American Journal of Potato Research (2024) 101:312–321
https://doi.org/10.1007/s12230-024-09960-8

Methods to Induce Sprouting in Dormant Potato Tubers for Direct Tuber Testing of Potato Virus Y

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Accepted: 26 June 2024 / Published online: 20 July 2024

Abstract

The ability to initiate sprouting soon after harvest to enable direct tuber testing for potato virus Y (PVY) could aid in acquiring more rapid results compared to the traditional winter grow out tests currently used. Methods to break dormancy for PVY detection using laboratory direct tuber testing by ELISA in commercially produced Ranger Russet, Clearwater Russet, and Umatilla Russet seed lots were tested over two years and compared to leaf testing results obtained from the winter grow out and spring grow out. At harvest, three 400 tuber samples from each cultivar were obtained for the trial and included (1) untreated control (UTC), (2) application of cold aerosol smoke, or (3) application of Rindite. Tuber samples were held at 18.3 C and sprout development was monitored weekly. Treatments were direct tuber tested for PVY when one treatment of that cultivar achieved three sprouts elongating to six millimeters. A fourth 400 tuber sample was collected, treated with Rindite, and 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 were analyzed for PVY by ELISA. Rindite treated tubers had greater sprout rating and number of sprouts elongating compared to UTC tubers and tubers receiving the smoke treatment at time of PVY testing. Smoke had a greater sprout rating but did not always significantly differ in the number of sprouts elongating compared to the untreated tubers. Overall, estimates of PVY prevalence from direct tuber testing showed limited significant differences to those obtained in the winter grow out for each cultivar, year, and PVY incidence. However, in year two, the incidence of PVY in the winter grow out (7% PVY) significantly differed from direct tuber testing (16% PVY) in Ranger Russet. In both years, the spring grow out PVY results for all cultivars were not significantly different than the direct tuber testing, except in year one the Ranger Russet direct tuber tested UTC showed 10% lower PVY detection compared to the spring grow out. This study identified a novel dormancy breaking treatment to promote earlier and accurate PVY detection by direct tuber testing using ELISA and provided data to support direct tuber testing for post-harvest evaluation of PVY in seed certification.

Keywords Smoke · Rindite · ELISA · PVY · Post-harvest Testing

Introduction

Seed certification is a service to the potato industry that assures available seed potatoes (*Solanum tuberosum* L.) are within thresholds for factors that may limit crop production, such as disease, varietal impurities, and chemical carryover (Callison et al. 1982; Frost et al. 2013). Certifying agencies in the United States (US) oversee establishing strict production guidelines for seed lot certification, recertification, and distribution (Gudmestad 1991; Frost et al. 2013). Potato virus Y (PVY) is an issue for potato producing regions around the world (Karasev and Gray 2013; Gray et al. 2010) and is currently the most common factor leading to downgrading or

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rejection of seed lots for certification (Frost et al. 2013; Tran et al. 2022; Lindner et al. 2015). PVY is spread through distribution of infected tubers. Limiting available inoculum by planting virus-free tubers is the best method for preventing further spread of PVY infection (Singh et al. 2013). Therefore, having an accurate estimation of virus levels in a seed lot is critical prior to seed potato distribution and planting. To predict virus levels in the subsequent crop, a post-harvest evaluation is conducted, which allows for visual and/or laboratory confirmations (Fox et al. 2005. The evaluation may include a winter grow out (WGO), a greenhouse grow-out, or laboratory-based methods which evaluate tissue directly from the tuber without the need to grow a plant and collect leaf tissue.

Currently, several US state seed certifying agencies, including Idaho, plant seed lot samples in Hawaii or Florida as a WGO location. Plants are grown until they reach adequate size and then inspected for mosaic symptoms primarily from PVY (Duellman et al. 2020). For many states, PVY in a seed lot is also tested using enzyme-linked immunosorbent assay (ELISA) or other methods by collecting a leaf tissue sample from each plant (or a composite) grown in the WGO. When potatoes are first harvested, they are often in a state of dormancy, or cessation of growth, where a sprout will not form even if the tubers are placed in optimal growing conditions (Sonnewald and Sonnewald 2014; Campbell et al. 2008; Mani et al. 2014; Suttle 2004). The duration of this state of dormancy varies for each cultivar but remains an issue for certification agencies that plant tubers shortly after harvest in the WGO (Liu et al. 2015). Due to dormancy issues, each sample is typically treated with Rindite (ethylene chlorohydrin, ethylene dichloride, and carbon tetrachloride 7:3:1 mixture by volume) or bromoethane to initiate sprouting in dormant tubers (Denny 1945; Akoumianakis et al. 2000; McDonald and Coleman 1988). The current WGO certification process can be time consuming, resource intensive, and the sustainability and availability of the dormancy breaking chemicals involved are questionable. Seed growers desire post-harvest testing results as early as possible to determine the volume and availability of viable seed stock, to capitalize on exporting to earlier markets, and making seed purchasing decisions (Fox et al. 2005; Singh et al. 2013). Direct tuber testing may provide an earlier assessment of PVY levels in the seed lot.

