were evaluated in growth chambers at the 6th Street Greenhouse in Moscow, ID using three reps per plant. PVY strain NE-11, isolate ID-20, was from the laboratory collection. All plants were maintained in tissue culture, in a virus-free environment, prior to transferring to ¾ gallon pots in a growth chamber (16 hour photoperiod, 68-72˚F). After three weeks of growth, plants were inoculated using carborundum with plant sap collected from positively infected White Barley Tobacco (Figure 1). Local (inoculated leaves) and systemic (upper, non-inoculated leaves) responses were evaluated visually, and using laboratory methods, ELISA and RT-PCR at 2 weeks and 4 weeks post-inoculation, respectively.

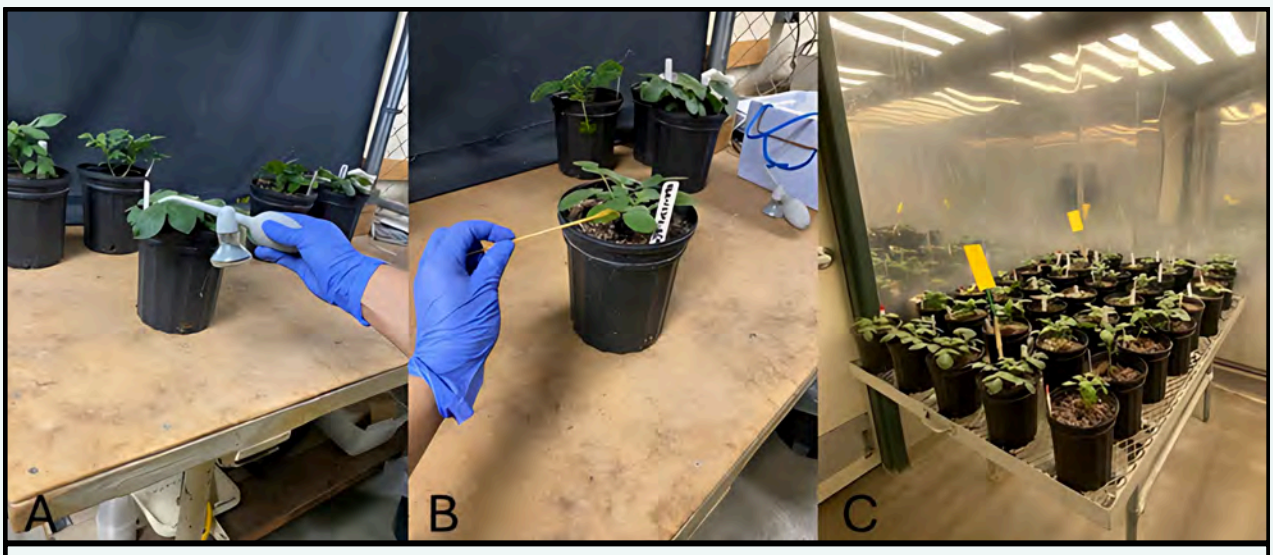


Figure 1. Inoculation of plants 3 weeks after transplanting from tissue culture to soil. A) Application of carborundum, B) rubbing inoculum into leaf, and C) plants arranged in growth chamber.

WHAT WE FOUND

Previous results identified 25 (14%) Yukon Gem x Russet Norkotah F1 progeny out of 176 total were resistant to systemic spread when inoculated with PVY isolated ID-20. Due to the low number of progeny with resistance, genetic analysis would be difficult. Two resistant progeny, YGRN147 and YGRN154, were backcrossed to Russet Norkotah to generate two BC1 populations. Thirty progeny from both populations were challenged with ID-20. While the YGRN147RN population yielded results similar to the original F1 population, 13% resistant progeny (4 out of 30), the YGRN154RN population exhibited 34% resistant progeny (10 out of 29, Table 1). While local and systemic responses were observed in similar numbers of progeny, the progeny identified were not the same, suggesting that the two responses segregate independently and may be controlled by different genes. Additional crosses were made using both F1 and BC1 progeny to generate populations with different segregation ratios. Interestingly, none of the crosses between F1 progeny were successful, however, successful crosses were made with BC1 progeny. In this case, only one BC1 progeny was successful as a male, YGRN154-RN9, which showed weakly local and systemic resistance. Other BC1 progeny were observed to have low pollen viability, which is not surprising considering the one round of inbreeding. Female BC1 progeny that produced seed are listed in Table 2 with their corresponding resistant response to ID-20.

Table 1. Evaluation of two BC1 populations with PVY isolated ID-20, evaluating two and four weeks post-inoculation (wpi) with ELISA and RT-PCR for local and systemic presence of PVY.

Population	#Evaluated	2 wpi local leaves	4 wpi systemic leaves
YGRN147RN	30	5	4
YGRN154RN	29	8	10

Table 2. Female BC1 progeny crossed with YGRN154-RN9 to generate intercrossed populations.

BC1 clone	Resistant response to ID-20	Number of Seeds
YGRN147-RN11	Systemic	2538
YGRN154-RN4	Weakly local & Systemic	1092
YGRN154-RN16	Local & Systemic	141
YGRN154-RN5	Weakly local & Systemic	172

Populations in Table 2 are currently being evaluated with PVY isolate ID-20.

TAKE-HOME HIGHLIGHTS

The BC1 YGRN154 population showed an increased number of progeny with resistance to systemic viral spread (10 out 29, 34%) compared to original F1 population (25 out 176, 14%). Preliminary data for the intercrossed population with YGRN147-RN11 suggests approximately 50% of progeny are resistant to systemic spread. Observations of higher numbers of resistant individuals with local and systemic resistance suggest that corresponding genetic region(s) will be identified.

NEXT STEPS

Continue to evaluate populations generated from intercrossing resistant BC1 progeny. Evaluate additional progeny for populations demonstrating simple segregation ratios. Map resistance to localize the corresponding resistance genes in the potato genome.

"Observations of higher numbers of resistant individuals with local and systemic resistance suggest that corresponding genetic region(s) will be identified."

Six recombinant variants of potato virus Y identified in North American potato cultivars grown in China

MARIANA RODRIGUEZ-RODRIGUEZ, MOHAMAD CHIKH-ALI, XUE FENG, AND ALEXANDER V. KARASEV, UNIVERSITY OF IDAHO

BACKGROUND

Potato virus Y (PVY) has flexuous filamentous particles and a positive-sense, single stranded RNA ca. 9.7-kb genome encoding a single open reading frame (ORF). PVY belongs to the genus Potyvirus (family Potyviridae) and exists as a complex of strains and genetic variants including multiple, more than 35, recombinants. China is one of the potato-growing countries with a great diversity of PVY recombinants described, but this diversity was addressed primarily for the local potato cultivars, and not studied in cultivars of North American origin.

OBJECTIVE

Here, a study was conducted to identify PVY strains circulating in North American potato cultivars grown in four provinces of China.

WHAT WE DID

A total of 26 potato samples from four North American potato cultivars, Blazer Russet, Ranger Russet, Russet Burbank, Shepody, and from unknown cultivar(s) were collected in June-July 2018 from potato originating in four provinces of China, Hebei, Inner Mongolia, Shaanxi, and Ningxia. Leaf samples from plants showing virus symptoms were collected from both seed and commercial fields and checked with the PVY-specific Immunostrip test. PVY-positive samples were printed on WhatmanTM FTATM PlantSaver Cards, these cards were dried at room temperature, sealed in a plastic bag with silica gel, and transported to the University of Idaho (UI) Virology Laboratory for further analysis.

Samples were typed to PVY strain using two multiplex RT-PCR assays. RNA was extracted from FTA cards and characteristic strain-specific bands were amplified using RT-PCR. Control isolates representing nine strains of PVY and additional recombinant variants were from the laboratory collection. RT-PCR assays confirmed PVY infection in 22 out of 26 samples printed onto the FTA cards and identified all of them as recombinants, but with unusual banding patterns in the RT-PCR that did not allow to type them to strain (Fig. 1). Six of the isolates representing these unusual banding patterns were selected for the whole genome sequencing and characterization.

The whole genome sequencing was conducted directly on overlapping RT-PCR fragments spanning the nearly complete PVY genome, using conventional Sanger methodology. Amplified PCR products were treated with Exosap-It and submitted for Sanger sequencing. Individual sequence reads were assembled using the SeqMan Pro program of the Lasergene14 Suite. Identity of the sequenced samples was determined using the BLAST program provided by the National Center for Biotechnology Information. For the multiple sequence alignment, MUSCLE implemented in MEGA 7 was used.

WHAT WE FOUND

Three recombinant types were revealed among the six sequenced PVY genomes (Fig. 2), one PVYN-Wi, three PVY-SYR-II, and two PVY-SYR-I, in agreement with the PVY strain composition determined for local Chinese potato cultivars. Examination of the sequences around the two main recombinant junctions (RJs), RJ2 and RJ3, revealed the shifts for the RJ breakpoint locations in Chinese PVY recombinants which may be responsible for the unusual banding patterns observed for the RT-PCR typing (Fig.1).

TAKE-HOME HIGHLIGHTS

Six genome sequences for potato virus Y (PVY) recombinants are reported from two North American potato cultivars grown in China. The coding complete sequences 9,609-9,634 nt-long encode a single open reading frame characteristic of potyviruses. The six sequenced PVY isolates represent three distinct recombinants of PVY, namely N-Wi, SYR-I, and SYR-II. Three of the recombinants had shifted recombinant break points accounting for unusual banding patterns in RT-PCR typing.

"Three of the recombinants had shifted recombinant break points accounting for unusual banding patterns in RT-PCR typing."

