ALTERING POTATO TUBER PHYSIOLOGY TO PROMOTE DORMANCY BREAK AND IMPLICATIONS OF PVY IN SEED CERTIFICATION

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Abstract

Potato virus Y (PVY) is a major pathogen in potato production that is spread through vegetative propagation of seed tubers and is the most common defect resulting in downgrading or rejection of seed lots. Implementation of seed certification practices has been a global response in attempt to limit the spread of viruses through seed distribution. The overall goal for this research was to provide methods and tools for seed certification agencies to continue ensuring that available seed for distribution is of high quality and further understand the impact of PVY on potato production. To accomplish this goal three major objectives were conducted for this project. The first objective was to understand distribution of PVY within a seed lot and to determine yield impact of seedborne PVY under commercial-like production conditions. The second objective was to identify post-harvest methods to promote sprout development in freshly harvested tubers. The final objective was to facilitate the development of direct tuber testing as a means to estimate PVY levels in seed lots.

Determining PVY distribution within a seed lot was carried out by planting seed pieces from several different mother tuber size categories in field plots and evaluating for PVY incidence. The effects of seedborne PVY on yield were assessed by planting seed from certified lots with various levels of seedborne PVY infection of Russet Burbank (0, 2 and 10% PVY), Ranger Russet (3 and 34% PVY), and Russet Norkotah (2 and 11% PVY) and evaluating final yield and grade. Objective two was conducted by applying several post-harvest treatments (cold temperature and temperature fluctuations, cold aerosol smoke, gibberellic acid, and combination treatments) to Russet Burbank, Clearwater Russet, and Umatilla Russet potato tubers approximately four, eight, or ten weeks after harvest and evaluating for sprout development. Objective three was conducted on Ranger Russet, Clearwater Russet, and Umatilla Russet. Treatments were applied to break dormancy soon after harvest (untreated, smoke, or Rindite) and evaluated for sprout development. PVY detection via ELISA was conducted on tissue directly from a non-dormant tuber and compared to PVY levels from leaf tissue samples in the winter grow out and subsequently compared to the directly tested seed planted in a field the following spring.

Major findings from this study were as follows. For objective one, distribution of PVY within a seed lot appeared to be uniform regardless of the mother tuber size used to produce a successive plant and the response was consistent with cultivar, PVY infection level, and year. Results indicate that selecting for tuber size, whether for the winter grow out or in commercial plantings, does not

influence the level of PVY observed. Effects of seedborne PVY infection on yield and grade were dependent upon cultivar. Russet Burbank and Russet Norkotah yields had an inverse relationship with seedborne PVY infection, as PVY infection increased, yields decreased, although yield reduction was not a linear function for Russet Burbank. Ranger Russet yield or grade was not significantly impacted by seedborne PVY infection in the current study. These results align with previous studies indicating seedborne PVY can impact yield, but the response is cultivar dependent.

For objective two, efficacy of treatments at promoting early sprout development were dependent upon treatment timing, cultivar, and year. The efficacy of most treatments at inducing sprout development increased with time after harvest, with the exception of temperature treatments, which resulted in equal or less sprout development compared to the untreated control. Aerosol smoke and gibberellic acid based treatments increased sprout development compared to the untreated control. The combination of aerosol smoke plus gibberellic acid promoted dormancy break and had the greatest sprout development in all treatment timings, years, and cultivars. Umatilla Russet was more responsive to gibberellic acid treatment, whereas Clearwater Russet and Russet Burbank were more responsive to 1h 20h smoke treatment in promoting sprouting. A novel method of breaking dormancy using cold aerosol smoke was identified and could be used alone or in combination with gibberellic acid to promote dormancy break and enhance sprout development to help facilitate direct tuber testing for PVY detection.

For objective three, treatments to enhance sprouting prior to direct tuber testing showed Rindite consistently produced the greatest sprout development. Smoke treatment encouraged sprout development more than untreated control. This established three levels of sprout development at the time of direct tuber testing for PVY. In general, utilizing direct tuber testing was comparable to the winter grow out for PVY detection in three russet cultivars (15% versus 14%). To observe PVY infection in a subsequent crop and further confirm accuracy of PVY detection in seed lots that were direct tuber tested after artificial dormancy break, a spring grow out of tested seed was conducted. Direct tuber testing of samples provided accurate PVY incidence results for three seed lots of russet cultivars 47 days earlier on average than the winter grow out results could be obtained. Findings from this research directly benefit potato seed certification agencies in determining proper management strategies for PVY detection in commercial seed lots.

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Dedication

I would like to dedicate this thesis to my fiancé, Chelsey, for her patience and encouragement throughout this project. I also dedicate this thesis to my mentors, friends, and family that have provided guidance and support throughout my life. I am beyond grateful to you all.

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Chapter 1: Introduction

The horticultural crop potato (*Solanum tuberosum* L.) is produced around the world and is a staple, nutrient rich food in many diets. Due to widespread production, the crop is subject to a plethora of production conditions and exposed to many different pathogens. In the United States (US), the sale of potatoes is worth $3.91 billion and the crop is produced on 373,525 hectares with an estimated 11% of the land dedicated to seed potato production (USDA 2021).

Potatoes are vegetatively propagated instead of planting true seed. The tuber portion of the potato plant is kept from previous crops to serve as the propagative "seed" tuber. This production method and cycle is subject to unique increases in diseases, such as viruses carried over from generation to generation. In the early 1900's potato growers were faced with what was referred to as the 'degeneration' effect on potato, where yields would decline after several years of continual production (Appel 1934; Leach 1938).

Loss in production was recognized to be caused primarily by the accumulation of viruses over time, resulting in the adoption of seed certification programs in many countries (Appel 1934; Leach 1938). The original objective of seed certification was to maintain varietal purity, reduce disease, and minimize spread of undesired traits through tuber propagation (Shepard and Claflin 1975). Virus levels can increase rapidly in a seed lot if left unmonitored or unmanaged. Currently, potato virus Y (PVY) is the most common virus resulting in the downgrading or rejection of seed lots for certification and recertification (Frost et al. 2013; Tran et al. 2022; Lindner et al. 2015); therefore, it may be the most important pathogen regulated by seed certification (Lindner et al. 2015).

PVY is a small, flexuous filamentous, positive sense single-stranded RNA virus belonging to the *Potyviridae* family (Fauquet et al. 2005; Huhnlein et al. 2013). It can infect plants in more than 31 different families including several Solanaceous crops (Kerlan and Moury 2008). The virus is well studied but remains a concern for potato growers in North America (Karasev and Gray 2013).

Seedborne PVY can express a wide variation in symptom severity that can range from mild mosaic of foliage to systemic plant necrosis, which can result in severe tuber yield loss and quality defects (Gray et al. 2010; Karasev and Gray 2013; Hane and Hamm 1999; Whitworth et al. 2006; Mackenzie et al. 2019, Chikh-Ali et al. 2020). Hane and Hamm (1999) determined seedborne PVY infection reduced marketable yield 79% in the cultivar Shepody compared to plants produced from a healthy non-infected seed tuber. Research on Russet Norkotah showed a total yield reduction of 45 to 48%, while marketable yields were reduced by 65% compared to plants produced from healthy

seed tubers (Hane and Hamm (1999). Whitworth et al. (2006) found Russet Norkotah, and cultivar CO800011-5 total yields were reduced by approximately 38% and Russet Burbank yields were reduced by 63% due to seedborne PVY infection. Further, Gundersen et al. (2019), Rykbost et al. (1999), Whitworth et al. (2010), Mackenzie et al. (2019), Kolychikhina et al. (2021), and Chikh-Ali et al. (2020) all reported significant yield losses due to seedborne PVY in several widely grown potato cultivars. While yield losses from seedborne PVY are significant, the extent of loss can be dependent upon potato cultivar and PVY strain, although these interactions are not fully understood.

PVY can also be transmitted to healthy potato plants during the growing season (in-season transmission) through an aphid vector (Gadhave et al. 2020). When plants are infected during the growing season, symptoms may range from no expression to very mild mosaic in leaves, to systemic plant necrosis depending upon the timing of infection, PVY strain, and potato cultivar (Mackenzie et al. 2019). Weber et al. (2021) evaluated current season infection of PVY and found yields of three chipping cultivars were negatively impacted if inoculated with PVY during the growing season. Whitworth et al. (2010) also compared the effects on yield between seedborne and current season PVY infection in Russet Norkotah. Seedborne infection caused greater yield loss compared to in-season PVY infection, but both produced lower yield than uninfected plants. Translating findings from many of these research studies to the effects of yield on a commercial production scale may be difficult since many of the studies focused on yield losses of an infected plant compared to a healthy plant. It is necessary for yield studies to be conducted under commercial production conditions to identify the true impact of seedborne PVY on a farm level, which was an objective of the present study.

To add to the complexity of infection, PVY has the capability of producing multiple recombinant strains (Lindner 2015; Kogovsek et al. 2008; Chikh-Ali et al. 2007; Visser et al 2012; Green et al. 2017; Green et al. 2018). Recombination, a survival mechanism employed by viruses to adapt to new environments and overcome host defenses, occurs when genetic information is exchanged during a co-infection of two or more viral genomes within a single host cell (Perez-Losada et al. 2015). Various studies have shown PVY strain and/or potato cultivar interaction have a significant influence on yield losses (Dupuis 2017; Gunderson et al. 2019; Weber et al. 2021; Whitworth et al. 2012). PVY^{O} was the predominant strain for many years (Gray et al. 2010), but Tran et al. (2022) and Mackenzie et al. (2019) showed strain composition has been changing over time and shifting to recombinant strains of PVY^{NTN} and $PVY^{N\text{-}Wi}$ which produce mild foliar symptoms compared to PVY^{O}. Also, PVY^{NTN} is known to cause potato tuber necrotic ringspot disease, a severe

quality defect in several potato cultivars. Many of the previous studies focused on the impact of PVY^{O} on yield. Research with current strains observed in commercial fields needs to be conducted to determine the impact new strains have on presently grown potato cultivars.

Seed certification agencies are responsible for assuring seed lots are within specified tolerances and relatively free from pests, varietal impurities, and defects (Callison et al. 1982; Duellman et al. 2020). The seed certification process evaluates issues deemed detrimental to potato production and is designed to encompass current production issues (Frost et al. 2013). In order to make decisions for regulating pathogens in seed production, it is imperative to understand the impact a pathogen has on a production system. Over time the seed certification process has grown to incorporate several production limiting issues including, but not limited to, varietal purity of the seed lot, chemical carryover (herbicide) in the seed tuber, level of potato leafroll virus (PLRV), and level of mosaic symptoms mainly attributed to presence of PVY (ICIA 2022a). Current and up-to-date research on pathogens, such as PVY, needs to be conducted to evaluate how changes in the production system and pathogenicity of the virus impact yield and quality of presently grown cultivars.

Seed certification programs have established strict production guidelines for seed lot recertification, certification, and distribution. Seed lots undergo five inspections throughout each production and storage season to ensure seed lots do not exceed set quality tolerances. Two field inspections are completed during the growing season followed by a storage inspection, a post-harvest crop inspection, and a final shipping point inspection (ICIA 2022a). An accurate estimation of pathogens and defects within a seed lot is necessary for commercial seed growers to make management and seed purchasing decisions.

Since PVY can be transmitted to plants late in the growing season (after in-season inspections are completed) or express latent symptoms in some cultivars (i.e. Russet Norkotah; Whitworth et al. 2010), a post-harvest test is necessary to accurately estimate the level of virus in a seed lot. Most seed producing states in the US use the winter grow out (WGO) method to conduct post-harvest testing during the storage months (soon after harvest) while other states use greenhouse grow-outs or rely solely on in-season field inspections.

In states that conduct a WGO, seed growers are responsible for collecting and summitting a representative sample of small seed tubers (42 to 113 g) to their state's certification agency (ICIA 2022b). The size of tubers within the seed lot may be greater or more variable, but smaller sized or

single drop tubers are desired for the WGO process for several reasons. The planting equipment cannot plant seed potatoes larger than approximately 113 grams without being cut or causing issues to the planting of the crop. Cut seed can increase the risk of potential cross contamination and spread of disease through mechanical inoculation (Inglis et al. 2013), and the logistics involved with cutting seed would add an extra step and complexity to the WGO system potentially further delaying the process. Although logistically easier to plant, smaller sized tubers may not represent the size range of tubers within a given seed lot.

Due to seed certification requiring smaller sized tubers to be submitted for WGO post-harvest testing, it is valuable to know if tuber size influences PVY infection level. If so, this could cause and under or over-estimation of PVY in seed lots. Fox et al. (2005) indicated the WGO may underestimate the level of infection of a seed lot due to uneven distribution of PVY between tubers. Numerous studies have shown variability in the potential for various plant parts, including tubers, to become infected with PVY. Dupuis (2017) indicated it is unknown if all stems emerging from a mother tuber will be infected with PVY. Kogovsek et al. (2011) studied the distribution of PVY^{NTN} in potato plant tissue and found virus accumulation differed throughout the plant with petiole and above ground stem tissue having higher levels of viral RNA compared to tubers. Dupuis (2017) studied the movement of PVY in the vascular system and discovered PVY can move through the xylem and phloem infecting both new foliage and daughter tubers. Also, PVY movement within a plant occurs more rapidly in young plants compared to older plants due to a restriction of cell-to-cell movement, therefore the maturity of the plant is an important component in PVY infection. Other studies suggest PVY is not evenly distributed in all daughter tubers of a plant (Rusetsky and Blotskaya 2001 as cited in Huhnlein et al. 2013; Fox et al. 2005). Whitworth et al. (2012) showed PVY distribution within a single tuber can be variable, with PVY strain and potato cultivar being significant factors. 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 timing of tuber initiation, tuber development, or final size of the tuber.

Collected seed for the WGO is planted in a permitting climate (i.e., Hawaii, Florida) soon after harvest for virus and other evaluations. However, tubers planted immediately after harvest will often not produce a plant even when optimal growing conditions are present (Sonnewald 2001). Potato tubers experience a state of dormancy or cessation of growth following harvest (Mani et al. 2014; Suttle 2004). The physiological state of dormancy, referred to as endo-dormancy, can last days, weeks, or even months depending upon the cultivar and pre- and post-harvest conditions (Mani et

al. 2014; Muthoni et al. 2014). Major phytohormones, ethylene, gibberellin, abscisic acid (ABA), auxin, and cytokinin are believed to be involved in maintenance and release of tuber dormancy (Suttle 2004; Mani et al. 2014; Campbell et al. 2008). Although exact biochemical and phytohormonal processes are not fully elucidated, it is well documented ABA and ethylene are involved in the induction and maintenance of dormancy while cytokinin is involved in releasing of dormancy, whereas auxin and GA are involved in sprout development upon dormancy release (Dogonadze et al. 2000; Mani et al. 2014; Campbell et al. 2008; Sonnewald 2001; Sonnewald and Sonnewald 2014; Suttle 1998). Once the endo-dormant period subsides, tubers may be in a state of arrested growth due to unfavorable conditions referred to as eco-dormancy (Aksenova et al. 2013; Mani et al. 2014). Eco-dormancy can be broken if environmental conditions become favorable, and sprout development may occur.

The status and length of dormancy varies for each cultivar, but dormancy remains an issue for certification agencies planting tubers soon after harvest (Liu et al. 2015). Due to dormancy delaying sprout development and plant growth, each sample destined for the WGO is treated with chemicals, typically 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). Treated tubers are then loaded into a climate-controlled cargo container and shipped to be planted in a permitting climate (Duellman et al. 2020). Difficult-to-sprout cultivars are dipped in a gibberellic acid treatment before planting to promote sprouting and increase the rate of emergence during the inspection period. Gibberellic acid is known to be involved in dormancy release and promote sprout elongation (Tavakoli et al. 2014; Dogonadze et al. 2000). Plants are grown until they reach adequate size, approximately 30 cm, and visually inspected for chemical (i.e. herbicide) carryover, PLRV, varietal mix, and mosaic symptoms primarily from PVY (Frost et al. 2013; ICIA 2022b; Duellman et al. 2020). Several states also collect leaf tissue samples to be lab tested for presence of PVY.

The current WGO certification process can be time consuming, resource intensive, and the availability and sustainability of chemicals involved in dormancy break may be questionable. Growers desire post-harvest testing results as early as possible to determine viable seed stock levels and to capitalize on exporting to earlier markets (Fox et al. 2005; Singh et al. 2013). In the Pacific Northwest, seed potatoes are typically harvested from September to October. The WGO produces seed lot health certificates around the middle of January. Extended periods between harvest and availability of winter test results are not desirable and may not allow growers ample time to adjust

marketing strategies to reach early markets or make decisions on purchasing seed (Fox et al. 2005; Singh et al. 2013). Further research into methods to reduce the time to produce plant health certificates as well as provide a safer yet effective method to break dormancy is needed.

Other methods for estimating PVY levels in seed lots include greenhouse grow-outs and laboratory-based methods for direct tuber testing. Laboratory methods are typically conducted using various reverse-transcription polymerase chain reaction (RT-PCR) formats or enzyme-linked immunosorbent assay (ELISA) methods, and are being explored as viable, accurate, high-throughput, cost-effective, and faster alternatives for the WGO (Singh et al. 2013; Avrahami-Moyal et al. 2017; Russo et al. 1999; Beissinger and Inglis 2018; Fox et al. 2005; Schumpp et al. 2021).

The laboratory ELISA and PCR methods utilize extract from tissue directly from the tuber, tissue from developing sprouts, or a combination of sprout and tuber tissue. Laboratory methods have been implemented on dormant tubers with variable results (Avrahami-Moyal et al. 2017; Barker et al. 1993; Russo et al. 1999; Hill and Jackson 1984; Sign and Singh 1996; Singh et al. 2013; Huhnlein et al 2013; Fox et al. 2005; Debokx and Mooi 1974; DeBokx and Cuperus 1987). Reliability and accuracy using direct tuber testing methods has varied with cultivar, sample location on the tuber, and length of time in storage.

ELISA methods have been shown to be inefficient at predicting PVY levels in dormant tubers (Barker et al. 1993; Gugereli and Gehriger 1980). However, the reliability of ELISA testing increases when tuber dormancy is artificially broken, and sprouting has begun (McDonald and Coleman 1988; Gugerli & Gehriger 1980; Vetten et al. 1983). Conversely, Fox et al. (2005) determined the reliability of ELISA testing for PVY significantly decreased after 10 weeks of storage, which coincided with natural dormancy break. Reduction in PVY detection after natural dormancy break was also observed by Hill and Jackson (1984). Gugerli and Gehriger (1980) found when using ELISA methods, PVY was not detected until after tuber dormancy was artificially broken with an application of Rindite. Vetten et al. (1983) observed a similar response of increased virus detection, and PVY was more uniformly distributed within a tuber after applying a Rindite treatment to break dormancy. McDonald and Coleman (1988) reported that treating with either bromoethane or Rindite increased virus detection in Russet Burbank tubers compared to the untreated control, but it is unclear to what extent development of spouted tissue is required. ICIA (2019) suggested reliability and accuracy of direct tuber testing increases when tubers have sprouts 6 mm in length or greater. Other certification agencies recommend using sprout lengths of 3 to 5 mm (UNECE 2019).

In general, tuber dormancy is a major issue for seed certification agencies since post-harvest testing requires actively growing tissue soon after harvest regardless of planting in a WGO, greenhouse, or directly testing the seed. Currently, most certification agencies use Rindite, which has shown to be effective at inducing sprouting in dormant tubers (Esztergaylyos and Polgar 2021; Denny 1945; Gugerli and Gehriger 1980; McDonald and Coleman 1988), but is considered to be extremely volatile, corrosive, dangerous (Bryan 1989), and highly toxic to mammals (McDonald and Coleman 1988). There is a demand for efficient and less hazardous methods for breaking potato dormancy soon after harvest (Haider et al. 2022; Wiltshire and Cobb 1996). Other chemical methods, such as thiourea, potassium thiocyanate, and carbon disulphide, have been studied for breaking tuber dormancy (Denny 1926; Bryan 1989). Several studies have found bromoethane is effective at breaking dormancy soon after harvest (Esztergalyos and Polgar 2021; Bryan 1989) and PVY detection using ELISA was similar to tubers treated with Rindite (McDonald and Coleman 1988). Bromoethane is less toxic than Rindite but is highly flammable and poses potential health hazards to animals and humans (McDonald and Coleman 1988; Safety Data Sheet 2022).

Using temperature manipulations and plant growth regulators (phytohormones) to break dormancy has been widely discussed with varying results. Wurr and Allen (1976) reported storing tubers at 2 to 3 C followed by warmer temperatures increased sprout development and improved emergence of the successive crop. Davidson (1958) found storing tubers at warm temperatures (26.7 C) induced sprouting sooner than tubers stored at cooler temperatures (1.7 C). Haider et al. (2022) showed a cold pre-treatment followed by an increase to ambient temperatures reduced the time to dormancy break. However, many of these studies were conducted over many months, which is not advantageous for certification agencies who need sprouting tubers soon after harvest.

Application of phytohormones have been used as an alternative to break dormancy. Soaking tubers in various solutions of a synthetic cytokinin (benzyl-adenine, benzyl amino purine, kinetin) has been shown to significantly reduce tuber dormancy in several cultivars (Esztergalyos and Polgar 2021; Haider et al. 2022; Majeed and Bano 2006). Dipping tubers in gibberellic acid (GA) has also been shown to reduce dormancy length and/or promote sprout elongation (Haider et al. 2022; Esztergalyos and Polgar 2021; Hartman et al. 2011; Kulen et al. 2011; Tavakoli et al. 2014; Wrobel et al. 2017). Although effective, many of these studies required wounding or excised tuber tissue, which is not desirable for large scale application as is necessary for seed certification.

Another hormonal treatment to alter dormancy is the application of ethylene. However, applications of ethylene can result in sprout inhibition or sprout promotion depending upon the concentration and duration of tuber exposure (Muthoni et al. 2014; Suttle 1998). Rylski et al. (1974) observed potatoes exposed to low rates of ethylene for 72 hours had greater sprout development compared to the untreated control, whereas longer exposures to ethylene inhibited sprout growth. Since sprout inducing effects of ethylene are variable and highly dependent on specific rates and timings, it is not suitable to be widely used to break dormancy for certification purposes.

Inconsistent results and lack of large-scale applicability for many treatments have left the seed potato industry desiring a consistent, effective, and safe method to break potato tuber dormancy soon after harvest (Haider et al. 2022). A butanolide compound, 3-methyl-2H-furo[2.3-c]pyran-2-one (Karrikinolide; a Karrikin plant growth regulator), isolated from smoke produced by combustion of plant-based materials has been reported to be responsible for seed germination in several true seed species (Flematti et al. 2004; Van Staden et al. 2004; Light et al. 2009; Chiwocha et al. 2009). Verschaeve et al. (2006) conducted tests to determine toxicity levels of Karrikinolide and determined it had no toxic nor genotoxic effects at the rates tested. Based upon toxicity screening and stimulatory effects on many true seed species, application of aerosol smoke was explored as a less toxic and effective method to break dormancy in potato tubers.

This project focused on providing strategies for managing and accessing PVY in seed certification. The first objective of this project was to evaluate if tuber size restrictions for the WGO may inadvertently be selecting higher or lower levels of PVY. Objective two was to evaluate the risk of a yield penalty associated with planting seed lots containing PVY on a commercial-like scale. Objective three was to evaluate multiple post-harvest treatments applied to dormant tubers to induce sprout development soon after harvest. The final objective of the project was to determine if effective dormancy breaking techniques would promote rapid sprout development for accurate direct tuber testing of PVY soon after harvest. The overall goals for this project were to identify methods for seed certification agencies to continue ensuring the supply of high-quality seed potatoes and to further understand the impacts of PVY on potato production.

References

Akoumianakis, K., C. M. Olympios, and H. C. Passam. 2000. Effect of "rindite" and bromoethane on germination, sprout emergence, number of sprouts, and total yield of tubers of potato cv. Spunta. *Advances in Horticultural Sciences* 14(1): 33-35.

Aksenova, N. P., L. I. Sergeeva, T. N. Konstantinova, S. A. Golyanovskaya, O. O. Kolachevskaya, and G. A. Romanov. 2013. Regulation of potato tuber dormancy and sprouting. *Russian Journal of Plant Physiology* 60(3): 301-312.

Appel, O. 1934. Vitality and vitality determination in potatoes. *Phytopathology* 24: 482-94.

Avrahami-Moyal, L., Y. Tam, M. Brumin, S. Prakash, D. Leibman, M. Pearlsman, M. Bornstein, N. Sela, M. Seidan, Z. Dar, U. Zig, A. Gal-On, and V. Gaba. 2017. Detection of potato virus Y in industrial quantities of seed potatoes by TaqMan Real Time PCR. *Phytoparasitica* 45: 591-598.

Barker H., K. D. Webster, and B. Reavy. 1993. Detection of potato virus Y in potato tubers: a comparison of polymerase chain reaction and enzyme-linked immunosorbent assay. *Potato Research* 36: 13-20.

Beissinger, A., and D. A. Inglis. 2018. Greenhouse comparison of two detection methods of Potato virus YN-Wi at four potato growth stages. *Plant Health Progress* 19: 71-75.

Bryan, J. E. 1989. Breaking dormancy of potato tubers. *CIP Research Guide* 16: 1-13.

Callison B. L., J. Harrington, and D. Douglas. 1982. Seed potato certification: Its purpose, capabilities, and limitations. *American Potato Journal* 59: 231-236.

Campbell, M., E. Segear, L. Beers, D. Knaber, and J. Suttle. 2008. Dormancy in potato tuber meristems: Chemically induced cessation in dormancy matches the natural process based on transcript profiles. *Functional and Integrative Genomics* 8: 317-328.

Chikh Ali, M., T. Maoka, and T. Natsuaki. 2007. The occurrence and characterization of new recombinant isolates of PVY displaying shared properties of PVY^{NW} and PVY^{NTN}. *Journal of Phytopatholgy* 155: 409-415.

Chikh-Ali, M., L. T. Tran, W. J. Price, and A. V. Karasev. 2020. Effects of the age-related resistance to potato virus Y in potato on the systemic spread of the virus, incidence of the potato tuber necrotic ringspot disease, tuber yield, and translocation rates into progeny tubers. *Plant Disease* 104: 269-275.

Chiwocha, S. D. S., K. W. Dixon, G. R. Flematti, E. L. Ghisalberti, D. J. Merritt, D. C. Nelson, J. A. M. Riseborough, S. M. Smith, and J. C. Stevens. 2009. Karrikins: A new family of plant growth regulators in smoke. *Plant Science* 117: 252-256.

Davidson, T. M. W. 1958. Dormancy in the potato tuber and the effects of storage conditions on initial sprouting and on subsequent sprout growth. *American Potato Journal* 35: 451-465.

DeBokx, J. A., and C. Cuperus. 1987. Detection of potato virus Y in early-harvest potato tubers by cDNA hybridization and three modifications of ELISA. *European and Mediterranean Plant Protection Organization Bulletin* 17: 73-79.

DeBokx, J. A., and J. C. Mooi. 1974. Methods of quality assessment of seed potatoes. *Potato Research* 17: 410-433.

Denny, F. E. 1926. Hastening the sprouting of dormant potato tubers*. American Journal of Botany* 13(2): 118-125.

Denny, F. E. 1945. Synergistic effects of three chemicals in the treatment of dormant potato tubers to hasten germination. *Boyce Thompson Institute for Plant Research, Inc*. 14: 1-14.

Dogonadze, M. Z., N. P. Korableva, T. A. Platoneva, G. L. Shaposhnikov. 2000. Effect of gibberellin and auxin on the synthesis of abscisic acid and ethylene in buds of dormant and sprouting potato tuber. *Applied Biochemistry and Microbiology* 36: 507-509.

Duellman, K. M., M. K. Thornton, and A. A. Westra. 2020. Potato seed certification and selection in Idaho. Extension Bulletin 983. *University of Idaho*, Moscow, ID.

Dupuis, B. 2017. The movement of potato virus Y (PVY) in the vascular system of potato plants. *European Journal of Plant Pathology* 147: 365-373.

Esztergalyos, A., and Z. Polgar. 2021. The effect of chemical treatments on the tuber dormancy of Hungarian potato cultivars. *Potato Research* 64: 327-337.

Fauquet, C. M., M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball. 2005. Virus taxonomy. *Eighth Report of the International Committee of Taxonomy of Viruses.* Academic Press 739-1128. ISBN 9780122499517.

Flematti, G. R., E. L. Ghisalberti, K. W. Dixon, and R. D. Trengove. 2004. A compound from smoke that promotes seed germination. *Science* 305(5686): 977.

Fox, A., F. Evans, and I. Browning. 2005. Direct tuber testing for potato Y potyvirus by real-time RT-PCR and ELISA: Reliable options for post-harvest testing? Bulletin 356: 93-97. *Scottish Agricultural Science Agency, Scotland.*

Frost, K., R. L. Groves, and A. O. Charkowski. 2013. Integrated control of potato pathogens through seed potato certification and provisions of clean seed potatoes. A*merican Phytopathological Society* 97(10): 1268-1280.

Gadhave, K. R., S. Gautam, D. A. Rasmussen, and R. Srinivasan. 2020. Aphid transmission of *potyvirus*: The largest plant-infecting RNA virus genus. *Viruses* 12(7): 773- 794.

Gray, S., S. De Boer, J. Lorenzen, A. Karasev, J. Whitworth, P. Nolte, R. Singh, A. Boucher, and H. Xu. 2010. Potato virus Y: an evolving concern for potato crops in the United States and Canada. *The American Phytopathological Society* 94(10): 1384-1397.

Green, K. J., C. J. Brown, and A. V. Karasev. 2018. Genetic diversity of potato virus Y (PVY): Sequence analyses reveal ten novel PVY recombinant structures. *Archives of Virology* 163: 23-32.

Green, K. J., C. J. Brown, S. M. Gray, and A. V. Karasev. 2017. Phylogenetic study of recombinant strains of *Potato virus Y*. *Virology* 507:40–52.

Gugerli, P., and W. Gehriger. 1980. Enzyme-linked immunosorbent assay (ELISA) for the detection of potato leafroll virus and potato virus Y in potato tubers after artificial break of dormancy. *Potato Research* 23: 353-359.

Gundersen, B., D. A. Inglis, M. Pavek, and A. V. Karasev. 2019. Foliar and tuber reactions of three strains of potato virus Y on five fresh market potato cultivars through three successive potato generations. *American Journal of Potato Research* 96:519-531.

Haider, M. W., M. Nafees, I. Ahmad, B. Ali, Maryam, R. Iqbal, D. C. Vodnar, R. A. Marc, M. Kamran, M. H. Saleem, A. A. Al-Ghamdi, F. M. Al-Hemaid, and M. S. Elshikh. 2022. Postharvest dormancy-related changes of endogenous hormones in relation to different dormancy-breaking methods of potato *(Solanum tuberosum L.)* tubers. *Frontiers in Plant Science* 13: 945253.

Hane, D. C., and P. B. Hamm. 1999. Effects of seedborne potato virus Y infection in two potato cultivars expressing mild disease symptoms. *Plant Disease* 83: 43-45.

Hartmann A., M. Senning, P. Hedden, U. Sonnewald, and S. Sonnewald. 2011. Reactivation of meristem activity and sprout growth in potato tubers require both cytokinin and gibberellin. *Plant Physiology* 155: 776-796.

Hill, S. A., and E. A. Jackson. 1984. An investigation of the reliability of ELISA as a practical test for detecting potato leaf roll virus and potato virus Y in tubers. *Plant Pathology* 33: 21-26.

Huhnlein, A., N. Drechsler, P. Steinback, T. Thieme, and J. Schubert. 2013. Comparison of three methods for the detection of Potato virus Y in seed potato certification. *Plant Diseases and Protection* 120(2): 57-69.

ICIA. 2019. ICIA procedure: Virus testing- ELISA method version 2019-01. *Idaho Crop Improvement Association, Inc*

ICIA. 2022a. Idaho Potato Certification Standards. *Idaho Crop Improvement Association, Inc*.

ICIA. 2022b. Policies and Procedures of Certification for Seed Potatoes in Idaho. *Idaho Crop Improvement Association, Inc*.

Inglis, D. A., D. Johnson, B. Schroeder, and C. Benedict. 2013. Bacterial ring rot on potatoes. FS Bulletin 102E, *Washington State University.* Pullman, WA.

Karasev, A., and S. M. Gray 2013. Continuous and emerging challenges of potato virus Y in potato. *Annual Review of Phytopathology* 51: 571-586.

Kerlan, C., and B. Moury. 2008. Potato virus Y. *Encyclopedia of Virology* 287-296.

Kogovsek, P., A. Kladnik, J. Mlakar, M. T. Znidaric, M. Dermastia, M. Ravnikar, and M. Pompe-Novak. 2011. Distribution of potato virus Y in potato plant organs, tissues, and cells. *American Phytopathological Society* 101: 1292-1300.

Kogovsek, P., L. Gow, M. Pompe-Novak, K. Gruden, G. D. Foster, N. Boonham, and M. Ravnikar. 2008. Single-step RT real-time PCR for sensitive detection and discrimination of potato virus Y isolates. *Virological Methods*. 149:1-11.

Kolychikhina, M. S., O. O. Beloshapkina, and C. Phiri. 2021. Change in potato productivity under the impact of viral diseases. *Earth and Environmental Science* 663: 012035

Kulen, O., C. Stushnoff, R. D. Davidson, and D. G. Holm. 2011. Gibberellic acid and ethephon alter potato minituber bud dormancy and improve seed tuber yield. *American Journal of Potato Research* 88: 167-174.

Leach, J. G. 1938. The biological basis for certification of seed potatoes. *American Journal of Potato Research* 15:117-130.

Light, M. E., M. I. Daws, and J. Van Staden. 2009. Smoke-derived butanolide: Towards understanding its biological effects. *South African Journal of Botany* 75: 1-7.

Lindner, K., F. Trautwein, A. Kellerman, and G. Bauch. 2015. Potato virus Y (PVY) in seed potato certification. *Journal of Plant Diseases and Protection* 122(3):109-119.

Liu B., N. Zhang, Y. Wen, X. Jin, J. Yang, S. Hauijun, and D. Wang. 2015. Transcriptomic changes during tuber dormancy release process revealed by RNA sequencing in potato. *Journal of Biotechnology* 198:17-30.

Mackenzie, T. D. B., Z. Nie, V. Bisht, and M. Singh. 2019. Proliferation of recombinant PVY strains in two potato-producing regions of Canada, and symptom expression in 30 important potato varieties with different PVY strains. *Plant Disease* 103(9): 2221-2230.

Majeed, A., and A. Bano. 2006. Role of growth promoting substances in breaking potato (*Solanum tuberosum* L.) tuber dormancy. *Journal of Agriculture and Social Sciences* 2(3): 175-178.

Mani, F., T. Bettaieb, N. Doudech, and C. Hannachi. 2014. Physiological mechanisms for potato dormancy release and sprouting: A review. *African Crop Science Journal* 22(2): 155-174.

McDonald, J. G., and W. K. Coleman. 1988. A reevaluation of bromoethane in comparison to rindite for the post-harvest detection of potato virus Y in tubers by ELISA. *American Potato Journal* 65: 547-550.

Muthoni, J., J. Kabira, H. Shimelis, and R. Melis. 2014. Regulation of potato tuber dormancy: A review. *Australian Journal of Crop Science* 8(5): 754-759.

Perez-Losada, M., M. Arenas, J. C. Galan, F. Palero, and F. Gonzalez-Candelas. 2015. Recombination in viruses: Mechanisms, methods of study, and evolutionary consequences. *Infection, Genetics and Evolution* 30: 296-307.

Rusetsky, N. V., and Z. V. Blotskaya. 2001. Distribution and accumulation of PVM and PVY, PVX in infected potato plants. *Archives of Phytopathology and Plant Protection* 34: 275-282.

Russo, P., L. Miller, R. P. Singh, and S. A. Slack. 1999. Comparison of PLRV and PVY detection in potato seed samples tested by Florida winter field inspection and RT-PCR. *American Journal of Potato Research* 76: 313-316.

Rykbost, D. A., D. A. Hane, P. B. Hamm, R. Voss, and D. Kirby. 1999. Effects of seedborne potato virus Y on Russet Norkotah performance. *American Journal of Potato Research* 75: 91-96.

Rylski, I., L. Rappaport, and H. K. Pratt. 1974. Dual effects of ethylene on potato dormancy and sprout growth. *Plant Physiology* 53: 658-662.

Safety Data Sheet (SDS). 2022. Bromoethane. *ThermoFisher Scientific* 1-8.

Schumpp, O., A. Brechon, J. Brodard, B. Dupuis, L. Farinelli, P. Frei, P. Otten, D. Pellet. 2021. Large-scale RT-qPCR diagnostics for seed potato certification. *Potato Research* 64: 553-569.

Shepard, J. F., and L. E. Claflin. 1975. Critical analyses of the principals of seed potato certification. *Annual Review of Phytopathology* 13:271-293.

Singh M., and R. P. Singh. 1996. Factors affecting detection of PVY in dormant tubers by reverse transcription polymerase chain reaction and nucleic acid spot hybridization. *Journal of Virological Methods* 60: 47-57.

Singh, M., R. P. Singh, M. S. Fageria, X. Nie, R. Coffin, and G. Hawkins. 2013. Optimization of a real-time RT-PCR assay and its comparison with ELISA, conventional RT-PCR, and the grow-out test for large scale diagnosis of potato virus Y in dormant potato tubers. *American Journal of Potato Research* 90: 43-50.

Sonnewald, S., and U. Sonnewald. 2014. Regulation of potato tuber sprouting. *Planta* 239: 27-38.

Sonnewald, U. 2001. Control of potato tuber sprouting. *Trends in Plant Science* 6(8): 333-335.

Suttle, J. C. 1998. Involvement of ethylene in potato microtuber dormancy. *Plant Physiology* 118: 843-848

Suttle, J. C. 2004. Physiological regulation of potato tuber dormancy. *American Journal of Potato Research* 81: 253-262.

Tavakoli, K., A. Razavi, and A. Sohani. 2014. Effects of different temperatures and hormone treatments on breaking dormancy in potato tubers. *Journal of Agricultural Sciences* 59(3): 255-264.

Tran, L., K. J. Green, M. Rodriguez-Rodriguez, G. E. Orellana, C. N. Funke, O. V. Nikolaeva, A. Quintero-Ferrer, M. Chikh-Ali, L. Woodell, N. Olsen, and A. V. Karasev. 2022. Prevalence of recombinant strains of potato virus Y in seed potato planted in Idaho and Washington states between 2011 and 2021. *Plant Disease* 106: 810-817.

