Managing and Monitoring Pythium Leak and Shatter Bruise of Russet Potato (*Solanum tuberosum* L.) Cultivars

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AUTHORIZATION TO SUBMIT DISSERTATION

This dissertation of Andrew Hollingshead, submitted for the degree of Doctor of Philosophy with a Major in Plant Sciences and titled "Managing and Monitoring Pythium Leak and Shatter Bruise of Russet Potato (Solanum tuberosum L.) Cultivars," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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ABSTRACT

Leak is a devastating storage disease caused by the opportunistic pathogen, *Pythium ultimum* var. *ultimum*. The disease is identified by dark grey lesions in the flesh of the potato that exude water from damaged cells. Oospores or other propagules typically infect potatoes through mechanical wounds (shatter bruises) during harvest. The disease contributes to loss in storage but also creates ideal environments for secondary pathogens to further decay. It is recommended to manage leak by implementing cultural controls such as harvesting at tuber temperatures below 21°C, avoiding bruise, and establishing a good skin set. The objectives of this research were to examine these general recommendations for several russet cultivars and understand why cultivars may differ in leak and bruise susceptibility and recommend means to prevent both. Specific studies evaluated (1) the effects of both pulp and storage temperature on leak susceptibility among several russet cultivars, (2) the association of leak development of russet cultivars and tuber shatter bruise and mechanical failure properties, (3) the efficiency of bacterial antagonists and other post-harvest fungicides in managing leak, and (4) methods and tools for bruise mitigation programs.

Although potato cultivars vary in susceptibility to leak, the standard recommended control method for all cultivars is to harvest potatoes when pulp temperatures are below 21°C. However, potatoes may be harvested with higher than ideal pulp temperatures or a cultivar may warrant stricter recommendations. Several russet cultivars were equilibrated to pulp temperatures of 12.8, 15.5, 18.3, and 21.1°C, bruised and inoculated with *P. ultimum* then evaluated four days later. To test how leak development in russet cultivars would respond to a change of temperature in storage, tubers were equilibrated to pulp temperatures of 15.5 and 21.1°C, bruised and inoculated then stored at 12.8, 15.5, 18.3, and 21.1°C for four days. Leak susceptibility was influenced by pulp temperature but the storage temperature had an overriding effect if altered from the original pulp temperature. Leak susceptibility differed among cultivars and by temperature. The results highlight the importance of harvesting susceptible cultivars when pulp temperatures are below 15.5℃ or cooling tubers below 15.5℃ when adequate refrigeration or cooling air are available to rapidly modify temperatures in storage.

Pythium ultimum infects a tuber via wounds or shatter bruises, which occur during mechanical harvesting and handling. To evaluate if cultivar susceptibility to leak is related to shatter bruise susceptibility and tuber mechanical failure properties, tubers were bruised and inoculated to mimic the state of potatoes during harvest. Cultivars varied in susceptibility to leak development, shatter bruise, and mechanical failure properties. Although, leak incidence was not highly correlated to shatter bruise area and number, a relationship between leak and mechanical failure properties of the tuber was observed. Shatter bruise incidence was correlated to tissue mechanical failure properties, and these measurements could provide insight into the shatter bruise potential for a cultivar. The prevalence or susceptibility of shatter bruise could not directly explain a cultivars susceptibility to leak and therefore additional research is needed to further understand the relationship between mechanical failure properties and leak.

Post-harvest fungicides can also be important tools in managing storage diseases such as leak. A mixture of bacterial control agents developed by the National Center for Agricultural Utilization Research (NCAUR, USDA-ARS, Peoria, IL) was tested for efficiency against leak compared to other commercial fungicides. The bacterial control agents varied in efficacy of leak control and were not as effective as a commercial chemical fungicide. The bacterial control agent may have contributed to the control of *P. ultimum* when rehydrated with a nutrient medium and mixed with the commercial chemical fungicide; however, *P. ultimum* control was not significantly greater than the conventional fungicide alone. Other post-harvest products studied were not effective and did not differ from the inoculated control. Biological antagonists may be an effective post-harvest tool but more research is warranted to realize its potential in managing leak alone or when mixed with conventional fungicides.

Minimizing bruise should be part of an integrated management program for storage diseases such as leak. Limiting bruise will also help maintain quality of potatoes. A bulletin was written to outline tools and protocols to monitor the potential for bruise within a potato handling operation. Tools include dipping potatoes in iodine to readily see the blue stained shatter bruises. Another tool outlined how to assess for blackspot bruise as the majority of the blackspot bruises could be visible and evaluated within 3 to 5 hours, which is earlier than previous recommendations. Instrumented spheres are another tool used to record damaging

impacts that occur during harvesting and handling operations. These tools and methods help to monitor bruise and aid in making timely mechanical adjustments to further prevent bruise.