Direct tuber testing is a laboratory method which utilizes extract directly from the tuber tissue, tissue from developing sprouts, or a combination of sprout and tuber tissue. Various RT-PCR (reverse transcriptase polymerase chain reaction) and ELISA methods have been implemented on dormant, non-sprouting tubers with variable results (Avrahami-Moyal et al. 2017; Barker et al. 1993; Russo et al. 1999; Hill and Jackson 1984; Singh and Singh 1996; Singh et al. 2013; Huhnlein et al. 2013; Fox et al. 2005; Schumpp et al. 2021). Others have reported that virus titer decreases during storage of potatoes, thereby reducing accuracy of testing (DeBokx and Cuperus 1987; Barker et al. 1993; Fox et al. 2005), and ELISA methods have shown to have lower PVY detection on dormant, non-sprouted, tubers compared to tubers that had broken dormancy (Barker et al. 1993; Gugereli and Gehriger 1980). Some studies have shown that reliability of ELISA testing for PVY increases when tuber dormancy is artificially broken and sprouting has begun (McDonald and Coleman 1988; Gugerli and Gehriger 1980; Vetten et al. 1983; ICIA 2020). Hill and Jackson (1984) suggested Rindite stimulates PVY detection in tubers. Vetten et al. (1983) found virus level was higher and uniformly distributed within a tuber after a treatment with Rindite. McDonald and Coleman (1988) reported treatment with either bromoethane or Rindite improved virus detection over the untreated control in Russet Burbank. United Nations Economic Commission for Europe (UNECE) suggested ELISA testing should be conducted on sprouting tubers (UNECE 2016). Idaho Crop Improvement Association (ICIA) suggests tubers should have sprouts 6 mm in length or greater (ICIA 2022) while others recommend using sprouts 3 to 5 mm in length for PVY detection (UNECE 2019). Reliability and accuracy of PVY detection using direct tuber testing has varied with laboratory method, cultivar, sample location on the tuber, state of dormancy, PVY strain, and time in storage. It appears published research supports artificial breaking of dormancy over natural breaking of dormancy for greater PVY detection when using ELISA methods on tuber tissue.

Several studies have examined methods to break dormancy in tubers, such as plant growth regulators, thiourea, temperature fluctuations, and carbon disulphide, but it is unclear whether these methods increase PVY detection in post-harvest testing for certification (Siregar et al. 2021; Rylski et al. 1974; Prange et al. 1998; Tavakoli et al. 2014; Thornton 1991; Denny 1926; Bryan 1989) and may not be suitable to seed certification programs due to undesirable application processes, inconsistent results, or lack of scalability. However, an application of cold aerosol smoke has shown to stimulate germination in several true seed crops (Drewes et al. 1995; Doherty and Cohn 2000; Ghebrehiwot et al. 2013) and previous studies by Gelles (2023) have demonstrated cold aerosol smoke promotes dormancy break in potato tubers and can be scalable to treat large quantities of seed potatoes.

The WGO is the benchmark standard for post-harvest PVY testing in many states, therefore it is imperative that new alternative testing methods consistently have comparable virus detection to the WGO for determining PVY in seed lots. Direct tuber testing could be a solution to provide

results sooner than the WGO but may require the necessity to break tuber dormancy to enhance the reliability of the testing. The objectives of this study were to (1) evaluate the use of a novel dormancy breaking technique of cold aerosol smoke application to initiate sprouting for PVY detection in treated tubers, (2) determine if the direct tuber testing method using ELISA (ICIA 2019) is comparable to the traditional Idaho WGO, and (3) compare PVY levels of a subsequent crop planted from tubers previously laboratory tested.