UNECE. 2019. Findings of the survey of seed potato virus testing methods associated with seed potato certification. *United Nations Economic Commission for Europe*.

USDA, United States Department of Agriculture. 2021. Potatoes 2021 summary. *National Agricultural Statistics Service*.

Van Staden, J., A. K. Jager, M. E. Light, and B. V. Burger. 2004. Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany* 70(4): 654-659.

Verschaeve, L., J. Maes, M. E. Light, and J. Van Staden. 2006. Genic toxicity testing of 3-methyl-2H-furo[2,3-c]pyran-2-one, an important biologically active compound from plant-derived smoke. *Mutation Research* 611: 89-95.

Vetten, H. J., U. Ehlers, and H. L. Paul. 1983. Detection of potato viruses Y and A in tubers by enzyme-linked immunosorbent assay after natural and artificial break of dormancy. *Journal of Phytopathology* 108(1): 41-53.

Visser, J. C., D. U. Bellstedt, and M. D. Pirie. 2012 The recent recombinant evolution of a major crop pathogen, *potato virus Y. PLOS ONE* 7(11): e50631

Weber, E. L., J. S. Busse, and P. C. Bethke. 2021. Current-season infection with ordinary and recombinant strains of potato virus Y has detrimental effects on chipping potato yield and tuber quality. *Plant Disease* 105(2): 450-455.

Whitworth, J. L., P. B. Hamm, and C. S. McIntosh. 2010. Effect of potato virus Y on yield of a clonal selection of Russet Norkotah. *American Journal of Potato Research* 87:310-314.

Whitworth, J. L., P. B. Hamm, and P. Nolte 2012. Distribution of potato virus Y strains in tubers during the post-harvest period. *American Journal of Potato Research* 89:136-141.

Whitworth, J. L., P. Nolte, C. McIntosh, and R. Davidson. 2006. Effect of potato virus Y on yield of three potato cultivars grown under different nitrogen levels. *Plant Disease* 90(1): 73-76.

Wiltshire, J. J. J., and A. H. Cobb. 1996. A review of the physiology of potato tuber dormancy. *Annals of Applied Biology* 129: 553-569.

Wrobel, S., J. Kesy, and K. Treder. 2017. Effect of growth regulators and ethanol on termination of dormancy in potato tubers. *American Journal of Potato Research* 94: 544-555.

Wurr, D. C. E., and E. J. Allen. 1976. Effects of cold treatments on the sprout growth of three potato varieties. *Journal of Agricultural Science* 86: 221-224.

Chapter 2: Distribution of Potato Virus Y (PVY) Within a Seed Lot and the Impact of Seedborne PVY Infection on Yield of Potato Under Simulated Commercial Production Conditions

Abstract

Potato virus Y (PVY) is an important pathogen in potato production that can cause yield losses and is the leading pathogen causing downgraded or rejected seed lots for seed certification. Two trials were conducted to determine the distribution of PVY within a seed lot associated with mother seed tuber size and to determine the yield impact of seedborne PVY infection on three russet cultivars. Trial one was conducted over two growing seasons with Russet Norkotah (60% seedborne PVY in year one and 11% in year two) and one growing season with Umatilla Russet (26% seedborne PVY). Predetermined seed tuber size categories were collected: a) single drop (42 to 112 g), b) small (113 to 169 g), c) medium (170 to 282 g), d) large (283 to 340 g), and e) a mixed sample (year one only). Trial two was conducted in one year with field plots (5 replicates x 15.2 m length per treatment) planted with low (0%), medium (5%), and high (8%) seedborne PVY Russet Burbank seed lots, low (3%) and high (34%) seedborne PVY Ranger Russet lots, and low (2%) and high (11%) seedborne PVY Russet Norkotah seed lots. Plots for both trials were planted at Kimberly Research and Extension Center, Kimberly, ID in a randomized block design. Visual evaluations for PVY symptoms were conducted when plants were approximately 30 cm tall, prior to flowering. Total yield, tuber number, yield profile, and USDA grade were evaluated. In trial one, visual seedborne PVY incidence did not significantly differ between plants based upon mother tuber size category for either year, cultivar, or seedborne PVY level. In trial two, the high seedborne PVY lot significantly reduced total yield by 14% in Russet Burbank and 13% in Russet Norkotah compared to the low PVY lots. Ranger Russet total yield was not significantly impacted by higher levels of seedborne PVY infection. The proportion of total yield in each size profile category was similar for the high and low PVY lots in each cultivar. It appeared PVY was evenly distributed within a seed lot based upon mother tuber size and the yield impacts due to seedborne PVY infection are dependent upon cultivar and level of seedborne PVY.

Introduction

Potato virus Y (PVY) is a well-studied pathogen but remains a concern for potato (*Solanum tuberosum* L.) growers in North America (Karasev and Gray 2013). It is a small, flexuous filamentous, positive sense single-stranded RNA virus belonging to the *Potyviridae* family (Fauquet et al. 2005;

Huhnlein et al. 2013) and can infect plants in more than 31 different families including several solanaceous crops (Kerlan and Moury 2008). The vegetative propagation of potato poses unique challenges to the production system. The tuber portion of the potato plant is kept from previous crops to serve as the propagative “seed” tuber for the following growing season. This production method and cycle is subject to unique and damaging increases of diseases carried over from generation to generation. The result is seed degeneration and there is potential to incur up to 100 percent of tubers becoming diseased if seed lots are not removed from the production system over time (Halterman et al. 2012; Khurana 2004).

Degeneration of potato production was recognized to be caused primarily by viruses and resulted in the adoption of seed certification practices in the early 1900’s (Appel 1934; Leach 1938). These practices resulted in limited number of years or ‘generations’ potatoes from a specific field or seed lot could be increased or replanted as seed (Duellman et al. 2020; Frost et al. 2013). The original goals of certification were to minimize disease spread, reduce varietal mixture, and improve varietal types (Shepard and Claflin 1975). Over time the seed certification process has evolved to encompass several production limiting issues including, but not limited to, varietal purity of the seed lot, chemical (i.e. herbicide) carryover in the seed tuber, level of potato leafroll virus (PLRV), and level of mosaic symptoms, mainly attributed to presence of PVY (ICIA 2022). PVY is the most common virus resulting in the downgrading or rejection of seed lots for certification (Frost et al. 2013; Tran et al. 2022; Lindner et al. 2015) and therefore may currently be the most important disease included in certification (Lindner et al. 2015).

In the United States (US), the governing power to manage potato seed certification has been granted to individual states, which have delegated the responsibility to either land grant universities, state departments of agriculture, or grower associations (Shepard and Claflin 1975; Gudmestad 1991). Within each certifying body, tolerances are decided upon for each defect, disease, and number of generations a seed lot can be increased or replanted (Shepard and Claflin 1975; Gudmestad 1991). The seed certification process evaluates new and evolving issues deemed to be detrimental to potato production (Frost et al. 2013). Each certifying body has the power to consider defects or diseases to be unfavorable for production and integrate concerns into the regulation of seed production and to set strict tolerances (Shepard and Claflin 1975).

Generally, the seed potato certification process has five steps to ensure quality seed is maintained throughout the system. There is a minimum of two in-season field inspections, a storage

inspection, a shipping point inspection for grade, and a post-harvest test (Duellman et al. 2020; Gudmestad 1991). Currently most seed producing states in the US use the winter grow out (WGO) method to conduct post-harvest testing during the storage months (soon after harvest) while others use greenhouse grow outs or rely solely on in-season field inspections.

The WGO is a process where whole- single drop tubers (42 to 113 g) 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 113 grams 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 (Inglis et al. 2013) 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 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. Fox et al. (2005) indicated the WGO may underestimate the level of infection of a seed lot due to uneven distribution of PVY within tubers. Numerous studies have shown variability in the potential for various plant parts, including tubers, to become infected with PVY. Dupuis (2017) indicated it is unknown if all stems emerging from an infected mother tuber will be infected with PVY. Kogovsek et al. (2011) studied the distribution of PVY^{NTN} in potato plant tissue and found virus accumulation differed throughout the plant with petiole and above ground stem tissue having greater viral RNA compared to tubers. Dupuis (2017) studied the movement of PVY in the vascular system and discovered PVY can move through the xylem and phloem, potentially infecting both new foliage and daughter tubers. Also, PVY movement within a plant occurs more rapidly in young plants compared to older plants due to a restriction of cell-to-cell movement, therefore the maturity of the plant is an important component to PVY infection (Dupuis 2017; Gibson 1991). Other studies suggest PVY is not evenly distributed in all daughter tubers of a plant (Rusetsky and Blotskaya 2001 as cited in Huhnlein et al. 2013; Fox et al. 2005; Bertschinger et al. 2017). Whitworth et al. (2012) showed PVY distribution within a single tuber can be variable with PVY strain and cultivar being significant factors. 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 timing of PVY infection or final size of the tuber.

Seedborne PVY can express a wide variation of symptoms and severity that range from mild mosaic of foliage to systemic plant necrosis, which can result in severe tuber yield loss and quality defects (Gray et al. 2010; Karasev and Gray 2013; Hane and Hamm 1999; Whitworth et al. 2006; Mackenzie et al. 2019; Chikh-Ali et al. 2020). There has been considerable research conducted on various cultivars regarding the impact of PVY on yield. Hane and Hamm (1999) determined seedborne PVY infection reduced marketable yield by 79% in the cultivar Shepody compared to plants produced from a healthy non-infected seed tuber. Russet Norkotah showed a 45 to 48% total yield reduction while marketable yields were reduced by 65% compared to plants produced from healthy seed tubers. Whitworth et al. (2006) found Russet Norkotah, and cultivar CO800011-5 total yields were reduced by approximately 38% and Russet Burbank yields were reduced by 63% due to seedborne PVY infection. Gundersen et al. (2019), Rykbost et al. (1999), Whitworth et al. (2010), Mackenzie et al. (2019), and Chikh-Ali et al. (2020) all observed yield losses due to seedborne PVY in several widely grown potato cultivars. Although yield losses from seedborne PVY are well documented, the extent of loss is dependent upon cultivar and PVY strain, therefore evaluation of the effects of current virus strains on presently grown cultivars is necessary.

To add to the complexity of infection, PVY has the capability of producing several recombinant strains (Lindner 2015; Kogovsek et al. 2008; Chikh-Ali et al. 2007; Visser et al. 2012). Recombination, a survival mechanism employed by viruses to adapt to new environments and overcome host defenses, occurs when genetic information is exchanged during a co-infection of two or more viral genomes within a single host cell (Perez-Losada et al. 2015). Various studies have shown that PVY strain and/or potato cultivar interaction have a significant response on yield losses (Dupuis 2017; Gunderson et al. 2019; Weber et al. 2021; Whitworth et al. 2012). PVY^{O} was the predominant strain for many years (Gray et al. 2010), but Tran et al. (2022) and Mackenzie et al. (2019) showed that the strain composition is changing over time and shifting to recombinant strains PVY^{NTN} and $PVY^{N\text{-}Wi}$ which produce more mild symptoms compared to PVY^{O}. Also, PVY^{NTN} is known to cause potato tuber necrotic ringspot disease, a severe quality defect in several cultivars. Many of the previous studies focused on the impact of PVY^{O} on yield. Research with current strains observed in commercial potato fields needs to be conducted to determine the impact commercial potato growers may experience if planting seed with seedborne PVY infection.

In addition to yield penalties of seedborne PVY infection, Weber et al. (2021) evaluated current season infection of PVY and found the yield of three chipping cultivars were negatively impacted if inoculated with PVY during the growing season. Whitworth et al. (2010) compared the

effects on yield between seedborne and current season PVY infection in Russet Norkotah; finding seedborne PVY infection caused greater yield loss compared to in-season PVY infection. However, many of the studies investigating the effects of seedborne PVY on yield compared individual plants (infected vs. non-infected; Whitworth et al. 2006; Hane and Hamm 1999; Mackenzie et al. 2019), very small plots (Gundersen et al. 2019; Whitworth et al. 2006), or mixtures of different seed lots (Rykbost et al. 1999; Nolte et al. 2004). Although a yield decrease due to PVY infection is anticipated, commercial growers question the overall impact seedborne PVY has on a farm level. There is often a gap between the research methods conducted and commercial farming practices. Research evaluating the effects of seedborne PVY on yield needs to be conducted on a larger scale and implement practices that commercial potato growers use to determine the relatable impacts of seedborne PVY on potato production.

The first 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. The second objective of this study was to determine the effects of seedborne PVY on yield with a focus on commercial production conditions.

Materials and Methods

Objective 1: Distribution of PVY within a seed lot

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 on February 22, 2021, and had an estimated 60% seedborne PVY infection based upon post-harvest testing (certified as seed). Umatilla Russet was sampled from KREC storage and had an estimated 26% seedborne PVY infection based upon previous growing season's visual PVY evaluations (not certified seed). Year two included one cultivar, Russet Norkotah (Northwest Norkotah 90), which was sampled from a commercial seed grower storage on March 4, 2022. The seed lot had an estimated 11% seedborne PVY based upon post-harvest testing (certified as seed). All samples collected from grower storages were placed into 4.4 C and 95% relative humidity (RH) storage at University of Idaho Kimberly Research and Extension Center, Kimberly, ID (KREC). Approximately five days prior to cutting, seed was warmed to 7.2 C. Five (year one) or four (year two) predetermined seed tuber size categories were collected. Mother tuber size categories were a) 42 to 112 g, b) 113 to 169 g, c) 170 to 282 g, d) 283 to 340 g, and e) a mixed

sample (year one only). Mother tuber size categories will be referred to as: single drop (42 to 112 g), small (113 to 169 g), medium (170 to 282 g), large (283 to 340 g), and mixed (even mixture of all sizes) for ease of discussion.

Approximately 72 hours prior to planting, all tubers were cut into 56 to 85 g seed pieces except for the 42 to 112 g sample (single drop). 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. Seed was stored at 7.2 C after cutting until planting. Plots were planted April 19, 2021 (year one) and April 21, 2022 (year two) in a randomized block design with five replicates of 15.2 m plots and 26.7 cm in-row spacing. Plots were grown at KREC fields according to University of Idaho nutrient, pest, and water management guidelines.

Emergence was evaluated periodically beginning May 5, 2021 (30 days after planting; DAP) through June 6, 2021 (49 DAP) in year one and May 25, 2022 (34 DAP) through June 13, 2022 (58 DAP) in year two (Appendix B). The number of stems per plant were counted on June 27, 2021, and June 13, 2022 (Appendix B).

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 (mosaic) in each plot (DeBokx and Mooi 1974; Karasev and Gray 2013). Plants with questionable symptoms were tested using Agdia Immunostrip test kits (Agdia Inc. Elkhart, IN). In addition, to confirm the accuracy of visual evaluation, each plant in the third replicate plot of Russet Norkotah was tested using Agdia Immunostrips. Additionally, leaf tissue samples were collected from plants expressing PVY symptoms throughout KREC field trials and sent to University of Idaho Virology Lab (Karasev) for PVY strain identification each year.

Vines were mechanically flailed eight (year 1) and 14 (year 2) days prior to harvest. Each plot was mechanically harvested to determine yield and yield profile on September 13, 2021 (145 DAP) and September 20, 2022 (152 DAP). All tubers from each plot were segregated into six industry standard size categories: less than 113 g, 113 to 170 g, 171 to 283 g, and greater than 283 g via a mechanical sorter (LectroTek Industries, Wenatchee, WA). Sorted tubers were graded by USDA industry standards of US no. 1 and US no. 2 (USDA, 2011). The sorting process counted the total number of tubers and total weight in each category. Random tuber samples were collected for destructive evaluation of external and internal PVY symptoms (year one only; Appendix B).

Objective 2: PVY effects on yield

The effect of seedborne PVY on potato yield was evaluated in 2022. Multiple seed lots of three cultivars were sampled from two commercial seed growers' storages. Russet Norkotah (Northwest Norkotah 90), Russet Burbank, and Ranger Russet were collected based upon post-harvest test results. 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. All samples were placed into 4.4 C and 95% RH storage at KREC. Approximately five days prior to cutting, seed was warmed to 7.2 C. Seed was cut into 56 to 85 g pieces, treated with Vibrance Ultra Potato (Syngenta Crop Protection LLC) at a rate of 0.3 ml per kg tubers, then stored at 7.2 C and 95% RH for 72 hours prior to planting. Cut seed was planted in replicated plots on April 21, 2022, in a randomized block design with five replicates of 15.2 m plots and 26.7 cm in-row spacing similar to Objective 1.

When plants reached approximately 30 cm tall (June 27, 2022; 67 DAP) visual evaluations were conducted based upon mosaic and rugose symptoms to determine the level of seed borne PVY (DeBokx and Mooi 1974; Karasev and Gray 2013). Plants in question were tested using Agdia Immunostrips. In addition, leaf tissue samples were collected from plants expressing PVY symptoms throughout KREC field trials 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 as described in Objective 1.

Statistical Analysis

PVY incidence, emergence, stem number, yield, tuber number, and grade were analyzed using the analysis of variance (ANOVA) procedures in R (RStudio, package car version 4.1.0, 2021; Fox and Weisberg 2019). Each year was analyzed separately for each objective. All trials' significant differences between means for response variables were compared at p-value of 0.05 by estimated marginal means procedures (RStudio, package emmeans version 1.6.1, 2020 Length 2021). Emergence and stem number are included in Appendix B (Figures B-1 to 5).

Results

Two PVY strains were identified in both years, NTN and N-Wi, at comparable levels in the collective plots. In year one, 57% of the samples were identified as PVY^{NTN} and 43% were $PVY^{N\text{-}Wi}$.

Year two, 47% of the samples were identified as PVY^{NTN} and 53% were identified as PVY^{N-Wi} (data not shown).

Objective 1: Distribution of PVY within a seed lot

Umatilla Russet

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 2-1).

Total yield from the mother tuber size treatments ranged from 63.7 to 72.1 t/ha^{-1} (Table 2-2). There was no significant difference in total yield between single drop, medium, and mixed treatments. The small treatment had significantly lower total yield compared to the single drop treatment. No differences between treatments were observed in harvested yields of less than 113 g or greater than 283 g tubers. The single drop, medium, and mixed treatments had higher yields in the 113 to 170 g tuber size profile category compared to the large mother tuber treatment. The single drop had significantly higher yields in the 113 to 170 g and 171 to 283 g size profile categories compared to the large tuber treatment, while other treatments had similar yields in these size categories. No differences were observed in US no. 1 yield between treatments. Single drop and mixed treatments had higher US no. 2 yield compared to the other treatments.

Total tuber number per plot for the mother tuber size treatments ranged from 427 to 505 (Table 2-3). Single drop and mixed treatments had significantly more tubers per plot compared to the large mother tuber treatment. Similar to the size profile yields, there were no significant differences between treatments in the less than 113 g and the greater than 283 g size profile categories. The large treatment had lower tuber number in the 113 to 170 g and 171 to 283 g size profile categories compared to the single drop treatment. No significant differences between treatments were observed in the number of US no. 1 tubers. Single drop had the greatest number of US no. 2 tubers compared to the small, medium, and large mother tubers.

Russet Norkotah

In 2021, Russet Norkotah (selection 3) plots were planted from a seed lot with an estimated 60% seedborne PVY. 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 2-1). In 2022, Russet Norkotah (Northwest Norkotah 90) 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 2-1). Agdia Immunostrip test kit results confirmed the accuracy of the visual evaluations of one replicate and showed 71% PVY compared to 72% visual PVY in year 2021 and 7% compared to 6% visual in 2022 (Table 2-4).

Total yield of tubers from 2021 Russet Norkotah mother tuber treatments ranged from 53.0 to 61.1 t/ha^{-1} (Table 2-5). The single drop, small, and medium mother tuber treatments had higher total yield compared to the large and mixed treatments. The single drop and small treatment had a higher proportion of yield in the less than 113 g size profile category compared to the large tuber treatment. The large tuber treatment had lower yield in the 113 to 170 g size profile category compared to the single drop, small, and mixed treatments. In the 171 to 283 g size profile category, the single drop treatment had higher yield compared to the small and the large treatments. No differences between treatments were observed in the greater than 283 g size profile category or US no. 1 yield. Single drop had higher US no. 2 yield compared to the small, medium, and large treatments but similar to the mixed treatment.

In 2021 Russet Norkotah, total number of tubers per plot ranged from 361 to 460 with the single drop mother tuber treatment having the highest number of tubers (Table 2-6). The large treatment had fewer tubers per plot compared to the single drop and small treatments, but similar to the medium and mixed treatments. Following the trend seen in yield distribution, single drop and small treatments had a greater number of tubers in the less than 113 g and the 113 to 170 g size profile categories compared to the large mother tuber treatment. Small and large treatments had fewer tubers per plot compared to the single drop treatment in the 171 to 283 g size profile category. There were no differences between treatments observed in the greater than 283 g size profile category. Single drop treatment had more US no. 1 tubers than the medium, large, and mixed treatments, but had similar numbers to the small treatment. In the US no. 2 category, the single drop treatment had more tubers than the small, medium, and large treatments but was similar to the mixed treatment.

Total yield from 2022 Russet Norkotah mother tuber treatments showed no significant differences between treatments (Table 2-7). The single drop treatment had a higher number of tubers per plot compared to other treatments (Table 2-8) There were no significant differences in yield or number of tubers per plot among treatments in the less than 113 g, 171 to 283 g, and greater than 283 g size profile categories. However, differences were noticed within the 113 to 170 g

size category with the single drop and large treatment having a higher yield and tuber number per plot compared to the medium treatment. No differences were observed between treatment in US no. 1 yields, but the single drop treatment had a higher number of tubers per plot compared to the other treatments. The single drop treatment had a higher US no. 2 yield and number of tubers than the other treatments. The medium treatment had a higher yield compared to the large treatment in US no. 2, but a similar number of tubers per plot.

Objective 2: PVY effects on yield

Russet Burbank

Russet Burbank plots were planted from 0% (low), 5% (medium), and 8% (high) seedborne PVY seed lots. In-season visual seedborne PVY evaluations indicated the low seed lot had no visual PVY symptoms, the medium seed lot had 2% visual PVY symptoms, and the high seed lot had 7% visual PVY symptoms (data not shown).

Total yield of Russet Burbank seed lots ranged from 55.6 (high PVY) to 64.1 (low PVY) t/ha^{-1} (Table 2-9). The yield produced from the high seedborne PVY treatment was significantly lower compared to the low and medium PVY seed lots. Significant differences in yield were observed between PVY seed lot treatments in all size profile categories. The medium PVY seed lot had lower yields in the less than 113 g and the 113 to 170 g size categories but higher yield in the greater than 283 g size category compared to the other PVY seed lots. Both the medium and high PVY treatments had lower yield compared to the low PVY treatment in the 171 to 283 g size profile category. USDA grade was affected by the PVY seed lot. The low PVY lot had higher US no. 1 yield than the medium and high PVY treatments. The medium PVY seed lot had higher yield of US no. 2 compared to the low and high treatments.

The low seedborne PVY lot produced a greater number of tubers per plot compared to the other treatments (Table 2-10). The medium PVY seed lot had the fewest number of tubers in the under 113 g and 113 to 170 g size profile categories and the greatest number of tubers in the above 283 g size category compared to the low and medium treatments. The medium and high PVY seed lot produced fewer tubers in the 171 to 283 g category compared to the low PVY treatment. The low and high PVY seed lots had a similar number of tubers in each size category apart from the 171 to 283 g category. The low PVY seed lot produced the highest number of US no. 1 tubers, and the medium lot produced the fewest US no. 1 tubers per plot and the highest US no. 2 tubers.

Ranger Russet

Ranger Russet plots were planted from seed lots with 3% (low) and 34% (high) PVY infection. In-season visual PVY evaluations indicated the low seedborne PVY seed lot showed 3% observable PVY seedborne incidence and the 34% seedborne PVY seed lot had 9% observable seedborne PVY incidence (data not shown).

Total yield and total tubers per plot were similar for both treatments (Table 2-11; Table 2-12). No significant differences between PVY seed lots were observed in the less than 113 g and the 171 to 283 g size profile categories. The low PVY seed lot had lower yield and fewer tubers in the 113 to 170 g size category but higher yield and higher number of tubers in the greater than 283 g category compared to the high PVY lot. No significant differences were observed between the PVY seed lots in US no. 1 yield or tuber number. The low PVY lot had a higher US no. 2 yield and number of tubers compared to the high PVY lot.

Russet Norkotah

Russet Norkotah plots were planted from seed lots with 2% (low) and 11% (high) seedborne PVY infection. In-season visual PVY evaluations indicated the low PVY seed lot had 5% observable PVY symptoms and the high PVY seed lot had 6% observable PVY symptoms (data not shown).

Total yield and number of tubers per plot were significantly different between treatments with the low PVY seed lot having higher yield and total tuber number compared to the high PVY seed lot (Table 2-13; Table 2-14). No significant differences between PVY seed lots were observed in the less than 113 g and greater than 283 g size categories. The low PVY seed lot produced more tubers and higher yields in the 113 to 170 g and the 171 to 283 g size categories compared to the high PVY seed lot. The high PVY seed lot had lower US no. 1 yield and number of tubers compared to the low PVY seed lot but no differences in US no. 2 grade were observed between the two PVY seed lots.

Discussion

PVY by Size

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. Seed certification is a service to the potato industry that assures available potato seed is within set thresholds and relatively free from pests, varietal impurities, and defects (Callison et al. 1982; Frost et al. 2013). Providing accurate information on the level of PVY in the seed lot is a mandate of the certification

process and is important to know how the virus is distributed within a seed lot to accurately assess virus levels.

Tuber size restrictions used during the post-harvest test for certification (small seed tubers) have raised questions if the process may be inadvertently selecting for higher or lower levels of PVY. Small (single drop) seed is desired for post-harvest testing to avoid the need for cutting. Cutting seed adds a level of logistical complexity to the certification process that could lead to delays and potential disease spread. Although not shown to be a significant concern for the mechanical spread of PVY (Duellman et al. 2020), cutting seed can spread pathogens such as bacterial ring rot, which is a zero-tolerance pathogen in many seed certification programs (Inglis et al. 2013). 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.

High levels of seedborne PVY were present in year one with Umatilla Russet having an estimated 26% seedborne PVY incidence and Russet Norkotah having 60% incidence. These higher levels of PVY may have 'overwhelmed' the plants, resulting in an increased accumulation of virus in all daughter tubers used in these trials. The extremely high levels of PVY may have increased the probability that a greater proportion of tubers would have PVY infection. However, year two was planted from a Russet Norkotah lot with 11% PVY infection and results remained consistent with no significant differences in observable PVY between mother tuber size categories. Additional research with lower levels of seedborne PVY infection may be beneficial to further understand if PVY accumulates in tubers equally under low virus pressure.

Boulard et al. (2021) assessed if early versus late infection of potato plants with PVY would influence the infection rate of daughter tubers. Although susceptibility of the plant to PVY infection decreased as the plant matured, the viral accumulation in daughter tubers of infected plants was equal. Virus titer was measured between infection dates with no differences in PVY levels. However, the size of tubers sampled were not taken into consideration. Beemster (1972), Dupuis (2017), Basky and Almasi (2005) have indicated different PVY strains move through a plant more efficiently than

others. Beemster (1972) indicated the time between inoculation and harvest dictated the number of daughter tubers infected with PVY, and PVY^{N} was transmitted to daughter tubers more efficiently than PVY^{O}. Dupuis (2017) showed $PVY^{N\text{-}Wi}$ transmitted to progeny tubers more readily than PVY^{NTN}. Timing of infection, in relation to age-related resistance or mature plant resistance, was involved in the movement of PVY within a plant (Dupuis 2017) and virus transmissibility of PVY to progeny tubers was reduced when inoculated after mature plant resistance was active (Chikh-Ali et al. 2020; Gibson 1991). Dupuis (2017) also indicated lower detection of PVY^{NTN} due to lower virus titer within the tuber. In the current study, PVY^{NTN} and $PVY^{N\text{-}Wi}$ were present in almost equal amounts in field plots. Latent symptom expression and/or low virus presence could have masked differences between mother tuber treatments. However, results from using Agdia Immunostrip test kits on replicate three of Russet Norkotah indicated that visual evaluations were sufficient in identifying the level of PVY infection. These studies indicated the need for further research into virus epidemiology and the accumulation of PVY in daughter tubers by size category rather than number of infected tubers in a random sub-sample from a plant as seen in Beemster (1972), Chikh-Ali et al. (2020), and Dupuis (2017) or the total number of tubers infected from a plant as described in Boulard et al. (2021). Future research is needed to assess virus titer levels in each tuber, with regards to size, produced from seedborne and current season PVY infected plants to determine if various sizes of daughter tubers have equal amounts of virus particles.

Based upon conclusions from Beemster (1972), time between inoculation of PVY and harvest dictates the number of tubers that will be infected. Inferences can be made that timing of tuber initiation and/or tuber bulking rate in relation to infection timing could play a role in the probability of daughter tubers accumulating virus. In Beemster's (1972) article, as the mother plant was growing, tubers initiated prior to infection with PVY may have had a higher chance of being infected compared to tubers initiated post-infection. Although knowing the timing of tuber initiation in comparison to inoculation date would be insightful, this would not directly translate to final daughter tuber size. Conversely, arguments could be made that tuber bulking rate could be a greater factor in the probability of PVY infection rather than timing of tuber initiation. During tuber bulking, plants are funneling photosynthates and other resources from the foliage into tuber production (Mihovilovich et al. 2014). The rate in which cells divide and elongate longitudinally influences the size of the tuber (Kondhare et al. 2020; Schnieders et al. 1988) and the hierarchy in which tubers enlarge or receive substrates is not constant over time (Struik et al. 1990). Larger tubers may have a greater sink strength and acquire more of these translocated substrates, leading to the hypothesis

that larger tubers may have an increased probability of having more virus particles. However, data from this study does not support this hypothesis. Findings of Moorby (1968) indicate that size of tuber does not dictate the amount of substrate that enters the tuber, which corroborates findings of this study. Evaluation of virus levels in different tuber sizes at various growth stages could clarify if PVY preferentially accumulates in tubers based upon bulking rate and final tuber size.

Plots planted from the various mother tuber sizes were grown to maturation and harvested for total yield and grade evaluation. Total yield, tuber number, harvested tuber size distribution, and USDA grade were impacted by the mother tuber treatment. Typically, the single drop mother tuber treatment had higher total yield and tuber numbers than the large mother tuber treatment but had more US no. 2 yield and tubers. 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. This is corroborated with studies conducted by Nielson et al. (1989) who concluded there was a negative trend for yield as seed tuber size, from which a seed piece was taken, increased. Conversely, Masarirambi et al. (2012) found yield increased when larger tubers were planted, however uncut seed tubers were used. Single drop seed used in this study could have had higher yields due to having more eyes per seed piece since the seed was not cut. The number of stems per plant has also been shown to alter the size profile and tuber distribution of a plant (Struik et al. 1990). The number of stems produced from a seed piece is partially linked to the number of eyes on a seed piece and physiological age (Bohl et al. 1995; Struik 2007). 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. 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 compared to whole seed tubers could be beneficial to the potato industry.

PVY Effect on Yield

This research provided an assessment for the consequences seed and commercial growers face when planting potato seed with varying levels of seedborne PVY. Seed certification is an evolving program that adapts to changes in the environment, production practices, and pathogen pressure. It is important that up to date research is conducted on the effect pathogens have on current cultivars and production practices in order to assess risk levels for potato production.

PVY is a well-studied pathogen and the effects of seedborne virus infection has been documented (Rykbost et al. 1999; Nolte et al. 2004; Whitworth et al. 2006; Whitworth et al. 2010;

Gundersen et al. 2019; Hane and Hamm 1999). However, PVY is an evolving pathogen that can produce recombinant strains (Karasev and Gray 2013). With strain composition changing, and an increased production of cultivars with latent symptom expression (i.e. Russet Norkotah, Shepody; Hane and Hamm 1999), it is important that studies evaluate the effect recombinant PVY strains have on commercially produced potatoes. This study was designed to mimic commercial production practices to evaluate the effects commercial growers would experience from seedborne PVY on total yield, tuber number, size distribution, and grade in Russet Burbank, Ranger Russet, and Russet Norkotah.

Plots in this study contained the two most prevalent strains of PVY (NTN and N-Wi) in the Pacific Northwest, US production region (Funke et al. 2017; Tran et al. 2022) and happened to occur in almost equal amounts throughout the KREC field plots. This study used cultivars with multiple levels of seedborne PVY incidence and expression of mild/latent and visible PVY symptoms. Potatoes were produced and harvested in a manner that mimicked commercial production practices to ensure certification agencies had a realistic assessment of PVY effects on the production system and the information would be relevant to industry members.

In Russet Burbank, 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. Nolte et al. (2004) indicated for each one percent of PVY infection in the seed lot, yield decreased by 0.18 t/ha^{-1}. Although yield was not shown to be significantly different between low and medium infection levels, the current study showed yield trended to decrease 0.06 t/ha^{-1} for each percent of expected PVY based upon post-harvest testing but decreased 0.15 t/ha^{-1} based upon observed seedborne PVY incidence. However, differences between low and high PVY levels were significant and showed a loss of 1.2 t/ha^{-1} for each percent of observed seedborne PVY incidence and 1.1 t/ha^{-1} for expected PVY. Commercial growers may experience discrepancies between post-harvest test PVY levels compared to what they experience during the growing season. Differences in yield loss from expected compared to observed PVY could indicate why growers may experience higher or lower yield reductions than anticipated when planting seed lots with PVY. These differences indicated total yield loss from PVY was significant and may not be a linear function in Russet Burbank.

Russet Burbank low PVY treatment produced 22% and 20% higher US no. 1 yield than the medium and high PVY treatments, respectively. This outcome agrees with findings from Rykbost et al. (1999), Hane and Hamm (1999), Whitworth et al. (2010), and Nolte et al. (2004) that marketable

yield is decreased when PVY infection is present. Low and high PVY treatments had a similar size profile distribution and US no. 2 yield. Whereas the medium PVY treatment size profile shifted to have significantly higher large tuber (>283 g) yield and a 69% increase of US no. 2 yield compared to the low treatment. Unusual differences observed in size profile and grade of the medium PVY lot may be attributed to differences in seed lot. The three seed lots were produced by the same grower and region, but the high and low PVY treatments were held in the same storage bay whereas the medium PVY lot was stored in a separate storage facility. Differences in storage methods prior to sampling could have caused variance in physiological age, which is known to affect seed performance (Struik 2007; Bohl et al. 1995). Typically, physiologically younger seed produces fewer tubers that are larger in size, similar to what was observed in the present study. Inferences can be made that physiological age of seed has a considerable impact on final yield and grade compared to seedborne PVY levels. Further research into the relationship of PVY impacts and seed age may be valuable to support growers in selecting seed lots for planting. Conversely, an argument could be made that an increase in large tubers and perhaps an increase in US no. 2 tubers may be due to plant compensation. In row spacing has shown to affect size profile and number of tubers (Struik et al. 1990; Zheng et al. 2016; Stewart 1921). A healthy non-infected plant adjacent to a smaller, weaker PVY infected plant could have compensated by producing more larger tubers as may have been the case in this study (Hirst et al. 1973; Stewart 1921). Excessive space for tubers to enlarge could have resulted in misshapen tubers explaining the increased yield and number of tubers downgraded to US no. 2. Plant compensation makes up for a greater proportion of yield loss with smaller gaps in row (Stewart 1921) and timing of disease pressure (Hirst et al. 1973). This could explain why total yield was comparable between low and medium PVY treatments, but the medium treatment had more larger tubers.

Contrary to Russet Burbank, significant yield differences were not observed in Ranger Russet. Seed lots were planted from two levels of seedborne PVY with seed lots produced by two different seed growers in different regions. Further, there was 33% greater US no. 2 yield with the low PVY treatment. A similar argument could be made that physiological age was different between the two seed lots and could be corroborated with higher yield within the large (>283 g) size profile category in the low PVY seed lot compared to the high PVY seed lot. Post-harvest test results indicated the high treatment had 31% more seedborne PVY than the low treatment but yield nor grade was significantly reduced. Visual evaluations indicated that only 9% PVY incidence was present, however adjacent plots planted from the same seed lot had 38% PVY incidence based upon

ELISA testing. This corroborates with Tran et al. (2022) that current strains of PVY produce mild symptoms and may be responsible for proliferation of these strains. Lack of distinct symptoms of new PVY strains accentuates the necessity for accurate post-harvest testing of PVY. Implications of this study suggest that further research needs to be conducted on commercially produced potatoes to accurately estimate the effect PVY will have on a farm level.

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 seedborne PVY incidence and the seed lot planted with high seedborne PVY incidence. Rykbost et al. (1999), Whitworth et al. 2006, Nolte et al. (2004), Hane and Hamm (1999) all reported total yield and marketable yield of Russet Norkotah were significantly impacted by PVY infection. This study validated previous research findings that total yield was significantly reduced by PVY infection. However, distribution of the total yield across size profiles was almost identical for the high and low PVY lots. Furthermore, the proportion of US no. 1 and US no. 2 yield was nearly identical. Lack of differences in size profile distribution and USDA grade indicates that current strains, and/or the commercial-like-production of plots in this study may negate the effect PVY has on marketable yields observed in previous studies. This study provides information supporting the need for further research into the effects current PVY strains have on yield in commercial production systems.

Overall, this research adds to the knowledge of the impact that seedborne PVY and current PVY strains have on three widely grown cultivars in the Pacific Northwest with varying expression of PVY symptoms. It was found that Russet Norkotah, a cultivar expressing mild/latent PVY symptoms, showed a greater yield response to PVY infection compared to Ranger Russet, which typically expresses foliar symptoms. Also, multiple seed lots were compared in this trial. This study emphasized managing plots in a similar manner to commercial potato growers and comparing the impact of yield using larger research plots. 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.

Conclusion

These studies showed PVY does not preferentially accumulate in tubers based upon final seed tuber size, thus implying current seed certification programs in the US are not inadvertently selecting higher or lower levels of PVY based upon tuber size restrictions for post-harvest testing. However, further research needs to be conducted to determine if PVY strains preferentially

accumulate depending upon the tuber size to ensure strain composition is not being altered through tuber size selection. The influence of seed lots containing various levels of two prevalent PVY strains in the Pacific Northwest growing region on yield were assessed. Findings of this research showed PVY infection impacts potato yield and should remain regulated as a significant limiting factor to potato production. However, 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. Further evaluation of current PVY strains and cultivars needs to be conducted to determine the risk associated with planting seed lots containing elevated levels of seedborne PVY. The effect of PVY was significant, however the differences in the physiological age of the seed lots used may have influenced the overall outcome on production. The current study contributed relevant information for commercial producers to make seed purchasing decisions and anticipate losses associated with PVY on a farm level.