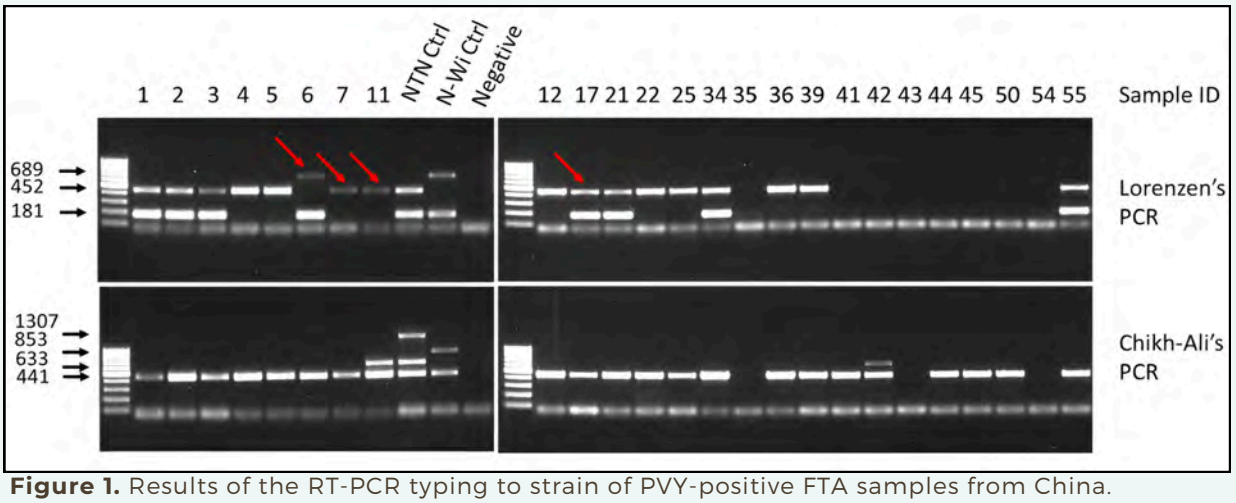


Figure 1. Results of the RT-PCR typing to strain of PVY-positive FTA samples from China.

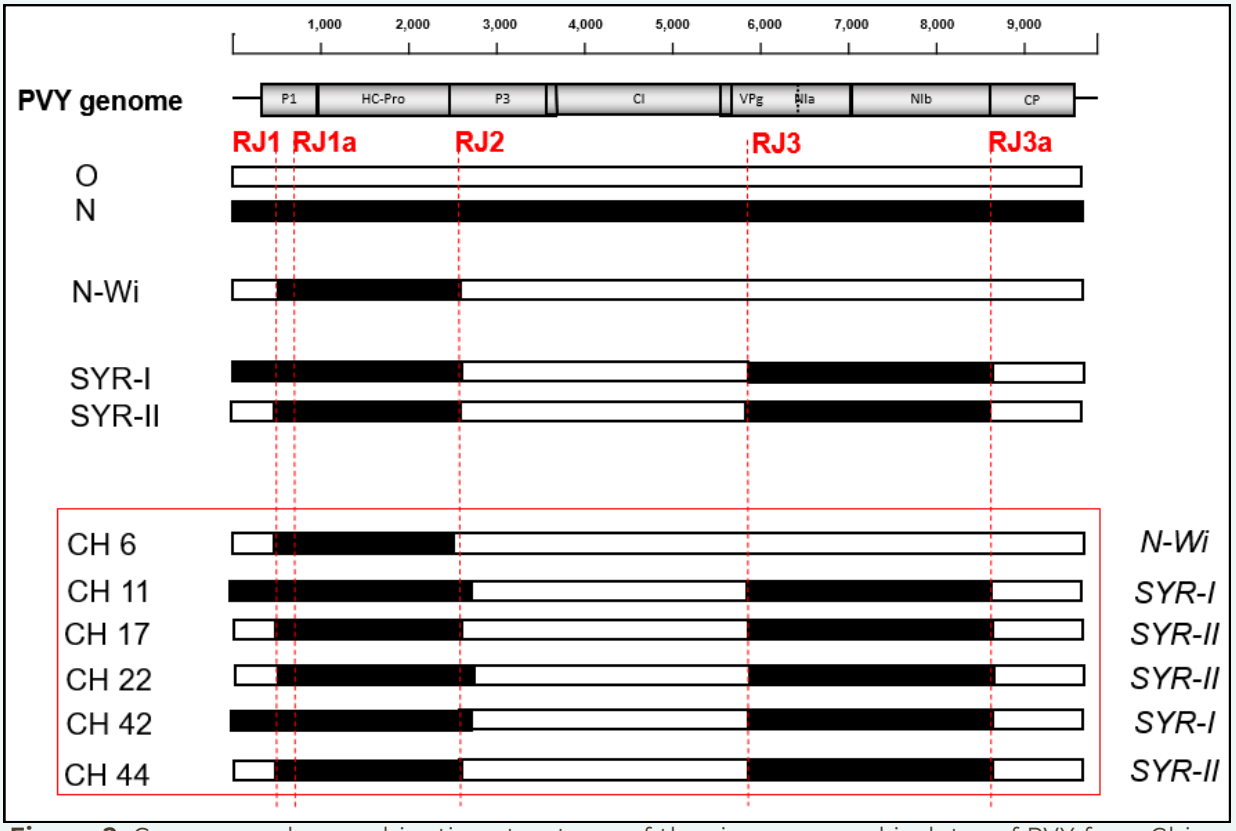


Figure 2. Genome and recombination structures of the six sequenced isolates of PVY from China.

NEXT STEPS

Given the high diversity of the PVY strains circulating in potato in China, resistance sources available in North American potato cultivars need to be tested against the local strains of the virus, specifically N-Wi, SYR-I, SYR-II, and SYR-III.

Screening wild potato germplasm for resistance to potato mop-top virus

KYLIE SWISHER GRIMM AND MAX FELDMAN, USDA-ARS

BACKGROUND

Potato mop-top virus (PMTV) induces internal tuber necrosis known as spraing that render a tuber unmarketable. While commercial cultivars may vary in their sensitivity to develop tuber necrotic symptoms, there are no commercially available cultivars with virus immunity. Therefore, even asymptomatic tubers can test positive for the virus by standard molecular detection methods. Currently, effective field treatments to diminish population levels of the PMTV vector, S. *subterranea*, in the soil are lacking, so the best long-term method to control against PMTV is host genetic resistance. Because no single resistance gene has been identified against PMTV, wild potato species could contain sources of resistance that, once integrated into cultivated potato, could be game changers for the commercial potato industry.

OBJECTIVE

The USDA Agricultural Research Service in Prosser, WA, conducted research trials to screen wild potato species for resistance to PMTV and/or S. *subterranea* in the greenhouse.

WHAT WE DID

Screen wild potato species for resistance to PMTV and/or S. subterranea in the greenhouse.

A total of 194 wild potato germplasm accessions were screened for PMTV resistance in the greenhouse. For 186 of these accessions, ≤20 plants were screened. For 8 accessions, seedlings were initially generated in tissue culture and ≤16 were transplanted into potting mix for the greenhouse screen. During the initial screen, 20 accessions (≤10 plants each) were planted in 4 inch pots containing potting mix (Figure 1a). For all subsequent accessions, conetainers were used to allow a higher number of plants to be screened simultaneously in the greenhouse (Figure 1b). The number of plants screened per accession was variable due to differences in germination rates and seedling quality. In addition to the wild potato germplasm accessions, 4 self-compatible diploid breeding lines and 13 tetraploid commercial cultivars or breeding clones were screened in conetainers. In every batch of plants screened, oatgrass and tobacco were screened as resistant and susceptible controls, respectively. All plants were inoculated with 20 sporosori/gram of soil when seedlings were approximately 3 to 6 inches in height. Plants were maintained in the greenhouse until 90 days post-inoculation (Figures 2). At this time, roots were harvested, symptoms of galling (Figure 3a and b) or lesions (Figure 3b) were recorded, roots were rinsed in tap water to remove all potting mix and stored in grinding bags at -20C for extraction. Total nucleic acid extraction of roots has begun, and testing for PMTV and S. *subterranea* is currently underway in the laboratory.

Figure 1. The greenhouse screen was successful with plants grown in both 4-inch pots (A) or in conetainers (B).

WHAT WE FOUND

Screen wild potato species for resistance to PMTV and/or S. subterranea in the greenhouse.

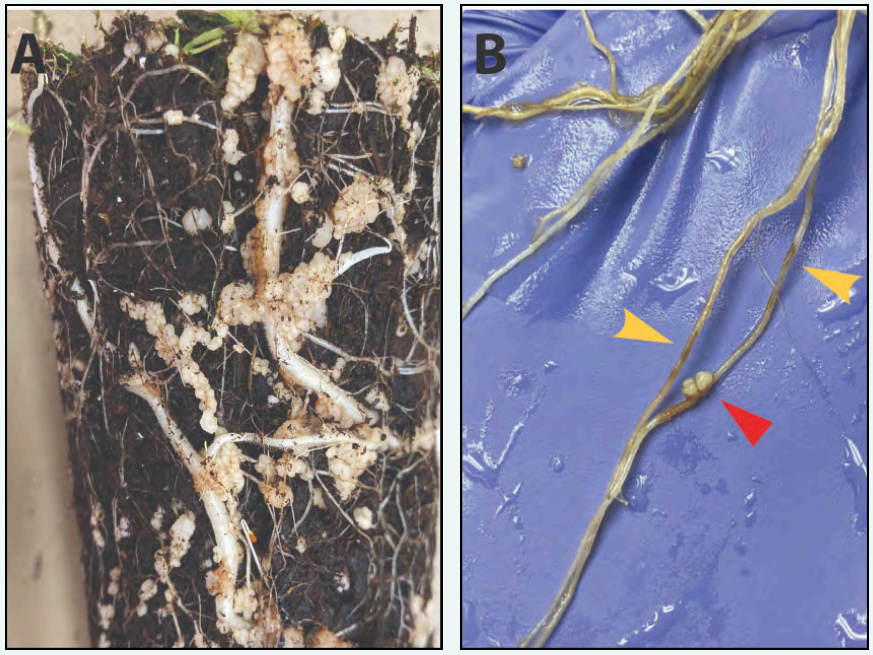
In all, 175 accessions successfully germinated and produced viable seedlings that could be screened using high disease pressure. A total of 1780 diploid wild potato germplasm plants, 68 self-compatible diploid breeding line plants, 165 tetraploid potato plants, and 107 resistant or susceptible control plants were successfully screened in batches, and roots were collected for pathogen testing. Of the 175 wild potato accessions, 56 did not show S. *subterranea* symptoms of root galling or lesions. Some level of galling and/or lesions were present on all other wild potato germplasm accessions, as well as the self-compatible diploid breeding lines and the tetraploid cultivars screened. Currently, 42% of roots have been tested for PMTV and S. *subterranea*. Of the accessions tested, nearly all became infected with S. *subterranea* (Figure 4a), signifying that the greenhouse screening protocol was successful. PMTV infection was found to be variable between different wild potato species (Figure 4b), as well as among accessions within an individual species. So far, 19 wild potato germplasm accessions were identified with potential resistance to PMTV (Table 1). Validation of the initial screening results will be conducted to confirm that these accessions have true resistance to PMTV or S. *subterranea*.