Materials and Methods

Tuber Sample Collection, Chemical Treatments, and Tuber Testing

Whole seed tubers of certified seed potato cultivars Ranger Russet, Umatilla Russet, and Clearwater Russet, with suspected PVY infection, were supplied by collaborating commercial seed potato growers in 2021 and 2022. Seed samples were collected when WGO samples are typically collected during harvest and loading into storage facilities (Table 1). Samples consisted of 1600 single drop (42 to 113 g) tubers per cultivar (400 tubers per treatment; 100 tubers per replicate; four replicates). Year two Ranger Russet had 348 tubers per treatment (88 tubers per replicate). Tubers within a cultivar were mixed and randomly assigned to four treatments. Samples were stored at the Kimberly Research and Extension Center, Kimberly, ID (KREC) at 12.8 C and 95% relative humidity (RH).

Three samples were used for direct tuber testing treatments including: (1) an untreated control (UTC), (2) an application of a cold aerosol smoke, and (3) an application of Rindite (according to ICIA standard procedures). Tuber samples were removed from the 12.8 C storage temperature on October 15, 2021 and October 17, 2022 for treatment applications. The UTC and smoke treatments were placed in 18.3 C (95% RH) prior to application. The Rindite treatment and the sample to be planted at the WGO (see below) were transported to ICIA on October 15, 2021 and October 17, 2022.

The smoke treatment consisted of a cold aerosol smoke application produced from the combustion of plant-based pellets (spruce, sugar pine, fir, poplar, and alder wood blend; Harvest Lane Honey, Salt Lake City, UT) in a 0.5 L custom cold smoke generator and injected with compressed air (Point Zero Airbrush; Tamarac, Florida) through tubing into a custom-built rectangular 1.2 m x 1.2 m x 2.4 m (L x W x H) wooden application chamber with two JISULIFE F8x handheld fans for circulation. Pellets (100 g) were ignited using a 0.4 L propane cylinder with brass torch (BenzOmatic, New York). Once the pellets were ignited, cold aerosol smoke was injected into the application chamber through a metal tube for one hour. The chamber was sealed to restrict smoke escape, and smoke was circulated by fans for 20 h (Gelles 2023). Smoke applications were initiated on October 21, 2021, and October 20, 2022, and samples were placed into 18.3 C and 95% RH storage upon completion.

Initially, the Rindite treatment potatoes were stored at ambient temperatures (approximately 18.3 C) for three days. Samples were then loaded into 246 × 264 × 1438 cm refrigerated container unit on October 19 where tubers were warmed to 21 to 23.9 C. Rindite (141 ml Rindite per m^3; ethylene chlorohydrin, ethylene dichloride and carbon tetrachloride in a 7:3:1 ratio by volume) was volatilized in air (21 to 23.9 C) from a plastic tub and circulated by the container fan unit with extra fans inside the application chamber according to ICIA standard protocol. Treatment was initiated on October 22 and completed on October 25 both years. Rindite application chambers were opened to ambient air and transported to KREC. After all applications were complete, treatment samples were stored at 18.3 C, 95% RH and evaluated for sprout development.

Sprout evaluations were conducted on a sub-sample ($n = 25$ tubers; four replicates) from treatments beginning approximately two weeks after treatment applications (November 8, 2021 and November 7, 2022). Sprout rating evaluations were conducted according to the University of Idaho sprout rating scale (1 to 4); where 1 = no bud activity; 2 = sprout initiating but not pointed; 3 = sprout pointed,

Table 1 Harvest date, direct tuber testing date, days after harvest and days after treatment when tubers were direct tuber tested for potato virus Y infection

Cultivar	Harvest date	Direct tuber testing date	Days after harvest	Days after treatment
	2021			
Ranger Russet	13-Sep-21	16-Nov-21	64	23
Clearwater Russet	27-Sep-21	29-Nov-21	63	37
Umatilla Russet	20-Sep-21	6-Dec-21	77	37
	2022			
Ranger Russet	3-Oct-22	14-Nov-22	42	24
Clearwater Russet	19-Sep-22	28-Nov-22	70	38
Umatilla Russet	26-Sep-22	28-Nov-22	63	45

but length not achieving five mm; and 4 = sprout elongating, length five mm or greater (Gelles 2023). The number of sprouts pointing (< 5 mm), number of sprouts elongating (≥ 5 mm), number of sprouts (≥ 6 mm), and length measurement of elongating sprouts (≥ 5 mm) were collected. Evaluations recurred weekly until at least one treatment of each cultivar reached 80% of the tubers showing three elongated sprouts (sprouts ≥ 6 mm). Once achieved, then all samples within the cultivar were delivered to ICIA for direct tuber testing (Table 1).