References

Appel, O. 1934. Vitality and vitality determination in potatoes. *Phytopathology* 24: 482-94.

Basky, Z., and A. Almasi. 2005. Differences in aphid transmissibility and translocation between PVY^{N} and PVY^{O} isolates. *Journal of Pest Science* 78: 67-75.

Beemster A. B. R. 1972. Virus translocation in potato plants and mature-plant resistance. In: Viruses of potatoes and seed-potato production, ed J. A. DeBokx. 144-106. Wageningen Center for Agricultural Publishing and Documentation, Wageningen, NL.

Bertschinger L., L. Buhler, B. Dupuis, B. Duffy, C. Gessler, G. A. Forbes, E. R. Keller, U. C. Scheidegger, and P. C. Struik. 2017. Incomplete infection of secondarily infected potato plants- An environment dependent underestimated mechanism in plant virology. *Frontiers in Plant Science* 8(74): 1-13.

Bohl, W. H., P. Nolte, G. E. Kleinkopf, and M. K. Thornton. 1995. Potato seed management: Seed size and age. Extension CIS 1031. *University of Idaho*, Moscow, ID.

Boulard F., G. Rohaut, D. Dupre, F. B. Barrailh, A. Person, M. Leclerc, E. Jacquot, and L. Glais. 2021. Impact of the potato inoculation date on potato virus Y load and viral distribution in daughter tubers at harvest. *Plant Pathology* 71: 1323-1334.

Callison B. L., and J. Harrington, D. Douglas. 1982. Seed potato certification: Its purpose, capabilities, and limitations. *American Potato Journal* 59: 231-236.

Chikh Ali, M., T. Maoka, and T. Natsuaki. 2007. The occurrence and characterization of new recombinant isolates of PVY displaying shared properties of PVY^{NW} and PVY^{NTN}. *Journal of Phytopatholgy* 155: 409-415.

Chikh-Ali, M., L. T. Tran, W. J. Price, and A. V. Karasev. 2020. Effects of the age-related resistance to potato virus Y in potato on the systemic spread of the virus, incidence of the potato tuber necrotic ringspot disease, tuber yield, and translocation rates into progeny tubers. *Plant Disease* 104: 269-275.

DeBokx, J. A., and J. C. Mooi. 1974. Methods of quality assessment of seed potatoes. *Potato Research* 17: 410-433.

Duellman, K. M., M. K. Thornton, and A. A. Westra. 2020. Potato seed certification and selection in Idaho. Extension Bulletin 983. *University of Idaho*, Moscow, ID.

Dupuis, B. 2017. The movement of potato virus Y (PVY) in the vascular system of potato plants. *European Journal of Plant Pathology* 147: 365-373.

Fauquet, C. M., M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball. 2005. Virus Taxonomy -- eighth report of the international committee of taxonomy of viruses, *Academic Press* 739-1128. ISBN 9780122499517.

Fox, A., F. Evans, and I. Browning. 2005. Direct tuber testing for potato Y potyvirus by real-time RT-PCR and ELISA: Reliable options for post-harvest testing? *European and Mediterranean Plant Protection Organization Bulletin* 356: 93-97.

Fox, J., and S. Weisberg. 2019. An {R} companion to applied regression, third edition, Thousand Oaks CA: Sage.

Frost, K., R. L. Groves, and A. O. Charkowski. 2013. Integrated control of potato pathogens through seed potato certification and provisions of clean seed potatoes. *American Phytopathological Society* 97(10): 1268-1280.

Funke, C. N., O. V. Nikolaeva, K. J. Green, L. T. Tran, M. Chikh-Ali, A. Quintero-Ferrer, R. Cating, K. E. Frost, P. B. Hamm, N. Olsen, M. J. Pavek, S. M. Gray, J. M. Crosslin, and A. V. Karasev. 2017. Strain-specific resistance to *Potato virus Y* (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. Plant Disease 101: 20-28.

Gibson, R. W. 1991. The development of mature plant resistance in four potato cultivars against aphid-inoculated potato virus Y^O and Y^N in four potato cultivars. *Potato Research* 34: 205-210.

Gray, S., S. De Boer, J. Lorenzen, A. Karasev, J. Whitworth, P. Nolte, R. Singh, A. Boucher, and H. Xu. 2010. Potato virus Y: An evolving concern for potato crops in the United States and Canada. *The American Phytopathological Society* 94(10): 1384-1397.

Gudmestad, N. C. 1991. A historical perspective to pathogen testing in seed potato certification. *American Potato Journal* 68:99-102

Gundersen, B., D. A. Inglis, M. Pavek, and A. V. Karasev. 2019. Foliar and tuber reactions of three strains of potato virus Y on five fresh market potato cultivars through three successive potato generations. *American Journal of Potato Research* 96:519-531.

Halterman, D., A. Charkowski, and J. Verchot. 2012. Potato, viruses, and seed certification in the USA to provide healthy propagated tubers. *Pest Technology* 6(1): 1-14.

Hane, D. C., and P. B. Hamm. 1999. Effects of seedborne potato virus Y infection in two potato cultivars expressing mild disease symptoms. *Plant Disease* 83: 43-45.

Hirst, J. M., G. A. Hide, O. J. Stedman, and R. L. Griffith. 1973. Yield compensation in gappy potato crops and methods to measure effects of fungi pathogenic on seed tubers. *Annals of Applied Biology*. 73(2): 119-241.

Huhnlein, A., N. Drechsler, P. Steinback, T. Thieme, and J. Schubert. 2013. Comparison of three methods for the detection of potato virus Y in seed potato certification. *Plant Diseases and Protection* 120(2): 57-69.

ICIA. 2022. Idaho potato certification standards. *Idaho Crop Improvement Association, Inc.*

Inglis, D. A., D. Johnson, B. Schroeder, and C. Benedict. 2013. Bacterial ring rot on potatoes. FS Bulletin 102E, *Washington State University.* Pullman, WA.

Karasev, A., and S. M. Gray. 2013. Continuous and emerging challenges of potato virus Y in potato. *Annual Review of Phytopathology* 51: 571-586.

Kerlan, C., and B. Moury. 2008. Potato virus Y. *Encyclopedia of Virology* 287-296.

Khurana, P. S. M. 2004. Potato viruses and their management. In: Diseases of fruits and vegetables, V2 389-440. *Kluwer Academic Publishers*, Netherlands.

Kogovsek, P., A. Kladnik, J. Mlakar, M. T. Znidaric, M. Dermastia, M. Ravnikar, and M. Pompe-Novak. 2011. Distribution of potato virus Y in potato plant organs, tissues, and cells. *American Phytopathological Society* 101: 1292-1300.

Kogovsek, P., L. Gow, M. Pompe-Novak, K. Gruden, G. D. Foster, N. Boonham, and M. Ravnikar. 2008. Single-step RT real-time PCR for sensitive detection and discrimination of potato virus Y isolates. *Virological Methods* 149:1-11.

Kondhare K. R., B. Natarajan, and A. K. Banerjee. 2020. Molecular signals that govern tuber development in potato. *International Journal of Developmental Biology* 64: 133-140

Leach, J. G. 1938. The biological basis for certification of seed potatoes. *American Journal of Potato Research* 15: 117-130.

Lindner, K., F. Trautwein, A. Kellerman, and G. Bauch. 2015. Potato virus Y (PVY) in seed potato certification. *Journal of Plant Diseases and Protection* 122(3):109-119.

Mackenzie, T. D. B., Z. Nie, V. Bisht, and M. Singh. 2019. Proliferation of recombinant PVY strains in two potato-producing regions of Canada, and symptom expression in 30 important potato varieties with different PVY strains. *Plant Disease* 103(9): 2221-2230.

Masarirambi, M. T., F. C. Mandisodza, A. B. Mashingaidze, and E. Bhebhe. 2012. Influence of plant population and seed tuber size on growth and yield components of potato (*Solanum tuberosum*). *International Journal of Agriculture and Biology* 14: 545-549.

Mihovilovich, E., C. Carli, F. De Mendiburu, V. Hualla, and M. Bonierbale. 2014. Tuber bulking maturity assessment of elite and advanced potato clones protocol. *International Potato Center,* Lima, Peru. 1-43.

Moorby, J. 1968. The influence of carbohydrate and mineral nutrient supply on the growth of potato tubers. *Annals of Botany* 32(125): 57-68.

Nielson, M., W. M. Iritani, and L. D. Weller. 1989. Potato seed productivity: Factors influencing eye number per seed piece and subsequent performance. *American Potato Journal* 66: 151-160.

Nolte, P., J. L. Whitworth, M. K. Thornton, and C. S. McIntosh. 2004. Effect of seedborne potato virus Y on performance of Russet Burbank, Russet Norkotah, and Shepody potato. *Plant Disease*. 88: 248-252.

Perez-Losada, M., M. Arenas, J. C. Galan, F. Palero, and F. Gonzalez-Candelas. 2015. Recombination in viruses: Mechanisms, methods of study, and evolutionary consequences. *Infection, Genetics and Evolution* 30: 296-307.

Rusetsky, N. V., and Z. V. Blotskaya. 2001. Distribution and accumulation of PVM and PVY, PVX in infected potato plants. *Archives of Phytopathology and Plant Protection* 34: 275-282.

Rykbost, K. A., D. C. Hane, P. B. Hamm, R. Voss, and D. Kirby. 1999. Effects of seedborne potato virus Y on Russet Norkotah performance. *American Journal of Potato Research* 75: 91-96.

Schnieders, B. J., L. H. J. Kerckhoffs, and P. C. Struik. 1988. Diel changes in tuber volume. *Potato Research* 31: 129-135.

Shepard, J. F., and L. E. Claflin. 1975. Critical analyses of the principals of seed potato certification. *Annual Review of Phytopathology* 13: 271-293.

Stewart, F. C. 1921. Further studies on the effect of missing hills in potato fields and on the variation in the yield of potato plants from halves of the same seed piece. Bulletin 489, *New York Agricultural Experiment Station*, Geneva, NY

Struik, P. C. 2007. The canon of potato science: 40. Physiological age of seed tubers. *Potato Research* 50: 375-377.

Struik, P. C., A. J. Haverkort, D. Vreugdenhil, C. B. Bus, and R. Dankert. 1990. Manipulation of tuber-size distribution of a potato crop. *Potato Research* 33: 417-432.

Tran, L., K. J. Green, M. Rodriguez-Rodriguez, G. E. Orellana, C. N. Funke, O. V. Nikolaeva, A. Quintero-Ferrer, M. Chikh-Ali, L. Woodell, N. Olsen, and A. V. Karasev 2022. Prevalence of recombinant strains of potato virus Y in seed potato planted in Idaho and Washington states between 2011 and 2021. *Plant Disease* 106: 810-817.

USDA. 2011. United States standards for grades of potatoes. *United States Department of Agriculture*.

Visser, J. C., D. U. Bellstedt, and M. D. Pirie. 2012. The recent recombinant evolution of a major crop pathogen, *potato virus Y. PLOS ONE* 7(11): e50631

Weber, E. L., J. S. Busse, and P. C. Bethke. 2021. Current-season infection with ordinary and recombinant strains of potato virus Y has detrimental effects on chipping potato yield and tuber quality. *Plant Disease* 105(2): 450-455.

Whitworth, J. L., P. B. Hamm, and C. S. McIntosh. 2010. Effect of potato virus Y on yield of a clonal selection of Russet Norkotah. *American Journal of Potato Research* 87: 310-314.

Whitworth, J. L., P. B. Hamm, and P. Nolte. 2012. Distribution of potato virus Y strains in tubers during the post-harvest period. *American Journal of Potato Research* 89: 136-141.

Whitworth, J. L., P. Nolte, C. McIntosh, and R. Davidson. 2006. Effect of potato virus Y on yield of three potato cultivars grown under different nitrogen levels. *Plant Disease* 90(1): 73-76.

Zheng, S. L., L. J. Wang, N. X. Wan, L. Zhong, S. M. Zhou, W. He, and J. C. Yuan. 2016. Response of potato tuber number and spatial distribution to plant density in different growing seasons in Southwest China. *Frontiers of Plant Science*. 7: 365-372.

Tables

Table 2-1. 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 56 to 85 g 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	-
Standard error	3	2	1

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g tubers, Mixed = equal amounts of each mother tuber category. Mixed size category was not planted in 2022 represented by dash.

Table 2-2. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of five mother tuber size treatments of Umatilla Russet in 2021. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	Yield (t/ha^{-1}) [1]									
	Total	*Size profile categories (g)*						*USDA grade*		
Mother tuber size[2]	**Yield**	**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**		**US no. 2**	
Single drop	72.1 c	8.0	a	11.7	b	22.9	b	29.4	a	60.6	a	11.5	b
Small	62.8 a	7.4	a	9.8	ab	20.1	ab	25.5	a	54.4	a	8.4	a
Medium	66.4 abc	7.2	a	10.6	b	22.3	ab	26.2	a	59.3	a	7.1	a
Large	63.7 ab	6.9	a	8.2	a	19.3	a	29.3	a	56.6	a	7.1	a
Mixed	71.4 bc	7.9	a	10.9	b	22.1	ab	30.6	a	60.6	a	10.8	b
Standard error	2.8	0.6		0.7		1.1		2.7		2.6		0.8	

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g, Mixed = equal amounts of each mother tuber category.

Table 2-3. Total tuber number per plot (15.3 m), categorized by harvested tuber size, and USDA grade of five mother tuber size treatments of Umatilla Russet in 2021. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	Tuber number per plot[1]											
	Total		*Size profile categories (g)*								*USDA grade*	
Mother tuber size[2]	**Number**		**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**	**US no. 2**
Single drop	505	b	144	a	115	b	145	b	101	a	450 a	55 c
Small	447	ab	134	a	98	ab	128	ab	87	a	404 a	43 ab
Medium	471	ab	130	a	105	b	142	ab	94	a	433 a	38 a
Large	427	a	125	a	82	a	122	a	98	a	393 a	34 a
Mixed	493	b	141	a	107	b	139	ab	106	a	444 a	49 bc
Standard error	20.3		11.3		7.0		6.8		7.6		20.2	3.3

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g, Mixed = equal amounts of each mother tuber category.

Table 2-4. Comparison of visual PVY evaluations to Agdia Immunostrip test kits for PVY detection in replicate three of Russet Norkotah during growing season. Percent infection for visual evaluation was averaged over all five replicates while Agdia Immunostrips were from replicate three only. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	2021		*2022*	
	PVY infection (%)			
Mother tuber size[1]	**Visual evaluation**	**Agdia Immunostrip**	**Visual evaluation**	**Agdia Immunostrip**
Single drop	76	71	6	7
Small	67	66	4	5
Medium	69	77	8	9
Large	74	70	5	7
Mixed	76	71	-	-
Average	72	71	6	7

[1]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g, Mixed = equal amounts of each mother tuber category. Mixed size category was not planted in 2022 represented by dash.

Table 2-5. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of five mother tuber size treatments of Russet Norkotah in 2021. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	Yield (t/ha^{-1}) [1]											
	Total		*Size profile categories (g)*								*USDA grade*	
Mother tuber size[2]	**Yield**		**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**	**US no. 2**
Single drop	61.1	b	8.7	c	9.5	b	19.5	b	23.5	a	55.9 a	5.2 b
Small	56.1	ab	7.8	bc	8.9	b	16.3	a	23.1	a	53.1 a	3.0 a
Medium	56.2	ab	6.8	ab	8.1	ab	17.4	ab	23.9	a	53.4 a	2.9 a
Large	53.0	a	5.8	a	7.0	a	16.7	a	23.6	a	50.2 a	2.8 a
Mixed	54.5	a	6.7	ab	8.6	b	18.0	ab	21.2	a	50.6 a	3.9 ab
Standard error	2.0		0.6		0.5		0.8		2.3		2.2	0.6

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g, Mixed = equal amounts of each mother tuber category.

Table 2-6. Total tuber number per plot (15.3 m), categorized by harvested tuber size, and USDA grade of five mother tuber size treatments of Russet Norkotah in 2021. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	Tuber number per plot[1]											
	Total		*Size profile categories (g)*								*USDA grade*	
Mother tuber size[2]	**Number**		**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**	**US no. 2**
Single drop	460	c	159	c	96	b	124	b	83	a	435 c	25 b
Small	416	b	141	bc	89	b	103	a	83	a	401 bc	15 a
Medium	398	ab	123	ab	79	ab	110	ab	85	a	385 ab	13 a
Large	361	a	108	a	68	a	105	a	81	a	349 a	13 a
Mixed	397	ab	122	ab	85	b	114	ab	76	a	378 ab	20 ab
Standard error	15.0		10.2		5.5		5.0		7.3		15.3	3.2

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g, Mixed = equal amounts of each mother tuber category.

Table 2-7. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of five mother tuber size treatments of Russet Norkotah in 2022. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	Yield (t/ha^{-1}) [1]									
	Total	*Size profile categories (g)*						*USDA grade*		
Mother tuber size[2]	**Yield**	**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**	**US no. 2**
Single drop	63.5 a	8.0	a	10.4	c	22.2	a	22.9	a	54.2 a	9.3 c
Small	60.9 a	6.2	a	8.6	ab	19.9	a	26.2	a	53.9 a	7.0 ab
Medium	60.2 a	6.9	a	8.2	a	20.0	a	25.0	a	52.3 a	7.8 b
Large	58.7 a	7.2	a	9.7	bc	20.1	a	21.8	a	52.5 a	6.2 a
Standard error	1.4	0.4		0.4		0.8		1.5		1.6	0.5

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g tubers.

Table 2-8. Total tuber number per plot (15.3 m), categorized by harvested tuber size, and USDA grade of five mother tuber size treatments of Russet Norkotah in 2022. All treatments were cut to 56 to 85 g seed pieces except single drop was left uncut.

	Tuber number per plot[1]									
	Total	*Size profile categories (g)*						*USDA grade*		
Mother tuber size[2]	**Number**	**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**	**US no. 2**
Single drop	479 b	150	a	104	c	140	a	85	a	427 b	52 b
Small	421 a	116	a	85	ab	125	a	96	a	385 a	37 a
Medium	428 a	130	a	81	a	126	a	91	a	387 a	41 a
Large	439 a	136	a	96	bc	126	a	81	a	404 a	35 a
Standard error	7	9		4		5		5		7	2

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g tubers.

Table 2-9. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of three seed lots with seedborne PVY infection of Russet Burbank in 2022.

	Yield (t/ha^{-1}) [1]									
	Total	*Size profile categories (g)*						*USDA grade*		
Seedborne PVY level[2]	**Yield**		**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**		**US no. 2**	
Low	64.1	b	7.7	b	9.9	b	21.8	b	24.6	a	53.9	b	10.1	a
Medium	63.8	b	4.8	a	7.0	a	17.5	a	34.6	b	43.1	a	20.8	b
High	55.6	a	7.9	b	9.3	b	17.4	a	20.9	a	44.1	a	11.5	a
Standard error	2.5		0.4		0.4		0.9		2.5		2.3		1.3	

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

Table 2-10. Total tuber number per plot (15.3 m), categorized by harvested tuber size, and USDA grade of three seed lots with seedborne PVY infection of Russet Burbank in 2022.

	Tuber number per plot[1]									
	Total	*Size profile categories (g)*						*USDA grade*		
Seedborne PVY level[2]	**Number**		**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**		**US no. 2**	
Low	472	c	146	b	99	b	138	b	89	a	419	c	53	a
Medium	384	a	89	a	70	a	109	a	116	b	300	a	84	b
High	430	b	152	b	92	b	111	a	75	a	372	b	58	a
Standard error	10		9		3		6		8		10		5	

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

Table 2-11. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of two seed lots with seedborne PVY infection of Ranger Russet in 2022.

	Yield (t/ha^{-1}) [1]									
	Total	*Size profile categories (g)*						*USDA grade*		
Seedborne PVY level[2]	**Yield**		**< 113**		**113-170**		**171-283**		**> 283**		**US no. 1**		**US no. 2**	
Low	58.6	a	4.2	a	6.1	a	17.1	a	31.2	b	51.6	a	7.0	b
High	54.5	a	4.7	a	7.2	b	16.7	a	26.0	a	49.5	a	5.0	a
Standard error	1.6		0.3		0.3		0.7		1.4		1.8		0.4	

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Low = 3% and High = 34% seedborne PVY seed lots based upon post-harvest test.

Table 2-12. Total tuber number per plot (15.3 m), categorized by harvested tuber size, and USDA grade of two seed lots with seedborne PVY infection of Ranger Russet in 2022.

	Tuber number per plot[1]						
	Total	*Size profile categories (g)*				*USDA grade*	
Seedborne PVY level[2]	**Number**	**< 113**	**113-170**	**171-283**	**> 283**	**US no. 1**	**US no. 2**
Low	352 a	77 a	60 a	107 a	108 b	318 a	34 b
High	352 a	83 a	72 b	104 a	92 a	325 a	27 a
Standard error	7	5	3	4	5	7	2

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Low = 3% and High = 34% seedborne PVY seed lots based upon post-harvest test.

Table 2-13. Total harvested tuber yield (t/ha^{-1}), yield size distribution, and USDA grade of two seed lots with seedborne PVY infection of Russet Norkotah in 2022.

	Yield (t/ha^{-1}) [1]						
	Total	*Size profile categories (g)*				*USDA grade*	
Seedborne PVY level[2]	**Yield**	**< 113**	**113-170**	**171-283**	**> 283**	**US no. 1**	**US no. 2**
Low	66.5 b	7.3 a	9.6 b	22.7 b	26.9 a	60.0 b	6.5 a
High	58.0 a	6.8 a	8.2 a	20.1 a	23.0 a	52.5 a	5.5 a
Standard error	16	2	3	7	13	15	4

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Low = 2% and High = 11% seedborne PVY seed lots based upon post-harvest test.

Table 2-14. Total tuber number per plot (15.3 m), categorized by harvested tuber size, and USDA grade of two seed lots with seedborne PVY infection of Russet Norkotah in 2022.

	Tuber number per plot[1]						
	Total	*Size profile categories*				*USDA grade*	
Seedborne PVY level[2]	**Number**	**< 113**	**113-170**	**171-283**	**> 283**	**US no. 1**	**US no. 2**
Low	473 b	136 a	96 b	142 b	99 a	436 b	37 a
High	423 a	131 a	81 a	125 a	86 a	392 a	31 a
Standard error	1.8	0.3	0.3	0.7	1.5	1.7	0.4

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Low = 2% and High = 11% seedborne PVY seed lots based upon post-harvest test.

Chapter 3: Promoting the Release of Potato (*Solanum tuberosum* L.) Tuber Dormancy and Encouraging Sprout Development Following Harvest

Abstract

Overcoming tuber dormancy soon after harvest is a major hurdle for seed certification agencies. Objectives of this study were to evaluate the efficacy of several documented treatments and explore novel (to potato) cold aerosol smoke application on tubers to break dormancy and induce sprouting soon after harvest. Eight treatments were applied to three separate groups of Russet Burbank, Clearwater Russet, and Umatilla Russet at three different timings after harvest. Treatments included: untreated control held at 18.3 C, cold-stratification held at 4.4 C for two weeks, temperature fluctuation held at 4.4 C for five days, 18.3 C for four days, then 4.4 C for five days, a dip in 20 ppm gibberellic acid (GA) solution, three different applications of cold aerosol smoke, and a combination of cold aerosol smoke followed by a dip in GA. Treatments were applied approximately four (October), six (November), and 10 (December) weeks after harvest. Sprout rating and sprout lengths were measured weekly following application. Treatments were more effective at promoting sprouting at later application timings. Cold-stratification and temperature fluctuations did not significantly alter sprout development for any application timing or cultivar, and in some cases retarded sprout development, compared to the untreated control. Smoke and GA based treatments increased early sprout development compared to the untreated control in each timing. The combination of smoke and GA significantly shortened the time to break dormancy and had higher successive sprout development compared to other treatments in each application timing. Combination treatment resulted in dormancy break as early as 15 days after treatment at the October application timing. This study provided foundational knowledge on the efficacy of several dormancy breaking techniques for seed certification to implement into the post-harvest testing program.

Introduction

Potato (*Solanum tuberosum* L.) is a major horticultural crop produced through vegetative propagation. The propagule (seed tuber) provides adequate resources to produce a plant. However, vegetative propagation renders the crop susceptible to elevated levels of diseases, such as viruses, potentially resulting in seed degeneration and accumulation of virus infection if seed lots are not removed from the production system over time (Halterman 2012; Khurana 2004). It is the responsibility of potato seed certification agencies to assure available seed potatoes are within

tolerances for pests, varietal impurities, and defects (Callison et al. 1982; Frost et al. 2013). To predict virus levels in the subsequent crop, a post-harvest crop inspection of seed potatoes is typically conducted soon after harvest and is referred to as the winter grow out (WGO; Fox et al. 2005). The WGO relies on growing plants to conduct visual and leaf tissue testing for regulated diseases and disorders. Other methods such as using polymerase chain reactions (PCR; Russo et al 1999; Singh et al. 2013), or enzyme-linked immunosorbent assays (ELISA; Hill and Jackson 1984; Avrahami-Moyal et al. 2017) to detect PVY directly from tubers have been studied. These laboratory methods may be used to estimate PVY levels in seed lots, but it is believed that non-dormant (sprouting) tubers increase accuracy of tuber testing (Coleman 1983; McDonald and Coleman 1988; Gugerli and Gehriger 1980; Vetten et al. 1983). However, it is unclear to what extent development of spouted tissue is required. ICIA (2019) suggested that reliability and accuracy of direct tuber testing increases when tubers have sprouts six millimeters in length or greater. Other certification agencies reported using sprout lengths of three to five millimeters (UNECE 2019). In any case, a non-dormant tuber with actively growing tissue is required for accurate PVY detection during post-harvest testing.

Potato tubers experience a state of dormancy or cessation of growth following harvest (Mani et al. 2014; Suttle 2004; Carvalho et al. 2021; Withers and Cooper 2008) where tubers will not produce a plant even when optimal growing conditions are present (Sonnewald 2001). The physiological state of dormancy, referred to as endo-dormancy, can last days, weeks, or even months depending upon the cultivar and pre- and post-harvest conditions (Mani et al. 2014; Muthoni et al. 2014). Major phytohormones, ethylene, gibberellin, abscisic acid (ABA), auxin, and cytokinin are believed to be involved in the maintenance and release of tuber dormancy (Suttle 2004; Mani et al. 2014; Campbell et al. 2008). Although exact biochemical and phytohormonal processes are not fully elucidated, it is well documented that ABA and ethylene are involved in the induction and maintenance of dormancy while cytokinin is involved in releasing of dormancy, and auxin and GA are involved in sprout development upon dormancy release (Dogonadze et al. 2000; Mani et al. 2014; Campbell et al. 2008; Sonnewald 2001; Sonnewald 2014; Suttle 1998). Once the endo-dormant period subsides, tubers may be in a state of arrested growth due to unfavorable conditions referred to as eco-dormancy (Aksenova et al. 2013; Mani et al. 2014). Tubers will remain in eco-dormancy until placed into favorable growth conditions or stimulated by exogenous applications of phytohormones or other chemical treatments.

The status and length of dormancy varies for each potato cultivar, but regardless, dormancy remains an issue for certification agencies that require actively growing tissue soon after harvest.

Due to dormancy delaying sprout development and plant growth, each seed lot sample is treated with chemicals, typically 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). While effective at promoting sprout development soon after harvest, Rindite is considered to be extremely volatile, corrosive, dangerous (Bryan 1989), and highly toxic to mammals (McDonald and Coleman 1988). A method to promote dormancy break in seed tubers safely and consistently would benefit the potato industry.

Additional chemical methods for breaking tuber dormancy have been studied. Campbell et al. (2008) found an application of bromoethane helped break tuber dormancy while mimicking similar gene expressions as natural dormancy break. McDonald and Coleman (1988) showed bromoethane applications produced similar sprouting results as Rindite and resulted in similar PVY detection efficiency when using ELISA. Others have reported success using bromoethane in breaking dormancy soon after harvest (Esztergalyos and Polgar 2021; Bryan 1989). While less toxic than Rindite, bromoethane is highly flammable and poses potential health hazards to animals and humans (McDonald and Coleman 1988; Safety Data Sheet 2022).

An efficient non-chemical method for breaking potato dormancy soon after harvest is desirable (Haider et al. 2022; Wiltshire and Cobb 1996). Using temperature manipulations, plant growth regulators, or phytohormones to break dormancy has been widely evaluated with varying results. Wurr and Allen (1976) reported storing tubers at 2 to 3 C followed by warmer temperatures increased sprout development and improved emergence rate of the successive crop. Davidson (1958) found storing tubers at warm temperatures (26.7 C) induced sprouting sooner than tubers stored at cooler temperatures (1.7 C). Haider et al. (2022) showed a cold pre-treatment followed by an increase to ambient temperatures reduced the time to dormancy break. However, many of these studies were conducted over many months, which is not advantageous for certification agencies who need non-dormant and sprouted tubers soon after harvest.

Exogenous applications of phytohormones have also been used as a method to break dormancy. Soaking tubers in various solutions of a synthetic cytokinin (benzyl-adenine, benzyl amino purine, kinetin) significantly reduced tuber dormancy in several cultivars (Esztergalyos and Polgar 2021; Haider et al. 2022; Majeed and Bano 2006). Dipping tubers in gibberellic acid (GA) also reduced length of dormancy and/or promoted sprout elongation (Haider et al. 2022; Esztergalyos and Polgar 2021; Hartman et al. 2011; Kulen et al. 2011; Tavakoli et al. 2014; Wrobel et al. 2017).

Hartmann et al. (2011) showed GA alone induced sprouting and found a treatment of cytokinin induced meristematic activity of the sprout but did not result in sprout elongation unless GA was added. Haider et al. (2022) reported similar results of GA plus cytokinin producing the greatest sprout development, but conversely experienced cytokinin alone broke dormancy and produced a sprout. Wrobel et al. (2017) showed the addition of ethanol to the combination of GA and cytokinin had a positive effect on dormancy break. However, many of these studies required excised tissue or wounded tubers with cuts to allow hormone uptake, which is not desirable for seed certification.

Another hormonal treatment to control dormancy is the application of ethylene, which does not require wounding of tubers. However, dormancy control with ethylene can result in sprout inhibition or sprout promotion depending upon the concentration and duration of tuber exposure (Muthoni et al. 2014; Suttle 1998). Rylski et al. (1974) observed potatoes exposed to low rates of ethylene for 72 hours had greater sprout development compared to the untreated control, whereas longer exposure to ethylene inhibited sprout growth. Denny (1926) indicated various rates of ethylene applied for differing time periods of one hour to seven days did not affect sprout development. Prange et al. (1998) successfully used long term ethylene exposure as a sprout suppressant. Application of ethylene-releasing agents (ethephon) resulted in longer dormancy (Korableva et al. 1989 and Cvikrova et al. 1994; as cited by Suttle 1998). Since sprout inducing effects of ethylene are variable and highly dependent on specific rates and timings, it is not suitable to be widely used to break dormancy for certification purposes.

Inconsistent results and lack of large-scale applicability for many treatments have left the seed potato industry desiring a consistent, effective, and non-toxic method to break potato tuber dormancy (Haider et al. 2022). De Lang and Boucher (1990) initiated a study to increase true seed germination of *Audouinia capitata*, a fynbos species (evergreen shrub), using plant derived smoke. It was determined that aerosol smoke and aqueous smoke extracts served as a germination cue for fynbos species. Later it was found that smoke stimulated germination in the agricultural crops' lettuce, red rice, and teff (Drewes et al. 1995; Doherty and Cohn 2000; Ghebrehiwot et al. 2013; respectively).

Several compounds found in smoke that could be involved in germination stimulation have been isolated and tested (Baldwin 1994; Van Staden and Brown 1995). In the process of identifying the active compounds in smoke, some have attributed the stimulation of seed germination to ethylene which is commonly found in combusting materials. However, Van Staden et al. (1995)

demonstrated ethylene is probably not the causal factor of germination. Keeley and Fotheringham (1998) concluded nitrogen oxides and nitric oxide (NO) were effective at stimulating germination in some species and most likely a key active compound in smoke responsible for stimulating seed germination. However, studies by Light and Van Staden (2004) showed two NO-releasing compounds did not stimulate seed germination in Grand Rapids lettuce seed, which is highly responsive to smoke applications. Furthermore, Preston et al. (2004) did not detect NO_2 in aqueous smoke solutions, which stimulated seed germination in two plant species.

A butanolide compound, 3-methyl-2H-furo[2.3-c]pyran-2-one (karrikinolide; a karrikin plant growth regulator), has been isolated from smoke and is believed to be responsible for the majority of the seed germination capabilities of smoke (Flematti et al. 2004; Van Staden et al. 2004; Light et al. 2009; Chiwocha et al. 2009), but plant-based smoke is likely to contain other unidentified compounds that stimulate germination (Chiwocha et al. 2009). Dixon et al. (2009) concluded synthesized karrikinolide promoted sprouting in several plant species, similar to smoke applications. Verschaeve et al. (2006) evaluated toxicity levels of karrikinolide and determined it had no toxic nor genotoxic effects at the rates tested, suggesting that smoke is non-toxic to humans. Many studies have been conducted on species grown from true seed, but no previous research has been conducted on the effects of aerosol smoke on freshly harvested seed potato tubers.

This project set out to 1) assess the efficacy of various environmental or chemical treatments at breaking tuber dormancy and promoting sprout elongation on potato cultivars grown in the Pacific Northwest of the United States, 2) explore the ability of novel (to potato) aerosol-smoke applications on potato tubers to break dormancy and induce sprouting soon after harvest, and 3) assess the efficacy of treatments at promoting dormancy release as time after harvest increases.

Materials and Methods

Sprout enhancement trials were conducted over two years (2021 and 2022) on three potato cultivars Umatilla Russet, Clearwater Russet, and Russet Burbank. In year one, Clearwater Russet and Russet Burbank were grown at University of Idaho Kimberly Research and Extension Center, Kimberly, Idaho, US (KREC). Potatoes were harvested and placed into 12.8 C and 95% relative humidity (RH) storage on September 13, 2021. Umatilla Russet was sampled from a commercial storage and delivered to KREC on October 7, 2021 and stored at 12.8 C. In year two, all three cultivars were grown at KREC and placed into 12.8 C and 95% RH on September 20, 2022. Potatoes

were ramped down at 0.3 C per day beginning on October 6, 2021 and October 3, 2022 until they reached a final holding temperature of 8.9 C on October 21, 2021 and October 17, 2022.

Treatments were applied to tuber samples at three different times in storage: October, November, and December. In year one application trials commenced 30, 49, and 79 days after harvest (DAH; October 13, November 1, and December 1, 2021) Year two, trials commenced 29, 50, and 77 DAH (October 18, November 8, and December 5, 2022). Trials will be referred to as October, November, and December treatment timings for ease of discussion.

October treatment was conducted prior to the storage bin reaching the final holding temperature. Approximately one week prior to each application date, unwashed tubers (n= 20; 3 replicates; tuber size 57-283 g) were sampled from bulk storage containers into 4.5 kg plastic mesh produce bags and placed into 12.8 C storage and 95% RH. A temperature recording device (Kestrel; Kestrel Instruments, Boothwyn, PA) was placed with each treatment (Appendix C). Each set of tubers were subjected to eight treatments for each application timing. After each treatment was completed, samples were stored at 18.3 C and 95% RH through the evaluation period.

Treatments

Untreated Control (UTC)

Tubers were removed from 12.8 C storage and placed into 18.3 C and 95% RH storage for 14 days.

Cold-Stratification

Tubers were removed from 12.8 C storage and placed into a 4.4 C and 95% RH storage for 14 days.

Temperature Fluctuations

Tubers were removed from 12.8 C storage and placed into 4.4 C storage for five days. On the fifth day tubers were removed from 4.4 C storage and placed into 18.3 C storage for four days. Day nine, tubers were removed from 18.3 C storage and placed back into 4.4 C storage for another five days.

Gibberellic Acid (GA) Dip

Deionized (DI) water (11.3L) was dispensed into 19-liter plastic buckets 12 to 24 hours prior to application date to ensure water acclimated to ambient room temperature (approximately 18.3 C). Immediately before GA dip application began, 5.7 ml Pro-Gibb 4% (Valent BioSciences, Illinois)

was added to the water and agitated until thoroughly mixed, resulting in a solution with 20 ppm GA. Tubers were removed from 12.8 C storage and submerged into the GA solution for 15 minutes then allowed to air dry for approximately three hours in ambient temperatures.

Smoke

Tubers were removed from 12.8 C storage and treated with smoke in a customized chamber. Smoke was 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 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 or 200 g) were ignited using a 0.4 L propane cylinder with brass torch (BenzOmatic, New York). Once the pellets were ignited, smoke was injected into the application chamber using a long metal tube to ensure smoke was cooled before entering chamber (Appendix A). The temperature of the application chamber remained within +/- 0.6 C of ambient temperatures (approximately 18.3 C).

Smoke treatments included: a) an injection of smoke for one hour with 20 hours of circulation (1h 20h) totaling 21 hours of exposure to smoke, b) injection of smoke for two hours with four hours of circulation (2h 4h), and c) injection of smoke for one hour with four hours of circulation (1h 4h).

Combination

Tubers were subjected to aerosol smoke (1h 20h) at the same time as smoke treatment ‘a’ (as defined above). After the circulation period was completed, tubers were placed into 18.3 C and 95% RH storage for 24 hours. After 24 hours, tubers were submerged into a 20 ppm GA solution for 15 minutes (as described above) and allowed to dry.

Sprout Evaluations

Sprout ratings were conducted according to the University of Idaho Sprout Rating Scale where: 1) no bud activity; 2) sprout initiating but not pointed; 3) sprout pointed, but length not achieving five mm; 4) sprout elongating, length five mm or greater (Figure 3-1). The number of sprouts pointing, the number of sprouts elongating to greater than 5 mm, and a length measurement of each sprout elongating was collected during evaluations. Evaluations of sprouts began approximately two weeks after treatments on October 28, November 17, and December 16, 2021 (year one) and November 1, November 22, and December 15, 2022 (year two). Sprout evaluations occurred weekly.

Sprout evaluations continued until the majority of the treatments achieved dormancy break (80% of tubers expressing a 3 rating or greater). All sprouts rated a 3 or 4 were removed from tubers and weighed collectively for each treatment during the final evaluation.