Table 1. Nineteen wild potato accessions have been identified with resistance to PMTV thus far. These accessions belong to 11 different species. Symptoms of galling or lesions were recorded for each plant and root tissue from each plant was tested for Spongospora *subterranea* (Sss) and PMTV. Validation of these potentially PMTV resistant accessions will be done soon. Abbreviations: Colorado potato beetle (CPB), Golden cyst nematode (GCN), Pale cyst nematode (PCN), Potato virus X (PVX), Potato virus Y (PVY), Root-knot nematode (RKN), Verticillium wilt (VERT).

Species	PI Accession	Total # of plants tested	Lesion or galling?	*Sss* (%)	PMTV (%)	Other traits
Solanum acaule	175395	11	None	45.5	0	PVX
Solanum berthaultii	414152	13	None	76.9	7.7	CPB
Solanum berthaultii	458364	10	None	90	0	CPB, PLRV
Solanum berthaultii	473218	12	None	75	0	CPB, VERT
Solanum berthaultii	473242	1	None	0	0	CPB, PLRV
Solanum berthaultii	473334	10	None	80	0	CPB, VERT
Solanum boliviense	210034	7	Unknown	100	0	GCN, PCN, VERT
Solanum boliviense	310928	11	None	63.6	9.1	RKN, PCN
Solanum brevicaule	205407	6	None	0	0	GCN, PCN, VERT
Solanum brevicaule	233692	2	None	0	0	GCN
Solanum brevicaule	275143	10	None	80	0	HAPLA
Solanum brevicaule	320299	12	None	50	8.3	PCN, VERT
Solanum candolleanum	365321	4	None	100	0	RKN
Solanum chacoense	133713	11	Some	100	0	Silver scurf
Solanum chacoense	195183	10	Unknown	100	0	PVY
Solanum medians	210045	5	None	60	0	VERT
Solanum microdontum	473174	16	None	81.3	6.3	NONE
Solanum multiinterruptum	498266	2	None	100	0	CPB, VERT
Solanum vernei	230468	10	Unknown	100	0	GCN, PCN
Solanum verrucosum	545747	12	None	100	0	Zebra chip

Figure 2. Containers were used to screen a majority of the potato germplasm, enabling a greater number of samples to be screened simultaneously.

Figure 3. Spongospora *subterranea* symptoms of galling (A and B, red arrow) and necrotic lesions (B, yellow arrows) were recorded for each root tissue harvested.



"A greenhouse assay to screen germplasm for PMTV and/or S. subterranea resistance was successfully generated at the USDA-ARS Prosser research laboratory."

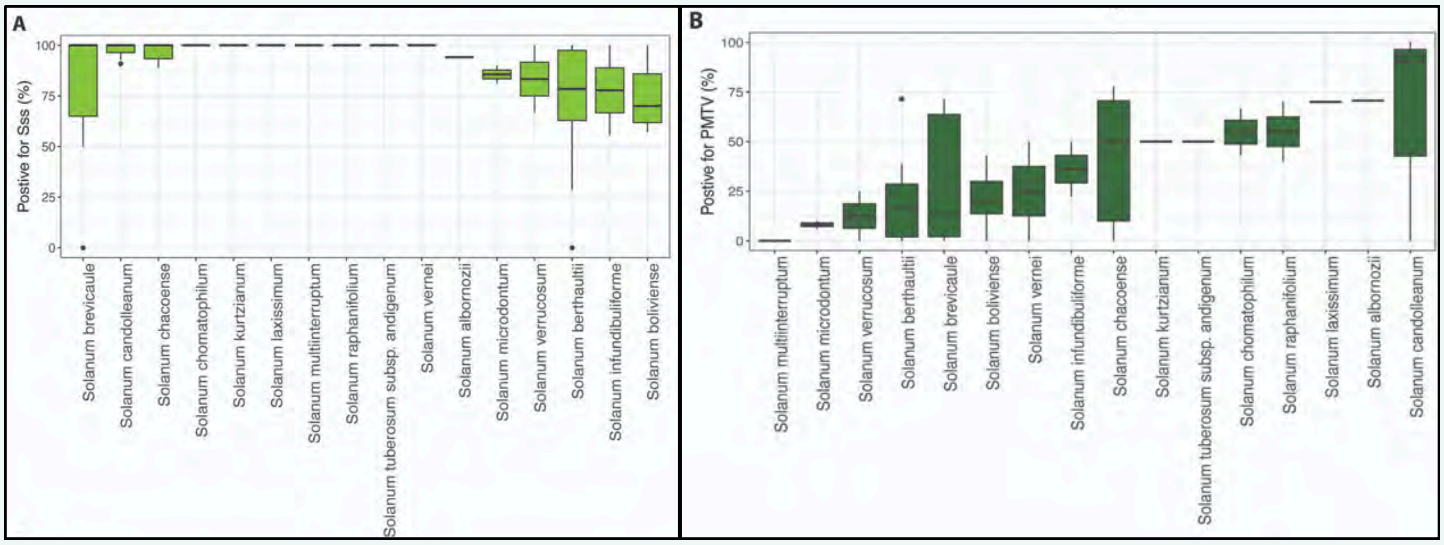


Figure 4. Among the wild potato germplasm tested for Spongospora *subterranea* and PMTV, nearly all were infected with S. *subterranea* (A), while PMTV infection among species was variable (B).

TAKE-HOME HIGHLIGHTS

A greenhouse assay to screen germplasm for PMTV and/or S. *subterranea* resistance was successfully generated and implemented at the USDA-ARS Prosser research laboratory. Diploid wild potato germplasm has been identified with possible resistance to PMTV, and it is anticipated that additional accessions will be identified in the near future upon completion of root testing. This information will be disseminated to breeders so that PMTV-resistant germplasm can be introduced into existing breeding programs.

NEXT STEPS

Remaining samples collected from the large greenhouse screen will be processed in the laboratory to determine PMTV and S. *subterranea* presence. Upon conclusion of this testing, potential PMTV-resistant accessions will be selected for validation by repeating the greenhouse PMTV screen. This assay can then be used to screen breeding populations to help identify specific genes conferring resistance to PMTV.

Potato mop-top virus in-field trials for resistance and symptom expression

JONATHAN WHITWORTH, NOELLE ANGLIN, USDA-ARS

BACKGROUND

Potato mop-top virus (PMTV) can cause tuber defects and reduce quality. This virus is transmitted by the organism, Spongospora *subterranea*, that causes powdery scab on potatoes. This organism can be found in the soil and can be transmitted whenever virus infected S. *subterranea* spore balls move from one field to another either in soil movement or potato movement. PMTV is an emerging disease which still requires a lot of research to understand symptoms, variety interactions, and methods for control.

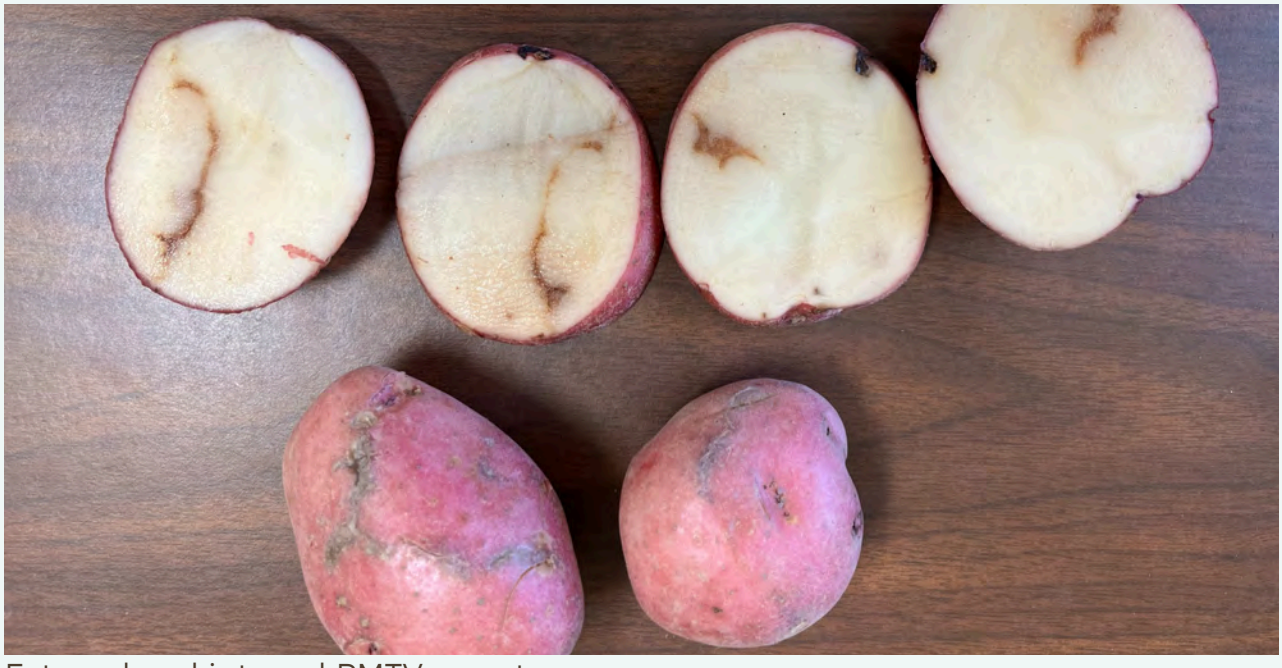
OBJECTIVE

For PMTV, field plots are used to grow popular varieties in infested fields. Samples are taken at harvest and tested for the presence of the virus and tuber symptoms. After long term storage, samples are again taken to record the level of virus and tuber symptoms. These measurements allow determination of the level of resistance, tolerance, and symptom expression.