Direct tuber testing for PVY was conducted by extracting tissue from an eye at the stem and bud ends and three eyes from the middle of the tuber using a cork borer (4 mm). Cores were homogenized using an Agdia tissue homogenizer (Elkhart, IN), and analyzed using ICIA's direct tuber testing ELISA protocol (ICIA 2019). Each plug contained tissue from the base of the sprout (if present) and tuber tissue directly below the eye. Each tuber from the three storage treatments were analyzed individually ($n = 100$; four replicates per cultivar).

Comparison of the Direct Tuber Testing by ELISA Versus Traditional WGO

The fourth 400 tuber sample per cultivar that was treated with Rindite as described above was subsequently planted in the traditional Idaho WGO plots in Hawaii. The WGO samples were transloaded into the shipping container for Hawaii on October 26. Once in Hawaii, WGO tuber samples were planted in a single row on a farm near Waialua, Hawaii on November 9, 2021, and November 8, 2022, in accordance with typical WGO procedures. Plants were allowed to grow until they reached approximately 30 cm in height. Once the plants achieved adequate size, a leaf tissue sample was taken from each plant and placed into a large plastic bag. Leaf tissue samples consisted of a terminal leaflet collected from a fully extended petiole near the top of the plant. The leaf tissue samples were shipped to ICIA's laboratory in Idaho Falls, ID and analyzed for PVY according to ICIA's protocol for ELISA testing using composites of five leaves (Tran et al. 2022). In year one, leaf samples of Ranger Russet were tested on January 13, 2022. Clearwater Russet and Umatilla Russet were tested on January 14, 2022. In year two, Ranger Russet leaf samples were tested on December 29, 2022, while Clearwater Russet and Umatilla Russet were tested on January 13, 2023. Estimation of PVY foliar infection in each plot was extrapolated from the five leaf composites, using the following equation (UNECE 2019):

$$percent\,virus = \left(1 - \left(1 - \left(\frac{Number\ Positives}{Number\ Tests}\right)^{0.2}\right)\right) X100$$

The WGO sample was then compared to the direct tuber tested samples to determine if PVY incidence significantly differed between testing methods.

The Spring Grow-Out of Plants from Laboratory-Tested Tubers

After direct tuber testing was completed, the sampled seed tubers were returned to KREC and stored at 4.4 C and 95% RH. Tubers were planted in the KREC potato field the following spring to further compare to direct tuber testing and WGO results (referred to as the spring grow out). The seed tubers were warmed to 7.2 C for approximately 72 h before planting on April 21, 2022 and April 20, 2023, respectively. The whole tuber, previously direct tuber sampled, for each treatment was planted in a single row plot with 26.7 cm in-row spacing and grown according to University of Idaho's nutrient, pest, and water management guidelines. There were 100 tubers per replicate and four replicates per treatment of each variety. A leaf sample was collected when plants were approximately 30 cm tall (as described above) and sent to ICIA laboratory for PVY detection in composites of five leaves according to the traditional WGO methodology (Tran et al. 2022). Ranger Russet plants were sampled June 15, 2022 and June 7, 2023. Umatilla Russet was sampled June 15, 2022 and June 13, 2023. Clearwater Russet was sampled June 27, 2022 and June 15, 2023.

Statistical Analysis

Sprout rating, number of sprouts per tuber ≥ 6 mm, and PVY incidence were analyzed using the analysis of variance (ANOVA) procedures in R (RStudio, package car version 4.1.0, 2021; Fox and Weisberg 2019). A linear model for all variables, except sprout rating, was fitted for each cultivar and year separately where treatment was considered the fixed effect. A linear model was fitted for sprout rating across both years where treatment was considered the fixed effect. All trials' means for response variables were compared and considered significantly different at p-value of 0.05 by estimated marginal means procedures (RStudio, package emmeans version 1.6.1, 2020).