Statistical Analysis

Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating (≥ 5 mm), average sprout lengths, and final sprout weight were analyzed using the analysis of variance (ANOVA) procedures in R Studio (RStudio, package car version 4.1.0, 2021; Fox and Weisberg 2019). Each application timing, year, and cultivar were analyzed separately for each objective. All trials' significant differences between means for response variables were compared at p-value of 0.05 by estimated marginal means procedures (RStudio, package emmeans version 1.6.1, 2020). Number of sprouts pointing, number of sprouts elongating, and final sprout weight are included in Appendix C (Figure C- 1 to 3; Table C- 1 to 4).

Results

Response variables for each cultivar were significantly impacted by treatment, application timing, and year. When year or cultivar was combined, significant interactions were observed and variability between treatments was lost, therefore results for each cultivar, application timing, and year were analyzed and discussed separately. Variables having similar treatment responses between years are explained.

October Treatment

Weekly evaluations in year one for Russet Burbank were conducted until 48 DAT, whereas in year two evaluations occurred until 77 DAT. The longer evaluation period occurred due to slower overall sprout development in the second year for this cultivar. Although it took longer for sprout development to occur in year two, treatments followed similar trends between years (Table 3-1; Table 3-2; Table 3-3). In year one, smoke-based treatments and the GA dip had significantly higher sprout rating and achieved dormancy break earlier compared to the UTC. The GA dip had significantly higher final sprout length compared to the UTC in year one but was similar in year two. Smoke based treatments had similar sprout lengths as the UTC and GA dip in year one but smoke 1h 20h and 2h 4h had higher sprout lengths compared to GA dip in year two. The combination treatment had significantly higher sprout rating during the first evaluations and final sprout length compared to the other treatments in both years. Cold-stratification and temperature fluctuation broke dormancy at the same time as UTC but had lower sprout ratings in year one. In year two, the

cold-stratification had a higher sprout rating, but similar sprout length compared to the UTC. Smoke 1h 4h and 2h 4h had higher sprout ratings compared to UTC from 23 to 40 DAT in year one but were often similar to the UTC in year two.

Clearwater Russet sprout development was similar between years (Table 3-1; Table 3-2; Table 3-3). The cold stratification and temperature fluctuation treatments were similar, but had significantly lower sprout rating and similar final sprout length compared to the UTC. GA dip, smoke 1h 4h, and smoke 2h 4h were similar regarding dormancy break. However, the GA dip had a higher sprout length compared to smoke 1h 4h in both years. Smoke 1h 20h had initiated sprout development earlier compared to most other treatments but had similar final sprout lengths as the GA dip. The combination treatment had the highest sprout rating during early evaluations and the longest final sprout length compared to the other treatments in both years.

In Umatilla Russet, year two had less sprout development compared to year one, but most of the treatments followed the same trend between years (Table 3-1; Table 3-2; Table 3-3). Cold-stratification and temperature fluctuation were similar and had lower final sprout length and retarded sprout development compared to the other treatments. In contrast, the combination treatment had higher sprout ratings and achieved dormancy break prior to the other treatments. The combination treatment final sprout length was similar to the smoke-based treatments in year one but was significantly higher in year two. GA dip and smoke 1h 20h achieved dormancy break at the same time in both years, but the GA dip had a higher sprout rating and sprout length in year one while the two treatments were similar in year two. Smoke 1h 4h and smoke 2h 4h were similar in sprout rating for both years but final sprout length differed between year with smoke 2h 4h being higher in year two.

November Treatment

Sprout development was delayed in year two compared to the first year in Russet Burbank, but many of the treatments followed similar trends between years (Table 3-4; Table 3-5; Table 3-6). Smoke-based treatments and GA dip each accelerated sprout development and achieved dormancy break sooner and final sprout lengths were significantly higher compared to the UTC. Smoke 1h 20h and GA dip both increased sprout development individually, but when combined, efficacy of initiating sprout development was significantly increased. The combination treatment had higher sprout ratings soon after treatment compared to the other treatments and was the first to break dormancy in both years. Combination treatment had significantly higher final sprout length than all

other treatments. Cold-stratification and temperature fluctuations both resulted in lower final sprout ratings than the UTC and did not reach dormancy break during the evaluation period. Neither temperature treatment showed any sprouts elongating at the final evaluation whereas the UTC had significantly longer average sprout length in year one. Year two sprout lengths were longer overall but were evaluated at 56 DAT compared to 29 DAT in year one.

November treated Clearwater Russet responded similarly in both years with minor variations in regard to sprout rating (Table 3-5; Table 3-6), but tended to have higher sprout lengths in the second year (Table 3-4). Smoke 1h 20h and GA dip alone both promoted early sprout development and resulted in higher final sprout lengths than the UTC. The combination treatment resulted in the highest sprout rating and had broken dormancy by the first evaluation. The combination treatment had the longest sprout lengths in both years. Cold-stratification and temperature fluctuation had significantly lower sprout ratings and sprout lengths compared to the other treatments during the final evaluation. Neither treatment achieved dormancy break during the evaluation period. Smoke 2h 4h was effective at promoting early sprout development and had greater final sprout lengths compared to the UTC in both years. The smoke 1h 4h had more variable results. In year one, smoke 1h 4h was similar to the UTC but had greater sprout rating and final sprout length in year two.

Treatment efficacy was variable between years for Umatilla Russet and did not follow the same trends as the other two cultivars (Table 3-4; Table 3-5; Table 3-6). The GA dip alone was as effective at promoting early sprout development as the combination treatment in year one. Both combination treatment and GA broke dormancy at the same time, but the combination treatment had significantly higher sprout length than the GA dip. In year two, smoke 1h 20h had similar early sprout development as the combination treatment, both achieving dormancy break at the same time. However, the combination treatment had significantly higher sprout lengths compared to the smoke 1h 20h treatment. Cold-stratification and temperature fluctuation had the lowest sprout development compared to the other treatments.

December Treatment

Overall, December application timing took less time to achieve dormancy break than the October and November treated tubers. Year two Russet Burbank had lower initial sprout development than year one, but treatments responded similarly between the years (Table 3-7; Table 3-8). Cold-stratification and temperature fluctuations had significantly lower final sprout ratings and sprout lengths compared to the other treatments in both years. In year one, UTC, GA dip, and

smoke-based treatments had achieved dormancy break by the first evaluation, but final sprout lengths were higher in smoke-based and GA treatments compared to the UTC. In year two, smoke 2h 4h and 1h 20h had similar sprout ratings for each evaluation, final sprout lengths, and achieved dormancy break at the same time. The combination treatment had the greatest sprout ratings and final sprout length compared to the other treatments but broke dormancy at the same time as smoke 1h 20h and smoke 1h 4h in year two. The GA dip did not stimulate early sprout development compared to the UTC in year two.

Clearwater Russet sprout ratings and sprout length differed between years (Table 3-7; Table 3-8). In year one, most treatments had achieved dormancy break by the first evaluation with the exception of cold-stratification and temperature fluctuation. The cold-stratification and temperature fluctuation achieved dormancy break in the final evaluation but had lower sprout ratings compared to the other treatments. The GA dip and smoke 1h 20h both had higher early sprout development compared to the other smoke treatments, but the combination treatment had the highest sprout rating overall. Year one sprout ratings were corroborated with the final sprout length. In year two, sprout evaluations were initiated at 9 DAT. The combination treatment had already achieved dormancy break by the first evaluation and had significantly longer sprouts compared to the other treatments. UTC, GA dip, and smoke-based treatments achieved dormancy break at the same evaluation period, but UTC had lower sprout rating and sprout length compared to the other treatments. Cold-stratification and temperature fluctuations did not achieve dormancy break prior to the UTC nor produce substantial sprout development in either year.

In year one, the UTC treatment achieved dormancy break by the first evaluation and had a similar sprout rating compared to GA dip, smoke 1h 20h, combination, and smoke 1h 4h in Umatilla Russet (Table 3-8). However final sprout length was significantly lower in the UTC compared to smoke-based treatments and GA dip (Table 3-7). The smoke 1h 20h did not break dormancy until the second evaluation but still produced sprouts comparable to the other smoke-based treatments. Temperature fluctuation achieved dormancy break during the evaluation period but did not produce significant sprout development compared to the other treatments. In year two, the smoke-based treatments and GA had similar sprout ratings, but the GA dip produced higher final sprout length. The combination treatment had comparable sprout rating to the GA dip and smoke 2h 4h but produced significantly higher sprout lengths than the other treatments.

Days to Dormancy Break

The number of days for each treatment to achieve dormancy break for each application timing varied between treatments (Table 3-9). Dormancy break is achieved when 80% of tubers in a treatment have begun to exhibit elongated sprouts (3 rating). The number of days until dormancy break were derived from the sprout ratings discussed previously. Data cannot be statistically analyzed since all tubers were used to generate the days to dormancy break, but general comments can be made about the results. Typically, as time after harvest increased, the time between treatment and dormancy break decreased. Treatments in year one typically achieved dormancy break sooner than in year two, especially in Russet Burbank. The combination treatment consistently achieved dormancy break in a shorter period of time than the majority of other treatments while cold-stratification and temperature fluctuation typically took longer to achieve dormancy break. The combination treatment achieved dormancy break an average of 22 and 14 days prior to the untreated during the October and November treatment timings, but only 5 days sooner in the December timing, although many of the treatments had broken dormancy prior to the first evaluation of the December applications. The cold-stratification and temperature fluctuations increased dormancy length by approximately 3 days while smoke 1h 20 reduced time to dormancy break by approximately 11 days and GA dip by 10 days for October treatments. Smoke 1h 4h and 2h 4h reduced time to dormancy break by 6 and 7 days respectively for October treatment.

Discussion

Potato dormancy poses a significant challenge to seed certification agencies that require actively growing sprout tissue to conduct post-harvest testing soon after harvest. Consistent, effective, and safe methods to break tuber dormancy are desired. The objective of this study was to evaluate methods to hasten dormancy break in tubers soon after harvest in accordance with typical winter grow out scheduling.

Treatment, timing of application, and cultivar influenced dormancy break. In general, treatments induced sprouting sooner after application as time after harvest increased. This was to be expected as some of the cultivars may have been approaching the end of their endo-dormant period. However, several of the smoke and GA treatments were effective at promoting early sprout development and had higher sprout lengths compared to the untreated control when treated even one month after harvest (October timing). Findings from this study indicate that smoke 1h 20h, GA dip, and the combination of smoke and GA are effective methods to induce sprout development

soon after harvest. Seed certification agencies could use these methods to break dormancy and facilitate early post-harvest testing by direct tuber testing of potatoes or by providing an alternative to Rindite for the winter grow out.

The cutoff for sample submission for the winter grow out is typically mid-October. Not every seed lot is harvested prior to this cut-off date and/or a seed lot may need re-tested after this date, so the samples must be laboratory tested. Due to the small volume of samples and the inconvenience of applying Rindite, late or re-test samples are typically dipped in GA or left in warm, 18 to 24 C, rooms until sprouting has begun, which may take weeks to months to occur. Results from the November and December treatment timings showed that samples submitted after the cutoff date for shipment to the winter grow out or re-test samples could be treated and have sprout development one to two weeks sooner compared to samples held at 18.3 C. Smoke and GA based treatments initiated sprout development soon after application and would be viable methods to use at this time. However, it has been reported that PVY detection is less efficient when tubers break dormancy naturally (De Bokx and Cuperus 1987; Barker et al. 1993; Fox et al. 2005), indicating late or re-test samples left in warm rooms may not accurately estimate levels of PVY in a seed lot. Gugerli and Gehriger (1980) and McDonald and Coleman (1988) found tubers treated with Rindite or bromoethane had higher PVY detection than untreated tubers when using ELISA methods on tubers, demonstrating chemical treatment may have improved PVY detection. These previous findings indicate that PVY detection studies need to be conducted on new dormancy breaking methods in order to accurately assess PVY levels in late or re-test samples. Further research needs to be conducted on whether smoke and/or GA treated tubers have similar PVY detection as tubers treated with Rindite.

As time after harvest increased, treatment efficacy increased at inducing sprouting with the exception of cold-stratification and temperature fluctuations. Previous studies demonstrated temperature fluctuations or cold treatments hastened dormancy break in potatoes (Bryan 1989; Wurr and Allen 1976; Tavakoli et al. 2014) and is a recommended practice for breaking tuber dormancy by International Potato Research Guide 16 (Bryan 1989). However, in the present study, temperature fluctuations and cold-stratification treatments did not increase sprouting compared to the untreated control at any application timing or cultivar, and in some cases retarded sprout development. These results could be due to the brief amount of time between harvest and treatment timings, the short period in which sprout development was desired for this study's objective, or cultivars used. In the case of Wurr and Allen (1976), temperature fluctuations were

conducted over many months in storage since sprouting was desired for planting in spring (following a fall harvest). Although temperature treatments have shown to be beneficial in previous long term storage trials, these treatments did not induce sprouting in the desired timeframe for the current objective of encouraging tubers to sprout soon after harvest for post-harvest testing in the seed certification process.

Maintenance and release of tuber dormancy is associated with several biochemical and phytohormonal processes (Suttle 2004; Mani et al. 2014; Campbell et al. 2008). Gibberellic acid is involved in release of tuber dormancy and successive sprout development upon dormancy release (Mani et al. 2014; Campbell et al. 2008; Sonnewald 2001; Suttle 1998; Kulen et al. 2011; Dogonadze et al. 200; Multhoni et al. 2014). The current study evaluated the effectiveness of submersion of whole, undamaged tubers in a 20 ppm GA solution. Submersion in a GA solution stimulated sprout development and resulted in dormancy break before the untreated control for most application timings. The effectiveness of GA dip was variable for each cultivar and year but consistently achieved dormancy break earlier and produced longer sprouts compared to the untreated control. These results corroborate findings from Kulen et al. (2011), Wrobel et al. (2017), and Travakoli et al. (2014). However, Wrobel et al. (2017) used excised tuber tissue and Kulen et al. (2011) and Travakoli et al. (2014) conducted studies on potato mini-tubers in growth chambers, which are known to respond differently than commercially produced tubers. Many seed certification agencies treat longer dormancy cultivars in a GA dip following application with Rindite or bromoethane to increase the rate of emergence and plant growth in the WGO. This study implies a dip in GA alone may be enough to break dormancy and induce sprouting in some cultivars. However, in some cases the GA dip produced etiolated sprouts (long and thin), which may be easily subjected to damage.

Application of cold aerosol smoke improved sprout development and decreased the amount of time necessary for tubers to achieve dormancy break in each of the cultivars. Smoke and smoke extracts derived from the combustion of plant material have been shown to stimulate germination in a wide range of true seed species (Dixon et at. 2009; De Lange and Boucher 1990; Chiwocha et al. 2009; Keeley and Fotheringham 1998; Light and Van Staden 2004; Ghebrehiwot et al. 2013, Light et al. 2009). The different injection periods and circulation times impacted the efficacy of smoke at accelerating sprout development in this study. The shorter circulation treatments were not beneficial and tended to have lower sprout development than the longer circulation treatment. The smoke 1h 20h often had greater sprout development and achieved dormancy break sooner than the UTC in each of the cultivars. A preliminary study (Appendix F) showed that increasing the injection time of

smoke did not improve sprout development and in some cases caused tuber injury. Although the direct mechanism of smoke's capacity to initiate sprouting in dormant tubers is not fully understood, it has been suggested that Karrikinolide, a Karrikin plant growth regulator isolated from smoke, serves as a cue for germination and/or increases sensitivity to other phytohormones (Light et al. 2009; Chiwocha et al. 2009; Dixon et al. 2009; Nelson et al. 2012). It has also been suggested that smoke can alter the permeability of seed coats and cuticle layers allowing for imbibition of water and other larger molecules (Keeley and Fotheringham 1998; Egerton-Warburton 1998).

Increased sensitivity to phytohormones and/or increased permeability may have been exemplified in this study. Application of cold aerosol smoke followed by a dip in GA solution significantly reduced the time to dormancy break and increased the length of developing sprouts compared to smoke alone and GA dip alone. In several cases, the final sprout length was more than the sum of each treatment alone, suggesting smoke and GA may have a synergistic effect on sprout development in potato. Further, the combination treatment produced more visually robust sprouts that appeared thicker, and more sturdy compared to a GA dip alone. Inclusion of smoke had positive effects on sprout development in each of the cultivars tested indicating the consistency of this application method. A preliminary study conducted by the author found that a dip in GA immediately prior to smoke application had similar sprout development as a GA dip following smoke application (Appendix F). This may indicate that a treatment with aerosol smoke could increase the ability of GA to penetrate into the potato tuber or that the tuber becomes more sensitive to exogenous hormone application. Using aerosol smoke to stimulate germination in crop species is a relatively new concept but appears to be a viable option to induce sprouting for direct tuber testing. Additional investigation into the mode of action of aerosol smoke treatments, and possibly synthesized Karrikinolide, on potato sprouting and the interrelated biochemical processes involved needs to be conducted. Application of smoke and combination of smoke plus GA needs to be conducted on a wide range of cultivars with varying dormancy ranges to determine specific protocols to induce sprouting in dormant potato tubers.

This study focused on developing consistent, non-toxic methods to promote sprouting in freshly harvested potato tubers. Preliminary and supplementary studies to develop the cold aerosol smoke application process were conducted to determine optimal application timing and exposure as well as evaluated the effects smoke application had on the quality of potato and the subsequent crop (Appendix E; Appendix F; Appendix G; Appendix H). These supplementary studies provided foundational knowledge in the development of a method for breaking tuber dormancy and

evaluated potential phytotoxicity risks for tubers. Verschaeve et al. (2006) reports Karrikinolide, the proposed active compound in smoke, is not genotoxic or toxic and is therefore considered non-hazardous to humans. Further research into other compounds found in smoke produced from combustion of plant materials should be conducted to ensure the safety of smoke application but, it appears application of aerosol smoke may be non-hazardous to humans and a consistent method to produce sprout development in recently harvest potato tubers. This study added to the growing collection of smoke induced sprouting data, however, this is the first report of using aerosol smoke to induce sprouting in potato, a vegetatively propagated crop.

Conclusion

Methods to induce sprouting in dormant potato tubers were investigated. Findings from this study suggest cold-stratification and temperature fluctuations of tubers is not an effective method to promote dormancy break nor increase sprout development soon after harvest. However, the application of cold aerosol smoke, generated from the combustion of plant material, may serve as a consistent, effective, and non-toxic (to humans) alternative to induce sprouting in dormant tubers. This is the first report of using aerosol smoke from combusted plant material to stimulate sprouting in dormant potato tubers. Although efficacy of several treatments studied were variable, the combination treatment of smoke and GA consistently had greater sprout development and produced more robust sprouts compared to GA dip alone and smoke alone in each of the cultivars and application timings studied. In order to produce sprout development soon after harvest, it would be recommended to apply smoke followed by a dip in GA. The combination treatment is equally effective regardless of the time between harvest and treatment. Further, the combination treatment appears to be a tuber and human-safe alternative method to promote dormancy break and induce sprout development soon after harvest. Not only will these treatments provide seed certification agencies with an alternative method to induce sprouting for post-harvest testing but may also provide opportunities for seed growers to treat dormant tubers and produce a successive crop soon after harvest in climates that allow multiple crops to be grown in a single year.

References

Akoumianakis, K., C. M. Olympios, and H. C. Passam. 2000. Effect of "rindite" and bromoethane on germination, sprout emergence, number of sprouts, and total yield of tubers of potato cv. Spunta. *Advances in Horticultural Sciences* 14(1): 33-35.

Aksenova, N. P., L. I. Sergeeva, T. N. Konstantinova, S. A. Golyanovskaya, O. O. Kolachevskaya, and G. A. Romanov. 2013. Regulation of potato tuber dormancy and sprouting. *Russian Journal of Plant Physiology* 60(3): 301-312.

Avrahami-Moyal, L., Y. Tam, M. Brumin, S. Prakash, D. Leibman, M. Pearlsman, M. Bornstein, N. Sela, M. Seidan, Z. Dar, U. Zig, A. Gal-On, and V. Gaba. 2017. Detection of potato virus Y in industrial quantities of seed potatoes by TaqMan Real Time PCR. *Phytoparasitica* 45: 591-598.

Baldwin, I. T., L. Staszak-Kozinski, and R. Davidson. 1994. Up in smoke: I. Smoke-derived germination cues for postfire annual, *Nicotiana attenuate* torr ex Watson. *Journal of Chemical Ecology* 20: 2345-2371.

Barker H., K. D. Webster, and B. Reavy. 1993. Detection of potato virus Y in potato tubers: a comparison of polymerase chain reaction and enzyme-linked immunosorbent assay. *Potato Research* 36: 13-20.

Bryan, J. E. 1989. Breaking dormancy of potato tubers. *CIP Research Guide* 16: 1-13.

Callison B. L., and J. Harrington, and D. Douglas. 1982. Seed potato certification: Its purpose, capabilities, and limitations. *American Potato Journal* 59: 231-236.

Campbell, M., E. Segear, L. Beers, D. Knaber, and J. Suttle. 2008. Dormancy in potato tuber meristems: Chemically induced cessation in dormancy matches the natural process based on transcript profiles. *Functional and Integrative Genomics* 8: 317-328.

Carvalho, C., D. Rees, G. Harper, A. Briddon, and R. Colgan. 2021. Research review: Potato tuber dormancy. *Agriculture and Horticulture Development Board* 1-37.

Chiwocha, S. D. S., K. W. Dixon, G. R. Flematti, E. L. Ghisalberti, D. J. Merritt, D. C. Nelson, J. A. M. Riseborough, S. M. Smith, and J. C. Stevens. 2009. Karrikins: A new family of plant growth regulators in smoke. *Plant Science* 117: 252-256.

Coleman, W. K. 1983. An evaluation of bromoethane for breaking tuber dormancy in *Solanum Tuberosum* L. *American Potato Journal* 60: 161-167.

Cvikrova, M., L. S. Sukhova, J. Eder, and N. P. Korableva. 1994. Possible involvement of abscisic acid, ethylene, and phenolic acids in potato tuber dormancy. *Plant Physiology and Biochemistry* 32: 685-691.

Davidson, T. M. W. 1958. Dormancy in the potato tuber and the effects of storage conditions on initial sprouting and on subsequent sprout growth. *American Potato Journal* 35: 451-465.

De Lange, J. H., and C. Boucher. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* 56(6): 700-703.

DeBokx, J. A., and C. Cuperus. 1987. Detection of potato virus Y in early-harvest potato tubers by cDNA hybridization and three modifications of ELISA. *European and Mediterranean Plant Protection Organization Bulletin* 17: 73-79.

Denny, F. E. 1926. Hastening the sprouting of dormant potato tubers. *American Journal of Botany* 13(2): 118-125.

Denny, F. E. 1945. Synergistic effects of three chemicals in the treatment of dormant potato tubers to hasten germination. *Contributions from Boyce Thomson Institute* 14: 1-14.

Dixon, K. W., D. J. Merrit, G. R. Flematti, and E. L. Ghisalberti. 2009. Karrikinolide-a phytoreactive compound derived from smoke with applications in horticulture, ecological restoration, and agriculture. *Acta Horticulturae* 813(40): 155-170.

Dogonadze, M. Z., N. P. Korableva, T. A. Platoneva, G. L. Shaposhnikov. 2000. Effect of gibberellin and auxin on the synthesis of abscisic acid and ethylene in buds of dormant and sprouting potato tuber. *Applied Biochemistry and Microbiology* 36: 507-509.

Doherty, L. C., and M. A. Cohn. 2000. Seed dormancy in red rice (Oryza sativa). XI. Commercial liquid smoke elicits germination. *Seed Science Research* 10: 415-421.

Drewes, F. E., M. T. Smith, and J. Van Staden. 1995. The effect of a plant-derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation* 16: 205-209.

Egerton-Warburton, L. M. 1998. A smoke-induced alteration of the sub-testa cuticle in seeds of the post-fire recruiter, *Emmenanthe penduliflora* Benth. (Hydrophyllaceae). *Journal of Experimental Botany* 49(325): 1317-1327.

Esztergalyos, A., and Z. Polgar. 2021. The effect of chemical treatments on the tuber dormancy of Hungarian potato cultivars. *Potato Research* 64: 327-337.

Flematti, G. R., E. L. Ghisalberti, K. W. Dixon, and R. D. Trengove. 2004. A compound from smoke that promotes seed germination. *Science* 305(5686): 977.

Fox, A., F. Evans, and I. Browning. 2005. Direct tuber testing for potato Y potyvirus by real-time RT-PCR and ELISA: Reliable options for post-harvest testing? *European and Mediterranean Plant Protection Organization Bulletin* 356: 93-97.

Fox, J., and S. Weisberg. 2019. An {R} companion to applied regression, third edition, Thousand Oaks CA: Sage.

Frost, K., R. L. Groves, and A. O. Charkowski. 2013. Integrated control of potato pathogens through seed potato certification and provisions of clean seed potatoes. *American Phytopathological Society* 97(10): 1268-1280.

Gebrehiwot, H., M. Kulkarni, M. Bairu, and J. Van Staden. 2013. Plant-derived aerosol-smoke and smoke solutions influence agronomic performance of traditional cereal crop, Tef. *Experimental Agriculture* 49(2): 244-255.

Gugerli, P., and W. Gehriger. 1980. Enzyme-linked immunosorbent assay (ELISA) for the detection of potato leafroll virus and potato virus Y in potato tubers after artificial break of dormancy. *Potato Research* 23: 353-359.

Haider, M. W., M. Nafees, I. Ahmad, B. Ali, Maryam, R. Iqbal, D. C. Vodnar, R. A. Marc, M. Kamran, M. H. Saleem, A. A. Al-Ghamdi, F. M. Al-Hemaid, and M. S. Elshikh. 2022. Postharvest dormancy-related changes of endogenous hormones in relation to different dormancy-breaking methods of potato (*Solanum tuberosum* L.) tubers. *Frontiers in Plant Science* 13: 945253.

Halterman, D., A. Charkowski, and J. Verchot. 2012. Potato, viruses, and seed certification in the USA to provide healthy propagated tubers. *Pest Technology* 6(1): 1-14.

Hartmann A., M. Senning, P. Hedden, U. Sonnewald, and S. Sonnewald. 2011. Reactivation of meristem activity and sprout growth in potato tubers require both cytokinin and gibberellin. *Plant Physiology* 155: 776-796.

Hill, S. A., and E. A. Jackson. 1984. An investigation of the reliability of ELISA as a practical test for detecting potato leaf roll virus and potato virus Y in tubers. *Plant Pathology* 33: 21-26.

ICIA. 2019. ICIA procedure: Virus testing- ELISA method version 2019-01. *Idaho Crop Improvement Association, Inc*

Keely, J. E., and C. J. Fotheringham. 1998. Smoke-induced seed germination in California chaparral. *Ecology* 79(7): 2219-2580.

Khurana P. S. M. 2004. Potato viruses and their management. In, Diseases of Fruits and Vegetables, V2, ed S. A. M. H. Naqvi, 389-440, *Kluwer Academic Publishers*, Netherlands.

Korableva, N. P., L. S. Dukhova, M. Z. Dogonadze, and I. Machackova. 1989. Hormonal regulation of potato tuber dormancy and resistance to pathogens. In: Signals in Plant Development, ed J. Kredule, R. Seidlova, 65-71. *SPB Academic Publishers*, The Hague.

Kulen, O., C. Stushnoff, R. D. Davidson and D. G. Holm. 2011. Gibberellic acid and ethephon alter potato minituber bud dormancy and improve seed tuber yield. *American Journal of Potato Research* 88: 167-174.

Light, M. E., and J. Van Staden. 2004. The potential of smoke in seed technology. *South African Journal of Botany* 70(1): 97-101.

Light, M. E., M. I. Daws, and J. Van Staden. 2009. Smoke-derived butanolide: Towards understanding its biological effects. *South African Journal of Botany* 75: 1-7.

Majeed, A., and A. Bano. 2006. Role of growth promoting substances in breaking potato (*Solanum tuberosum* L.) tuber dormancy. *Journal of Agriculture and Social Sciences* 2(3): 175-178.

Mani, F., T. Bettaieb, N. Doudech, and C. Hannachi. 2014. Physiological mechanisms for potato dormancy release and sprouting: A review. *African Crop Science Journal* 22(2): 155-174.

McDonald, J. G., and W. K. Coleman. 1988. A reevaluation of bromoethane in comparison to rindite for the post-harvest detection of potato virus Y in tubers by ELISA. *American Potato Journal* 65: 547-550.

Muthoni, J., J. Kabira, H. Shimelis, and R. Melis. 2014. Regulation of potato tuber dormancy: A review. *Australian Journal of Crop Science* 8(5): 754-759.

Nelson, D. C., G. R. Flematti, E. L. Ghisalberti, K. W. Dixon, and S. M. Smith. 2012. Regulation of seed germination and seedling growth by chemical signals from burning vegetation. *Annual Review of Plant Biology* 63: 107-130.

Prange, R. K., W. Kalt, B. J. Daniels-Lake, C. L. Liew, R. T. Page, J. R. Walsh, P. Dean, and R. Coffin. 1998. Using ethylene as a sprout control agent in stored 'Russet Burbank' potatoes. *Journal of American Society for Horticultural Science* 123(3): 463-469.

Preston, C. A., R. Becker, and I. T. Baldwin. 2004. Is 'NO' news good news? Nitrogen oxides are not components of smoke that elicits germination in two smoke-stimulated species, *Nicotiana attenuate* and *Emmenanthe penduliflora. Seed Science Research* 14: 73-79.

Russo, P., L. Miller, R. P. Singh, and S. A. Slack. 1999. Comparison of PLRV and PVY detection in potato seed samples tested by Florida winter field inspection and RT-PCR. *American Journal of Potato Research* 76: 313-316.

Rylski, I., L. Rappaport, and H. K. Pratt. 1974. Dual effects of ethylene on potato dormancy and sprout growth. *Plant Physiology* 53: 658-662.

Safety Data Sheet (SDS). 2022. Bromoethane. *ThermoFisher Scientific* 1-8.

Singh, M., R. P. Singh, M. S. Fageria, X. Nie, R. Coffin, and G. Hawkins. 2013. Optimization of a real-time RT-PCR assay and its comparison with ELISA, conventional RT-PCR, and the grow-out test for large scale diagnosis of potato virus Y in dormant potato tubers. *American Journal of Potato Research* 90: 43-50.

Sonnewald, S., and U. Sonnewald. 2014. Regulation of potato tuber sprouting. *Planta* 239: 27-38.

Sonnewald, U. 2001. Control of potato tuber sprouting. *Trends in Plant Science* 6(8): 333-335.

Suttle, J. C. 1998. Involvement of ethylene in potato microtuber dormancy. *Plant Physiology* 118: 843-848.

Suttle, J. C. 2004. Physiological regulation of potato tuber dormancy. *American Journal of Potato Research* 81: 253-262.

Tavakoli, K., A. Razavi, and A. Sohani. 2014. Effects of different temperatures and hormone treatments on breaking dormancy in potato tubers. *Journal of Agricultural Sciences* 59(3): 255-264.

UNECE. 2019. Findings of the survey of seed potato virus testing methods associated with seed potato certification. *United Nations Economic Commission for Europe*.

Van Staden J., F. E. Drewes, and A. K. Jager, 1995. The search for germination stimulants in plant-derived smoke. *South African Journal of Botany* 61: 260-263.

Van Staden, J., A. K. Jager, M. E. Light, and B. V. Burger. 2004. Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany* 70(4): 654-659.

Van Staden, J., and N. A. C. Brown. 1995. Some chromatographic characteristics of germination stimulants in plant-derives smoke extracts. *Plant Growth Regulation* 17: 241-249.

Verschaeve, L., J. Maes, M. E. Light, and J. Van Staden. 2006. Genic toxicity testing of 3-methyl-2H-furo[2,3-c]pyran-2-one, an important biologically active compound from plant-derived smoke. *Mutation Research* 611: 89-95.

Vetten, H. J., U. Ehlers, and H. L. Paul. 1983. Detection of potato viruses Y and A in tubers by enzyme-linked immunosorbent assay after natural and artificial break of dormancy. *Journal of Phytopathology* 108(1): 41-53.

Wiltshire, J. J. J., and A. H. Cobb. 1996. A review of the physiology of potato tuber dormancy. *Annals of Applied Biology* 129: 553-569.

Withers, P. C., and C. E. Cooper. 2008. Dormancy. In: Encyclopedia of Ecology, ed. S. E. Jorgensen, B. D. Fath, 952-957. *Elsevier B.V*. Netherlands.

Wrobel, S., J. Kesy, and K. Treder. 2017. Effect of growth regulators and ethanol on termination of dormancy in potato tubers. *American Journal of Potato Research* 94: 544-555.

Wurr, D. C. E., and E. J. Allen. 1976. Effects of cold treatments on the sprout growth of three potato varieties. *Journal of Agricultural Science* 86: 221-224.

Tables

Table 3-1. Average sprout length of three potato cultivars at the final evaluation after the October treatments.

	Russet Burbank[1]		Clearwater Russet		Umatilla Russet	
Treatment[2]	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
	Average sprout length (mm)					
UTC	0.0 a	1.9 a	0.9 a	2.2 a	9.2 b	2.8 b
Cold-stratification	0.0 a	4.1 ab	0.2 a	0.5 a	2.8 a	0.4 a
Temp. fluctuation	0.4 a	2.0 a	0.4 a	0.4 a	4.8 a	0.4 a
GA dip	3.1 b	3.9 a	2.6 bc	9.8 c	16.6 d	8.5 d
Smoke 1h 20h	2.2 ab	7.5 bc	3.7 c	9.9 c	10.6 bc	7.2 cd
Combination	9.3 c	20.5 d	10.3 d	22.2 d	11.6 c	15.1 e
Smoke 2h 4h	1.3 ab	10.9 c	1.4 ab	7.9 bc	10.1 bc	6.2 c
Smoke 1h 4h	1.1 ab	5.4 ab	0.4 a	5.8 b	10.9 bc	4.4 b
Standard error	0.7	1.2	0.5	0.8	0.8	0.6

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

Table 3-2. Sprout ratings over time of three potato cultivars for tubers treated in October 2021.

	Days after October treatment[1]											
Treatment[2]	**15**		**23**		**28**		**33**		**40**		**48**	
	Russet Burbank sprout ratings[3,4]											
UTC	1.4	a	1.4	a	2.0	a	2.4	b	**3.1**	**b**	3.5	a
Cold-stratification	1.6	ab	1.5	a	1.9	a	2.1	a	**2.8**	**a**	3.2	a
Temp. fluctuation	1.7	bc	1.4	a	1.8	a	2.1	a	**2.8**	**a**	3.4	a
GA dip	1.9	cd	**3.0**	**c**	3.2	c	3.8	de	3.9	cd	4.0	b
Smoke 1h 20h	2.0	d	**3.3**	**d**	3.5	d	3.9	e	3.9	d	3.9	b
Combination	**2.9**	**e**	3.9	e	4.0	e	4.0	e	4.0	d	4.0	b
Smoke 2h 4h	1.6	abc	2.7	b	**3.2**	**c**	3.6	cd	3.8	cd	3.9	b
Smoke 1h 4h	1.6	ab	2.5	b	**2.9**	**b**	3.4	c	3.7	c	3.8	b
Standard error	0.09		0.08		0.08		0.08		0.06		0.09	
	Clearwater Russet sprout ratings											
UTC	1.5	ab	1.8	a	2.4	b	**2.9**	**b**	3.5	b	3.8	b
Cold-stratification	1.5	ab	1.5	a	1.8	a	2.1	a	2.7	a	**3.3**	**a**
Temp. fluctuation	1.6	ab	1.6	a	1.9	a	2.0	a	**2.9**	**a**	3.3	a
GA dip	1.8	bc	2.6	b	**3.1**	**cd**	3.6	d	3.9	de	4.0	b
Smoke 1h 20h	1.9	c	**3.2**	**c**	3.7	e	3.9	e	4.0	e	4.0	b
Combination	**2.9**	**d**	3.9	d	4.0	f	4.0	e	4.0	e	4.0	b
Smoke 2h 4h	1.4	a	2.7	b	**3.1**	**d**	3.5	cd	3.8	cd	4.0	b
Smoke 1h 4h	1.5	ab	2.4	b	**2.8**	**c**	3.3	c	3.7	bc	3.8	b
Standard error	0.12		0.11		0.09		0.08		0.07		0.08	
	Umatilla Russet sprout ratings											
UTC	1.1	a	1.2	a	1.7	ab	2.5	b	**3.3**	**b**	3.9	cd
Cold-stratification	1.1	a	1.2	a	1.3	a	1.4	a	2.4	a	**3.4**	**a**
Temp. fluctuation	1.1	a	1.1	a	1.2	a	1.5	a	2.6	a	**3.5**	**b**
GA dip	1.2	a	1.9	b	3.3	d	**3.9**	**e**	4.0	d	4.0	d
Smoke 1h 20h	1.1	a	1.3	ab	2.3	bc	**3.0**	**bcd**	3.7	cd	4.0	cd
Combination	1.3	a	1.9	b	**2.8**	**cd**	3.5	de	3.8	cd	3.9	cd
Smoke 2h 4h	1.1	a	1.3	ab	1.9	ab	2.7	bc	**3.5**	**bc**	3.8	c
Smoke 1h 4h	1.2	a	1.6	ab	2.3	bc	**3.1**	**cd**	3.7	bcd	4.0	cd
Standard error	0.07		0.20		0.25		0.21		0.12		0.05	

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

[2]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

[3]Bolded numbers within the table indicate treatment achieved dormancy break (80% tubers expressing a 3 rating).

[4]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 3-3. Sprout ratings over time of three potato cultivars for tubers treated in October 2022.