WHAT WE DID

For PMTV, identical plots were planted in two field locations in growing regions in Idaho with different growing degree days (GDD) (SE Idaho 1670 GDD; S Idaho 2301 GDD in 2023). Seven widely grown russet varieties were planted and samples were collected at harvest. Slices from the stem and bud end of each tuber were taken and tested with qPCR to detect the level of virus in the sample. Additional tubers were cut and visual symptoms were recorded. After storage of approximately 200 days samples were again taken and tested for virus and symptoms were recorded.

"PMTV is an emerging disease which still requires a lot of research."



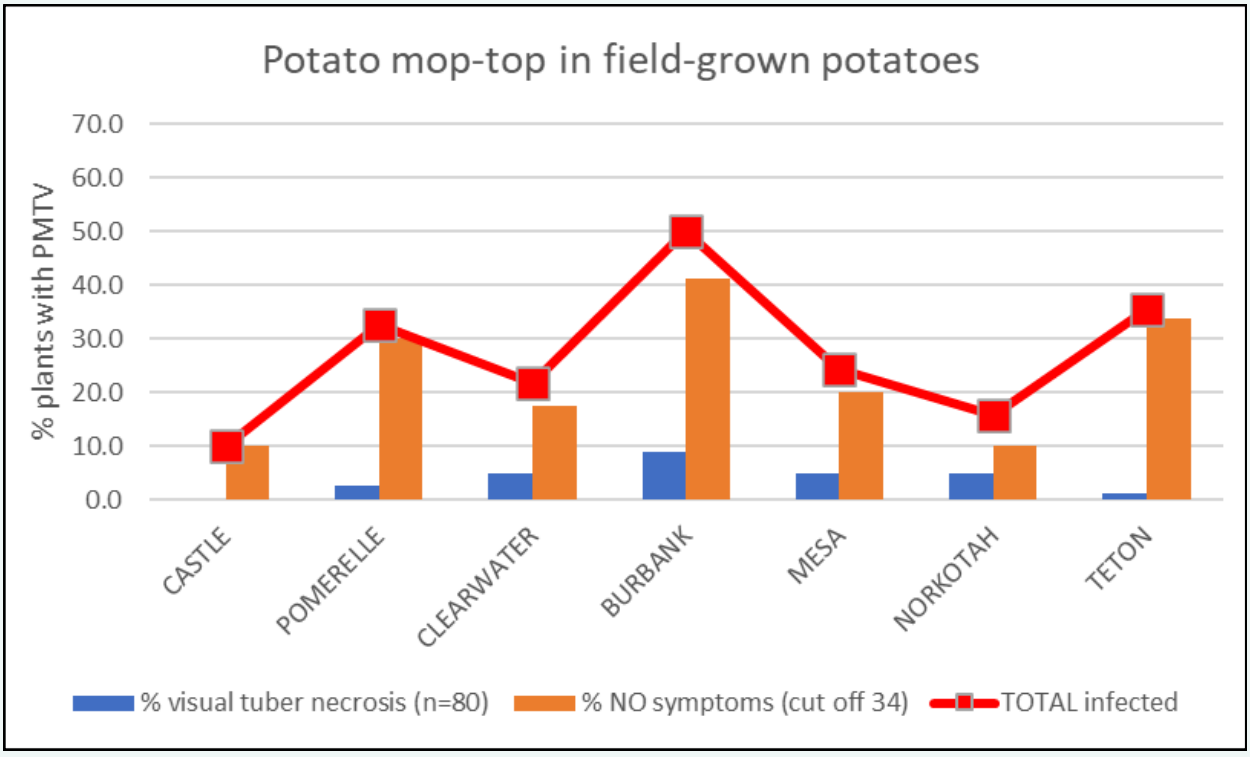
External and internal PMTV symptoms

WHAT WE FOUND

For PMTV, the field plots showed that all seven varieties had some level of PMTV infection with no tuber symptoms present. In the variety Castle Russet, no symptoms were present but a low % of tubers were infected with the virus. In the remaining varieties tuber symptoms ranged from 1.3% to 8.8%. For virus presence with no symptoms the range for all seven varieties was from 10% to 41.3%.

"Resistance to PMTV needs to be incorporated into new varieties but resistance to the virus and not just the symptoms must be found."

Necrotic tuber symptoms caused by PMTV



TAKE-HOME HIGHLIGHTS

For PMTV, the field plots showed that complete resistance to the virus doesn't exist in the widely grown russet varieties. It also showed the problem that exists in trying to control this virus by relying on visual symptoms. Resistance to PMTV needs to be incorporated into new varieties but first resistance to the virus and not just the symptoms must be found.

NEXT STEPS

PMTV work will continue with breeding accessions and varieties with reported resistance screened in a virus infested field. Populations and selections from PMTV targeted crosses will be screened in this field.

Payette Russet, harboring Rysto resistance gene, is immune to 12 genetic variants of potato virus Y

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BACKGROUND

PVY exists as a complex of strains and genetic variants which can be defined molecularly and, sometimes, biologically. Molecular characterization of PVY strains revealed that PVYO, PVYN, and PVYC had non-recombinant genomes that formed three separate phylogenetic clades. PVYZ, on the other hand, was classified as either PVYNTN or PVYNTN-NW recombinant based on molecular characteristics. There are multiple other recombinants, at least 35, most often built of PVYO and PVYN parental sequences, named PVYN-Wi, PVYN:O, PVY-NE11 and others, but these were not defined genetically and were classified only based on molecular properties.

Figure 1. Payette Russet plant inoculated with PVY isolate N1 (strain N-Wi), 29 days post-inoculation (photo credit: C. Funke).

Two types of genes confer resistance to potato virus Y (PVY) in potato. R genes confer an extreme resistance (ER) or immunity which is very durable and is effective against a broad range of virus strains. Phenotypically, ER manifests itself as lack of any symptoms in an inoculated leaf and no detectable virus infection. N genes confer a hypersensitive resistance (HR) response where a small group of plant cells infected with the virus die, forming a necrotic lesion which often restricts further movement of the virus outside of this lesion. Occasionally, when the virus spread is not completely restricted, the infection may spread through the entire plant, and in this case the HR reaction becomes systemic, visible as various types of systemic necrosis, such as vein necrosis, leaf drop syndrome, and stem streaking. Unlike ER, HR is strain specific, and very sensitive to environmental factors, especially temperature – it can be broken due to changes in the temperature. A few years ago, Payette Russet, a dual-use commercial cultivar, was released harboring Rysto resistance gene (Novy et al. 2017). The presence and inheritance of this Rysto gene was inferred based on molecular markers linked to this resistance genes, and although in field experiments Payette Russet was found to be PVY-resistant, the strain of the challenging virus was not disclosed, and hence additional testing of the susceptibility of this cultivar to a multitude of PVY strains and genetic variants was desirable.

OBJECTIVES

Here, a study was conducted to screen three American cultivars for various resistance sources to PVY strains, including N and R resistance genes. Specifically, three potato cultivars were studied under greenhouse conditions for their ability to elicit a resistance response against four of the most common strains of PVY. The cultivars Dark Red Norland, Chieftain, and Payette Russet were tested against strains PVYO, PVYNTN, PVYN-Wi, and PVYEu-N in search of the HR reaction or immunity to a virus challenge.

Payette Russet, known to have an extreme resistance gene Rysto in its genetic background (Novy et al. 2017), was challenged with additional five strains and three genetic variants of PVY to evaluate robustness of its broad PVY resistance due to the presence and efficiency of the Rysto gene.

WHAT WE DID

The collection of 12 PVY strains and genetic variants maintained in the Virology Laboratory of the University of Idaho, included virus isolates from the Pacific Northwest of the U.S., but also some PVY isolates collected in other states and obtained from collaborators in other countries. These genetic variants of PVY represented a large set of PVY isolates from potato and non-potato hosts exhibiting various pathotypes in potato, tobacco, pepper, cape gooseberry, and tamarillo (Table 1). Infected tobacco tissue was used as an inoculum source for the potato plants. Potato plants were mechanically inoculated at the six- to ten-leaf stage; three terminal leaflets on three leaves per plant were inoculated. PVY presence in inoculated and non-inoculated leaves, was tested in a TAS-ELISA format. All tests included control PVY isolates from the laboratory collection, with distinct serological patterns characteristic of PVYO and PVYN strains.

WHAT WE FOUND

Cultivars Dark Red Norland and Chieftain exhibited strain-specific, hypersensitive resistance to PVYO and PVYNTN strains. These same two cultivars, Dark Red Norland and Chieftain, appeared to have an additional resistance source in their genomes providing partial resistance against PVYN-Wi but were found fully susceptible to the non-recombinant PVYEu-N strain. Payette Russet was found immune to the same four strains of PVY; PVYO, PVYEu-N, PVYN-Wi, and PVYNTN, and was additionally challenged with the total of 18 isolates of PVY representing 12 genetic variants of the virus from potato and non-potato solanaceous hosts. None of the 18 isolates of the virus was found able to replicate in the inoculated or upper non-inoculated leaves of Payette Russet (fig. 1), confirming the broad specificity of the Rysto gene present in the Payette Russet genome.

TAKE-HOME HIGHLIGHTS

This suggested that the Rysto resistance gene present in the genome of Payette Russet indeed conferred an ER against nine strains and additional three genetic variants of PVY maintained in our PVY collection. These tested isolates of PVY represented all genetic diversity of PVY found so far in the U.S., and thus Payette Russet may be deemed fully PVY-resistant or completely immune to PVY within the boundaries of the U.S. or even in North America.

Table 1. Molecular and phenotypic traits of the PVY isolates used in this study.