Results

Use of Novel Dormancy Breaking Technique

Ranger Russet tubers achieved the desired sprout development (three sprouts per tuber ≥ 6 mm) and were delivered for direct tuber testing in mid-November both years (Table 1). The samples were delivered 23 and 24 days after

Table 2 Average sprout rating (2021–2022 combined) for each treatment when sampled for direct tuber testing of potato virus Y infection via ELISA laboratory methods at Idaho Crop Improvement Association

Treatment[1]	Ranger Russet	Clearwater Russet	Umatilla Russet
	Sprout rating[2,3]		
UTC	2.1 a	2.2 a	1.4 a
Smoke	3.0 b	3.2 b	1.8 b
Rindite	4.0 c	4.0 c	3.8 c
Standard error	0.04	0.03	0.03

[1]Treatments: UTC = untreated control held at 18.3 C; Smoke = application of aerosol smoke 1 h injection 20 h circulation; Rindite = application of volatized Rindite

[2]Values followed by the same letter are not significantly different (α = 0.05) within each column

[3]University of Idaho sprout rating scale; (1) no bud activity; (2) sprout initiating but not pointed; (3) sprout pointed, but length not achieving 5 mm; (4) sprout elongating, length 5 mm or greater

Table 3 Average number of sprouts elongating (≥ 6 mm) per tuber, for each treatment, when sampled for direct tuber testing (DTT) of potato virus Y infection via ELISA laboratory methods at Idaho Crop Improvement Association

Treatment[1]	Ranger Russet	Clearwater Russet	Umatilla Russet
	Number of sprouts elongating at DTT 2021[2]		
UTC	0.1 a	0.3 a	0.4 a
Smoke	0.4 a	0.9 a	0.6 a
Rindite	3.0 b	3.0 b	1.5 b
Standard error	0.2	0.41	0.07
	Number of sprouts elongating at DTT 2022		
UTC	0.0 a	0.6 a	0.1 a
Smoke	0.2 a	1.1 b	0.3 b
Rindite	4.3 b	1.9 c	2.0 c
Standard error	0.16	0.08	0.06

[1]Treatments: UTC = untreated control held at 18.3 C; Smoke = application of aerosol smoke 1 h injection 20 h circulation; Rindite = application of volatized Rindite

[2]Values followed by the same letter are not significantly different (α = 0.05) within each column and year

treatment in each year studied, but an additional 18 days after harvest in the first year. Clearwater Russet tubers were delivered for testing before the end of November for each year. In both years Clearwater tubers were delivered 37 and 38 days after treatment but seven days earlier after harvest in year one. Umatilla Russet tubers were delivered for testing the first week of December in year one and the last week of November in year two. In year two, Umatilla Russet was direct tuber tested eight days earlier after treatment and 14 days earlier after harvest compared to year one.

Rindite treated tubers had the highest sprout rating and number of sprouts elongating compared to UTC and smoke in both years for each cultivar (Tables 2 and 3). Smoke treated tubers had significantly higher sprout rating in all

Table 4 Percent potato virus Y (PVY) infection detected by direct tuber testing (DTT) and the winter grow out (WGO) as influenced by treatment and cultivar in 2021 and 2022

Treatment[1]	Testing method[2]	Ranger Russet	Clearwater Russet	Umatilla Russet
		2021 PVY infection (%)[3]		
UTC	DTT	30 a	1 a	18 a
Smoke	DTT	37 a	1 a	19 a
Rindite	DTT	36 a	4 a	23 a
Rindite	WGO	33 a	3 a	22 a
Standard error		2	1	2
		2022 PVY infection (%)		
UTC	DTT	16 bc	0.2 a	16 a
Smoke	DTT	14 b	0.2 a	17 a
Rindite	DTT	20 c	0.3 a	18 a
Rindite	WGO	7 a	0.6 a	18 a
Standard error		2	0.4	2

[1]Treatments: UTC = untreated control held at 18.3 C; Smoke = application of aerosol smoke 1 h injection 20 h circulation; Rindite = application of volatized Rindite

[2]DTT= direct tuber testing via ELISA on nondormant tubers; WGO = winter grow out

[3]Values followed by the same letter are not significantly different (α = 0.05) within each column and year

three cultivars compared to the UTC (Table 2). Ranger Russet and Clearwater Russet achieved dormancy break (80% of tubers expressing a 3 rating) prior to being delivered for testing. However, the smoke and UTC treatments did not reach dormancy break in Umatilla Russet prior to testing. Neither smoke nor UTC treated tubers achieved the goal of three elongated sprouts (≥ 6 mm) in either year (Table 3). In year one, smoke and UTC did not significantly differ in the number of elongated sprouts in each of the cultivars at the time of testing, but smoke had significantly higher number of elongated sprouts in Clearwater Russet and Umatilla Russet in year two (Table 3).