	Days after October treatment[1]													
Treatment[2]	**14**		**22**		**27**		**34**		**44**		**55**		**77**	
	Russet Burbank sprout rating[3,4]													
UTC	1.1	a	1.3	ab	1.4	ab	1.4	a	1.6	a	1.7	ab	2.6	a
Cold-stratification	1.1	a	1.3	ab	1.3	a	1.3	a	1.6	a	1.6	a	**3.0**	**b**
Temp. fluctuation	1.1	a	1.2	a	1.4	ab	1.4	a	1.6	a	1.6	a	**2.8**	**ab**
GA dip	1.1	a	1.4	b	1.6	b	1.9	b	2.0	b	2.0	bc	**3.1**	**b**
Smoke 1h 20h	1.1	a	1.3	ab	1.5	b	1.7	b	2.0	b	2.2	cd	**3.5**	**c**
Combination	1.3	b	1.8	c	2.2	c	2.4	c	**2.8**	**c**	3.1	e	3.9	d
Smoke 2h 4h	1.1	a	1.2	a	1.4	ab	1.5	a	1.9	b	2.3	d	**3.4**	**c**
Smoke 1h 4h	1.1	a	1.4	ab	1.5	ab	1.4	a	1.6	a	1.9	ab	**3.1**	**b**
Standard error	0.04		0.06		0.08		0.08		0.07		0.1		0.1	
	Clearwater Russet sprout ratings													
UTC	1.3	a	1.5	a	1.9	b	2.4	b	**3.2**	**b**	-		-	
Cold-stratification	1.2	a	1.4	a	1.6	a	1.8	a	**2.8**	**a**	-		-	
Temp. fluctuation	1.3	a	1.4	a	1.5	a	1.7	a	2.7	a	-		-	
GA dip	1.6	b	2.6	c	**3.2**	**d**	3.6	d	3.8	c	-		-	
Smoke 1h 20h	1.4	ab	**3.0**	**d**	3.5	e	3.7	d	3.9	cd	-		-	
Combination	2.3	c	**3.8**	**e**	4.0	f	4.0	e	4.0	d	-		-	
Smoke 2h 4h	1.3	a	2.7	c	**3.4**	**de**	3.7	d	3.9	cd	-		-	
Smoke 1h 4h	1.3	a	2.3	b	**3.0**	**c**	3.4	c	3.7	c	-		-	
Standard error	0.08		0.08		0.08		0.06		0.06		-		-	
	Umatilla Russet sprout ratings													
UTC	1.1	a	1.2	a	1.3	a	1.8	b	2.7	b	-		-	
Cold-stratification	1.1	a	1.2	a	1.5	a	1.5	ab	2.3	a	-		-	
Temp. fluctuation	1.2	ab	1.3	ab	1.4	a	1.4	a	2.1	a	-		-	
GA dip	1.3	bc	1.6	bc	2.0	b	**2.9**	**de**	3.7	de	-		-	
Smoke 1h 20h	1.1	a	1.8	c	2.5	c	**3.0**	**e**	3.4	cd	-		-	
Combination	1.4	c	**2.9**	**d**	3.6	d	3.8	f	3.9	e	-		-	
Smoke 2h 4h	1.1	a	1.5	abc	1.9	b	2.6	cd	**3.5**	**cd**	-		-	
Smoke 1h 4h	1.1	a	1.2	a	1.7	ab	2.2	c	**3.2**	**c**	-		-	
Standard error	0.05		0.11		0.13		0.12		0.11		-		-	

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

[2]Bolded numbers within the table indicate treatment achieved dormancy break (80% tubers expressing a 3 rating). Dashes ("-") indicate cultivar was not evaluated at that time period.

[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.

[4]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

Table 3-4. Average sprout length of three potato cultivars at the final evaluation after the November treatments.

	Russet Burbank		Clearwater Russet		Umatilla Russet	
Treatment[2]	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
	Average sprout length (mm)[1]					
UTC	1.0 b	2.2 a	0.1 a	3.2 b	1.4 b	3.1 b
Cold-stratification	0.0 a	1.0 a	0.0 a	0.0 a	0.0 a	0.1 a
Temp. fluctuation	0.0 a	0.6 a	0.0 a	0.0 a	0.0 a	0.0 a
GA dip	3.4 c	11.8 c	3.2 b	8.1 c	5.3 c	7.6 d
Smoke 1h 20h	5.1 d	11.1 c	5.0 c	8.2 c	2.2 b	6.6 cd
Combination	12.3 e	23.3 d	15.2 d	18.3 d	9.9 d	11.6 e
Smoke 2h 4h	4.8 d	9.4 bc	3.1 b	8.6 c	1.7 b	4.2 b
Smoke 1h 4h	2.5 c	7.2 b	1.1 a	6.9 c	2.1 b	4.8 bc
Standard error	0.3	1.2	0.6	0.8	0.4	0.6

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

Table 3-5. Sprout ratings over time of three cultivars for tubers treated in November 2021.

	Days after November treatment[1]		
Treatment[2]	**16**	**22**	**29**
	Russet Burbank sprout rating[3,4]		
UTC	2.0 b	2.6 b	**3.2 b**
Cold-stratification	1.4 a	1.5 a	2.0 a
Temp. fluctuation	1.3 a	1.5 a	2.0 a
GA dip	2.4 bc	**3.0 c**	3.7 cd
Smoke 1h 20h	2.7 c	**3.5 e**	3.9 de
Combination	**3.4 d**	4.0 f	4.0 e
Smoke 2h 4h	2.4 bc	**3.4 de**	3.9 de
Smoke 1h 4h	2.3 bc	**3.1 cd**	3.6 c
Standard error	0.14	0.10	0.81
	Clearwater Russet sprout ratings		
UTC	1.4 a	1.9 b	**2.8 b**
Cold-stratification	1.3 a	1.5 a	1.9 a
Temp. fluctuation	1.3 a	1.5 a	1.7 a
GA dip	2.3 c	**3.1 d**	3.6 d
Smoke 1h 20h	2.5 c	**3.3 d**	3.9 ef
Combination	**3.6 d**	4.0 e	4.0 f
Smoke 2h 4h	1.9 b	**3.1 d**	3.7 de
Smoke 1h 4h	1.8 b	2.6 c	**3.3 c**
Standard error	0.11	0.10	0.07
	Umatilla Russet sprout ratings		
UTC	1.2 a	2.0 b	**3.0 b**
Cold-stratification	1.1 a	1.2 a	1.4 a
Temp. fluctuation	1.1 a	1.2 a	1.5 a
GA dip	1.6 b	**3.0 c**	3.9 d
Smoke 1h 20h	1.2 a	2.3 b	**3.4 c**
Combination	1.7 b	**3.5 d**	4.0 d
Smoke 2h 4h	1.2 a	1.9 b	**3.4 c**
Smoke 1h 4h	1.2 a	2.3 b	**3.4 c**
Standard error	0.10	0.12	0.10

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

[2]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

[3]Bolded numbers within the table indicate treatment achieved dormancy break (80% tubers expressing a 3 rating).

[4]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 3-6. Sprout ratings over time of three cultivars for tubers treated in November 2022.

	Days after November treatment[1]				
Treatment[2]	**14**	**21**	**29**	**34**	**56**
			Russet Burbank sprout ratings[3,4]		
UTC	1.2 a	1.3 a	1.5 ab	1.9 abc	2.6 b
Cold-stratification	1.1 a	1.5 ab	1.4 a	1.7 ab	1.8 a
Temp. fluctuation	1.3 ab	1.4 ab	1.7 abc	1.6 a	1.7 a
GA dip	1.4 b	1.6 ab	2.2 d	2.2 c	**3.5 cd**
Smoke 1h 20h	1.3 ab	1.8 b	2.4 e	2.2 c	**3.4 cd**
Combination	2.0 c	2.6 c	**3.1 f**	3.3 d	3.9 d
Smoke 2h 4h	1.1 a	1.5 ab	1.8 c	2.2 c	**3.4 cd**
Smoke 1h 4h	1.2 ab	1.5 ab	1.7 bc	2.1 bc	**3.3 c**
Standard error	0.08	0.14	0.08	0.13	0.18
			Clearwater Russet sprout ratings		
UTC	1.9 abc	2.6 b	**3.3 b**	-	-
Cold-stratification	1.7 ab	1.8 a	2.2 a	-	-
Temp. fluctuation	1.6 a	1.7 a	2.4 a	-	-
GA dip	2.2 c	**3.5 cd**	3.7 c	-	-
Smoke 1h 20h	2.2 c	**3.4 cd**	3.8 c	-	-
Combination	**3.3 d**	3.9 d	4.0 c	-	-
Smoke 2h 4h	2.2 c	**3.4 cd**	3.9 c	-	-
Smoke 1h 4h	2.1 bc	**3.3 c**	3.8 c	-	-
Standard error	0.11	0.18	0.13	-	-
			Umatilla Russet sprout ratings		
UTC	1.3 a	2.0 bc	**3.0 b**	-	-
Cold-stratification	1.3 a	1.4 a	1.7 a	-	-
Temp. fluctuation	1.3 a	1.4 ab	1.7 a	-	-
GA dip	1.7 abc	2.6 de	**3.5 cd**	-	-
Smoke 1h 20h	1.9 bc	**3.0 e**	3.6 de	-	-
Combination	2.0 c	**3.6 f**	3.9 e	-	-
Smoke 2h 4h	1.3 a	2.1 cd	**3.1 bc**	-	-
Smoke 1h 4h	1.4 ab	2.2 cd	**3.4 bcd**	-	-
Standard error	0.18	0.19	0.14	-	-

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

[2]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

[3]Bolded numbers within the table indicate treatment achieved dormancy break (80% tubers expressing a 3 rating). Dashes "-" indicate cultivar was not evaluated at that time period.

[4]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 3-7. Average sprout length of three potato cultivars at the final evaluation after the December treatment in 2021 and 2022.

	Russet Burbank		Clearwater Russet		Umatilla Russet	
Treatment[1]	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
	Average sprout length (mm)[2]					
UTC	4.9 b	0.6 a	4.6 b	6.9 b	12.0 b	6.7 b
Cold-stratification	0.7 a	0.0 a	0.4 a	0.0 a	1.5 a	0.2 a
Temp. fluctuation	1.4 a	0.0 a	1.0 a	0.0 a	2.6 a	0.3 a
GA dip	8.9 c	1.3 a	11.3 d	11.4 bc	14.5 c	11.7 c
Smoke 1 h 20 h	14.5 d	8.7 c	12.0 d	13.3 c	11.2 b	7.6 b
Combination	18.1 e	19.5 d	16.9 e	19.5 d	14.8 c	16.0 d
Smoke 2 h 4 h	10.3 c	7.7 bc	9.4 c	8.5 bc	11.2 b	8.1 b
Smoke 1 h 4 h	10.1 c	5.3 b	8.2 c	11.1 bc	12.8 bc	8.0 b
Standard error	0.7	1.1	0.5	1.7	0.7	1.2

[1]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.
[2]Values followed by the same letter are not significantly different (α=0.05) within each column.

Table 3-8. Sprout ratings over time of three cultivars for tubers treated in December 2021 and 2022.

	Days after December Treatment[1,2,3]				
Treatment[4]	**15**	**26**	**9**	**21**	**28**
	2021 Russet Burbank sprout ratings		*2022 Russet Burbank sprout ratings*		
UTC	**3.0 c**	3.6 b	1.6 a	1.9 bc	2.7 b
Cold-stratification	1.9 a	**2.9 a**	1.5 a	1.4 a	1.6 a
Temp. fluctuation	2.2 b	**3.1 a**	1.6 a	1.5 ab	1.8 a
GA dip	**3.3 d**	3.9 c	1.5 a	2.1 cd	2.7 b
Smoke 1h 20h	**3.4 d**	4.0 c	1.6 a	**3.2 f**	3.7 cd
Combination	**3.8 e**	4.0 c	2.2 b	**3.8 g**	3.9 d
Smoke 2h 4h	**3.2 cd**	4.0 c	1.5 a	**2.9 ef**	3.6 c
Smoke 1h 4h	**3.3 d**	3.9 c	1.7 a	2.6 de	**3.5 c**
Standard error	0.09	0.06	0.09	0.15	0.11
	2021 Clearwater Russet sprout ratings		*2022 Clearwater Russet sprout ratings*		
UTC	**2.9 c**	3.7 c	2.4 bc	**3.8 b**	-
Cold-stratification	1.8 a	**2.8 a**	1.9 a	2.3 a	-
Temp. fluctuation	2.1 b	**3.0 b**	1.9 a	2.2 a	-
GA dip	**3.4 ef**	4.0 d	2.6 c	**3.9 bc**	-
Smoke 1h 20h	**3.2 de**	4.0 d	2.5 c	**4.0 c**	-
Combination	**3.6 f**	4.0 d	**3.0 d**	4.0 c	-
Smoke 2h 4h	**3.0 cd**	4.0 d	2.2 b	**3.9 bc**	-
Smoke 1h 4h	**3.1 cd**	4.0 d	2.4 bc	**3.9 bc**	-
Standard error	0.09	0.03	0.07	0.07	-
	2021 Umatilla Russet sprout ratings		*2022 Umatilla Russet sprout ratings*		
UTC	**2.9 cd**	3.9 c	1.9 abc	**3.4 c**	-
Cold-stratification	1.3 a	2.6 a	1.7 ab	1.7 a	-
Temp. fluctuation	1.6 a	**3.1 b**	1.6 a	2.1 b	-
GA dip	**3.5 e**	4.0 c	2.1 cd	**3.8 de**	-
Smoke 1h 20h	2.6 bc	**4.0 c**	2.0 bc	**3.7 d**	-
Combination	**3.1 d**	4.0 c	2.3 d	**4.0 e**	-
Smoke 2h 4h	2.5 b	**3.9 c**	1.8 abc	**3.7 de**	-
Smoke 1h 4h	**3.1 d**	4.0 c	1.9 abc	**3.7 d**	-
Standard error	0.11	0.06	0.11	0.08	-

[1]Values followed by the same letter are not significantly different (α=0.05) within each column.
[2]Bolded numbers within the table indicate treatment achieved dormancy break (80% tubers expressing a 3 rating). Dashes ("-") indicate cultivar was not evaluated at that time period.
[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.
[4]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.

Table 3-9. Number of days from treatment to dormancy break for each treatment. Dormancy break is achieved when 80% of tubers in a treatment have at least one sprout elongating (3 rating).

Treatment[1]	2021			2022		
	October	November	December	October	November	December
	Russet Burbank days to dormancy break[2]					
UTC	40	29	15	-	-	-
Cold-stratification	40	-	26	77	-	-
Temp. fluctuation	40	-	26	77	-	-
GA dip	23	22	15	77	56	-
Smoke 1h 20h	23	22	15	77	56	21
Combination	15	16	15	44	29	21
Smoke 2h 4h	28	22	15	77	56	21
Smoke 1h 4h	28	22	15	77	56	28
	Clearwater Russet days to dormancy break					
UTC	33	29	15	44	29	21
Cold-stratification	48	-	26	44	-	-
Temp. fluctuation	40	-	26	-	-	-
GA dip	28	22	15	27	21	21
Smoke 1h 20h	22	22	15	22	21	21
Combination	15	16	15	22	14	9
Smoke 2h 4h	28	22	15	27	21	21
Smoke 1h 4h	28	29	15	27	21	21
	Umatilla Russet days to dormancy break					
UTC	40	29	15	-	29	21
Cold-stratification	48	-	-	-	-	-
Temp. fluctuation	48	-	26	-	-	-
GA dip	28	22	15	34	29	21
Smoke 1h 20h	33	29	26	34	21	21
Combination	28	22	15	22	21	21
Smoke 2h 4h	40	29	26	44	29	21
Smoke 1h 4h	33	29	15	44	29	29

[1]UTC: 18.3 C 14 day (d); Cold-stratification: 4.4 C 14 d; Temp. fluctuation: 4.4 C 5 d → 18.3 C 4 d → 4.4 C 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 18.3 C post-treatment.
[2]Dashes ("-") indicate treatment did not achieve dormancy break during evaluation period.

Figures

1. No bud activity	2. Peeping	3. Point initiated < 5mm	4. Sprout elongation ≥ 5mm

Figure 3-1.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.

Chapter 4: Investigating Methods to Induce Sprouting in Dormant Tubers and Evaluating Direct Tuber Testing for Potato Virus Y Detection

Abstract

Potato seed certification agencies regulate the allowable potato virus Y (PVY) levels in seed lots for certification to control the spread of PVY in seed production. This study evaluated methods to initiate sprouting soon after harvest to enable direct tuber testing for PVY and compare to leaf testing results obtained from the winter grow out. Methods to break dormancy for reliable PVY detection using laboratory direct tuber testing by ELISA in Ranger Russet, Clearwater Russet, and Umatilla Russet were tested over two years. Three 400 tuber samples from each cultivar were treated with: 1) untreated control, 2) application of cold aerosol smoke, or 3) application of Rindite. Samples were held at 18.3 C and sprout development monitored weekly by measuring the number and length of elongating sprouts. 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 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 through the winter months and planted in a spring grow out. Leaf samples were collected and analyzed for PVY by ELISA. Rindite treated tubers had greater sprout rating and number of sprouts elongating compared to untreated control and smoke at time of PVY testing. Smoke had a greater sprout rating, but a similar number of sprouts elongating compared to the untreated control. Overall, direct tuber testing was similar to winter grow out results for each cultivar, year, and PVY incidence; however, in year two, winter grow out (7% PVY) significantly differed from direct tuber testing (16% PVY) in Ranger Russet. Umatilla Russet and Clearwater Russet direct tuber testing PVY results were similar to the winter grow out in both years. Rindite and smoke treated samples evaluated in the spring grow out had comparable PVY results as the direct tuber testing. The Ranger Russet direct tuber tested untreated control showed 10% lower PVY detection compared to the spring grow out. Clearwater Russet and Umatilla Russet spring grow out results were comparable to direct tuber testing. This study evaluated the efficacy of dormancy breaking treatments to promote earlier and accurate PVY detection by direct tuber testing using ELISA and provided data supporting direct tuber testing as a reliable alternative to the winter grow out for post-harvest evaluation of potato virus Y in seed certification.

Introduction

Seed certification is a service to the potato industry that assures available potato seed (*Solanum tuberosum* L.) is 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). Seed certification programs were initiated in the early 1900's in many countries with the original objective to maintain varietal purity and reduce disease and spread of undesired traits through tuber propagation (Shepard and Claflin 1975). At the time, potato growers were facing what was referred to as the 'degeneration' effect on potato, where yields would decline after several years of production (Appel 1934; Leach 1938). This was later attributed to the accumulation of viruses in tubers kept for seed from year to year (Halterman 2012; Khurana 2004). The limited generation system and seed certification programs were implemented to remove seed lines from production after several years of re-production as seed (Duellman et al. 2020; Frost et al. 2013).

In the United States, the governing power to manage seed certification has been granted to individual states, who have delegated responsibility to either land grant universities, state departments of agriculture, or grower associations (Gudmestad 1991; Shepard and Claflin 1975). Within each certifying body, tolerances are decided upon for each defect, disease, and the number of generations a seed lot can be increased (Gudmestad 1991; Shepard and Claflin 1975). The seed certification process evaluates issues deemed to be detrimental to potato production and encompasses current and emerging production issues (Frost et al. 2013). With exception to national quarantine pests, each certifying body has the power to identify defects to be included into regulation of seed production and set tolerances for their respective state (Shepard and Claflin 1975).

Idaho is the largest seed growing state in the country with approximately 12,140 ha^{-1} in production annually (USDA 2021). Idaho Crop Improvement Association, Inc (ICIA) is responsible for ensuring that Idaho seed potatoes meet a designated quality tolerance (Duellman et al. 2020). ICIA oversees establishing strict production guidelines for seed lot certification, recertification, and distribution. In addition to production requirements, seed lots undergo five inspections throughout each production and storage season to ensure seed lots do not exceed set quality tolerances. Two field inspections are completed during the growing season followed by a storage inspection, a post-harvest crop inspection, and a final shipping point inspection (ICIA 2022). This research focused on the post-harvest crop inspection as it relates to potato virus Y (PVY).

To predict virus levels in the subsequent crop, a post-harvest crop inspection is conducted soon after harvest and is referred to as the winter grow out (WGO; Fox et al. 2005). The WGO is the standard method for post-harvest testing in Idaho and many other states. To conduct the WGO, seed growers are responsible for collecting and summitting a representative sample of single drop (42 to 113 g) seed tubers to their state's certification agency to be planted in a field for assessment of PVY and other certifiable defects (ICIA 2022). However, 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). This state of dormancy varies for each cultivar but remains an issue for certification agencies that plant tubers shortly after harvest (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). Treated tubers to be field planted are loaded into climate-controlled cargo containers and shipped to a permitting climate location (Duellman et al. 2020). In addition to Rindite application, difficult-to-sprout cultivars are dipped in a gibberellic acid (GA) treatment before planting to promote sprouting and improve emergence during the inspection period.

Currently, Idaho and several other states' seed lots are planted and grown in Hawaii as a WGO location. Plants are grown until they reach adequate size, approximately 30 cm tall, and then inspected for chemical (herbicide) carryover, potato leaf roll virus, varietal mix, and mosaic symptoms primarily from PVY (Duellman et al. 2020). 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 virus resulting in downgrading or rejection of seed lots for certification (Frost et al. 2013; Tran et al. 2022; Lindner et al. 2015). PVY is spread rapidly through distribution of infected tubers so 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 in a seed lot is critical prior to distribution and planting. For many states, PVY is evaluated in seed lots by collecting a leaf tissue sample from each plant grown in the WGO. It is costly and time prohibitive to test each individual leaf, so leaves are grouped into five or 10 leaf samples (depending on the state) and sent to a qualified laboratory to be tested for PVY using enzyme-linked immunosorbent assay (ELISA). Since samples are completed in composites of five or 10, results must be extrapolated to provide an

estimate of PVY within a seed lot using the following equation (for five leaf composites; UNECE 2019):

$$percent\ virus = (1 - \left(1 - \frac{Number\ Positives}{Number\ Tests}\right)^{0.2}\right)\ X\ 100.$$

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 and to capitalize on exporting to earlier markets and making seed purchasing decisions (Fox et al. 2005; Singh et al. 2013). In the Pacific Northwest seed potatoes are typically harvested in September and October. The WGO typically provides results within the first three weeks of January. Extended periods between harvest and availability of post-harvest test results are not desirable and may not allow growers ample time to adjust seed purchase decisions, marketing strategies, or reach foreign markets (Fox et al. 2005; Singh et al. 2013) therefore, alternatives to produce post-harvest test results rapidly are being investigated.

Other methods besides the WGO for estimating PVY levels in seed lots include greenhouse grow-outs or laboratory-based methods which evaluate tissue directly from the tuber without the need to grow a plant and collect leaf tissue. Laboratory tuber testing is typically conducted using reverse-transcription polymerase chain reactions (RT-PCR) or ELISA methods. These laboratory methods are being explored with the goal of being viable, accurate, high-throughput, and faster alternatives for the WGO (Singh, et al. 2013; Avrahami-Moyal et al. 2017; Russo et al. 1999; Beissinger and Inglis 2018; Fox et al. 2005; Schumpp et al. 2021).

Direct tuber testing is a laboratory method which utilizes extract from tissue directly from the tuber, tissue from developing sprouts, or a combination of sprout and tuber tissue. However, there is very little research comparing the type of tissue used for PVY detection. Further, RT-PCR and ELISA methods have been implemented on dormant tubers with variable results (Avrahami-Moyal et al. 2017; Barker et al. 1993; Russo et al. 1999; Hill and Jackson 1984; Sign and Singh 1996; Singh et al. 2013; Huhnlein et al 2013; Fox et al. 2005; Schumpp et al. 2021). 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. Singh and Singh (1996) found PVY^{O} virus titer was higher in cultivars Atlantic and Russet Norkotah compared to Shepody using RT-PCR on dormant tubers. Studies have shown PVY was more prevalent in the bud end of the tuber compared to the stem end (Singh and Singh 1996; Vetten et al. 1983; Dupuis 2017; Whitworth et al.

2012), indicating PVY strain and/or sample location could impact PVY detection when using direct tuber testing. 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 (Barker et al. 1993; Gugereli and Gehriger 1980).

Studies using laboratory methods for PVY detection on tubers have shown that reliability of ELISA testing increases when tuber dormancy is artificially broken and sprouting has begun (McDonald and Coleman 1988; Gugerli & Gehriger 1980; Vetten et al. 1983; ICIA 2020), but Fox et al. (2005) determined the reliability of ELISA testing for PVY significantly decreased after 10 weeks of storage, which coincided with natural dormancy break and sprout development. A decrease in PVY detection after natural dormancy break was also observed by Hill and Jackson (1984). However, these studies are not clear if, or how much sprout tissue was present on the tuber at the time of sampling. Gugerli and Gehriger (1980) found when using ELISA methods, PVY was not detected until after dormancy was artificially broken with Rindite. Hill and Jackson (1984) suggested Rindite stimulates PVY detection in tubers. Vetten et al. (1983) found virus detection was higher and uniformly distributed within a tuber after a treatment with Rindite. McDonald and Coleman (1988) reported treating with either bromoethane or Rindite increased virus detection over the untreated control in Russet Burbank. Although the level of sprout development necessary for accurate direct tuber testing is not well documented, UNECE (2016) suggested ELISA testing should be conducted on sprouting tubers but does not mention using the developed sprout tissue when sampling.

It appears research supports artificial breaking of dormancy over natural breaking of dormancy for greater PVY detection when using ELISA methods on tuber tissue. Certain chemicals seem to improve the distribution of PVY within a tuber, but it is unclear the extent of sprout development needed for accurate testing. Although the necessary level of sprout development is not fully understood, ICIA suggests reliability and accuracy of direct tuber testing for PVY increases when tubers have sprouts that are 6 mm in length or greater (ICIA 2022). Others recommend using sprouts 3 to 5 mm in length for PVY detection (UNECE 2019). Neither ICIA nor UNECE report using the developed sprout tissue in their tests. Although not clearly stated in the literature, an assumption could be made that not only is a chemical treatment beneficial in stimulating PVY concentrations within a tuber, but there may also be a need for a certain level of sprout development.

Several studies have examined methods to break dormancy in tubers, such as plant growth regulators, thiourea, temperature fluctuations, carbon disulphide, and others, 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; Travakoli et al 2014; Thornton 1991; Denny 1926; Bryan 1989) and may not be suitable to improve 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 (Chapter 3) 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 testing in many states, therefore it is imperative that new alternatives consistently have comparable virus detection to the WGO for determining PVY in seed lots. However, the WGO system takes many months to complete and seed growers desire results as soon as possible. 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 are to 1) evaluate the use of a novel dormancy breaking technique of cold aerosol smoke application to initiate sprouting for PVY detection, 2) determine if the direct tuber testing method using ELISA (ICIA 2019) is comparable to the traditional WGO, and 3) compare PVY levels of a subsequent crop planted from tubers previously laboratory tested.

Materials and Methods

Objective 1: Use of novel dormancy breaking techniques

Samples from three seed potato cultivars: Ranger Russet, Umatilla Russet, and Clearwater Russet with suspected PVY infection were supplied by collaborative commercial seed potato growers in Idaho over two years, 2021 and 2022. The seed samples were collected at the time of the traditional WGO sampling; during harvest and loading into storage operations (Table 4-1). The samples consisted of four 400 single drop (42 to 113 g) tuber samples (1600 total per cultivar; 400 tubers per treatment). Year two Ranger Russet had 350 tubers per sample. Objective one utilized three of the samples and objective two utilized the fourth sample. Samples were transferred to Kimberly Research and Extension Center, Kimberly, ID (KREC) and placed at 12.8 C and 95% relative humidity (RH).

Three treatments included 1) an untreated control (UTC) held at 18.3 C and 95% RH, 2) an application of a cold aerosol smoke, and 3) an application of Rindite (according to ICIA standard procedures). Samples were removed from storage 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 was transported to ICIA on October 15, 2021 and October 17, 2022. Temperatures were evaluated during application of treatments (Figure 4-1).

The smoke treatment was subjected to an 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 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 hours (as described in Appendix A). Smoke applications were initiated on October 21, 2021, and October 20, 2022, and placed into 18.3 C and 95% RH storage upon completion.

The Rindite treatment was stored at ambient temperatures (approximately 18.3 C) for three days then loaded into 246 x 264 x 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 unit and 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 air and treatment 3 was transported to KREC. After all applications were complete, treatments were stored at 18.3 C 95% RH and evaluated for sprout development. Once desired sprout development was achieved, samples were delivered to ICIA for direct tuber testing via ELISA laboratory methods for PVY.

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); whereas 1) no bud activity; 2) sprout initiating but not pointed; 3) sprout pointed, but length not achieving five mm; 4) sprout elongating, length five mm or greater

(Figure 4-2). 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; sprout rating of 4) and all samples within a cultivar were delivered on a given date to ICIA for direct tuber testing (Table 4-1).

To conduct the direct tuber testing for PVY, one eye was cored from the stem end, bud end, and three middle eyes on each tuber using a cork borer (4 mm), homogenized using 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 storage treatments were analyzed individually (1200 tubers per cultivar; 400 tubers per treatment).

Objective 2: Direct tuber testing method using ELISA (ICIA 2019) compared to the traditional WGO

The additional 400 tuber sample was treated with Rindite at the same time as the Rindite treatment 3 in Objective 1 and subsequently planted at the WGO. The Rindite treated sample was delivered to Hawaii to be included in the Idaho WGO plots. Temperatures were monitored using sensors (Kestrels, Boothwyn, PA) during the application of treatments and while samples were shipped to Hawaii (Figure 4-3). 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 were an adequate size, a leaf sample was taken from each plant and placed into large plastic bags. Tissue samples were shipped back to ICIA lab in Idaho Falls, ID and analyzed for PVY according to ICIA's protocol for ELISA testing in composites of five leaves (Tran et al. 2022). In year one, leaf samples of Ranger Russet were tested on January 13, 2021. Clearwater Russet and Umatilla Russet were tested on January 14, 2021. In year two, Ranger Russet leaf samples were tested December 29, 2022, while Clearwater Russet and Umatilla Russet were tested January 13, 2023. Estimation of PVY foliar infection in plots was extrapolated from five leaf composites using the following equation (UNECE 2019):

$$percent\ virus = (1 - \left(1 - \frac{Number\ Positives}{Number\ Tests}\right)^{0.2}\Big)\ X\ 100$$

The WGO sample was then compared to the direct tuber tested samples in Objective 1 to determine if PVY incidence was similar between testing methods.

Objective 3: Evaluate PVY levels of a subsequent crop planted from tubers that were laboratory tested

After direct tuber testing was completed from Objective 1, the sampled seed was returned to KREC and stored at 4.4 C 95% RH to be planted into 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 was warmed to 7.2 C for approximately 72 hours before planting on April 21, 2022. Seed of 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. Plants were allowed to grow until approximately 30 cm tall. A leaf sample was then taken from each plant and sent to ICIA laboratory for PVY detection in composites of five leaves according to the traditional WGO methodology (Tran et al. 2022). These results were compiled in the same manner as objective 2 leaf samples. Ranger Russet and Umatilla Russet were sampled June 15, 2022, and Clearwater Russet was sampled on June 27, 2022.

Statistical Analysis

Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating (≥ 5 mm), number of sprouts per tuber ≥ 6 mm, average sprout lengths, 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' significant differences between means for response variables were compared at p-value of 0.05 by estimated marginal means procedures (RStudio, package emmeans version 1.6.1, 2020). Number of sprouts pointing, number of sprouts elongating (≥ 5 mm), and final sprout weights are included in Appendix D (Table D- 1 to 6).

Results

The Rindite treatment created an environment where treated tubers were subjected to cooler, followed by warmer temperatures compared to smoke and UTC treatments (year 1; Figure 4-1). Despite the fluctuating temperatures of Rindite treated tubers, the average temperature during application periods were 18.9 C while the smoke treatment and UTC averaged 17.8 C. Year two application temperatures of smoke and Rindite treatments were similar to the previous year (data

not shown). Sharp declines in temperatures observed after the Rindite treatments were from chamber doors being opened to outside air for handling and removal. Elevated temperatures during transport to Hawaii indicated potatoes retained heat over four days following the Rindite application (Figure 4-3).

Objective 1: Use of novel dormancy breaking technique

Ranger Russet achieved the desired sprout development (three sprouts per tuber ≥ 6 mm) and were delivered to ICIA for direct tuber testing in mid-November both years. Samples were delivered a similar number of days after treatment in each year studied, but an additional 18 days later after harvest in the first year (Table 4-1). Clearwater Russet tubers were delivered to ICIA for testing before the end of November for each year. In both years tubers were delivered in a similar number of days after treatment but 7 days earlier after harvest in year one. Umatilla Russet tubers were delivered to ICIA the first week of December in year one and the last week of November in year two. 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 of the cultivars (Table 4-2). Neither smoke nor UTC achieved the goal of three elongating sprouts (≥ 6 mm) in either year (Table 4-3). Smoke treated tubers had significantly higher sprout rating in all three cultivars compared to the UTC. Ranger Russet and Clearwater Russet achieved dormancy break (80% of tubers expressing a 3 rating) prior to being delivered to ICIA for testing. However, the smoke and UTC treatments did not reach dormancy break in Umatilla Russet prior to testing. In year one smoke and UTC had a similar number of elongating sprouts in each of the cultivars at the time of testing but smoke had significantly higher number of sprouts elongating in Clearwater Russet and Umatilla Russet in year two.

Objective 2: Direct tuber testing method using ELISA (ICIA 2019) compared to the traditional WGO

Overall, year two had significantly lower levels of PVY in each of the cultivars. Ranger Russet had greater variability in PVY detection between treatments compared to Clearwater Russet and Umatilla Russet in year two (Table 4-4). In year one, Ranger Russet had no differences among direct tuber tested treatments. Collectively the average PVY detection of the three direct tuber testing treatments was 34% and was similar to the WGO (33% PVY) in year one. Ranger Russet in year two showed differences among PVY detection of direct tuber tested treatments with Rindite having significantly higher PVY levels than the smoke treatment. The WGO sample was significantly lower in

year two (7% PVY) compared to direct tuber tested samples (16% PVY). Clearwater Russet had similar PVY detection between direct tuber testing (1.1%) and WGO (1.8%) combined over years. Direct tuber testing of Umatilla Russet detected 18.5% PVY while the WGO had similar PVY detection of 20% averaged over both years. Overall, PVY detection of direct tuber tested samples was similar to the WGO in both years.

Objective 3: Evaluate PVY levels of a subsequent crop planted from tubers that were laboratory tested

Direct tuber tested samples were stored and planted in a spring grow out. 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 (Table 4-5). Each of the direct tuber tested treatments aligned with the spring grow out PVY levels. However, Ranger Russet spring grow out PVY levels differed from direct tuber testing samples. The Ranger Russet UTC treatment had 10% less PVY detected in the direct tuber tested sample compared to the spring grow out, but the smoke and Rindite treated tubers were not significantly different between the direct tuber testing and spring grow out.

Discussion

Seed growers rely upon plant health certificates produced from certification agencies to market and sell their crop as certified seed. Knowledge of PVY levels in seed is critical, but plant health certificates are not produced until post-harvest testing is completed. Current WGO post-harvest testing involves breaking tuber dormancy and a lengthy plant grow-out period. To streamline the process, methods to break dormancy and test tissue directly from tubers were evaluated. To be a viable solution for PVY detection, industry demands ELISA based direct tuber testing results be comparable to the standard, which is the WGO.

It is required that direct tuber testing methods be accurate, cost effective, and have capabilities of processing high volumes of samples in a short amount of time. Direct tuber testing primarily uses RT-PCR or ELISA testing methods to process samples for PVY detection. ELISA testing is a relatively easy and rapid process for virus detection and is already used to analyze large quantities of leaf tissue samples from the winter grow out by many certification agencies. All of the equipment, materials, and trained personnel are in place for this ELISA testing procedure. The current ELISA process was easily adapted to detect PVY from tuber tissue. Previous research has indicated PVY detection is improved when tubers have broken dormancy, therefore if ELISA methods are to be used for direct tuber testing, tubers should be sprouting.

Sprout development (rating, and number of sprouts 6 mm) was significantly higher in the Rindite treated tubers compared to smoke and UTC. Tubers treated with Rindite were subjected to warmer temperatures for several days which has shown to promote dormancy break (Wurr and Allen 1976) but was not considered a major factor contributing to sprout development in this study. In most cases, smoke achieved dormancy break whereas the UTC did not. These differences between treatments provided three levels of sprout development at the time of direct tuber testing. Although the smoke treatment had fewer sprouts elongating compared to Rindite, PVY detection was comparable between the two treatments. Previous studies demonstrated that treating tubers with chemicals for dormancy break may improve PVY detection (Gugerli and Gehriger 1980; McDonald and Coleman 1988). The relationship of PVY detection between smoke and Rindite treated samples suggests that smoke, similar to Rindite, may also increase virus detection.

Previous studies evaluating the viability of direct tuber testing as an alternative to the WGO have shown mixed results. Fox and Browning (2005) and Avrahami-Moyal et al. (2017) stated PCR methods have similar PVY detection to the WGO compared to ELISA tuber testing on dormant potatoes. Singh et al. (2013) showed ELISA methods are comparable to PCR but take longer from harvest to produce results since dormancy must be broken. In the current study, direct tuber testing had similar PVY detection compared to the WGO. Past comparisons of direct tuber testing methods have been conducted at different times after harvest in order to enable natural dormancy break. It has been shown PVY detection decreases with time in storage (Barker et al. 1993; DeBokx and Cuperus 1987) and is lower when potatoes break dormancy naturally (Hill and Jackson 1984). Applications of aerosol smoke and Rindite provided varying levels of sprout development for a simultaneous comparison. Artificial dormancy break with Rindite and bromoethane has demonstrated greater PVY detection when using ELISA (Vetten et al. 1983; Gugerli and Gehriger 1980; McDonald and Coleman 1988) and may be comparable to the WGO. Similar results of enhanced PVY detection were observed with Rindite and smoke treated samples. 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. When supply costs, time of conducting tests, and training of personnel are taken into consideration, ELISA may be an effective and efficient option for laboratory testing of large quantities of tuber samples. This study clearly demonstrates that ICIA's direct tuber testing protocol combined with dormancy breaking techniques can provide similar PVY detection as the WGO.

The Rindite application process is considered undesirable due to length of application timing, health hazards of the application, and acquiring components of the three-way mixture can prove difficult. Aerosol smoke may be an effective and more convenient alternative to promote sprouting for accurate PVY detection when using direct tuber testing. Nevertheless, it remains unclear if lower levels of sprout development can be used for accurate PVY assessment than previously suggested or if smoke stimulates an increase in PVY detection similarly to what has been exhibited by Rindite and bromoethane (Gugerli and Gehriger 1980; McDonald and Coleman 1988). It is also unclear if higher PVY detection in these treatments is a result of increased virus titer within the tuber from viral replication or from a concentration of existing virus particles into the eyes beginning to develop a sprout. 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 concentrations would imply sample location is important for accurate PVY detection. Due to the sampling method of ICIA (2019), where cores are taken from multiple areas on the tuber, 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. Further evaluation into the cause of increased virus detection with smoke treated tubers needs to be conducted. The untreated control had naturally broken dormancy at the time of direct tuber testing in several cases, yet PVY detection did not significantly differ from the WGO sample. 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 used by ICIA, or level of sprout development at the time of testing.