Isolates	Strain[a)]	Genotype	Tobacco bioassay[b)]	Serotype	Genome sequence[c)]	Reference
Tb60	PVY^{O}	PVY^{O}	Mos	O	NA	Lorenzen et al. 2006a
Oz	PVY^{O}	PVY^{O}	Mos	O	EF026074	Baldauf et al. 2006
ID269	PVY^{O}	PVY^{O}-O5	Mos	O5	FJ643477	Karasev et al. 2010
N1	PVY^{N-Wi}	PVY^{N-Wi}	VN	O	HQ912863	Karasev et al. 2011
Alt	$PVY^{N:O}$	$PVY^{N:O}$	VN	O	AY884985	Lorenzen et al. 2006a
Pondo4	261-4	261-4	VN	O	KY848023	Green et al. 2017a
Mont	PVY^{N}	PVY^{N}	VN	N	AY884983	Lorenzen et al. 2006a
HR1	PVY^{Z}	PVY^{NTN} (*syn.* PVY^{Z}-NTN)	VN	N	FJ204166	Hu et al. 2009
L26	PVY^{Z}	PVY^{NTN} (*syn.* PVY^{Z}-NTN)	Mos	N	FJ204165	Hu et al. 2009
NE-11	NE-11	NE-11 (long)	VN	N	DQ157180	Piche et al. 2008; Green et al. 2017a
ID20	NE-11	NE-11 (short)	VN	N	HQ912867	Karasev et al. 2011; Green et al. 2017a
PVY-AGA	E	E	VN	N/AST	JF928459	Galvino-Costa et al. 2012
HI-14	C	C1	Mos	O	KX580384	Chikh-Ali et al. 2016
Poha2	C	C-Poha	Mos	O	MF134862	Green et al. 2017b
Poha6	C	C-Poha	Mos	-	MF134866	Green et al. 2017b
Tam13	SA-N	Tamarillo	VN	N	MT380736	Green et al. 2020a
Tam15	SA-N	Tamarillo	VN	-	MT380738	Green et al. 2020a
Tam17	SA-N	Tamarillo	NS	N	MT380740	Green et al. 2020a

NEXT STEPS

Since Payette Russet harbors a very robust, broad resistance source against PVY, presented by the Rysto gene, this cultivar may be confidently recommended as a parent for further breeding efforts introgressing this valuable source of resistance into commercial potato cultivars.

"Although in [previous] field experiments Payette Russet was found to be PVY-resistant, the strain of the challenging virus was not disclosed."

Breeding for resistance to tuber necrotic viruses

RICH NOVY, USDA-ARS

BACKGROUND

PVY and PMTV resistance genes have been identified and are being utilized in the Aberdeen, ID potato breeding for the development of new potato varieties. Host plant resistance is a valuable component in an integrated approach for the management of these tuber necrotic viruses.

OBJECTIVE

Identify and utilize genes for resistance to tuber necrotic viruses in the development of new potato varieties.

WHAT WE DID

Directed hybridizations between PMTV and PVY resistant potato clones resulted in new progenies that can become new potato varieties with resistance to these viruses. Host plant resistance is an important component of an integrated approach for the management of these two tuber necrotic viruses.

WHAT WE FOUND

Genes for extreme resistance to all strains of PVY have been successfully incorporated into breeding clones that have potential for release as new potato cultivars. One of the more promising PVY-resistant breeding clones from our program is A12305-2adg. This breeding clone is in its third and final year of evaluations in the Western Regional Potato Variety Trials and is being considered for release as a new variety in 2025 that could be used for both processing and fresh-pack. It is notable for its high yields in both early and full-season trials, its high field merit, and it has successfully completed all three years of the National Fry Processing Trials (NFPT) with acceptable fry processing characteristics. A12305-2adg has a gene, Ryadg, which confers extreme resistance to all strains of PVY. It is also notable for having resistance to Verticillium/early die and late blight.

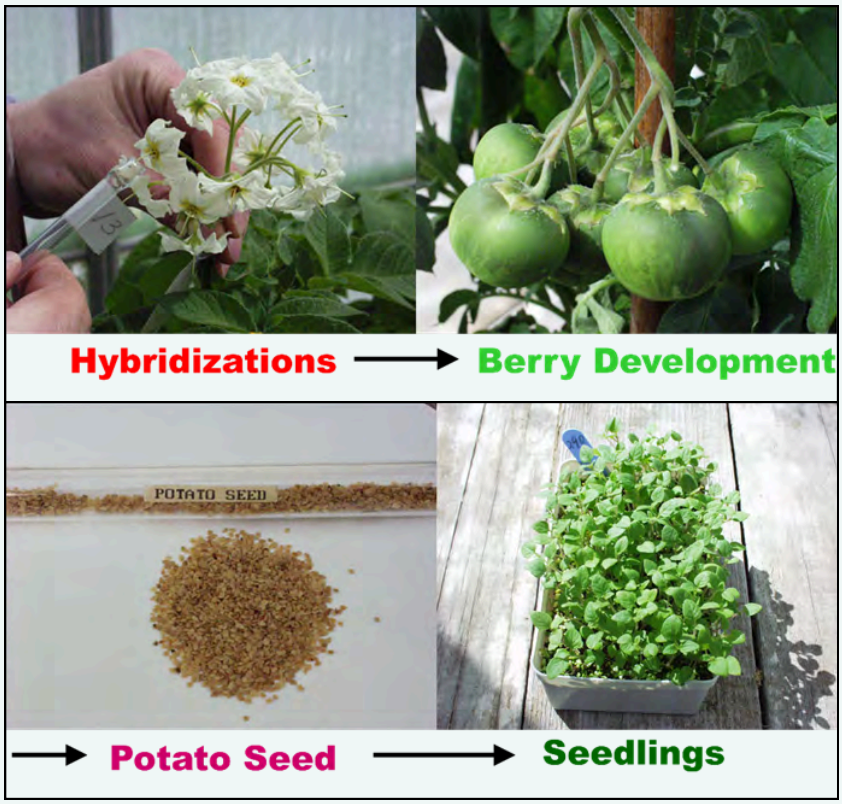


Photo 1. The generation of new PVY and PMTV resistant potato varieties involves hybridizing parental clones in the greenhouse, the development of potato berries, extraction of true potato seed from the berries, germination of potato seedlings, and years of trialing of promising new breeding clones relative to industry standard varieties such as Russet Burbank.

TAKE-HOME HIGHLIGHTS

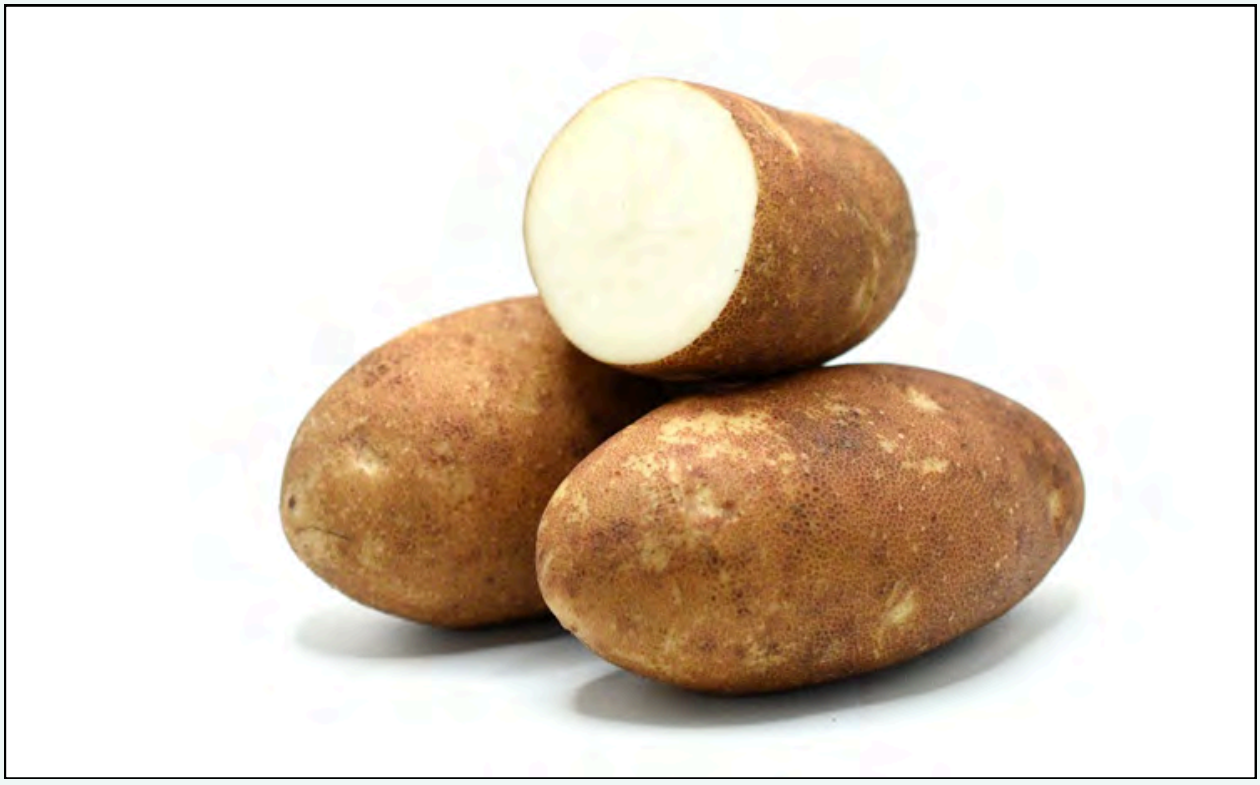
Genes for extreme resistance to PVY are successfully being utilized in the Aberdeen, Idaho and Tri-State potato breeding programs. Molecular markers associated with these genes for resistance have been useful in more rapidly identifying and incorporating PVY resistance into potato breeding clones having potential for release as new PVY-resistant potato varieties.

Research efforts have also been made in characterizing PMTV resistance derived from 'Castle Russet'. Unlike PVY resistance mediated by single Ry genes, PMTV resistance involves many chromosomal regions which is proving more challenging in the development of PMTV-resistant potato varieties.

NEXT STEPS

The continued development of PVY and PMTV resistant potato varieties, with A12305-2adg being a potential new PVY-resistant variety to be released in 2025 that can prove useful in the management of PVY.

Photo 2. Tubers of A12305-2adg: A promising new breeding clone having extreme resistance to PVY. Photo courtesy of Jenny Durrin-Gentry, Potato Variety Management Institute (PVMI).