Direct Tuber Testing Using ELISA Compared to the Traditional WGO

PVY detection in Ranger Russet in year two showed significant differences between treatments, whereas no significant differences between treatments were observed in Clearwater Russet and Umatilla Russet in either year (Table 4). In year one, Ranger Russet had no differences in PVY detection among treatments. In year two, Ranger Russet tubers treated with Rindite had significantly higher PVY levels than tubers from the smoke treatment. The WGO foliar sample had significantly lower PVY in year two compared to direct tuber tested samples for Ranger Russet. There were no significant differences in the detection of PVY between the WGO and the direct tuber testing treatments for Clearwater Russet and

Umatilla Russet in both years tested and Ranger Russet in 2021 (Table 4).

PVY Levels of a Subsequent crop Planted from Tubers that were Laboratory Tested

Overall, there were no significant differences in PVY detection in the spring grow out compared to the direct tuber testing in Clearwater Russet and Umatilla Russet in both years, and in Ranger Russet in 2022 (Tables 5 and 6). However, spring grow out PVY levels from Ranger Russet plants significantly differed from the UTC direct tuber testing samples in 2021.

Discussion

To be a viable WGO replacement option for PVY detection, direct tuber testing methods need to be accurate, cost effective, and have capabilities of processing high volumes of samples in a short period of time. The current ELISA process used at ICIA was easily adapted to detect PVY from tuber tissue due to the equipment, materials, and trained personnel already in place for this testing procedure. Previous research has indicated PVY detection is improved when tubers have broken dormancy (Gugerli and Gehriger 1980; McDonald and Coleman 1988), therefore if ELISA methods are to be used for direct tuber testing, tubers should be sprouting. This study focused on developing novel methods to promote sprouting soon after harvest, which may allow the industry to avoid waiting for natural dormancy to break and/or avoid the use of Rindite. The Rindite application process is considered undesirable due to length of application timing, health hazards of the application, and difficulty in acquiring components of the three-way mixture.

Sprout development was significantly higher in the Rindite treated tubers compared to smoke and UTC and emphasized the usefulness of this standard product to initiate and promote sprout development. However, in most cases, smoke achieved dormancy break (80% of tubers having a 3 rating) whereas the UTC did not, and smoke had significantly greater sprout ratings than the UTC. Although smoke treated tubers did not have as developed sprouts compared to Rindite treated tubers, a smoke treatment showed the ability to promote sprout development beyond using storage temperature alone. Aerosol smoke may be an effective and convenient alternative to promote sprouting for PVY detection when using direct tuber testing.

Samples were treated and delivered for direct tuber testing approximately the same date in both years, however harvest dates were significantly different between years. Time to dormancy break appeared to be dependent upon time after treatment (~2 weeks) rather than days after harvest. This timing indicated that treatments may have promoted endo-dormancy break, which is controlled through physiological mechanisms (Mani et al. 2014; Suttle 2004). Alternatively, tubers may have already ended their natural

Table 5 Percent potato virus Y (PVY) infection detected by direct tuber testing in 2021 of three treatments compared to composite leaf samples of the same tubers planted in the spring grow out in 2022

Testing method[1]		
Treatment[2]	Direct tuber testing	Spring grow-out
	Ranger Russet PVY infection (%)	
UTC	30 a	40 b
Smoke	37 b	41 b
Rindite	36 ab	39 b
Standard error	2	
	Clearwater Russet PVY infection (%)	
UTC	1 a	1 a
Smoke	1 a	2 a
Rindite	4 a	4 a
Standard error	1	
	Umatilla Russet PVY infection (%)	
UTC	18 a	22 a
Smoke	19 a	26 a
Rindite	23 a	22 a
Standard error	2	

[1]Values followed by the same letter are not significantly different (α = 0.05) within each cultivar