Using non-dormant tubers is recommended for PVY detection when using ELISA laboratory methods (UNECE 2016), but the level of sprout development needed for accurate PVY detection is not fully understood nor well documented. Recommendations vary to have tubers with 3 to 6 mm sprouts. 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 was further developed. It appeared tubers with the lowest sprout development, less than 0.5 sprouts per tuber elongating, had lower levels of PVY detection. Tubers that had one or more sprouts per tuber elongating tended to have PVY detection levels closer to the WGO and were

similar to the spring grow out samples. However, there did not appear to be an additional benefit to having more than one sprout elongating per tuber. These observations on the necessary number of sprouts per tuber for accurate PVY detection could have also been influenced by the artificial treatments. Further research should investigate sprout development on a single treatment of tubers at various sprouting levels.

Samples were treated and delivered to ICIA 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 time 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 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 and similar results would be expected, further expediting the sprouting process and ability to obtain PVY results.

The use of direct tuber testing was explored as an alternative solution to detect PVY sooner than the WGO results could be obtained. Results from direct tuber tested samples were produced on average 47 days earlier compared to samples sent to the WGO and had comparable PVY detection except one seed lot. Further, direct tuber testing was comparable to the WGO with seed lots having very low levels of PVY (< 0.7%) and high levels of PVY (> 30%). PVY detection of the direct tuber tested samples were similar to their respective WGO sample for each cultivar in both years, with the exception of Ranger Russet in 2022. The Ranger Russet direct tuber tested samples in 2022 had an average of 56 tubers out of each 350-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 bulk of five leaves could have one to five PVY positive leaves and give the same percent PVY result in the sample. This means the actual 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 accuracy of testing must be taken into consideration.

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, but this was not necessarily

observed in the other 5 seed lots evaluated. On average, the PVY incidence of all direct tuber testing was 15% compared to 14% in the WGO. Growers who provided seed for the trial shared the official WGO PVY results from samples they submitted. The grower results from the WGO and this study's results from the WGO differed from 1 to 4%. Variations in the two samples sent to the WGO from the same seed lot potentially indicated sampling error could contribute up to 4% difference in seed lot WGO PVY values. Given the +/- 4% leeway from sampling error, direct tuber testing would have comparable results to the WGO in the 2022 Ranger Russet sample. Consideration of sampling error provides further evidence that ICIA's direct tuber testing protocol is an accurate alternative for determining PVY in seed lots. Despite possible differences in one sample, this study indicates that using ICIA's ELISA protocol on non-dormant, sprouted tubers has potential to be an accurate comparison to the WGO. The ICIA laboratory was able to sample and analyze all tubers delivered within days of delivery indicating a relatively quick and efficient process for detecting PVY in seed lots for certification.

Results from the spring grow out allowed further confirmation in the accuracy of direct tuber testing. Smoke and Rindite treated tubers had similar PVY detection as the spring grow-out samples in the three cultivars. This consistency indicates that application with either Rindite or aerosol smoke to break dormancy resulted in accurate PVY detection using ELISA methods on sprouted tubers. In Ranger Russet, 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 broken dormancy naturally (Hill and Jackson 1984). Since a discrepancy in results was not observed in Clearwater Russet nor Umatilla 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 the field. Further investigating PVY distribution within a tuber for many cultivars would be worthwhile to help understand the variability seen 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 was studied. The application of Rindite produced greater sprout development compared to the smoke treatment and untreated control. However, the aerosol smoke treatment yielded similar PVY detection as the Rindite treated tubers. 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. In addition, direct tuber tested samples had comparable PVY detection as samples sent to the WGO. ICIA's direct tuber testing ELISA protocol 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 that direct tuber testing using ELISA methods on sprouted non-dormant tubers treated with Rindite or aerosol smoke could be a reliable, high-throughput, and faster alternative to the WGO for evaluating post-harvest PVY incidence in seed lots.

References

Akoumianakis, K., C. M. Olympios, and H. C. Passam. 2000. Effect of "rindite" and bromoethane on germination, sprout emergence, number of sprouts and total yield of tubers of potato cv. Spunta. *Advances in Horticultural Sciences* 14: 33-35.

Aksenova, N. P., L. I. Sergeeva, T. N. Konstantinova, S. A. Golyanovskaya, O. O. Kolachevskaya, and G. A. Romanov. 2013. Regulation of potato tuber dormancy and sprouting. *Russian Journal of Plant Physiology* 60(3): 301-312.

Appel, O. 1934. Vitality and vitality determination in potatoes. *Phytopathology* 24: 482-94.

Avrahami-Moyal L., Y. Tam, M. Brumin, S. Prakash, D. Leibman, M. Pearlsman, M. Bornstein, N. Sela, M. Zeidan, Z. Dar, U. Zig, A. Gal-On, and V. Gaba. 2017. Detection of potato virus Y in industrial quantities of seed potatoes by TaqMan Real Time PCR. *Phytoparasitica* 45: 591-598.

Barker H., K. D. Webster, and B. Reavy. 1993. Detection of potato virus Y in potato tubers: A comparison of polymerase chain reaction and enzyme-linked immunosorbent assay. *Potato Research* 36: 13-20.

Beissinger A., and D. A. Inglis. 2018. Greenhouse comparison of two detection methods of potato virus $Y^{N\text{-}Wi}$ at four potato growth stages. *Plant Health Progress* 19:71-75.

Bryan, J. E. 1989. Breaking dormancy of potato tubers. *CIP Research Guide* 16: 1-13.

Callison B. L., J. Harrington, and D. Douglas. 1982. Seed potato certification: Its purpose, capabilities, and limitations. *American Potato Journal* 59: 231-236.

Campbell M., E. Segear, L. Beers, D. Knauber, and J. Suttle. 2008. Dormancy in potato tuber meristems: Chemically induced cessation in dormancy matches the natural process based on transcript profiles. *Functional and Integrative Genomics* 8: 317-328.

De Bokx, J. A., and C. Cuperus. 1987. Detection of potato virus Y in early-harvest potato tubers by cDNA hybridization and three modifications of ELISA. *European and Mediterranean Plant Protection Organization Bulletin* 17: 73-79.

Denny, F. E. 1926. Hastening the sprouting of dormant potato tubers. *American Journal of Botany*. 13(2): 118-125.

Denny, F. E. 1945. Synergistic effects of three chemicals in the treatment of dormant potato tubers to hasten germination. *Boyce Thompson Instituter for Plant Research, Inc*. 14: 1-14.

Doherty, L. C., and M. A. Cohn. 2000. Seed dormancy in red rice (Oryza sativa). XI. Commercial liquid smoke elicits germination. *Seed Science Research* 10: 415-421.

Drewes, F. E., M. T. Smith, and J. Van Staden. 1995. The effect of a plant-derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation* 16: 205-209.

Duellman, K. M., M. K. Thornton, and A. A. Westra 2020. Potato seed certification and selection in Idaho. Extension Bulletin 983. *University of Idaho*, Moscow, ID.

Dupuis, B. 2017. The movement of potato virus Y (PVY) in the vascular system of potato plants. *European Journal of Plant Pathology* 147: 365-373.

Fox, A., F. Evans, and I. Browning. 2005. Direct tuber testing for potato Y potyvirus by real-time RT-PCR and ELISA: Reliable options for post-harvest testing? *European and Mediterranean Plant Protection Organization Bulletin* 356: 93-97.

Fox, J., and S. Weisberg. 2019. An {R} companion to applied regression, third edition, Thousand Oaks CA: Sage.

Frost, K., R. L. Groves, and A. O. Charkowski. 2013. Integrated control of potato pathogens through seed potato certification and provisions of clean seed potatoes. *American Phytopathological Society*. 97(10): 1268-1280.

Gebrehiwot, H., M. Kulkarni, M. Bairu, and J. Van Staden. 2013. Plant-derived aerosol-smoke and smoke solutions influence agronomic performance of traditional cereal crop, Tef. *Experimental Agriculture* 49(2): 244-255.

Gray, S., S. De Boer, J. Lorenzen, A. Karasev, J. Whitworth, P. Nolte, and H. Xu. 2010. Potato virus Y an evolving concern for potato crops in the United States and Canada. *The American Phytopathological Society* 94(10): 1384-1397.

Gudmestad, N. C. 1991. A historical perspective to pathogen testing in seed potato certification. *American Potato Journal* 68:99-102.

Gugerli, P., and W. Gehriger. 1980. Enzyme-linked immunosorbent assay (ELISA) for the detection of potato leafroll virus and potato virus Y in potato tubers after artificial break of dormancy. *Potato Research* 23: 353-359.

Halterman, D., A. Charkowski, and J. Verchot. 2012. Potato, viruses, and seed certification in the USA to provide healthy propagated tubers. *Pest Technology* 6(1): 1-14.

Hill, S. A., and E. A. Jackson. 1984. An investigation of the reliability of ELISA as a practical test for detecting potato leaf roll virus and potato virus Y in tubers. *Plant Pathology* 33: 21-26.

Huhnlein A., N. Drechsler, P. Steinbach, T. Thieme, and J. Schubert. 2013. Comparison of three methods for the detection of potato virus Y in seed potato certification. *Journal of Plant Diseases and Protection* 120(2): 57-69.

ICIA. 2019. ICIA Procedure: Virus testing- ELISA method. *Idaho Crop Improvement Association*.

ICIA. 2020. ICIA Potato Inspection Manual*. Idaho Crop Improvement Association, Inc.*

ICIA. 2022. Policies and Procedures of Certification for Seed Potatoes in Idaho. *Idaho Crop Improvement Association, Inc.*

Karasev, A., and S. M. Gray. 2013. Continuous and emerging challenges of potato virus Y in potato. *Annual Review of Phytopathology* 51: 571-586.

Khurana P. S. M. 2004. Potato viruses and their management. Diseases of Fruits and Vegetables, V2 389-440, *Kluwer Academic Publishers*, Netherlands.

Leach, J. G. 1938. The biological basis for certification of seed potatoes. *American Journal of Potato Research* 15: 117-130

Lindner, K., F. Trautwein, A. Kellerman, and G. Bauch. 2015. Potato virus Y (PVY) in seed potato certification. *Journal of Plant Diseases and Protection* 122(3): 109-119.

Liu B., N. Zhang, Y. Wen, X. Jin, J. Yang, S. Hauijun, and D. Wang. 2015. Transcriptomic changes during tuber dormancy release process revealed by RNA sequencing in potato. *Journal of Biotechnology* 198: 17-30.

Mani, F., T. Bettaieb, N. Doudech, and C. Hannachi. 2014. Physiological mechanisms for potato dormancy release and sprouting: A review. *African Crop Science Journal* 22(2): 155-174

McDonald J. G., and W. K. Coleman. 1988. A reevaluation of bromoethane in comparison to rindite for the post-harvest detection of potato virus Y in tubers by ELISA. *American Potato Journal*. 65: 547-550.

Prange R. K., W. Kalt, B. J. Daniels-Lake, C. L. Liew, and R. T. Page. 1998. Using ethylene as a sprout control agent in stored ‘Russet Burbank’ potatoes. *Journal of American Society for Horticultural Science* 123(3): 463-469.

Russo, P., L. Miller, R. P. Singh, and S. A. Slack. 1999. Comparison of PLRV and PVY detection in potato seed samples tested by Florida winter field inspection and RT-PCR. *American Journal of Potato Research* 76: 313-316.

Rylski L., L. Rappaport, and H. K. Pratt. 1974. Dual effects of ethylene on potato dormancy and sprout growth. *Plant Physiology* 53:658-662.

Schumpp, O., A. Brechon, J. Brodard, B. Dupuis, L. Farinelli, P. Frei, P. Otten, D. Pellet. 2021. Large-scale RT-qPCR diagnostics for seed potato certification. *Potato Research* 64: 553-569.

Shepard, J. F., and L. E. Claflin. 1975. Critical analyses of the principals of seed potato certification. *Annual Review of Phytopathol*ogy 13:271-293.

Singh M., and R. P. Singh. 1996. Factors affecting detection of PVY in dormant tubers by reverse transcription polymerase chain reaction and nucleic acid spot hybridization. *Journal of Virological Methods* 60: 47-57.

Singh, M., R. P. Singh, M. S. Fageria, X. Nie, R. Coffin, and G. Hawkins. 2013. Optimization of a Real-Time RT-PCR Assay and its comparison with ELISA, conventional RT-PCR, and the grow-out test for large scale diagnosis of potato virus Y in dormant potato tubers. *American Journal of Potato Research* 90: 43-50.

Siregar L. A. M., L. Turnip, and I. M. R. Damanik. 2021. Immersion in 6-Benzylaminopurine for dormancy release and initiation of potato sprouts at various tuber weight and storage duration. *Indonesian Journal of Agronomy* 49(1): 60-67.

Sonnewald, S., and U. Sonnewald. 2014. Regulation of potato tuber sprouting. *Planta.* 239: 27-38.

Suttle, J. C. 2004. Physiological regulation of potato tuber dormancy. *American Journal of Potato Research* 81: 253-262.

Tavakoli K., A. Razavi, and A. Sohani. 2014. Effects of different temperatures and hormone treatments on breaking dormancy in potato tubers. *Journal of Agricultural Sciences* 59(3): 255-264.

Thornton M. K. 1991. Potato Dormancy. *Proceedings of the University of Idaho Winter Commodity Schools*. 23: 210-213.

Tran, L., K. J. Green, M. Rodriguez-Rodriguez, G. E. Orellana, C. N. Funke, O. V. Nikolaeva, A. Quintero-Ferrer, M. Chikh-Ali, L. Woodell, N. Olsen, and A. V. Karasev. 2022. Prevalence of recombinant strains of potato virus Y in seed potato planted in Idaho and Washington states between 2011 and 2021. *Plant Disease* 106: 810-817.

UNECE. 2016. UNECE standard S-1: Concerning the marketing and commercial quality control of seed potatoes. *United Nations Economic Commission for Europe.*

UNECE. 2019. Findings of the survey of seed potato virus testing methods associated with seed potato certification. *United Nations Economic Commission for Europe*.

USDA, United States Department of Agriculture. 2021. Potatoes 2021 summary. *National Agricultural Statistics Service*.

Vetten, H. J., U. Ehlers, and H. L. Paul. 1983. Detection of potato viruses Y and A in tubers by enzyme-linked immunosorbent assay after natural and artificial break of dormancy. *Journal of Phytopathology* 108(1): 41-53.

Whitworth, J. L., P. B. Hamm, and P. Nolte. 2012. Distribution of potato virus Y strains in tubers during the post-harvest period. *American Journal of Potato Research* 89: 136-141.

Wurr, D. C. E., and E. J. Allen. 1976. Effects of cold treatments on the sprout growth of three potato varieties. *Journal of Agricultural Science* 86: 221-224.

Tables

Table 4-1. Harvest date, direct tuber testing date, days after harvest, and days after treatment when tubers were direct tuber tested for potato virus Y infection.

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

Table 4-2. 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 (ICIA).

	Ranger Russet	Clearwater Russet	Umatilla Russet
Treatment[1]	*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 1h injection 20h 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 4-3. 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.

	Ranger Russet	Clearwater Russet	Umatilla Russet
Treatment[1]	*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	0.20	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 1h injection 20h 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.

Table 4-4. 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.

		Ranger Russet	Clearwater Russet	Umatilla Russet
Treatment[1]	**Testing method[2]**	*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 (%)[1]		
UTC	DTT	16 bc	0.2 a	16 a
Smoke	DTT	13 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 1h injection 20h 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.

Table 4-5. Percent potato virus Y (PVY) infection detected by direct tuber testing of three treatments compared to composite leaf samples of the same tubers planted in the spring grow out in 2021.

	Testing method[1]	
	Ranger Russet PVY infection (%)	
Treatment[2]	**Direct tuber testing**	**Spring grow-out**
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 1h injection 20h circulation; Rindite= application of volatized Rindite.

Figures

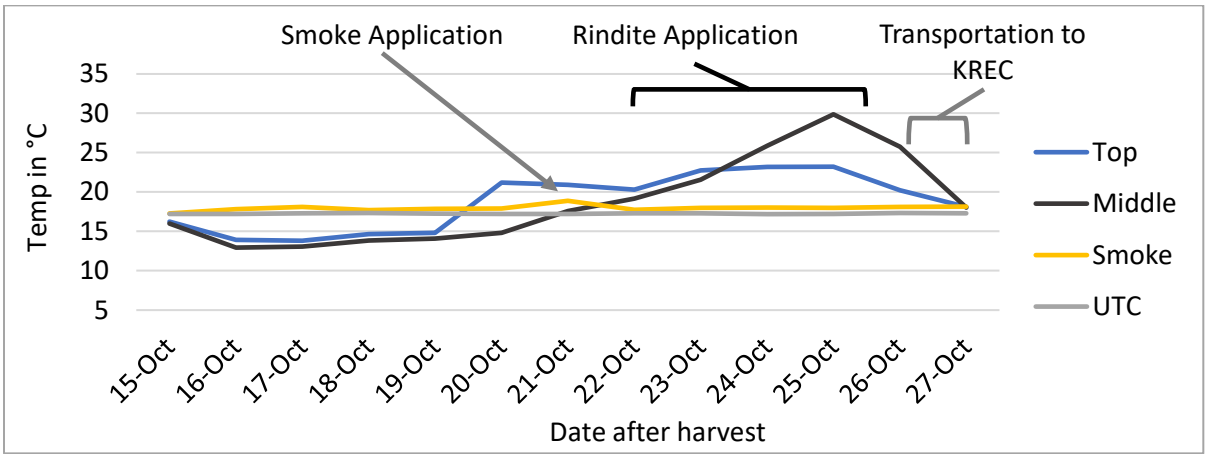

Figure 4-1. Temperatures during the application process of each treatment in 2021. Temperature on the top and middle of pallet during Rindite application. Smoke: temperature during application period and storage. UTC: temperature of the storage bin of the untreated control.

1. No bud activity	2. Peeping	3. Point initiated < 5mm	4. Sprout elongation ≥ 5mm

Figure 4-2.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.

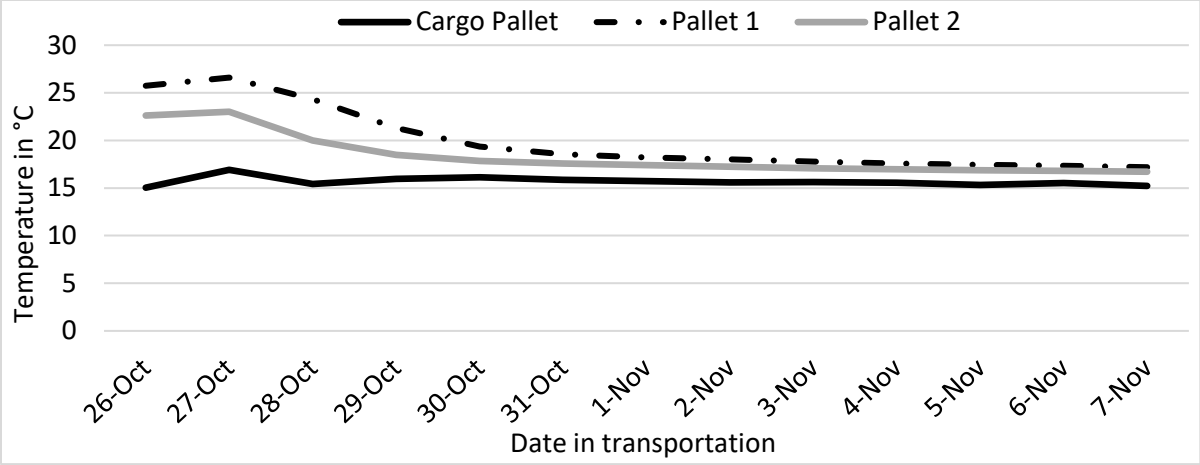

Figure 4-3. Temperatures of storage containers holding samples for the winter grow out transported from the Idaho Crop Improvement Association facility (Idaho Falls) to Hawaii in 2021. Cargo pallet: temperature of cargo container during shipping. Pallet 1 position was buried towards the middle of the pallet while pallet 2 position was closer to the top of the pallet.

Appendix A. Construction of Treatment Chamber, Smoke Generator, and Application Procedure of Cold Aerosol Smoke.

Application chamber: A custom-built rectangular 1.2 m x 1.2 m x 2.4 m (L x W x H) application chamber was constructed from 1.9 cm thick plywood with a 1.3 cm thick plexiglass door. An injection port was created by drilling a 2 cm hole in the bottom right wall (centered). All-purpose weather stripping was used to seal around the plexiglass door to ensure minimal smoke escape.

Cold-smoke generator: A custom-built container was constructed to produce cold aerosol smoke using a 0.5 L metal can with removable lid. Three 0.8 cm holes were drilled near the bottom outside rim of the can. Three 0.8 cm by 1.9 cm bolts were screwed into holes to raise the container off the ground to allow airflow. Additionally, six holes approximately 0.5 cm in diameter were drilled in a circular pattern in the bottom of the paint can for air flow. A 1.3 cm diameter hole was drilled into the center of the metal can lid to accommodate the 'application T'.

Application 'T': A 2.5 cm long by 1.3 cm diameter galvanized pipe with threaded ends was screwed into the bottom of a 1.3 cm diameter metal T joint. A 7.6 cm long, 1.3 cm diameter galvanized pipe with threaded ends was attached to the top left of the T joint while a 30.5 cm long, 1.3cm diameter galvanized pipe with threaded ends was attached to the top right of the T joint. The bottom portion of the 'application T' was inserted into the hole drilled in the metal can lid and secured using heat resistant tape.

Injection: A Point Zero 1/5 HP Airbrush compressor- Portable Quiet Hobby Tankless Oil-less Air Pump (Point Zero Airbrush; Tamarac, Florida) was used to force smoke into application chamber (20 to 23 liters per minute airflow). A 182 cm long by 0.63 cm rubber air hose was attached to compressor outlet. On opposite end of the air-hose a 15 cm long by 0.6 cm diameter galvanized pipe with threaded ends was attached using a galvanized female-female adapter.

Fuel: Plant-based pellets comprised from a blend of spruce, sugar pine, fir, poplar, and alder (Harvest Lane Honey; Salt Lake City, Utah) were burned to produce smoke. Approximately 100 g wood pellets were used to produce 1 hour of smoke and about 200-225 g to produce 2 hours of smoke.

Ignition: A BenzOmatic 0.4 L propane cylinder with brass torch (BenzOmatic; New York) was used to ignite the wood pellets.

Circulation: Two JISULIFE F8x Bear handheld Foldable Fan (Jisu Technology; Shanghai, China) was used to ensure adequate air and smoke movement through the application chamber.

Shelves: Two HDX 5-Tier steel wire shelving unit 123 cm x 46 cm x 183 cm (W x D x H) suspended potatoes in the chamber to allow smoke penetration above and below tubers.

Application:

1) Open the plexiglass door and load potatoes to be treated onto wire racks. Place two Jisulife fans on the floor of the application chamber, speed setting two. One fan in the back left corner and one fan in the front right corner when facing the chamber. Close the plexiglass door.

2) Add 100 g Harvest Lane Honey pellets to the metal can. With lid off, begin heating the bottom of the metal can with BenzOmatic propane torch until several pellets are ignited and a consistent stream of smoke is rising from the can (approximately 5 minutes). Remember to wear heat resistant insulated gloves as the can gets very hot. Attach lid with application 'T' inserted through the application port on the bottom of the right wall of the application chamber.

3) Turn on the Point Zero airbrush compressor with attached air hose and metal pipe attachment and ensure air is flowing. Insert the metal pipe on the air compressor hose inside of the application 'T' hole until the end of the compressor pipe is approximately 1.3 cm past the joint of the T joint connection.

4) Allow compressor to force smoke (inject) into the application chamber for one hour or desired period. Occasionally check to make sure pellets are burning and that smoke is billowing through the application T. Agitate the can every 15 minutes or so to ensure wood pellets continually burn. Some smoke will leak from the application chamber while injecting. This is okay since you are forcing air into a closed container.

5) Once the smoke injection is completed remove the application 'T' from application port in the chamber. Cover the hole with tape to prevent excess smoke from escaping. At this point tape any areas that smoke is escaping. There should be minimal smoke escaping the chamber at this point. Allow to circulate for desired period, typically four or 20 hours. Once circulation period is complete, open the plexiglass door and vent for 5 to 10 minutes. Remove treated potatoes from application chamber and place into desired storage bin.

*** For a two-hour application: follow same starting procedure as the one-hour application but add an additional 90g of wood pellets to metal can approximately 45 minutes after smoking begins and an additional 10 to 20 grams at 1.5 hours into the application period based upon the amount of pellets left.

Appendix B. Supplemental Data for Chapter 2

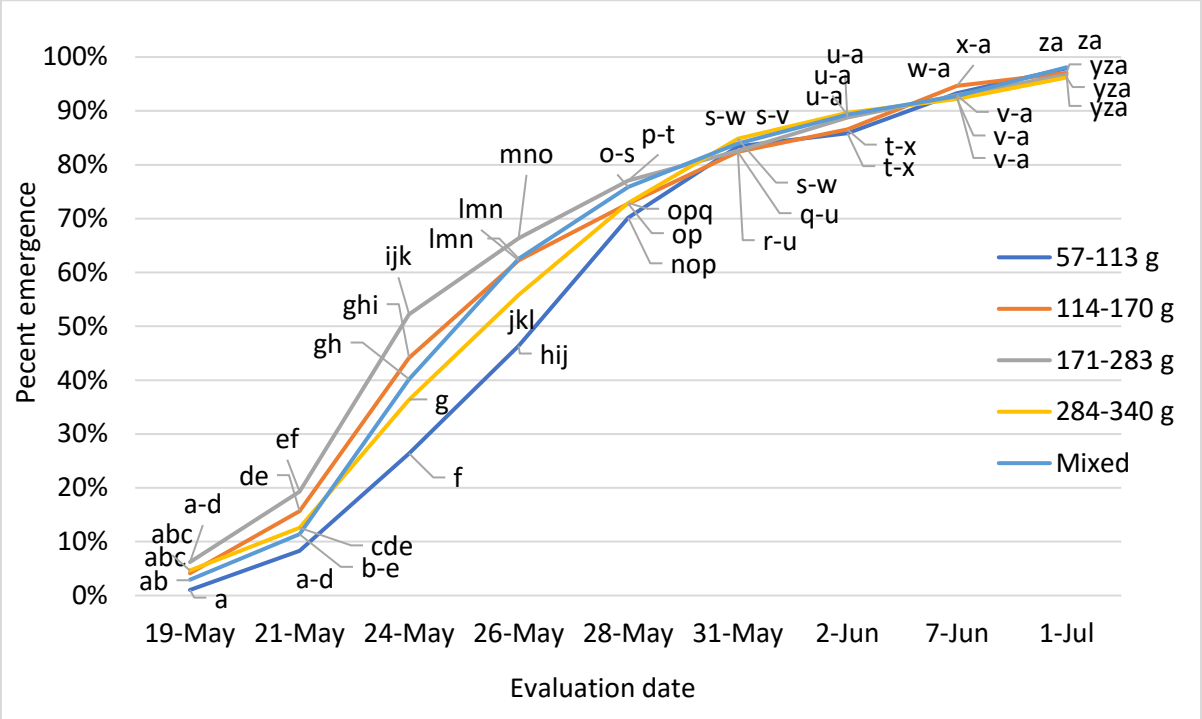

Figure B-1. Percent of plants emerged over time for five different mother tuber size categories in Russet Norkotah, 2021. Values followed by the same letter are not significantly different (α=0.05) for each variable. Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g tubers, Mixed = equal amounts of each mother tuber category.

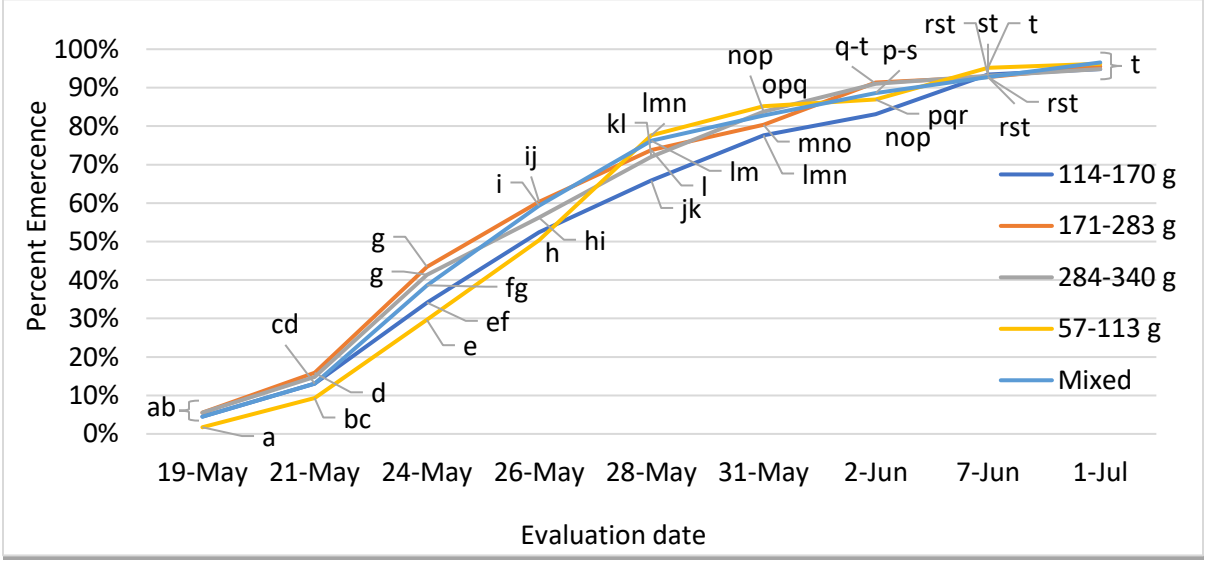

Figure B-2. Percent of plants emerged over time for five different mother tuber size categories in Umatilla Russet, 2021. Values followed by the same letter are not significantly different (α=0.05) for each variable. Single Drop = 42 to 112 g, Small = 113 to 169 g, Medium = 170 to 282 g, Large = 283 to 340 g tubers, Mixed = equal amounts of each mother tuber category.

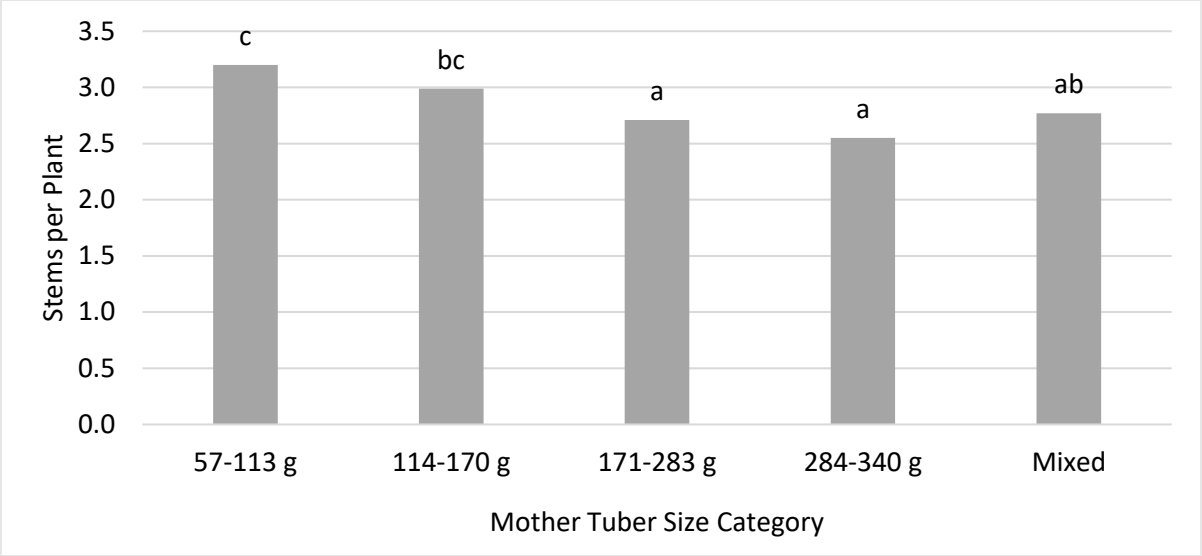

Figure B-3. Number of stems per plant for each mother tuber size category in Russet Norkotah, 2021. Values followed by the same letter are not significantly different (α=0.05) for each variable.

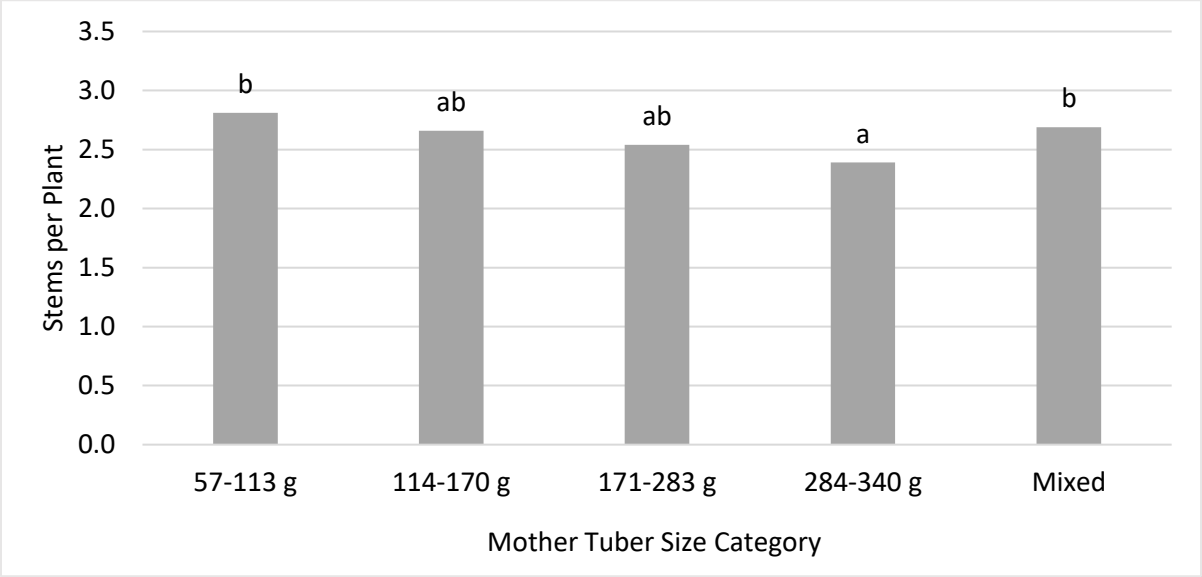

Figure B-4. Number of stems per plant for each mother tuber size category in Umatilla Russet, 2021. Values followed by the same letter are not significantly different (α=0.05) for each variable.

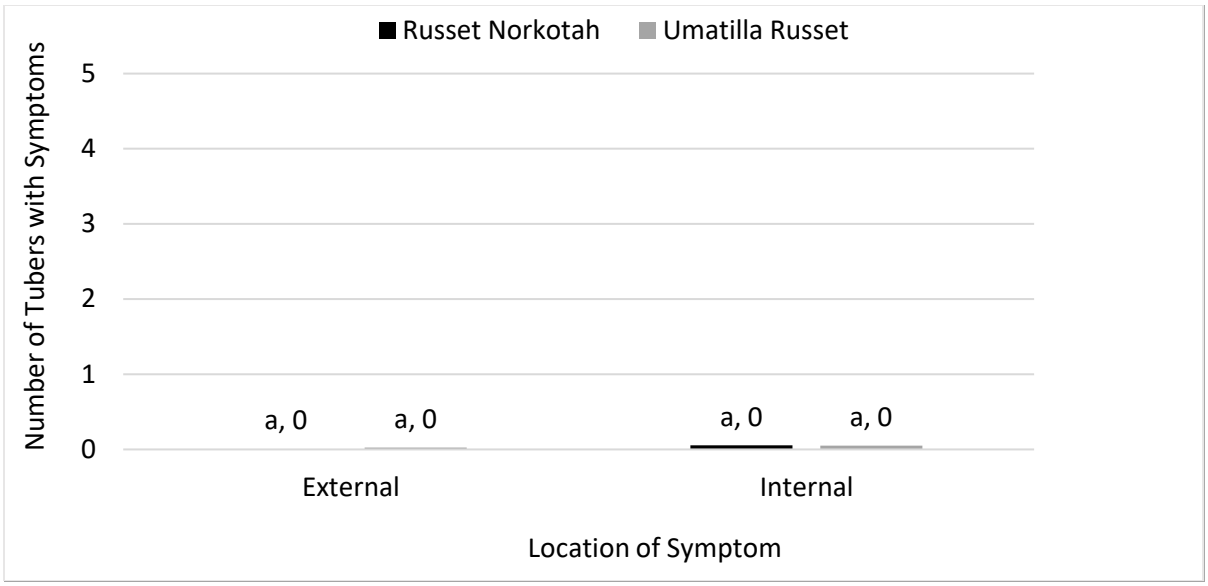

Figure B-5. Number of tubers with internal and external PVY symptoms in Umatilla Russet and Russet Norkotah, 2021. Values followed by the same letter are not significantly different (α=0.05) for each variable.

Appendix C. Supplemental Data for Chapter 3.

Table C-1. Number of sprouts pointing, 3 rating or greater, for October treated Russet Burbank, 2021.

	Number of Sprouts per Tuber Rated as a 3 or Greater[2]									
	Days After Treatment									
October Treatment	**15[1]**		**23**		**28**		**33**		**40**	
UTC	0.0	-	0.0	a	0.2	a	0.6	ab	1.3	abc
Cold-Stratification	0.0	-	0.1	a	0.5	ab	0.3	a	1.0	a
Temp. Fluctuation	0.0	-	0.0	a	0.0	ab	0.4	a	1.1	ab
GA Dip	0.0	-	1.2	b	1.3	bc	1.4	bc	1.9	abcd
Smoke 1h 20h	0.0	-	2.1	c	2.3	c	2.6	d	3.0	d
Combination	0.0	-	4.9	d	5.4	d	5.1	e	4.9	e
Smoke 2h 4h	0.0	-	1.1	b	1.4	bc	1.9	cd	2.2	cd
Smoke 1h 4h	0.0	-	1.1	b	1.3	bc	1.7	cd	2.1	bcd
Standard Error	-		0.24		0.34		0.35		0.35	

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

[2]A 3 rating indicates that the sprout has begun to elongate but less than 5 mm.