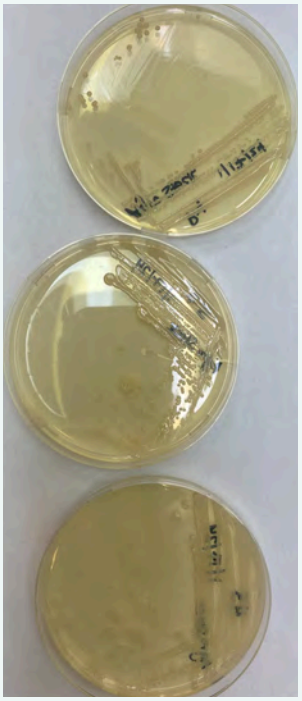
"Molecular markers associated with these genes for resistance have been useful in more rapidly identifying and incorporating PVY resistance into potato breeding clones having potential for release as new PVY-resistant potato varieties."

Progress towards cloning Ry-adg: A resistance gene against potato virus Y

PIA SPYCHALLA, WALTER DE JONG, CORNELL UNIVERSITY
SHENGWEI HU, SAGAR SATHUVALLI, OREGON STATE UNIVERSITY

BACKGROUND

Potato Virus Y (PVY) is a major pathogen of potato worldwide. PVY has the potential to reduce yield by 80% through stunting growth and/or eliciting necrosis in tubers, so it is important to develop effective control measures. One straightforward approach is to breed cultivars that don't become infected with PVY. A gene that confers such immunity to PVY is Ry-adg. Originating from Solanum tuberosum subspecies andigena, Ry-adg confers extreme resistance to all strains of PVY. Cloning this gene would help breeders develop PVY resistant cultivars in two different ways: first, by making it possible to develop assays that can track its presence in conventional crossing and selection schemes with 100% accuracy, and second, because the gene could be inserted into existing successful cultivars so that they are no longer susceptible to PVY. Ry-adg has previously been mapped to chromosome 11 and has been successfully tracked in breeding programs, albeit imperfectly, with various markers including RYSC3, M45, and M6.



OBJECTIVES

In conjunction with researchers at Oregon State University, Cornell researchers have conducted research to:

1) identify candidate genes for Ry-adg

2) test whether these candidate genes confer resistance to PVY

WHAT WE DID

CLONING CANDIDATE GENES INTO PLANT TRANSFORMATION VECTOR

Previous research conducted at the International Potato Center (CIP) narrowed the location of Ry-adg down to an interval in between markers M45 and M6. Within this region, we identified two genes that share sequence similarities to other known virus resistance genes. We synthesized these genes and after amplifying them through PCR, cloned each into the plant transformation vector pGWB414 using the Gateway Cloning method (Figure 1). This vector utilizes a CaMV 35S promoter to drive gene expression. These constructs were then transformed into Agrobacterium, which in turn delivers genes into plants, enabling us to test their function.

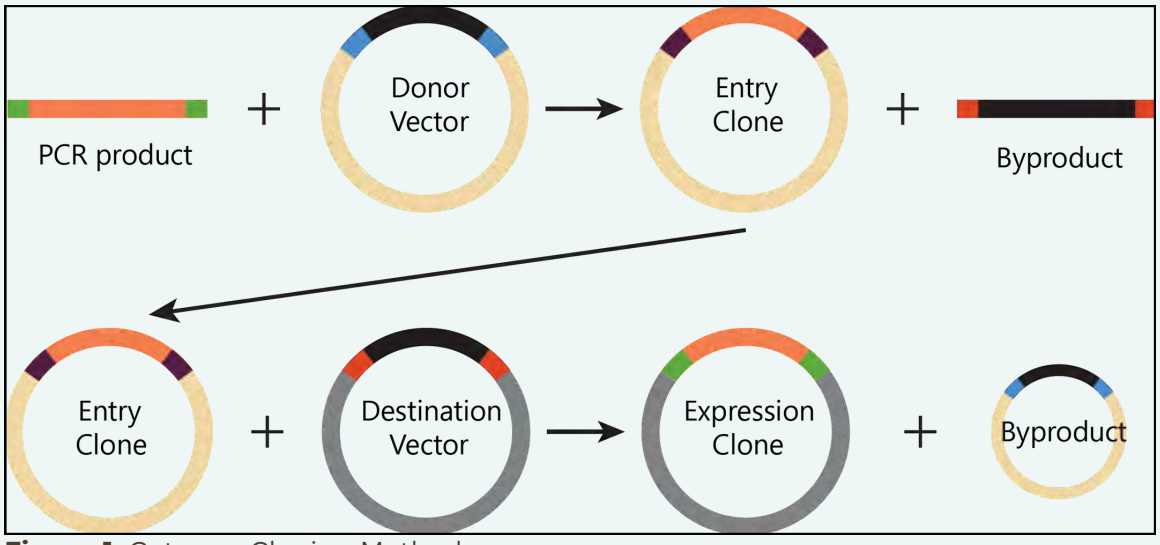


Figure 1. Gateway Cloning Method

ASSAYS FOR RESISTANCE TRANSIENT EXPRESSION ASSAY

We used a transient expression assay to temporarily express candidate genes by injecting Agrobacterium into the inside of tobacco leaves that had previously been infected with PVY. As a positive control, we injected a known PVY resistance gene called Ry-sto, and as a negative control, we injected a potato virus X resistance gene, Rx. Our expectation: Ry-adg would elicit a hypersensitive reaction after injection, as Ry-sto has previously been shown to do by other researchers, while Rx would have no effect.

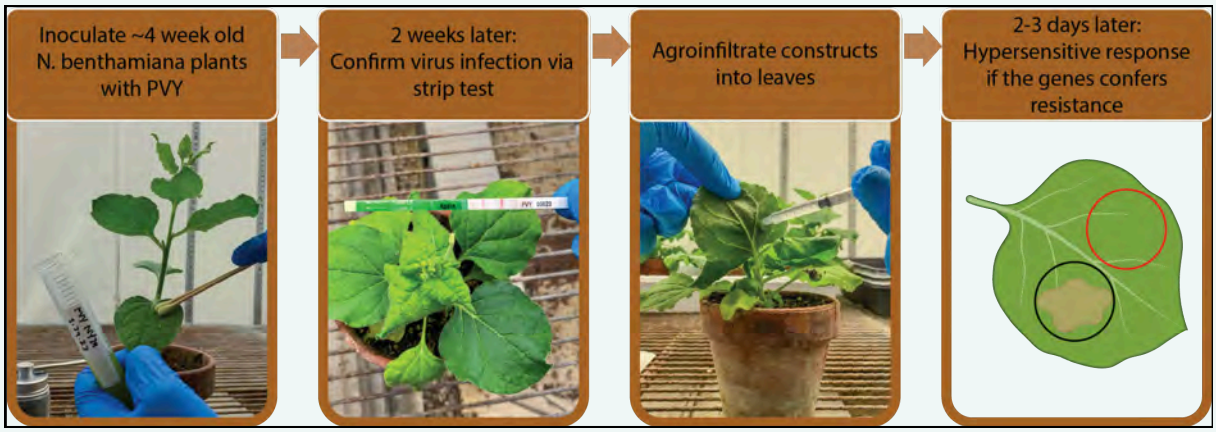


Figure 2. Transient Expression Assay Process

WHAT WE FOUND

The transient assay has proven more difficult to interpret than we expected. In particular, Ry-sto, the positive control, often failed to elicit cell death. Different environmental conditions will be tested in the hope that we can obtain more conclusive results going forward. In the meantime we are simultaneously pursuing a much slower, stable transformation assay to test candidate gene function.

STABLE POTATO TRANSFORMATION

The potato cultivar Desiree is often used for testing gene function since it is easy to transform. Although the cultivar Desiree is resistant to PVY-O, it is susceptible to all other strains of PVY, making it acceptable for this study. Both candidate genes and the positive control Ry-sto were transformed into the cultivar Desiree.

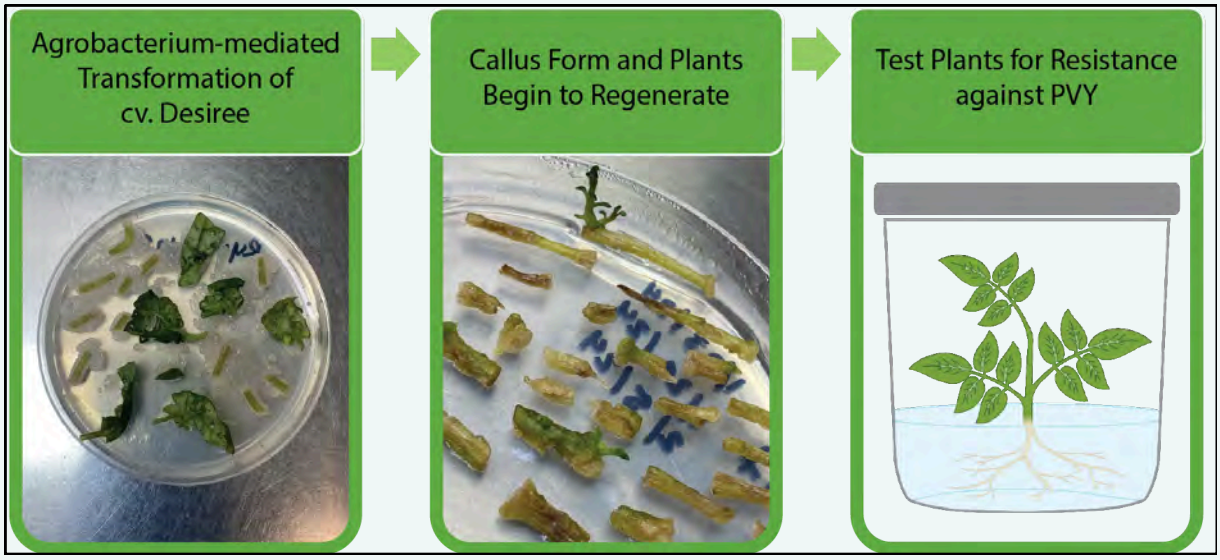


Figure 3. Stable Potato Transformation Process

NEXT STEPS

Once the transformed Desiree plants are large enough, they will be inoculated with PVY-NTN to test if either of the candidate genes confers resistance to the virus.

"Once the transformed Desiree plants are large enough, they will be inoculated with PVY-NTN to test if either of the candidate genes confers resistance to the virus."