[2]Treatments: UTC = untreated control held at 18.3 C; Smoke = application of aerosol smoke 1 h injection 20 h circulation; Rindite = application of volatized Rindite

Table 6 Percent potato virus Y (PVY) infection detected by direct tuber testing in 2022 of three treatments compared to composite leaf samples of the same tubers planted in the spring grow out in 2023

Testing method[1]		
Treatment[2]	Direct tuber testing	Spring grow-out
	Ranger Russet PVY infection (%)	
UTC	16 a	17 a
Smoke	14 a	14 a
Rindite	20 a	20 a
Standard error	2	
	Clearwater Russet PVY infection (%)	
UTC	0.2 a	0.3 a
Smoke	0.2 a	0.3 a
Rindite	0.3 a	0.0 a
Standard error	0.3	
	Umatilla Russet PVY infection (%)	
UTC	16 a	20 a
Smoke	17 a	20 a
Rindite	18 a	20 a
Standard error	2	

[1]Values followed by the same letter are not significantly different (α = 0.05) within each cultivar

[2]Treatments: UTC = untreated control held at 18.3 C; Smoke = application of aerosol smoke 1 h injection 20 h circulation; Rindite = application of volatized Rindite

endo-dormant state and were in a state of eco-dormancy at the time of applications, which allows sprouting behavior to be influenced by environmental or chemical conditions (Aksenova et al. 2013; Mani et al. 2014). Regardless of the dormancy status, treatments can be applied at any time after harvest, further expediting the sprouting process and ability to obtain PVY results.

Using non-dormant tubers is recommended for PVY detection when using ELISA laboratory methods and recommendations vary from having tubers with three to six mm sprouts and one or multiple sprouts elongating. This study was conducted with tubers treated with different compounds, however, additional information on the level of sprout development necessary for accurate direct tuber testing using ELISA should be further developed. The necessary number of sprouts per tuber for accurate PVY detection could be influenced by the artificial treatments and further research should investigate sprout development on a single treatment of tubers at various sprouting levels.

Treatments in this study provided various levels of sprout development at the time of direct tuber testing and PVY detection. Interestingly, PVY detection was not significantly different between the treatments except for Ranger Russet in year two, where the smoke treated tubers had significantly lower PVY than the Rindite treated tubers. It remains unclear if lower levels of sprout development can be used for accurate PVY assessment than previously suggested or if there is an increase in PVY detection as previously described with Rindite and bromoethane (Gugerli and Gehriger 1980; McDonald and Coleman 1988). Upon dormancy release, the tuber becomes a source to supply nutrients and metabolites to developing sprouts (Aksenova et al. 2013). It may be speculated that virus particles move in conjunction with the metabolites. This type of movement would create a concentration of virus in and around sprouting eyes. Increased virus concentrations near the eyes would imply sample location is important for accurate PVY detection. In the sampling method of ICIA (2019), cores were taken from multiple areas on the tuber, so it is unknown if virus was more prevalent in the bud or stem end of the tuber as seen in Gugerli and Gehriger (1980) and Whitworth et al. (2012) or if virus was distributed evenly throughout the tuber. The untreated control was stored at elevated temperatures (18.3 C) and had broken dormancy at the time of direct tuber testing in several cases, yet PVY detection did not significantly differ from the other treatments. Results from this study were inconsistent with outcomes from Fox et al. (2005) and Hill and Jackson (1984) who found PVY detection was significantly lower when using ELISA after tubers broke dormancy naturally. Differences in observations could be attributed to the cultivars used, tuber sampling, ELISA procedures, or level of sprout development at the time of testing.

Previous studies evaluating the viability of direct tuber testing as an alternative to the WGO leaf sampling have shown mixed results. One reason for variable results may be due to the methodology used to detect PVY. Fox and Browning (2005) indicated a more reliable association between PCR testing methods of dormant tubers and the WGO, whereas testing via ELISA was less reliable. Avrahami-Moyal et al. (2017) indicated qPCR testing methods of dormant tubers were categorically similar to WGO results. Singh et al. (2013) showed ELISA methods were comparable to PCR but can take longer from harvest to produce results since dormancy must be broken. Past published research evaluating direct tuber testing methods has been conducted at different times after harvest in order to enable natural dormancy break. Although previous studies have indicated a slight advantage of PCR over ELISA in accuracy of PVY detection, the increased cost, time associated with conducting tests and data processing, and skills required for PCR testing are often overlooked. This study demonstrated that the direct tuber testing protocol using ELISA combined with dormancy breaking techniques can provide commercially applicable PVY detection.