UTC=18.3C 14 days (d) storage; Cold Stratification=4.4C 14d; Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip=dipped into 20ppm for 15 minutes; Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; Combination=1h20h + 24h 18.3C + GA Dip; Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

Table C-2. Number of sprouts pointing, 3 rating or greater, for October treated Russet Burbank, 2022.

	Number of Sprouts per Tuber Rated as a 3 or Greater[2]													
	Days After Treatment													
October Treatment	**14[1]**		**22**		**27**		**34**		**44**		**55**		**77**	
UTC	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a	0.1	a	0.9	a
Cold-Stratification	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a	0.1	a	2.1	bc
Temp. Fluctuation	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a	0.1	a	1.7	ab
GA Dip	0.0	a	0.0	a	0.1	a	0.1	ab	0.4	a	0.6	ab	3.8	de
Smoke 1h 20h	0.0	a	0.0	a	0.2	a	0.3	b	0.5	a	1.0	bc	3.7	de
Combination	0.0	a	0.5	b	0.7	b	1.1	c	2.3	b	4.0	d	9.2	f
Smoke 2h 4h	0.0	a	0.1	a	0.1	a	0.2	ab	0.4	a	1.2	c	4.8	e
Smoke 1h 4h	0.0	a	0.0	a	0.0	a	0.1	ab	0.0	a	0.4	a	3.1	cd
Standard Error	0.01		0.07		0.08		0.11		0.19		0.19		0.37	

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

[2]A 3 rating indicates that the sprout has begun to elongate but less than 5 mm.

UTC=18.3C 14 days (d) storage; Cold Stratification=4.4C 14d; Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip=dipped into 20ppm for 15 minutes; Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; Combination=1h20h + 24h 18.3C + GA Dip; Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

Table C-3. Number of sprouts elongating, 4 rating, for October treated Russet Burbank, 2021

	Number of Sprouts per Tuber Rated as a 4[2]				
	Days After Treatment				
October Treatment[3]	**15[1]**	**23**	**28**	**33**	**40**
UTC	0.0 -	0.0 a	0.0 a	0.0 a	0.0 a
Cold-Stratification	0.0 -	0.0 a	0.0 a	0.0 a	0.0 a
Temp. Fluctuation	0.0 -	0.0 a	0.0 a	0.0 a	0.0 a
GA Dip	0.0 -	0.0 a	0.0 a	0.1 a	0.2 a
Smoke 1h 20h	0.0 -	0.0 a	0.0 a	0.1 a	0.2 a
Combination	0.0 -	0.7 b	1.2 b	1.2 b	1.2 b
Smoke 2h 4h	0.0 -	0.0 a	0.0 a	0.1 a	0.3 a
Smoke 1h 4h	0.0 -	0.0 a	0.1 a	0.1 a	0.2 a
Standard Error	-	0.08	0.14	0.14	0.15

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

[2]A 4 rating is equivalent to a sprout elongating to 5 mm or greater.

[3]UTC=18.3C 14 days (d) storage; Cold Stratification=4.4C 14d; Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip=dipped into 20ppm for 15 minutes; Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; Combination=1h20h + 24h 18.3C + GA Dip; Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

Table C-4. Number of sprouts elongating, 4 rating, for October treated Russet Burbank, 2022.

	Number of Sprouts per Tuber Rated as a 4[2]				
	Days After Treatment				
October Treatment[3]	**15[1]**	**23**	**28**	**33**	**40**
UTC	0.0 -	0.0 a	0.0 a	0.0 a	0.0 a
Cold-Stratification	0.0 -	0.0 a	0.0 a	0.0 a	0.0 a
Temp. Fluctuation	0.0 -	0.0 a	0.0 a	0.0 a	0.0 a
GA Dip	0.0 -	0.0 a	0.0 a	0.1 a	0.2 a
Smoke 1h 20h	0.0 -	0.0 a	0.0 a	0.1 a	0.2 a
Combination	0.0 -	0.7 b	1.2 b	1.3 b	1.2 b
Smoke 2h 4h	0.0 -	0.0 a	0.0 a	0.1 a	0.3 a
Smoke 1h 4h	0.0 -	0.0 a	0.1 a	0.1 a	0.2 a
Standard Error		0.08	0.14	0.14	0.15

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

[2]A 4 rating is equivalent to a sprout elongating to 5 mm or greater.

[3]UTC=18.3C 14 days (d) storage; Cold Stratification=4.4C 14d; Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip=dipped into 20ppm for 15 minutes; Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; Combination=1h20h + 24h 18.3C + GA Dip; Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

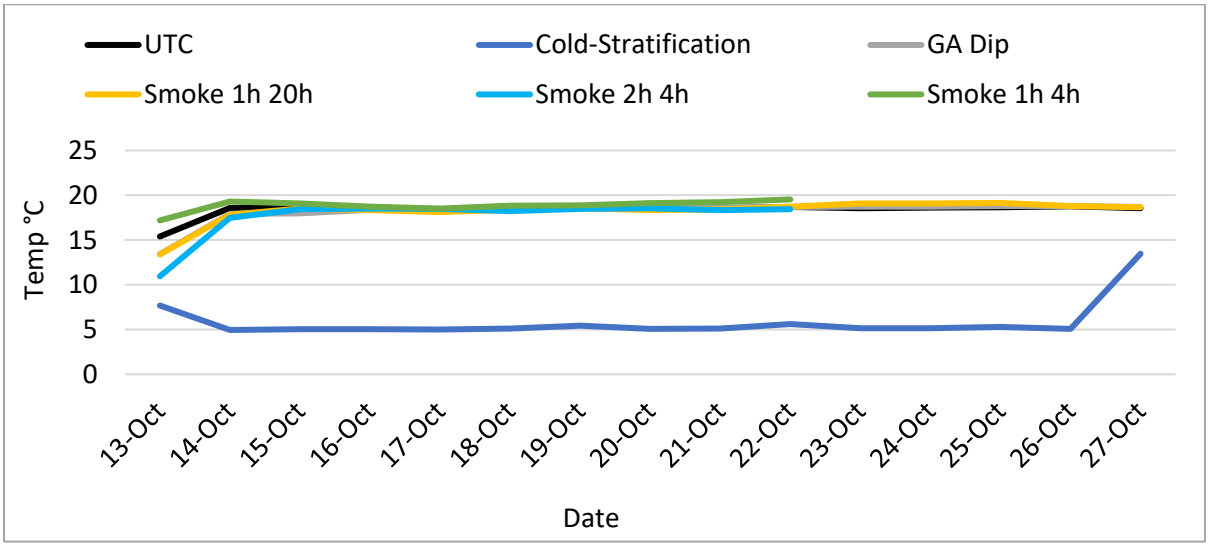

Figure C-5. Average daily temperature during application process for each treatment in 2021 October treated tubers. UTC=18.3C 14 day (d); Cold-stratification=4.4C 14d; Temp. fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip at 20ppm; Smoke 1h 20h=smoke injected 1 hour (h), recirculated 20 h; Combination=1h20h + GA Dip; Smoke 2h 4h= smoke injected 2 h, recirculated 4 h; Smoke 1h 4h= smoke injected 1 h, recirculated 4h. All tubers were stored at 18.3C post-treatment.

[1]Temperature fluctuation and combination treatment are missing data.

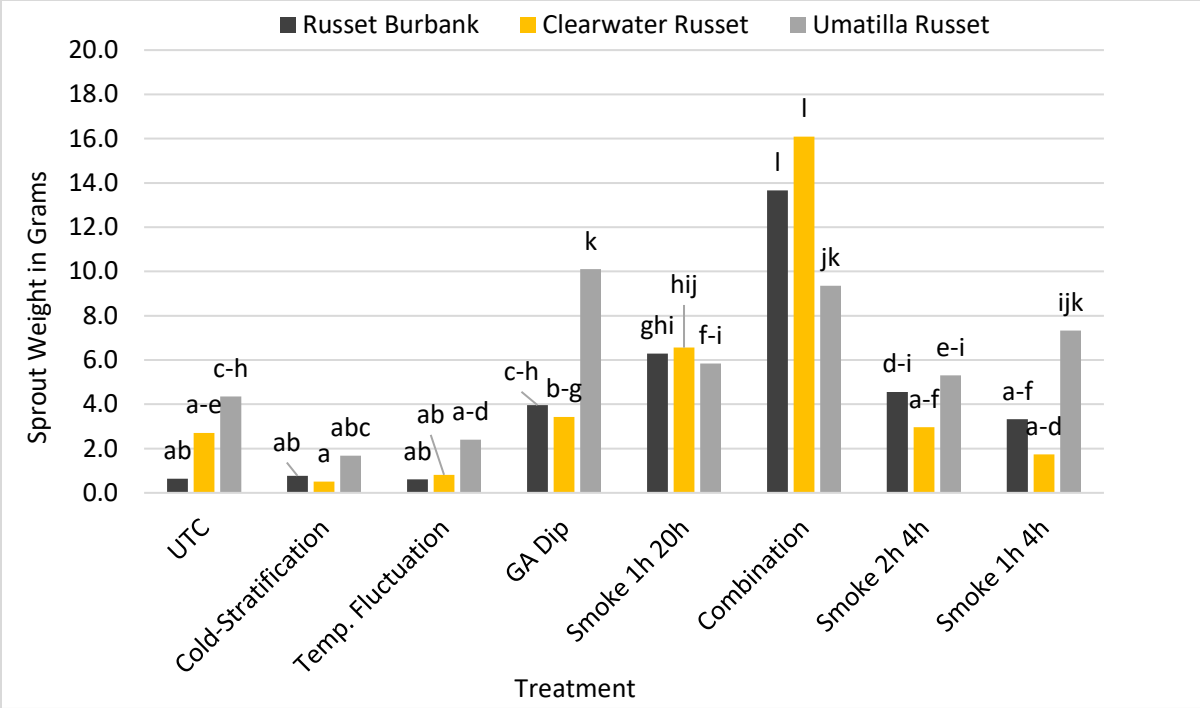

Figure C-6. Final weight of removed sprouts from October treatment timing of Russet Burbank, Clearwater Russet, and Umatilla Russet 2021 (n=20). UTC=18.3C 14 days (d) storage; Cold Stratification=4.4C 14d; Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip=dipped into 20ppm for 15 minutes; Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; Combination=1h20h + 24h 18.3C + GA Dip; Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

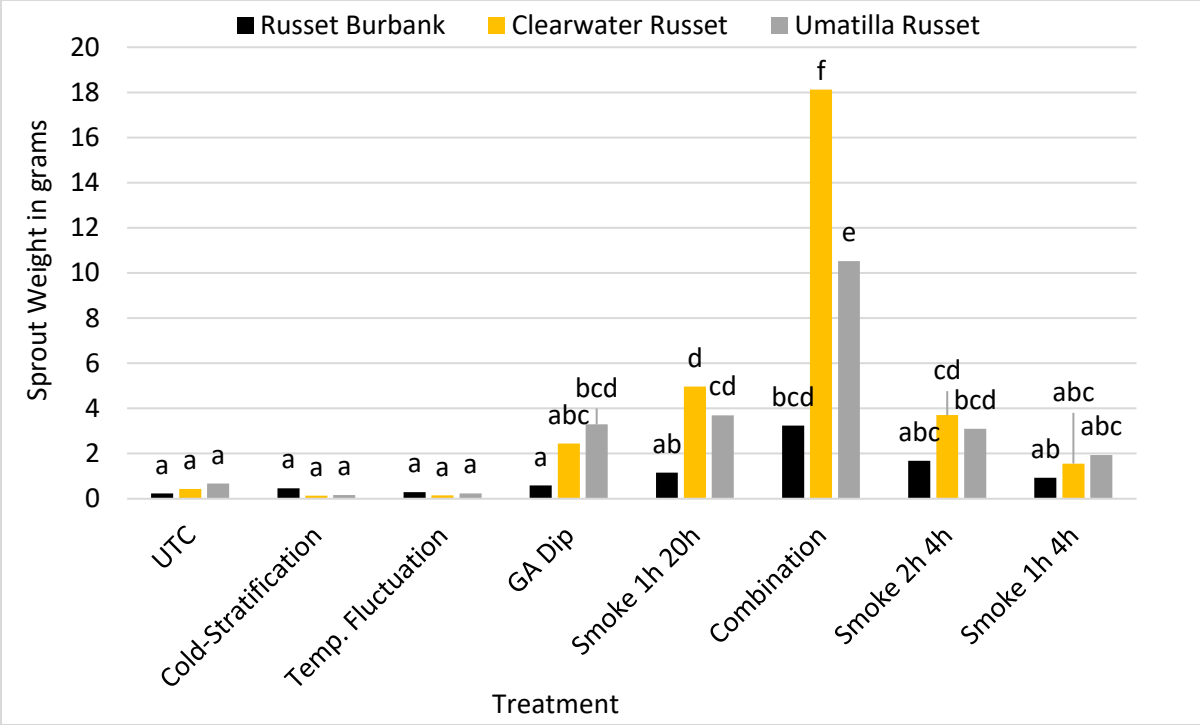

Figure C-7. Final weight of removed sprouts from October treatment timing of Russet Burbank, Clearwater Russet, and Umatilla Russet 2022 (n=20). UTC=18.3C 14 days (d) storage; Cold Stratification=4.4C 14d; Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; GA dip=dipped into 20ppm for 15 minutes; Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; Combination=1h20h + 24h 18.3C + GA Dip; Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

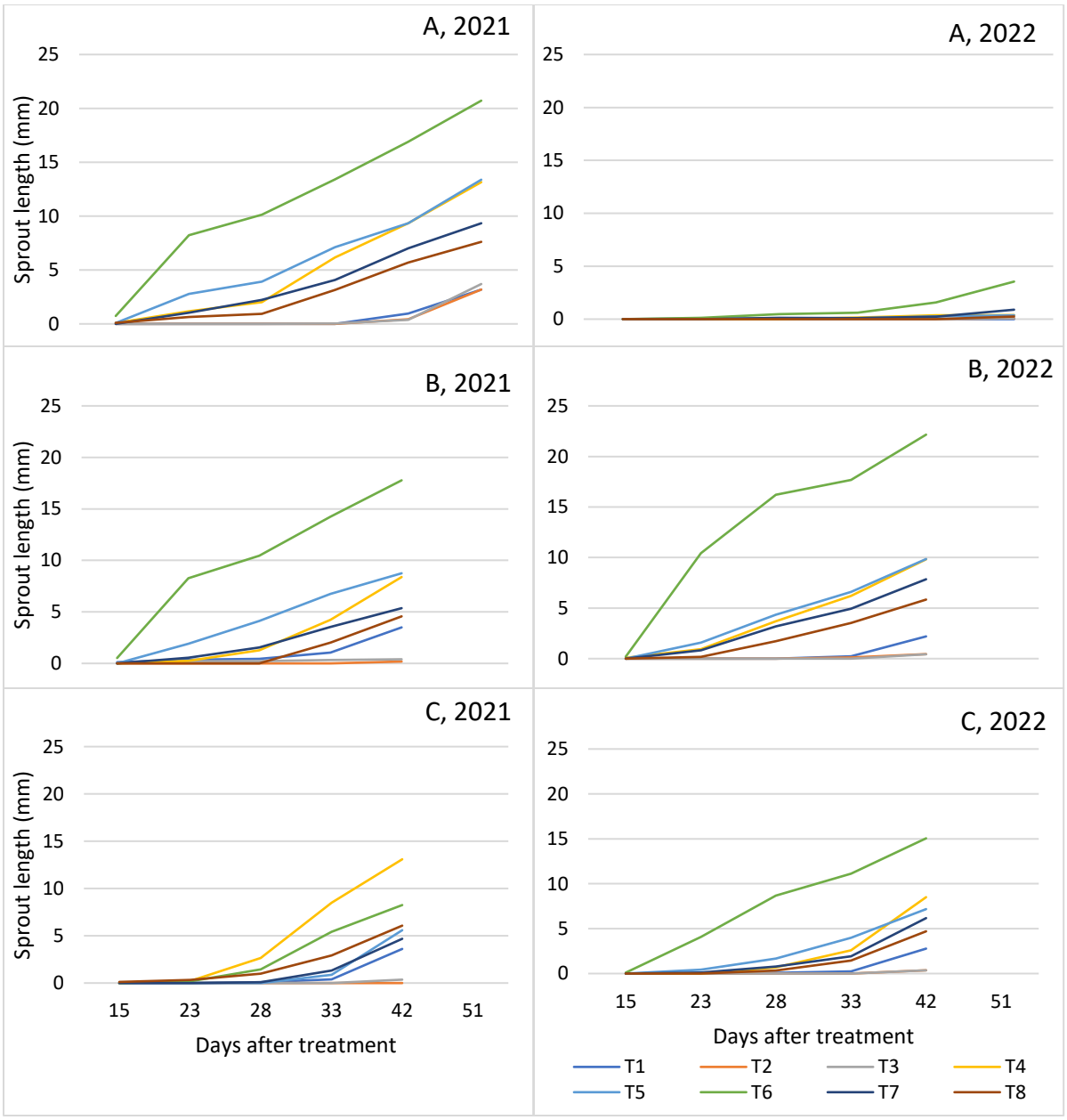

Figure C-8. Average sprout length over time for A) Russet Burbank, B) Clearwater Russet, and C) Umatilla Russet for October treated tubers. T1:UTC=18.3C 14 days (d) storage; T2:Cold Stratification=4.4C 14d; T3:Temp Fluctuation=4.4C 5d->18.3C 4d->4.4C 5d; T4:GA dip=dipped into 20ppm for 15 minutes; T5:Smoke 1h 20h=smoke injected for 1 hour, recirculated 20 hours; T6:Combination=1h20h + 24h 18.3C + GA Dip; T7:Smoke 2h 4h= smoke injected for 2 hours, recirculated 4 hours; T8:Smoke 1h 4h= smoke injected for 1 hour, recirculated 4 hours. All tubers stored in 18.3C post-treatment.

Appendix D. Supplemental Data for Chapter 4.

Table D-1. Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating in Ranger Russet, 2021.

Days after treatment	Treatment[1]	Sprout rating[2,3]	Number sprouts per tuber 3 rating*	Number sprouts per tuber 4 rating**
17	UTC	1.8 a	0.3 a	0.0 a
17	Smoke	2.3 b	1.2 a	0.1 a
17	Rindite	3.8 d	10.4 a	2.7 a
24	UTC	2.4 b	1.0 a	0.1 a
24	Smoke	3.2 c	1.9 a	0.5 a
24	Rindite	4.0 d	9.5 a	3.4 a
Standard error		0.1	0.5	0.3

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

[2]Values followed by the same letter are not significantly different (α=0.05) for each variable within each column and day after treatment.

[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.

*Interaction was not significant (p 0.1579); however, treatment was significant ($p < 0.0001$) UTC: 0.7 a, smoke 1.5 b, Rindite: 9.9 c. Days after treatment (DAT) was not significant (p 0.6370).

**Interaction was not significant (p 0.6818) however, treatment was significant ($p < 0.0001$) UTC: 0.1 a, smoke: 0.3 a. Rindite: 3.0 b. DAT was not significant (p 0.1248).

Table D-2. Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating in Ranger Russet, 2022.

Days after treatment	Treatment[1]	Sprout rating[2,3]	Number sprouts per tuber 3 rating*	Number sprouts per tuber 4 rating **
16	UTC	1.4 a	0.1 a	0.0 a
16	Smoke	1.8 b	0.2 a	0.0 a
16	Rindite	4.0 d	8.6 a	4.1 a
23	UTC	1.8 b	0.4 a	0.1 a
23	Smoke	2.8 c	1.3 a	0.3 a
23	Rindite	4.0 d	9.3 a	4.6 a
Standard error		0.1	0.2	0.1

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

[2]Values followed by the same letter are not significantly different (α=0.05) for each variable within each column and day after treatment.

[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.

*Interaction was not significant (p 0.2694); however, treatment was significant (p < 0.0001) UTC: 0.2 a, smoke 0.7 b, Rindite: 9.0 c. Days after treatment (DAT) was significant (p 0.0009) 17 DAT: 3.0 a, 24 DAT: 3.6 b.

**Interaction was not significant (p 0.15372) however, treatment was significant (p < 0.0001) UTC: 0.0 a, smoke: 0.2 a. Rindite: 4.3 b. DAT was significant (p 0.0100) 17 DAT: 1.4 a, 24 DAT 1.6 b.

Table D-3. Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating in Clearwater Russet, 2021.

Days after treatment	Treatment[1]	Sprout rating[2,3]	Number sprouts per tuber 3 rating*	Number sprouts per tuber 4 rating **
17	UTC	1.3 a	0.0 a	0.0 a
17	Smoke	2.1 c	0.6 a	0.0 a
17	Rindite	3.7 g	8.2 a	1.9 a
24	UTC	1.9 b	0.2 a	0.0 a
24	Smoke	3.0 e	1.7 a	0.2 a
24	Rindite	4.0 h	7.9 a	2.7 a
31	UTC	2.6 d	0.7 a	0.1 a
31	Smoke	3.7 g	1.8 a	0.7 a
31	Rindite	4.0 h	7.9 a	2.9 a
38	UTC	3.4 f	1.3 a	0.5 a
38	Smoke	3.9 h	1.9 a	1.0 a
38	Rindite	4.0 h	7.7 a	3.2 a
Standard error		0.051	0.498	0.370

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

[2]Values followed by the same letter are not significantly different (α=0.05) for each variable 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.

*Interaction was not significant (p 0.4853), however treatment was significant ($p < 0.0001$) UTC: 0.6 a, smoke 1.5 b, Rindite: 7.9 c. DAT was not significant (p 0.3457)

**Interaction was not significant (p 0.877) however, treatment was significant ($p < 0.0001$) UTC: 0.1 a, smoke: 0.5 a. Rindite: 2.7 b. Days after treatment (DAT) was significant (p 0.0309) 17 DAT: 0.6 a, 24 DAT 1.0 ab, 31 DAT: 1.2 ab, 38 DAT: 1.6 b.

Table D-4. Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating in Clearwater Russet, 2022.

Days after treatment	Treatment[1]	Sprout rating[2,3]	Number sprouts per tuber 3 rating	Number sprouts per tuber 4 rating
16	UTC	1.6 a	0.1 a	0.0 a
16	Smoke	2.4 b	0.6 ab	0.1 a
16	Rindite	3.9 fg	5.2 d	1.9 e
23	UTC	2.5 b	0.7 ab	0.1 a
23	Smoke	3.4 d	1.5 c	0.5 b
23	Rindite	4.0 g	5.4 d	2.0 ef
30	UTC	3.2 c	1.1 bc	0.4 b
30	Smoke	3.8 f	1.6 c	1.0 d
30	Rindite	4.0 g	5.1 d	2.2 g
37	UTC	3.7 e	1.2 bc	0.8 c
37	Smoke	4.0 g	1.5 c	1.1 d
37	Rindite	4.0 g	5.0 d	2.2 fg
Standard error		0.035	0.232	0.066

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

[2]Values followed by the same letter are not significantly different (α=0.05) for each variable 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 D-5. Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating in Umatilla Russet, 2021.

Days after treatment	Treatment[1]	Sprout rating[2,3]	Number sprouts per tuber 3 rating	Number sprouts per tuber 4 rating
17	UTC	1.2 a	0.0 a	0.0 a
17	Smoke	1.4 ab	0.0 a	0.0 a
17	Rindite	3.4 h	5.6 g	0.7 d
24	UTC	1.4 bc	0.1 a	0.0 a
24	Smoke	1.6 c	0.2 ab	0.0 a
24	Rindite	3.7 ij	5.3 fg	1.3 e
31	UTC	1.8 d	0.3 ab	0.1 ab
31	Smoke	2.2 e	0.6 b	0.1 ab
31	Rindite	3.9 jk	5.3 fg	1.4 ef
38	UTC	2.5 f	0.6 b	0.2 ab
38	Smoke	2.9 g	1.3 c	0.3 bc
38	Rindite	3.9 k	5.0 f	1.6 f
45	UTC	3.1 g	1.1 c	0.5 c
45	Smoke	3.6 hi	1.8 d	0.7 d
45	Rindite	4.0 k	4.5 e	1.6 f
Standard error		0.067	0.151	0.078

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

[2]Values followed by the same letter are not significantly different (α=0.05) for each variable 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 D-6. Sprout rating, number of sprouts per tuber pointing, number of sprouts per tuber elongating in Umatilla Russet, 2022.

Days after treatment	Treatment[1]	Sprout rating[2,3]	Number sprouts per tuber 3 rating	Number sprouts per tuber 4 rating
16	UTC	1.2 a	0.0 a	0.0 a
16	Smoke	1.2 ab	0.0 a	0.0 a
16	Rindite	3.9 f	5.8 de	2.1 d
23	UTC	1.4 b	0.0 a	0.0 a
23	Smoke	2.0 c	0.3 a	0.0 a
23	Rindite	4.0 f	6.1 e	2.2 e
30	UTC	1.8 c	0.3 a	0.0 a
30	Smoke	2.9 d	1.2 c	0.2 b
30	Rindite	4.0 f	5.8 de	2.4 f
37	UTC	2.7 d	0.8 b	0.2 b
37	Smoke	3.5 e	1.3 c	0.6 c
37	Rindite	4.0 f	5.6 d	2.3 ef
Standard error		0.071	0.144	0.051

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

[2]Values followed by the same letter are not significantly different (α=0.05) for each variable 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.

Appendix E. Comparing PVY Detection and Growth Behaviors of Potential Alternatives to Rindite in the Winter Grow Out.

A study was conducted in collaboration with Potato Certification Association of Nebraska (PCAN) to evaluate alternative methods to break dormancy for the winter grow out. Treatment efficacy trials were conducted on four cultivars of seed potatoes (Vizelle, Russet Norkotah (Selection 278), Lamoka, Vanguard Russet) with varying levels of dormancy. Samples were provided by collaborative seed grower(s) in Nebraska. Samples were collected by PCAN during the time of traditional winter grow out (WGO). Sampling required six, 500 single drop (42-113 g) tuber samples be collected, homogenized, then divided equally (6 trt x 500 tubers = 3000 tubers per seed lot). The grower was responsible for their own WGO sample for certification purposes.

Treatments included 1) dip in 20 ppm gibberellic acid (GA) solution for 15 minutes prior to shipment, 2) application of cold aerosol smoke, 1 h injection 20 h recirculation (1h20h) prior to shipment, 3) application of 1h20h cold aerosol smoke followed by a dip in GA prior to shipment, 4) application of 1h20h cold aerosol smoke followed by a dip in GA after shipment, 5) application of Rindite prior to shipment followed by a dip in GA after shipment, and 6) application of Rindite prior to shipment.

Collected samples were stored at PCAN until all samples of each cultivar were collected according to standard practices. Temperature monitors (Kestrels, Boothwyn, PA) were placed with each of the samples after collection. Once collection was complete, all samples were delivered to Kimberly Research and Extension Center, Kimberly, ID (KREC) for storage at 18.3 C 95% RH.

Sample 1 was dipped in a 20 ppm GA solution for 15 minutes and dried. Samples 2, 3, and 4 were treated with a cold aerosol smoke. After smoke application sample 3 was dipped in GA solution and dried. October 12, 2022 all samples were delivered to PCAN. Samples 5 and 6 were loaded and treated with Rindite according to PCAN standard practices beginning October 13, 2022.

Upon completion of Rindite application, all samples were loaded into a climate-controlled cargo container (18.3 to 21 C) and shipped to Hawaii. Upon arrival, samples 4 and 5 were dipped in a 10 ppm GA solution according to PCAN standard practices.

All samples were planted in a complete random block design (four replicates) in the field in Waialua, Hawaii. To compare efficacy of treatments at breaking dormancy and promoting plant growth, a pre-plant sprout score, emergence, stem number, date in which seed-lots achieve adequate size for sampling was recorded. Pre-plant sprout score was conducted on a scale of 1 to 10 with 1 being tubers had no visible sprout development and 10 being tubers had several sprouts per

tuber elongating. A leaf tissue sample was collected from each lot in composites of 10 leaves per sample. The number of leaves collected served as the final stand count. PVY analysis was conducted on each sample to compare detection levels.

Tables

Table E-1. Final emergence, stem number, sprout score at planting, and the number of days after planting (DAP) samples were large enough to be leaf picked for PVY testing for each treatment. included 1) dip in 20 ppm gibberellic acid (GA) solution for 15 minutes prior to shipment, 2) application of cold aerosol smoke, 1 hour injection 20 hour recirculation (1h20h) prior to shipment, 3) application of Rindite prior to shipment 4) application of 1h20h cold aerosol smoke followed by a dip in GA after shipment, 5) application of 1h20h cold aerosol smoke followed by a dip in GA prior to shipment, and 6) application of Rindite prior to shipment followed by a dip in GA after shipment.

Treatment	Final emergence (%)[1]	Stem number per plant	Sprout score at planting[2]	DAP of sampling
		Vizelle		
Idaho GA	70 a	4.5 bc	4.8 a	49 b
Smoke	66 a	3.9 ab	4.5 a	49 b
Rindite	68 a	3.4 a	5.3 a	49 b
Smoke + Hawaii GA	68 a	5.4 d	4.8 a	49 b
Smoke + Idaho GA	80 a	5.6 d	10.0 b	43 a
Rindite + Hawaii GA	71 a	5.0 cd	5.3 a	49 b
Standard error	5.0	0.295	0.799	0.816
		Vanguard Russet		
Idaho GA	20 a	-	1.0 a	68 a
Smoke	10 a	-	1.0 a	68 a
Rindite	14 a	-	1.3 a	68 a
Smoke + Hawaii GA	17 a	-	1.0 a	68 a
Smoke + Idaho GA	20 a	-	1.8 a	68 a
Rindite + Hawaii GA	19 a	-	1.3 a	68 a
Standard error	3.2	-	0.243	0.000
		Russet Norkotah 278		
Idaho GA	49 abc	1.0 ab	1.5 a	69 a
Smoke	34 a	1.0 a	2.3 a	69 a
Rindite	63 c	1.1 a	2.3 a	69 a
Smoke + Hawaii GA	40 ab	1.1 a	2.0 a	69 a
Smoke + Idaho GA	37 a	1.0 a	3.0 a	69 a
Rindite + Hawaii GA	61 bc	1.4 b	2.3 a	69 a
Standard error	7.3	0.070	0.517	0.000
		Lamoka		
Idaho GA	68 ab	1.4 abc	7.0 a	46 ab
Smoke	54 a	1.2 ab	5.7 a	46 ab
Rindite	64 ab	1.1 a	6.3 a	52 b
Smoke + Hawaii GA	72 bc	1.4 abc	6.0 a	44 a
Smoke + Idaho GA	86 c	1.5 c	9.5 b	42 a
Rindite + Hawaii GA	76 bc	1.5 bc	6.0 a	42 a
Standard error	4.8	0.114	0.487	2.360

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

[2]Sprout score scale 1 to 10: 1=no sprout development, 10= several sprouts per tuber elongating.

Figures

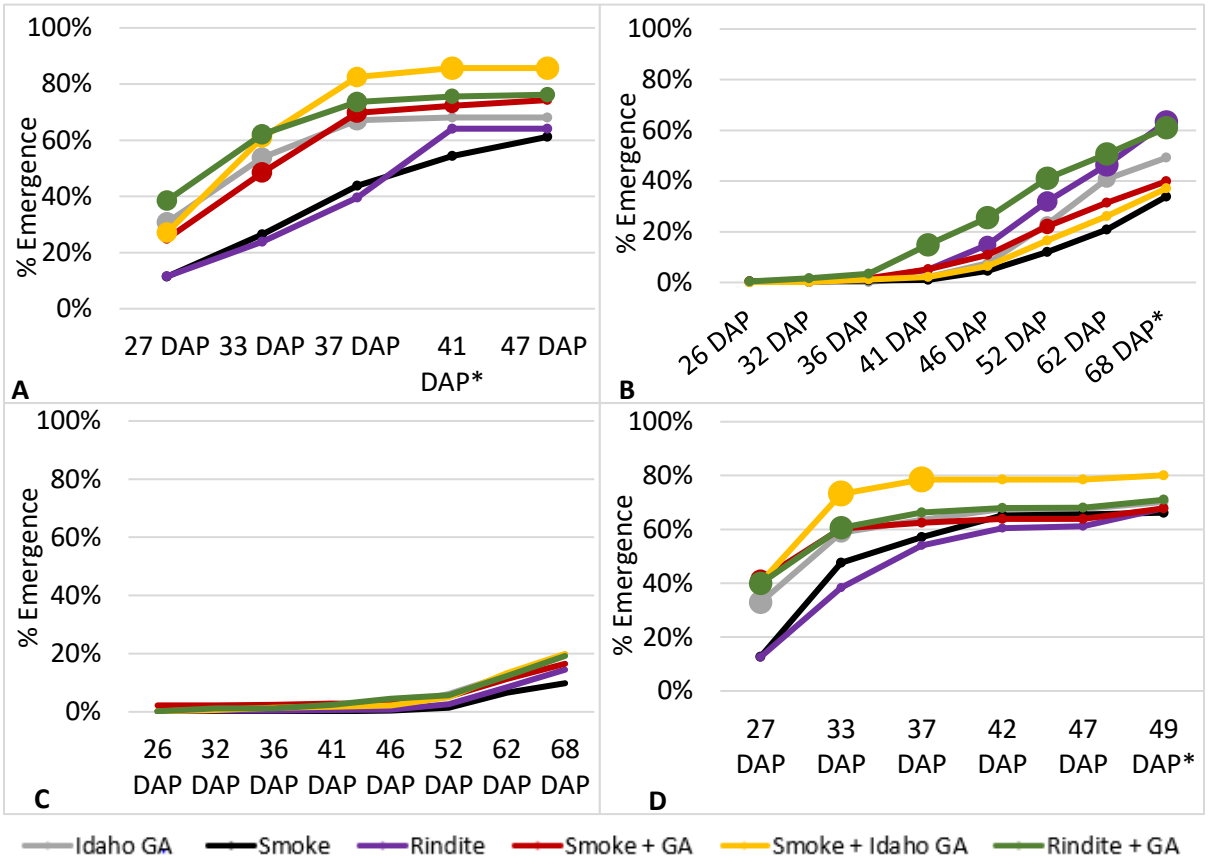

Figure E-1. Percent emergence over time for A) Lamoka, B) Russet Norkotah 278, C) Vanguard Russet, D) Vizelle in Nebraska winter grow out plots after treatment with dormancy breaking treatments. DAP= days after planting. Idaho GA= 20ppm for 15 min dip in gibberellic acid (GA), smoke= 1 hour injection 20 hour recirculation of cold aerosol smoke (1h20h), Rindite = 4ppm Rindite application, Smoke + GA = smoke application 1h20h followed by GA dip in Hawaii, Smoke + Idaho GA= smoke 1h20h followed by GA dip in ID, Rindite + GA= application of Rindite followed by GA dip in Hawaii. Larger dots within each line indicate treatment was significantly different at that evaluation period (α=0.05).

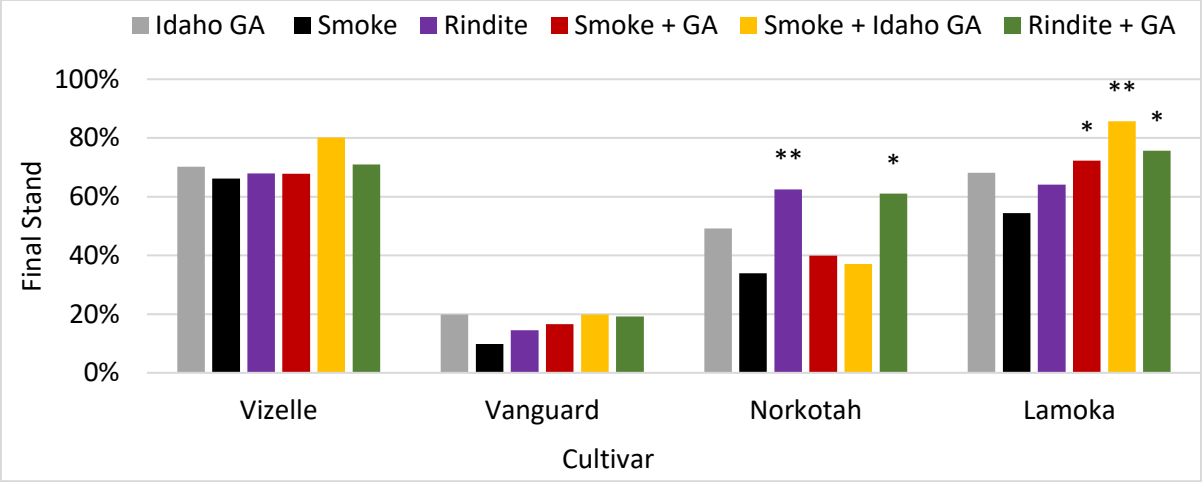

Figure E-2. Final emergence of dormancy breaking treatments in four cultivars in the Nebraska winter grow out plots in Waialua, HI, 2021. Idaho GA= 20ppm for 15 min dip in gibberellic acid (GA), smoke= 1 hour injection 20 hour recirculation of cold aerosol smoke (1h20h), Rindite = 4ppm Rindite application, Smoke + GA = smoke application 1h20h followed by GA dip in Hawaii, Smoke + Idaho GA= smoke 1h20h followed by GA dip in ID, Rindite + GA= application of Rindite followed by GA dip in Hawaii. Asterisk indicates treatment significance in that cultivar (α=0.05).

Appendix F. Preliminary Data on Development of Cold Aerosol Smoke Application Process.

A study to determine the effect of timing of the GA dip in combination with smoke and if GA dip was necessary to increase sprout development was conducted. Tubers were dipped immediately before smoke application or 20 hours after application. Dip was a 20 ppm solution of GA for 15 minutes. Water was a submersion into DI water for 15 minutes. An additional study was conducted on the effect of a smoke application to freshly harvested or suberized potatoes.

Tables

Table F-1. Sprout rating, number of sprouts pointing, and number of sprouts elongating per tuber for Umatilla Russet and Clearwater Russet tubers treated with 1) untreated control (UTC) held at 18.3 C, 2) application of aerosol smoke 1 hour injection 20 hour recirculation (smoke) followed by a dip in deionized (DI) water for 15 minutes, 3) smoke application followed by a dip in 20 ppm gibberellic acid solution for 15 minutes (GA), 4) GA dip followed by smoke application, and 5) dip in water followed by smoke application.