OBJECTIVE 4

To identify economic or incentive barriers to effective disease management and to use this information to aid in industry adoption of improved management strategies and harmonized regulations

PVY dormant tuber diagnostic workshop

NINA ZIDACK, MONTATA STATE UNIVERSITY
BROOKE BABLER, UNIVERSITY OF WISCONSIN-MADISON

BACKGROUND

How can US seed potato certification programs address the increasing demand for direct tuber testing as an alternative or in conjunction to current postharvest test methods? This was the underlying question that drove the mission of a workshop on direct tuber testing methods that was held April 4-5, 2023 and hosted by Wisconsin Seed Potato Certification at the University of Wisconsin Institute for Discovery on the University of Wisconsin campus. The workshop was sponsored by the Certification Section of the Potato Association of America, and the USDA Specialty Crop Research Initiative (SCRI) project on necrotic viruses of potato. Additional support was provided by Agdia Inc., Promega and Thermo Fisher Scientific.

The University of Wisconsin Institute for Discovery served as the perfect location for this workshop. We had the run of a state-of-the art biotech teaching lab that allowed 45 participants a hands-on experience. In this environment, everyone was able to roll up their sleeves and participate in the actual performance of different tuber testing methods. Informational sessions were led by seed potato certification personnel and researchers from University of Wisconsin-Madison, Cornell University, Montana State University, and the University of Idaho. Attendees included staff from seed certification programs in Nebraska, Oregon, Montana, Colorado, North Dakota, Wisconsin, Idaho, Maine, Michigan and Alberta, Canada. USDA- APHIS and ARS staff were also on hand.

WHAT WE DID

The postharvest test is the definitive measure by which seed potato quality is measured by certification agencies. In the United States, it has traditionally been a field test performed in either a tropical or southern desert location, or in greenhouses. Depending on the state program, visual inspections are performed, leaf samples are collected and tested for virus, in tandem or as stand-alone tests. Over the past 40 years, emphasis has been placed on assessing infection levels of PVY, but data on varietal mixtures, herbicide injury and physiological disorders is also gathered and provides important information for both re-certification and commercial seed sales. While a field grow out is advantageous due to the broad spectrum of diseases and disorders that can be assessed in one test, it can be fraught with catastrophic weather events, scarce land availability, and ever-increasing costs. It is a time-consuming process with planting in November and results collected mid-January at the earliest.

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WHAT WE FOUND

The Wisconsin workshop had a lecture/discussion component, but more importantly, provided a hands/on opportunity to perform tuber testing protocols in the lab. Jason Ingram from USDA-ARS Cornell led participants in the performance of a method where potatoes cores are pressed onto special paper that preserves nucleic acids. Paper punches containing the sample can then go through an RNA extraction process using a robotic instrument called a KingFisher which was demonstrated by representatives from Thermo Fisher Scientific. This instrument is widely used in high-through- put diagnostics for both human, animal and plant pathogens. An advantage of this testing method is that once the nucleic acid has been extracted, multiple pathogens can be detected. A disadvantage of this system is the processing time to smash potato cores onto FTA cards. Brooke Babler from the Wisconsin program demonstrated another nucleic acid extraction instrument called the Maxwell that their program uses for extractions for Pectobacterium and Dickeya testing. Alice Pilgeram from the Montana Seed Potato Certification Program instructed participants on all of the steps to perform immunocapture, which is a PVY specific test where antibodies are used to capture the virus, and PCR can is performed on the captured virus. An advantage of this protocol is that it does not require nucleic acid extraction which eliminates the need for robotic nucleic acid extraction. A disadvantage is that it is limited to a single pathogen.

Lisa Tran from Idaho Crop and University of Idaho performed a cost analysis on all of the protocols. This was extremely informative and illustrated to participants the investment that would be required to establish a direct tuber testing program. Many programs already have PCR instruments but those who don't would have to make a minimal investment of $28,000 for a Real-Time machine. The robotic nucleic acid extraction robots start at $41,000 for the 16 sample Maxwell and go from $60,000-$75,000 for a King Fisher which process 96 samples, the equivalent of an ELISA plate.

"Going forward, certification labs will need to work together to establish recognized standard protocols that facilitate transferability of direct tuber test results between states."

TAKE-HOME HIGHLIGHTS & NEXT STEPS

The most valuable aspect of this workshop was the development of connections that will facilitate future communication and collaboration. Going forward, certification labs will need to work together to establish recognized standard protocols that facilitate transferability of direct tuber test results between states. This workshop was a great first step in this process. This group will continue to work towards the objective of establishing standard protocols through research funded by the Specialty Crop Research Initiative, and vetted by the Certification Section of the Potato Association of America.

Potato virus Y demonstration plots and field days

JONATHAN WHITWORTH, USDA-ARS

BACKGROUND

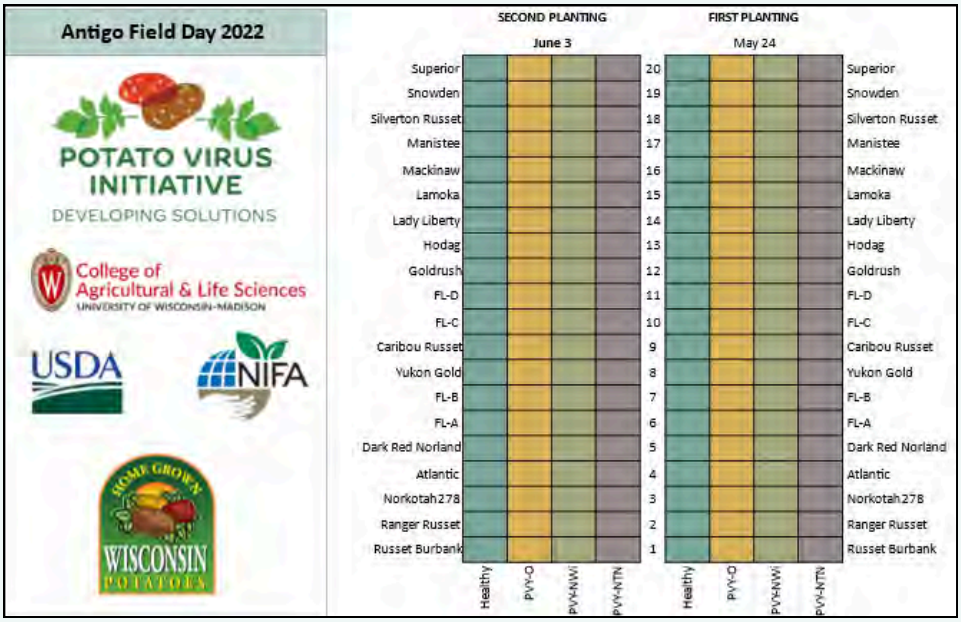
Potato virus Y demonstration plots have been conducted in 2022 and are being held again in 2024. PVY is a yield limiting disease that affects most of the widely grown potato varieties. Different strains of the virus can also cause tuber defects lowering the quality of the potatoes. This virus is moved most efficiently by aphids that feed on potato and other crops and weeds. Control of the aphids (and virus) is difficult with pesticides as aphid acquisition time and transmission time is much quicker than the action of the pesticide used. An understanding of the symptoms caused by different strains of the virus in different varieties is helpful to growers in general and specifically to seed potato growers. Seed growers can remove infected plants during the growing season to reduce the amount of virus in a seed lot.

OBJECTIVE

For PVY, demonstration plots using standard varieties and region-specific varieties are conducted in three locations across the US. Comparison of healthy and PVY infected potatoes are compared in side-by-side plots. Field days are held to allow growers and researchers to observe differences in foliar symptom expression among varieties.

WHAT WE DID

For PVY, demonstration plots were held in Washington, Wisconsin, and Maine in 2022 and are planned again for 2024. Each location has the same 7 varieties and then a set of 13 varieties specific to their respective regions. Each variety is planted as a plot of 5 plants of healthy, 5 plants of PVYO, 5 plants of PVYNWi, and 5 plant of PVYNTN. These side-by-side plots allow immediate comparison of symptom expression.



WHAT WE FOUND

For PVY, demonstration plots showed that certain varieties showed very strong symptoms when infected with PVYO, while others showed little to no symptoms when infected with PVYO, PVYNWi, and PVYNTN. In some varieties such as Silverton Russet, symptoms of all three PVY strains were difficult to distinguish.

"The demonstration plots were a valuable learning experience that showed how difficult it now is for seed growers to identify and remove virus infected plants from their field."

TAKE-HOME HIGHLIGHTS

For PVY, the demonstration plots were a valuable learning experience that showed how difficult it now is for seed potato growers to identify and remove virus infected plants from their field. This practice of taking out the "rogue" plants has been an effective tool for maintaining seed lots with very low to no PVY, something that is now more infinitely difficult with the N type PVY strains.

NEXT STEPS

The 2024 field demonstration plots will be held across the United States in Othello, Washington on June 27, 2024, Antigo Wisconsin on July 18, 2024, and in Maine (TBD).

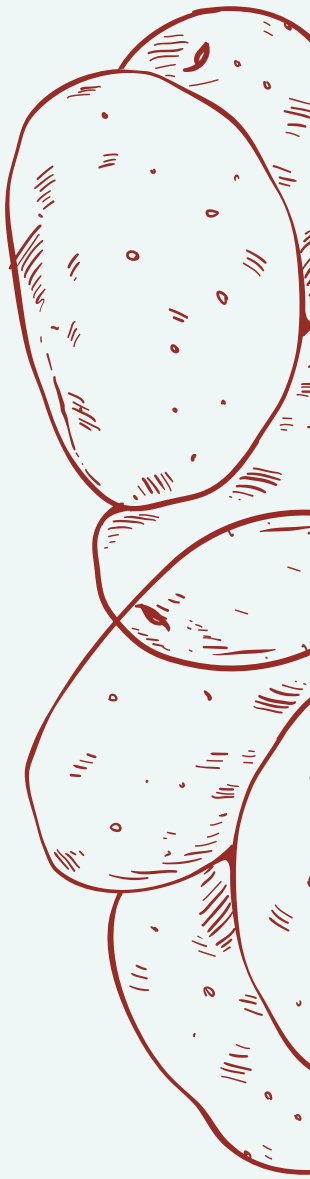
Highlighting extension activities for the SCRI Potato Virus Initiative Developing Solutions grant

NORA OLSEN, RABECKA HENDRICKS, AND ALEX KARASEV, UNIVERSITY OF IDAHO

BACKGROUND

Researchers, extension personnel, and the potato industry have partnered on a multi-year project to tackle the issue of viruses in the potato industry. The 'Potato Virus Initiative: Developing Solutions' is a federally funded Specialty Crop Research Initiative (SCRI), multi-state research and extension-based program with the mission to develop sustainable system-based management strategies and decisions aids, specifically for Potato mop top virus and Potato virus Y (PVY), by enhancing diagnostic and detection methods, breeding for resistance, and creating applied solutions for direct use by the potato industry to create economical and efficient means to manage the viruses.