The use of direct tuber testing was explored as an alternative method to detect PVY sooner than the WGO results. Results from direct tuber tested samples were produced on average 47 days earlier compared to samples sent to the WGO and did not significantly differ in PVY detection except one seed lot (Ranger Russet in 2022). The result of the Ranger Russet seed lot in 2022 coincides with Fox et al. (2005) stating the WGO may underestimate the level of PVY infection in seed lots The Ranger Russet direct tuber tested samples in 2022 had an average of 56 tubers out of each 348-tuber sample test positive (16%) whereas the WGO sample had 21 PVY positive composite samples out of 69 composites (7%). Bulked samples cannot be separated into individual samples. Therefore, a positive test on a bulk sample of five leaves could have one to five PVY positive leaves and present the same percent PVY result in the sample. Using simple math and the equation, the number of PVY positive plants in the WGO sample could have ranged from 21 to 105 (6 to 30% PVY infection). Discrepancies from sample bulking could have been avoided if each WGO leaf was tested individually, however, cost of sampling and mimicking the current Idaho seed certification method of testing was taken into consideration for this study.

Results from the spring grow out allowed further confirmation in the accuracy of direct tuber testing. Smoke and Rindite treated tubers did not significantly differ in PVY detection compared to the spring grow-out samples in the three cultivars. This consistency indicated that

application with either Rindite or aerosol smoke to break dormancy resulted in PVY detection using ELISA methods on sprouted tubers comparable to what a grower may see in the field planted with the seed lot. For Ranger Russet in year 1, the UTC differed from the spring grow out results by 10%. This discrepancy could be due to ELISA testing being less accurate on tubers that have naturally broken dormancy (Hill and Jackson 1984). Since a discrepancy in results was not observed in Clearwater Russet, Umatilla Russet, nor in the second year for Ranger Russet, the inconsistency in Ranger Russet could be attributed to estimation of PVY levels from bulking leaves in the spring grow out compared to individual testing of tubers. Alternatively, cultivar could have an influence on PVY detection when using direct tuber testing. Ranger Russet may have greater variability of PVY distribution within the tuber, which could impact the ability to detect PVY both in the tuber and in leaves. Further investigating PVY distribution within a tuber for multiple cultivars would be worthwhile to help understand the variability observed in the literature and methods for accurate direct tuber testing of PVY. Inaccuracy or inconsistency may be a function of PVY distribution as well as sprout development. Additional research into comparing direct tuber testing on sprouted tubers to subsequent leaves from plants emerging from the same tuber sample needs to be conducted to further confirm if artificial dormancy breaking treatments influence the ability to detect PVY in tuber samples.

Conclusion

The influence of several methods to break dormancy of potato tubers to facilitate early and accurate PVY testing using laboratory-based ELISA techniques on tubers were studied. The application of Rindite produced greater sprout development compared to the smoke treatment and untreated control, but did not yield significantly different PVY incidence. 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. The direct tuber testing ELISA protocol used in this study on non-dormant tubers produced final PVY results an average of 47 days prior to the WGO. Further understanding of sprout development needed for accurate PVY detection, provided by this study, indicates tuber samples could have been tested even sooner. This study showed the novel use of cold aerosol smoke to encourage sprout development and that direct tuber testing using ELISA methods on sprouted non-dormant tubers could be a reliable, high-throughput, and faster alternative to the WGO for evaluating post-harvest PVY incidence in seed lots.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12230-024-09960-8.

Acknowledgements The authors are grateful to Lynn Woodell, Tyler Spence, Rabecka Hendricks, Lisa Tran, and Alan Westra for help and assistance during this project.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nathan Gelles and Nora Olsen. The first draft of the manuscript was written by Nathan Gelles and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The experiments described comply with the current laws of the United States.

Funding This study was funded in part through the USDA-NIFA-SCRI grant 2020-51181-32136, "Development of Sustainable System-based Management Strategies for Two Vector-borne, Tuber Necrotic Viruses in potato."

Declarations

Competing Interests The authors have no competing interests to declare that are relevant to the content of this article.

Disclaimers None

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