Cultivar	Treatment	Sprout rating	No. sprouts per tuber 3 rating	No. sprouts per tuber 4 rating
Clearwater Russet	UTC	3.7 cd	3.8 b	1.0 bc
Clearwater Russet	Smoke + water	3.9 ef	5.5 d	1.6 de
Clearwater Russet	Smoke + GA	4.0 f	7.0 e	2.9 f
Clearwater Russet	GA + smoke	3.9 f	6.8 e	2.8 f
Clearwater Russet	Water + smoke	3.9 ef	7.0 e	1.9 e
Umatilla Russet	UTC	3.3 a	2.9 a	0.6 a
Umatilla Russet	Smoke + water	3.6 bc	4.5 bc	1.0 bc
Umatilla Russet	Smoke + GA	3.7 cd	5.0 cd	1.6 de
Umatilla Russet	GA + smoke	3.8 de	5.0 cd	1.4 cd
Umatilla Russet	Water + smoke	3.5 b	5.5 d	0.9 ab

[1]Values followed by different letters are significantly different in each column and cultivar (α=0.05).
[2]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 F-2. Smoke studies conducted on freshly harvested and suberized potatoes (12.8 C for one week) prior to maturation of Russet Norkotah in 2021 to determine optimal application timing, injection rate, and circulation time of aerosol smoke. Various aerosol smoke injection rates and circulation timings were evaluated to determine tuber injury and sprout development.

Smoke trials conducted on Russet Norkotah to determine optimal timing and rate of aerosol smoke application							
Date harvest	**Date smoked**	**Treatment ID**	**Time smoke injected**	**Time recirculated**	**Tuber status at smoking**	**Observed tuber injury from smoke application**	**Final sprout rating**
7/19/2021	UTC	T1	0 hrs	0 hrs	Fresh	Minor lenticel damage, black ends, skin cracking	2.1
7/19/2021	7/19/2021	T2	2 hrs	20 hrs	Fresh	Extreme phytotoxicity, started molding	discarded
7/19/2021	7/20/2021	T3	1 hr	20 hrs	Fresh	Severe lenticel damage, minor tip burn, mild mold	3.7
7/19/2021	7/21/2021	T4	1 hr	0 hrs	Fresh	Moderate lenticel damage, minor tip burn	2.4
7/21/2021	UTC	T5	0 hrs	0 hrs	Suberized 1 wk	Minor lenticel damage	1.1
7/21/2021	7/23/2021	T6	2 hrs	0 hrs	Fresh	moderate to severe lenticel damage, mild to moderate tip burn, mild mold	1.5
7/21/2021	7/28/2021	T7	1 hr	0 hrs	suberized 1 wk	Minor lenticel damage	1.5
7/21/2021	7/28/2021	T8	1 hr	20 hrs	suberized 1 wk	Moderate lenticel damage, minor to moderate tip burn	2.3
7/21/2021	7/29/2021	T9	2 hrs	0 hrs	suberized 1 wk	Moderate to severe lenticel damage, mild to moderate tip burn, mild mold	2.6
7/21/2021	8/2/2021	T10	2 hrs	20 hrs	suberized 1 wk	Minor Lenticel damage, minor to moderate tip burn	2.1
7/21/2021	7/30/2021	T11	1 hr	4 hrs	suberized 1 wk	Minor lenticel damage, minor tip burn	1.2

Table F-3. Smoke studies conducted on freshly harvested, suberized potatoes prior to maturation of Russet Burbank in 2021 to determine optimal application timing, injection rate, and circulation time of aerosol smoke and dip in gibberellic acid (GA; 10ppm 5 minutes). Various aerosol smoke injection rates, circulation timings, and GA dips were evaluated to determine tuber injury and sprout development.

Smoke trials conducted on Russet Burbank to determine optimal timing and rate of aerosol smoke application and GA dip						
Date harvest	**Date smoked**	**Treatment ID**	**Time smoke injected**	**Time recirculated**	**Observed tuber injury from smoke application**	**Final sprout rating**
8/5/2021	UTC	T1	0 hr	0 hr	minor tip burn	1.2
8/5/2021	A.M. 8/10/21	T2	0.5 hr	0 hr	softening ends, minor tip burn	1
8/5/2021	A.M. 8/10/21	T3	1 hr	0 hr	no comments	1.2
8/5/2021	A.M. 8/10/21	T4	2 hr	0 hr	moderate pitting, moderate tip burn, ends softening	1.1
8/5/2021	8/11/21	T5	0.5 hr	4 hr	moderate pitting, mild to moderate tip burn, end softening	1.1
8/5/2021	8/11/21	T6	1 hr	4 hr	minor to moderate pitting, mild to moderate tip burn	1.1
8/5/2021	8/11/21	T7	2 hr	4 hr	mild to moderate pitting, minor to moderate tip burn, end softening	1.1
8/5/2021	P.M 8/10/21	T8	0.5 hr	20 hr	minor to moderate pitting, moderate tip burn, starting to mold	1.2
8/5/2021	P.M 8/10/21	T9	1 hr	20 hr	minor to moderate pitting, minor tip burn, few molding	1.5
8/5/2021	P.M 8/10/21	T10	2 hr	20 hr	moderate to severe pitting, moderate tip burn, starting to mold	1.5
8/5/2021	A.M. 8/10/21	T11	0 hr	0 hr, GA	minor tip burn	1.3
8/5/2021	A.M. 8/10/21	T12	0.5 hr	0 hr, GA	no comments	1.4
8/5/2021	A.M. 8/10/21	T13	1 hr	0 hr, GA	minor pitting, minor tip burn	1.7
8/5/2021	A.M. 8/10/21	T14	2 hr	0 hr, GA	minor to moderate pitting, minor tip burn	2.2

Appendix G. Effects of Cold Aerosol Smoke on the Control of Post-Harvest Storage Diseases and Processing Quality of Potato.

Preliminary study: A preliminary study was initiated in November 2021 to determine the efficacy of a post-harvest application of aerosol smoke at controlling pink rot *(Phytophthora erythroseptica)* and Fusarium dry rot in stored potatoes. Three replicates of 10 (n=30) Clearwater Russet tubers were used in the study. Tubers were inoculated with pink rot and Fusarium dry rot using the procedures described below. Tubers were loaded into a smoke application chamber and distributed by replicate on shelving on 11/2/21. The inoculated untreated control was placed into a plastic tote with large ventilation hole and left at ambient temperature and humidity for duration of smoke application (1 hour injection 20 hour recirculation). Following smoke application, pink rot inoculated tubers were transferred into small disease boxes and placed into 18.3 C storage while dry rot was placed on shelves at 7.2 C on 11/3/21 and evaluated 78 days later. Pink rot evaluations were conducted eight days after inoculation according to University of Idaho protocol described below.

Trial two: Post-harvest disease control trials were conducted on Russet Burbank potatoes to determine the efficacy of novel post-harvest treatments. Treatments were applied as described below. Russet Burbank was harvested September 13, 2021 and September 14, 2022 and brought into Kimberly R and E Center (KREC) storage. Potatoes were placed into 12.8 C to begin wound healing process. Storage temperatures were ramped from 12.8 C to 5.6 C beginning October 6, 2021 and reached a final holding temperature on November 1, 2021. Tubers were hand sampled and sorted into four reps of 10 tubers (r=40). Disease study was initiated December 15, 2021 and December 14, 2022 (124 Days After Harvest: DAH). Tubers were subjected to inoculation of pink rot and fusarium dry rot, procedures below. Tubers were loaded into an application chamber and distributed by replicate on shelving. Pink rot untreated control was placed into a plastic tote with large ventilation hole while Fusarium dry rot untreated control was placed on a slotted shelf with a plastic covering. Both were left at ambient temperature and humidity for duration of smoke application (1 hour injection 20 hour recirculation). Application began the afternoon of December 15, 2021 and December 14, 2022 and commenced on the following day.

Following smoke application, pink rot inoculated tubers were transferred into small disease boxes and placed into 18.3 C storage while dry rot was placed on shelves at 7.2 C. Pink rot evaluations were conducted 14 days after inoculation according to University of Idaho protocol described below.

Fusarium dry rot evaluations were conducted approximately 78 days after inoculation using method described below.

Analysis of variance (ANOVA) was performed on percent incidence and severity and means were separated by LSD at α=0.05.

Inoculation

Fusarium dry rot: Unwashed tubers (Russet Burbank) were dropped through a potato wounding box (Schisler *et al*., 2000). This simulated wounding that can occur at harvest and contributes to increased tuber susceptibility to dry rot development. Tubers are inoculated by spraying each side with 1.6 ml of 2.4×10^5 conidia/ml (50/50 mixture sensitive to resistant) of *Fusarium sambucinum* (primary cause of dry rot in storage). Inoculated tubers were stored at ambient air temperature wrapped in plastic for one-half hour prior to treatment and placed into treatment boxes.

Pink rot: This post-harvest inoculation procedure is designed to mimic the tumbling of healthy and infected (pink rot) tubers that occurs during the harvest and handling operations as tubers were placed into storage. Unwashed tubers (Russet Burbank) were tumbled in a modified cement mixer for 2 minutes as a suspension of 2.5×10^4 zoospores (2.5 ml/lb of potatoes) of *P. erythroseptica* (cause of pink rot) are sprayed onto the tubers. The cement mixer was modified by removing the baffles. Inoculated tubers were stored at ambient air temperature wrapped in plastic for one-half to one hour prior to treatment and/or placing into treatment boxes.

Treatments: Treatments included an untreated control and an application of aerosol smoke. Treatments consisted of four replications of 15 tubers (n=60). Untreated controls were stored in a vented box near the application chamber to maintain similar temperature conditions.

Untreated control treatment: inoculated but not treated.

Aerosol smoke: Plant based pellets (105g of spruce, sugar pine, fir, poplar, and alder blend) are heated until smoldering to release smoke. Smoke is forced by compressed air into the application chamber for one hour and circulated for 20 hours (ambient temperature; approximately 17.2 C) and then vented. Pink rot treatments were placed in small plastic disease boxes and stored at 18 C 95% RH for 8 or 14 days. Dry rot treatments were placed into plastic mesh bags and stored on shelves at 7.2 C for 78 days.

Evaluations:

At the end of the storage period tubers were evaluated for percent incidence and severity of infection. For dry rot, tubers were sliced longitudinally into quarters and evaluated for percent incidence and severity of dry rot infection. For pink rot, tubers were sliced longitudinally into halves and evaluated for percent incidence and severity of pink rot infection.

Results:

Pink Rot

Preliminary study: Untreated control had 48% incidence with 82% severity rating of pink rot (Figure G-1). Smoke significantly reduced incidence and severity of disease to zero. Results from preliminary study led us to explore the potential of smoke as a post-harvest storage treatment for pink rot.

Trial two: Application of aerosol smoke on pink rot inoculated Russet Burbank tubers appeared to have significant suppression of pink rot infection. Untreated control had a higher incidence and severity of disease. Smoke significantly reduced the incidence and severity of disease to zero (Figure G-1; Figure G-2). Multiple studies results indicate aerosol smoke may be an effective candidate for disease control in stored potatoes.

Methodologies to ensure that treatment is causing disease reduction and not the handling of tubers should be explored.

Dry rot

Incidence of Fusarium dry rot infection in smoke treated tubers was greater than that of untreated control (Figure G-4; Figure G-5). However, severity of the infection was significantly lower in smoke treated tubers.

Discussion: Application of aerosol smoke as a post-harvest disease control method for two common storage diseases, pink rot, and Fusarium dry rot, was explored. Preliminary and supplemental studies suggest that smoke application may be an effective post-harvest application to control pink rot infection. Incidence and severity were significantly reduced with the application of aerosol smoke. Further studies are being conducted to confirm the methodology of handling the inoculated tubers.

Preliminary studies indicate that smoke may be beneficial in reducing the severity of Fusarium dry rot. Incidence of disease was significantly greater in smoke treated tubers. This is believed to be due to the phytotoxicity of the smoke on freshly wounded tubers resulting in small infection sites. When minor infection sites are not evaluated (severity less than 5%) then the incidence of infection is

significantly lower in smoke treated tubers. Further investigation needs to be conducted to confirm the efficacy of aerosol smoke as a post-harvest control method for Fusarium dry rot.

Reference

Schisler, D. A., P. J. Slininger, G. E. Kleinkopf, R. J. Bothast, and R. C. Ostrowski. 2000. Biological control of Fusarium dry rot of potato tubers under commercial storage conditions. *American Journal of Potato Research* 77: 29-40.

Figures

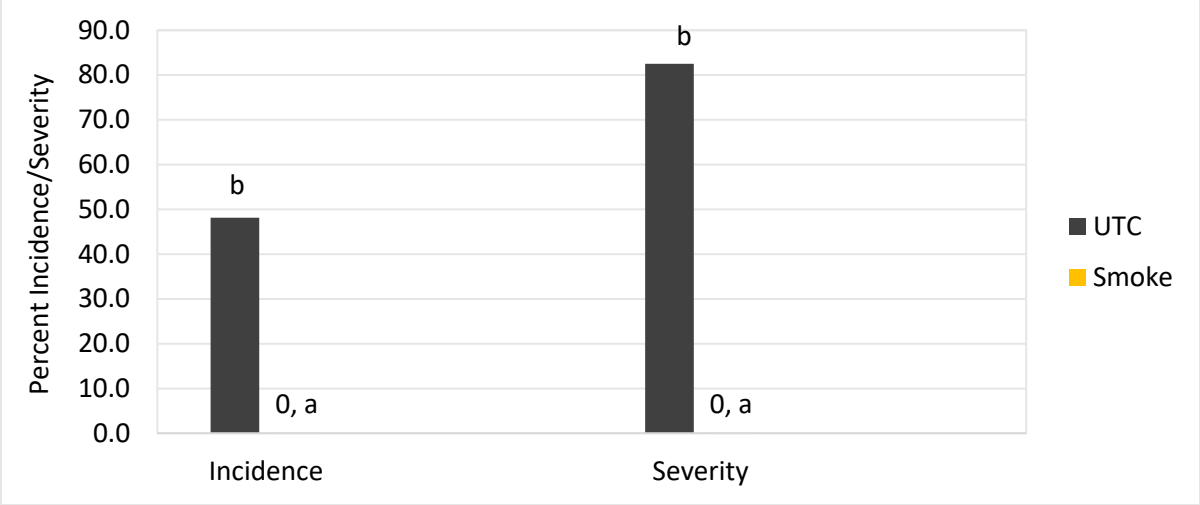

Figure G-1. Preliminary trial incidence and severity of pink rot in Clearwater Russet. Values followed by different letters are significantly different for each variable (α=0.05).

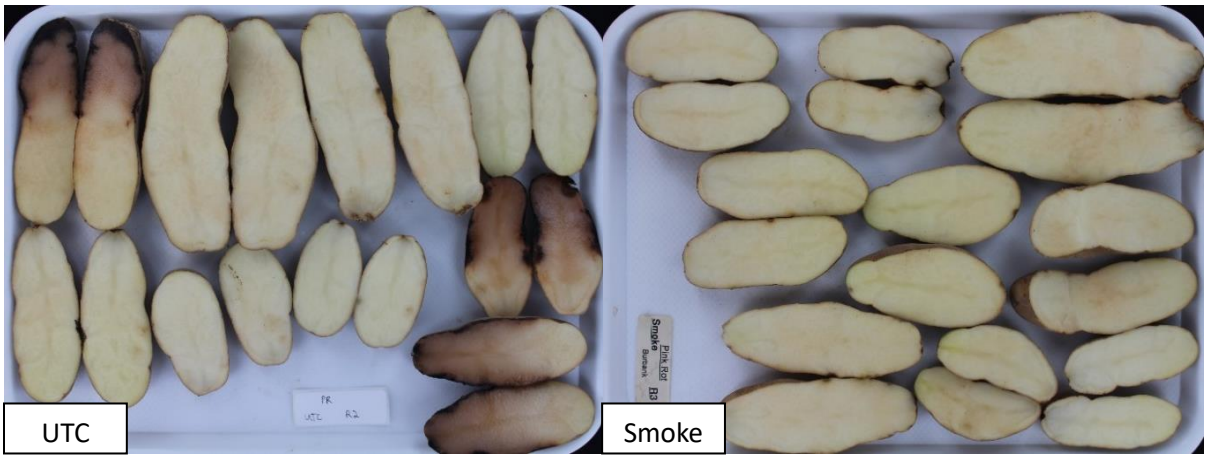

Figure G-2. Russet Burbank pink rot untreated control and smoke treatment 14 days after inoculation.

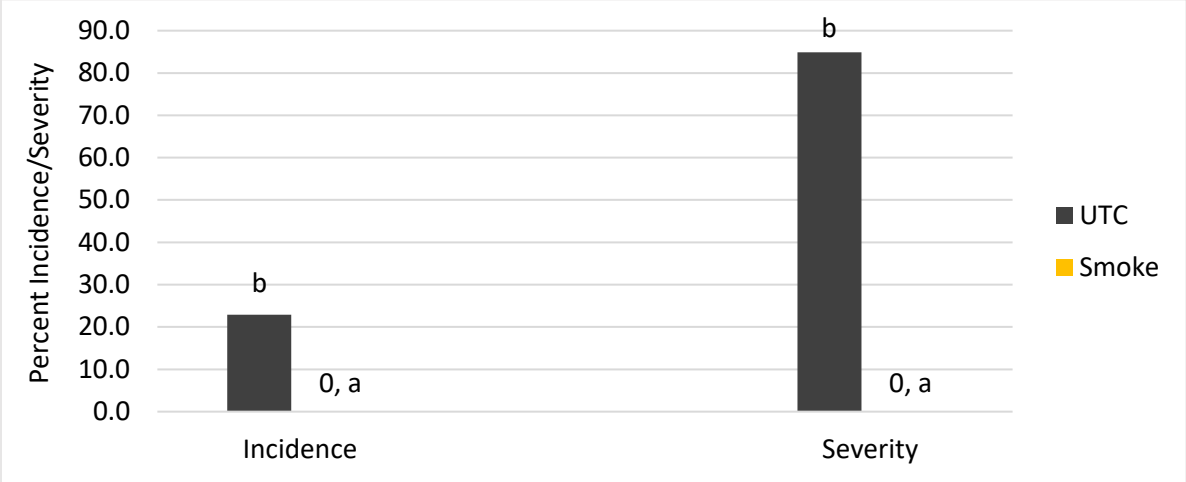

Figure G-3. Russet Burbank pink rot disease incidence and severity of untreated control and smoke treated tubers in trial two. Values followed by different letters are significantly different for each variable (α=0.05).

Figure G-4. Fusarium dry rot incidence of untreated control and smoke treated Russet Burbank.

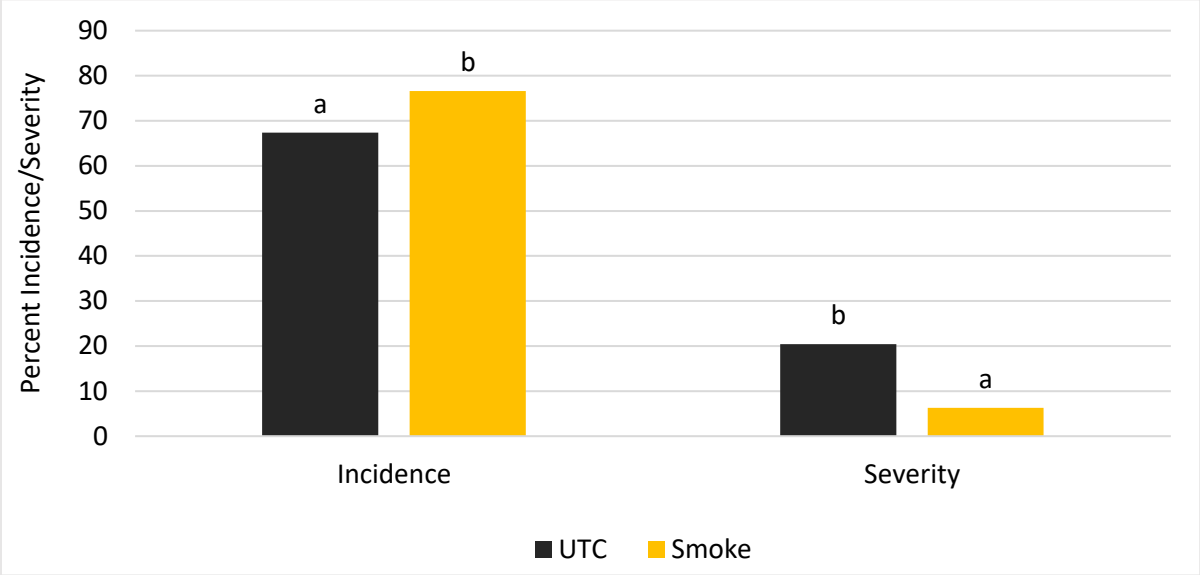

Figure G-5. Fusarium dry rot incidence and severity of untreated control and smoke treated tubers of Russet Burbank over two. Values followed by different letters are significantly different for each variable (α=0.05)

Results indicate that aerosol smoke may be a viable tool to control pink rot and Fusarium dry rot in stored potatoes. However, further research needs to be conducted on the methodology in which pink rot studies are conducted to determine if smoke is causing the reduction of disease or if the lack of humidity in the smoke chamber is affecting the inoculation proceedure. Another study conducted in 2023 indicates it may be the latter, but it is still uncertain if aerosol smoke provides disease control of *P. erythroseptica*.

2022 sugar fry data

Studies were conducted to determine the effects of aerosol smoke on processing quality of Clearwater Russet tubers. An application of aerosol smoke (1 hour injection 20 hour recirculation; 1h 20h) was applied to washed Clearwater Russet tubers held in 8.9 C 95% RH for approximately three weeks.

Sucrose and glucose concentrations were determined from a 10-tuber sample per replicate (3 replicates) using the method of Sowokinos et al. (2000) with modifications. Tubers were cut using a Keen Kut Shoe Stringer French fry cutter. Two hundred grams of tuber tissue collected from the center of the tubers were macerated in an Acme Juicerator (Acme Equipment, Spring Hill, FL). During processing, tuber tissue was washed with 150 mL of sodium-phosphate buffer (0.05 M, pH 7.5) for a final homogenate volume of 275 mL. Glucose and sucrose concentrations were determined using a

YSI model 2900 Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) and expressed on a percent fresh weight basis.

One plank (3.0 cm x 0.8 cm) from each tuber used in the sugar extraction procedure was used for fry color determination (10 strips per replicate). Strips were fried in canola oil at 190.5 C for 3.5 minutes. Fry color was determined within 3 minutes using a model 577 Photovolt Reflection Meter (model 577, Photovolt Instruments Inc., Minneapolis, MN). A green filter was used and calibrated using a black-cavity standard as 0.0% reflectance and a white plaque (Cat. No. 26-570-08) as 99.9% reflectance. Measurements were taken on the bud and stem ends of each strip. A relationship between USDA fry color and photovolt reflectance as measured by our instrument and methodology was previously established. The data produced a scale of a USDA fry color rating where USDA 1 was equal to a 44.0 or greater reflectance rating, a USDA 2 rating was less than 44.0 to 35.0 reflectance reading, a USDA 3 rating was less than 35.0 to 26.0 reflectance reading, and a USDA 4 rating was less than 26.0 reflectance rating. The lower the reflectance measurement, the darker the fry color.

Table G-1. Sucrose and glucose (% fresh weight basis) levels of Clearwater Russet tubers treated with aerosol smoke and untreated control held at 8.9 C.

Treatment	**Sucrose[1]**	**Glucose**
UTC	0.041 a	0.008 a
Smoke	0.085 b	0.041 b
Standard error	0.004	0.003

[1]Values within each column followed by different letters are statistically significant (α=0.05).

Table G-2. Fry color and quality of Clearwater Russet treated with aerosol smoke and untreated control stored at 8.9 C.

	Reflectance[2]		Mottling[3]		Sugar end incidence (%)
Treatment	Bud end	Stem end	Incidence	Severity	
UTC	64.4 a	62.1 a	32.5 b	1.3 b	5 b
Smoke 1h 20h	56.8 b	48.2 b	92.2 b	2.5 b	18 a
LSD	1.3	2.3	34.4	0.6	16.2

[1]Values in the same column followed by the same letter are not significantly different at α=0.05.
[2]USDA fry color rating #1≥ 43, #2 < 43 but ≥ 35, #3 < 35 but ≥26, #4 < 26 reflectance.
[3]Mottling severity: 1=no mottling 2=mild 3=moderate 4=severe

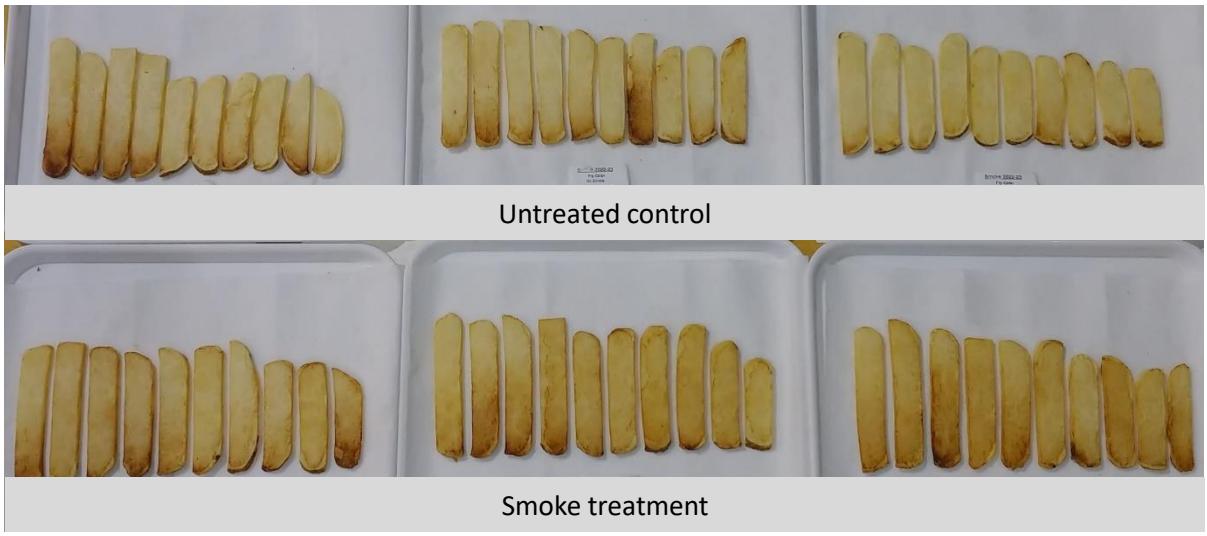

Figure G-6. Planks of Clearwater Russet tubers treated with aerosol smoke and untreated control held at 8.9 C and fried at 190 C for three minutes thirty seconds.

Appendix H. Using Aerosol Smoke as a Pre-Plant Growth Enhancement

A study was initiated to determine if aerosol smoke application one- or two weeks prior to planting would improve plant emergence and overall performance of Clearwater Russet and Russet Burbank (year one only) in a field setting (following approximately 6 month storage). One hour injection 20 hour recirculation period (1h 20h) of cold aerosol smoke was applied to whole seed tubers one or two weeks prior to planting (year one) and held at 12.2 C and 95% RH until planting preparation. In year two treatments were 1) an untreated control maintained at 12.2 C, 2) 1h 20h cold aerosol smoke was applied to whole seed tubers one week prior to planting, and 3) smoke application (1h 20h) followed by a dip in a 20 ppm GA solution for 15 minutes one week prior to planting and held at 12.2 C and 95% RH until planting preparation. Approximately 72 hours prior to planting whole seed tubers were cut into 56 to 85 g seed pieces. Cut seed was planted into 15.3 m single row plots in a completely randomized block design. Crop was grown according to University of Idaho pest, nutrient, and irrigation management practices. Emergence was evaluated periodically beginning 30 days after planting (DAP). The number of stems per plant and final yield and grade were collected.

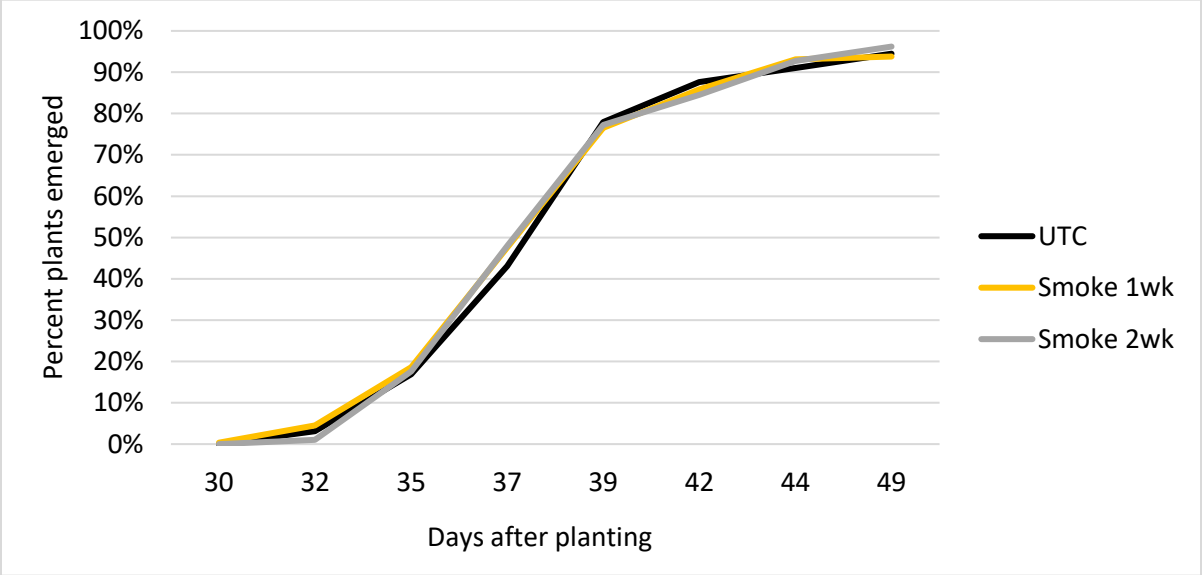

Figure H-1. Emergence over time of Clearwater Russet tubers treated with smoke one or two weeks prior to planting, 2021. No significant differences were observed between treatments (α=0.05).

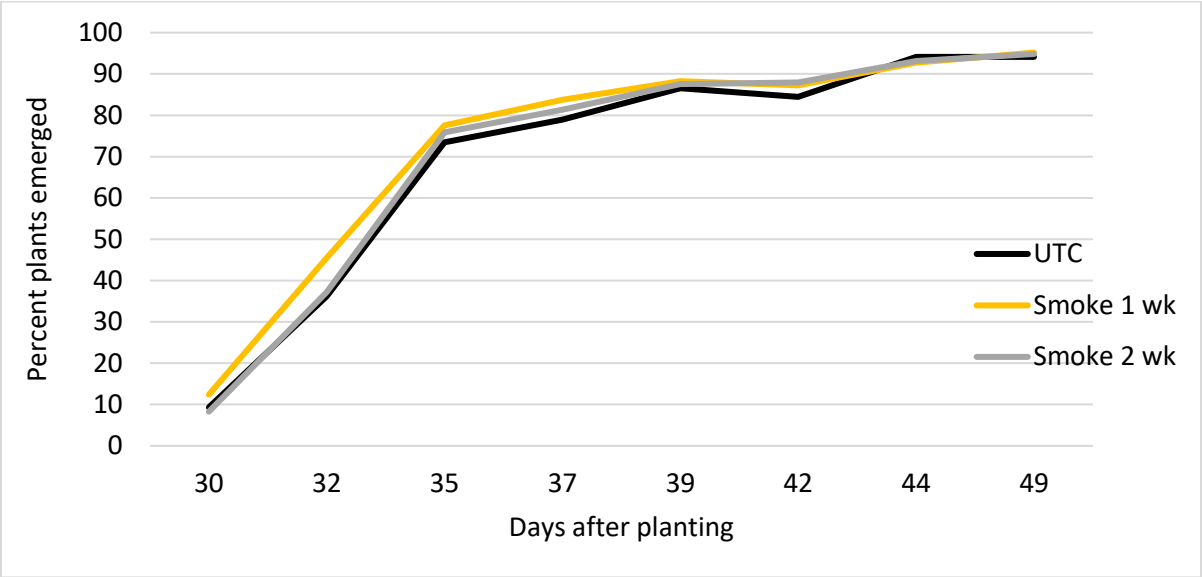

Figure H-2. Emergence over time of Russet Burbank tubers treated with smoke one or two weeks prior to planting, 2021. No significant differences were observed between treatments (α=0.05).

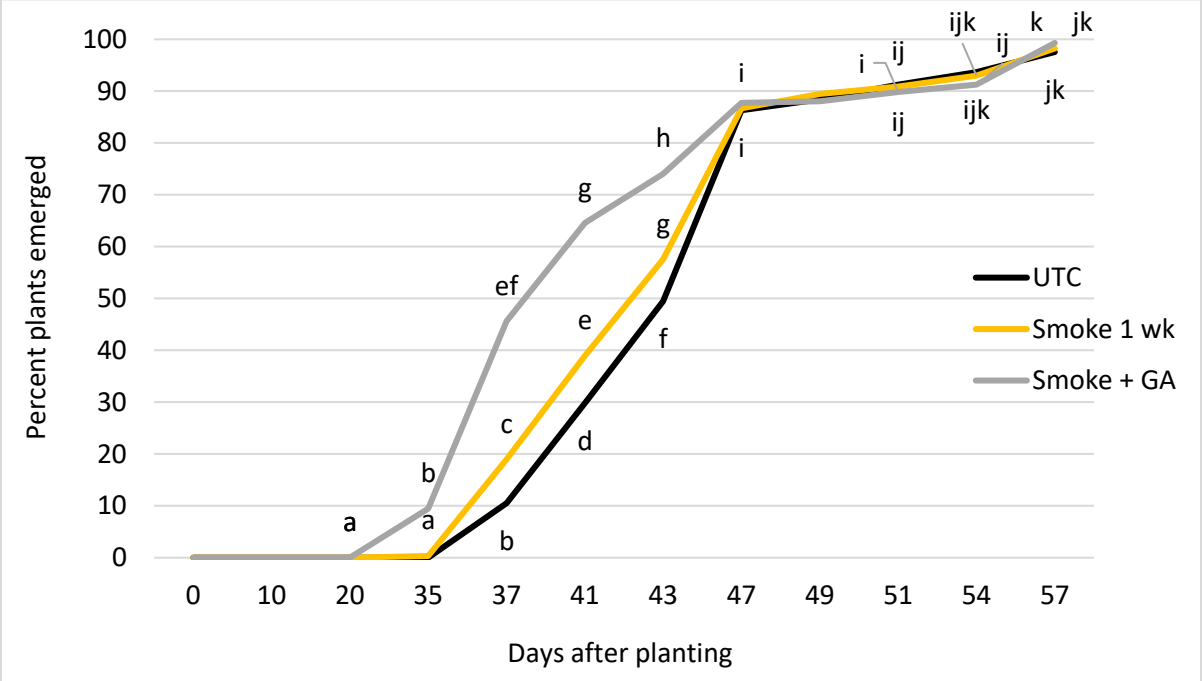

Figure H-3. Emergence over time of Clearwater Russet tubers treated with smoke one week prior to planting or smoke one week prior to planting followed by a dip in 20 ppm GA for 15 minutes, 2021.[1]Values followed by the same letter are not significantly different (α=0.05) within each time period.

Table H-1. Number of emerged stems per plant for Russet Burbank and Clearwater Russet treated with aerosol smoke one or two weeks (1hr injection 20 hour recirculation) prior to planting or smoke one week prior to planting followed by a dip in 20 ppm GA for 15 minutes.

	Number of stems per plant	
Treatment	**Russet Burbank**	**Clearwater Russet**
	2021 stem number per plant[1]	
UTC	2.8 a	3.0 a
Smoke 1 week	2.9 a	3.2 a
Smoke 2 week	3.0 a	3.0 a
P-value	0.341	0.5084
2022 stem number per plant		
UTC	-	2.6 a
Smoke 1 week	-	2.7 a
Smoke + GA dip	-	3.6 b
P-value	-	> 0.0001

[1]Values followed by the same letter are not significantly different (α=0.05) within each column and year. Dashes "-" indicate cultivar was not included in study that year.

Table H-2. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of smoke treated tubers prior to planting of Clearwater Russet in 2021.

	Yield (t/ha-1)						
Treatment	*Total*	*Size profile categories (g)*[1]				*USDA grade*	
	Total Yield	**< 113**	**113-170**	**171-283**	**> 283**	**US no. 1**	**US no. 2**
UTC	60.2 a	9.5 a	13.7 a	22.2 a	14.8 a	58.1 a	2.1 a
1wk	63.4 a	9.5 a	13.8 a	23.4 ab	16.7 a	60.1 a	3.3 a
2wk	63.2 a	9.0 a	14.7 a	25.8 b	13.7 a	60.7 a	2.5 a
Standard error	2.5	0.4	0.7	1.0	2.4	2.4	0.4

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

Table H-3. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of smoke treated tubers of Russet Burbank in 2021.

	Yield (t/ha-1)						
Treatment	*Total*	*Size profile categories (g)*[1]				*USDA grade*	
	Yield	**< 113**	**113-170**	**171-283**	**> 283**	**US no. 1**	**US no. 2**
UTC	72.4 a	8.3 a	11.3 a	27.8 a	25.1 a	58.5 a	14.0 a
1wk	66.7 a	7.9 a	11.4 a	25.0 a	22.4 a	56.5 a	10.2 a
2wk	68.7 a	8.7 a	10.3 a	24.8 a	24.9 a	58.5 a	10.0 a
Standard error	4.1	0.6	0.6	2.0	3.0	3.3	1.9

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

Table H-4. Total harvested tuber yield (t/ha^{-1}), size distribution, and USDA grade of smoke treated tubers of Clearwater Russet 2022.

	Yield (t/ha-1)						
	Total	*Size profile categories (g)*[1]				*USDA grade*	
Treatment	**Yield**	**< 113**	**113-170**	**170-283**	**>283**	**US no. 1**	**US no. 2**
UTC	57.1 a	5.1 a	7.2 a	18.1 a	26.7 b	53.9 b	3.2 a
Smoke 1 wk	55.1 a	5.5 a	7.9 a	17.8 a	23.9 b	51.8 b	3.3 a
Smoke + GA	53.4 a	8.7 b	11.5 b	21.8 a	11.5 a	41.9 a	11.5 b
Standard error	3.4	0.4	0.5	1.4	0.0	2.8	0.8

[1]Values followed by the same letter are not significantly different (α=0.05) within each column and year. Smoke 1 wk: application of 1h smoke 20h circulation one week prior to planting. Smoke + GA: smoke (1h 20h) followed by a dip in 20 ppm GA for 15 minutes one week prior to planting.