As the grant enters its fourth year, a tremendous amount of basic and applied information has been generated. For the results to have an impact on the potato industry, the information needed to be packaged in multiple formats and dispersed throughout the industry. Extending the value of the Potato Virus Initiative research projects and the major scientific findings will help create solutions and impact how the industry manages the two potato viruses. Using traditional efforts, in conjunction with innovative means, to disseminate the data will ensure the industry has access to obtain and utilize science-based information.



OBJECTIVE

To develop extension outlets to disseminate information generated from this grant in multiple formats to provide maximum exposure to stakeholders and the scientific community.

POTATO VIRUS INITIATIVE
DEVELOPING SOLUTIONS

Logo designed by Daniella Echeverria, Oregon State University graduate student

WHAT WE DID

The initial step was to adopt a name of the grant, develop a logo, write a mission statement, and devise a plan of action on how to spread the word about the grant activities. We used multiple traditional and non-traditional outlets to extend research and extension activities associated with the Potato Virus Initiative.

Manuscripts were submitted to peer reviewed journals, scientific and extension presentations were given, workshops and field days were organized, and social media outlets were developed. A website was created as a "one stop shop" to learn about the grant, post videos and articles, highlight the PVY demonstration plots and field days, and link to additional resources on PVY and PMTV. Instagram, X, and LinkedIn social media outlets were used to further extend highlights and activities. Stickers were produced with catchy sayings like "Save a Potato: Manage for Virus" and "Out Smart Viruses: Be Part of the Solution." A newsletter was created to collate all the impactful research and extension activities into an easy to disseminate document.

SCAN

and follow us on Instagram!

POTATOVIRUSINITIATIVE

Follow us on:

@PotatoVirusIni1

@potatovirusinitiative

@potatovirusinitiative

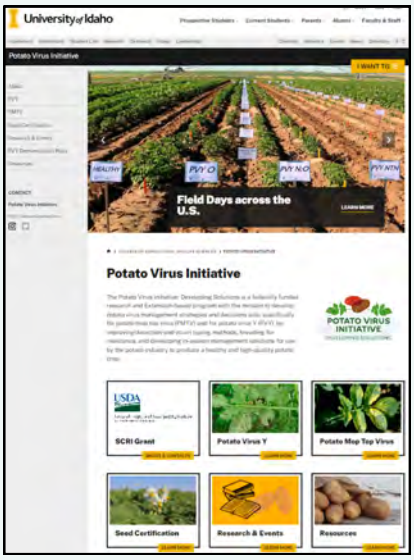
"Creating digital formats and summaries of major findings allowed for easy dispersal of impactful activities."

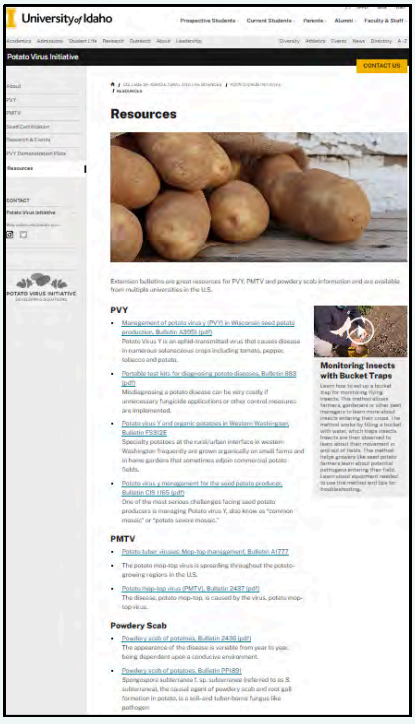
WHAT WE FOUND

Disseminating information in multiple formats helped reach the scientific community and industry partners. Within the first 3 years of this grant, 18 scientific articles were published in journals such as Plant Disease, Phytopathology, American Journal of Potato Research, and Frontiers in Plant Sciences. Publishing the research generated is providing foundation science to develop solutions to combat PVY and PMTV.

There were over 80 scientific and extension presentations at a variety of scientific and grower meetings and field days. Magazine articles are a great way to reach the potato industry and at least 9 articles were published in Spudman, Potato Grower, and Potato Country Magazines. Tremendous interaction and discussion took place within the potato industry, helping to shape the direction and impact of the research.

Creating digital formats and summaries of major findings allowed for easy dispersal of impactful activities. Hundreds of people visit the website each year and there are over 400 followers on Instagram, 200 followers on X, and 180 followers on LinkedIn. We made 43 posts on LinkendIn last year which gained close to 9,000 impressions. The website is the 4th link listed when you "google" 'potato virus.' The presence of the Potato Virus Initiative on multiple digital formats is spreading the word on the impact from this grant.





Example of a few stickers created to help create name recognition of the grant to stakeholders.

TAKE-HOME HIGHLIGHTS

Continuous interaction with the stakeholders has allowed the research and extension efforts to stay relevant to the issues of the industry. Highlights from the research and extension activities are being packaged into multiple formats and disseminated in a wide variety of outlets to reach the potato industry and scientific communities.

Visit https://www.uidaho.edu/cals/potato-virus-initiative to access information about the grant.

NEXT STEPS

Extension activities will continue to build as additional results from research in the 4th year of the grant are finalized. New and creative means to disseminate the information will be explored.

FOR MORE INFORMATION PLEASE CONTACT:

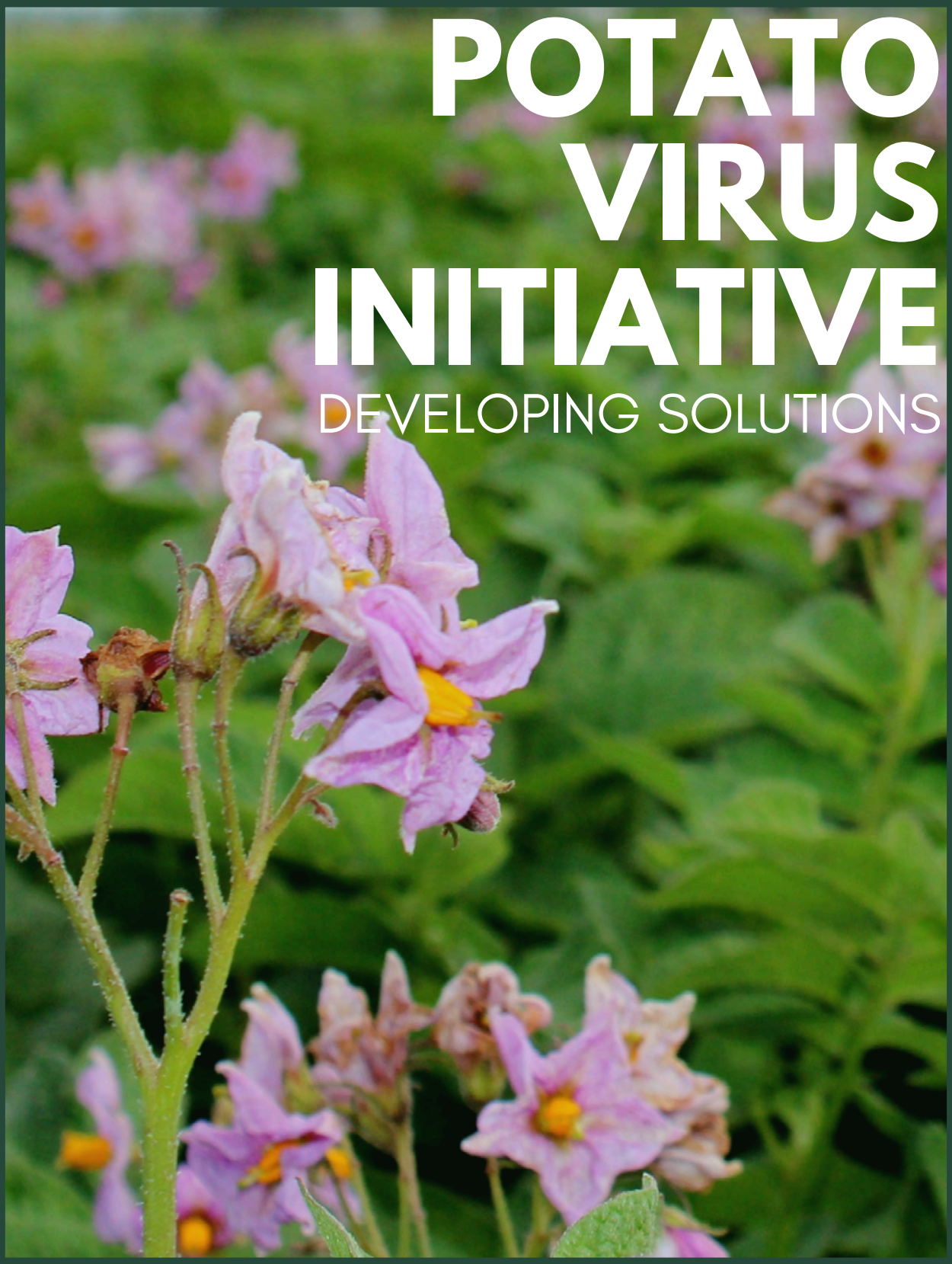
Scan QR code to visit our website and browse!

potatovirusinitative@uidaho.edu

www.uidaho.edu/cals/potato-virus-initiative

Designed and compiled by Rabecka Hendricks, Mikaela McSweeney, and Nora Olsen, University of Idaho

POTATO VIRUS INITIATIVE: DEVELOPING SOLUTIONS



POTATO VIRUS INITIATIVE

DEVELOPING SOLUTIONS