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DEDICATION

I dedicate this dissertation to my wife, Whitney, for her patience and love throughout these long years. I also dedicate this dissertation to Anelle, Carwin, and Winston and look forward to spending more of my time with you.

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INTRODUCTION

The United States produced more than 20 million metric tonnes of potatoes in 2018 and lost 1.2 million metric tonnes due to shrinkage and disease in storage (USDA-NASS 2019). Potato tuber impacts sustained at harvest can greatly influence the quality of potatoes from harvest and throughout the storage season. Wounded and bruised tubers are prone to greater weight loss and disease development (Hesen 1960; Hudson and Orr 1977; Lulai and Corsini 1998). Open wounds or shatter bruises are produced by mechanical handling and create ideal environments for diseases such as Pythium leak and Fusarium dry rot, which both can lead to substantial losses in storage (Powelson and Rowe 2008). Leak is caused by the soil-borne oomycete, *Pythium ultimum*, which infection develops dark grey to black lesions associated with water readily leaking out of the tuber flesh (Powelson and Rowe 2008; Salas and Secor 2001). Leak has also been referred to as "shell" rot because in some cases only the cortex and periderm remains after significant decay occurs (Blodgett and Ray 1945).

Control measures for leak are mainly focused on cultural practices such as harvesting while pulp temperatures are below 21°C and minimizing shatter bruising caused in the harvest and handling operations (Powelson and Rowe 2008; Secor and Gudmestad 1999). Metalaxyl or mefenoxam is labeled for use against *P. ultimum* and applied in-furrow or as foliar treatments. However, resistant populations of the disease have been present across the US since 1998 and in the Pacific Northwest since 2004 (Porter et al. 2009; Taylor et al. 2002). Efficacy of metalaxyl or mefenoxam may be dependent upon the distribution of the chemical found within the tuber (Bruin et al. 1982). Bruin et al. (1982) found concentrations of mefenoxam in the following tissues of a potato: 0.055 µg mL^{-1} periderm, 0.034 µg mL^{-1} medullary tissue, and 0.022 µg mL^{-1} in the cortex and vascular ring. The concentrations of mefenoxam found in the tuber tissue was lower than the mean EC_{50} (0.12 µg mL^{-1}, (Taylor et al. 2004). The efficacy of metalaxyl may vary in field trials due to soil moisture (Kirk et al. 2001), active ingredient concentration in tubers (Barak et al. 1984; Bruin et al. 1982), and resistant isolates (Porter et al. 2009).

Taylor et al. (2004) observed a 17% reduction in leak incidence when metalaxyl was applied and questioned if cultural controls would be more efficient for controlling leak. The

primary recommendation was to stop harvesting when pulp temperatures reached 21°C. However, potatoes have been observed to be infected with leak at temperatures below 21°C or potatoes may be below 21°C at harvest but warm above that temperature as they are windrowed, transported, or in storage prior to cooling ventilation. Goss and Jensen (1944) observed 'water rot' symptoms on tubers coming out of the field with initial infection primarily on the stem end. They also noted that temperatures of 5 to 10°C had less leak than tubers at 20 to 30°C, but it was not specified if that was air or pulp temperature. The ideal temperature for *P. ultimum* growth and propagation is above 21°C (Triki et al. 2001). Potatoes can transition from a warmer pulp temperature in the field to a cooler temperature in storage with the goal to reach ideal wound healing temperatures between 10 and 13°C (Kleinkopf 1995). The effects of pulp and storage temperatures and shatter bruise susceptibility are two factors that have not been studied for disease management of leak. Chapter 1 examined how pulp and storage temperatures impact leak susceptibility of russet cultivars as potatoes are warmed, maintained, or cooled after infection.

Leak development will not occur unless a shatter bruise or entry point is available for *P. ultimum* to infect. The susceptibility of a cultivar to leak may be influenced by the cultivar susceptibility to shatter bruise (Salas et al. 2003; Taylor et al. 2008; Taylor et al. 2004). Mechanical harvesting can cause the periderm of a potato to break resulting in a shatter bruise. Hawkins and Harvey (1919) showed cultivars that were able to resist greater pressure before the periderm broke were also more resistant to leak. However, the relationship between leak, shatter bruise and tuber mechanical failure properties have not been further studied but could aid in assessing cultivars for disease and bruise susceptibility.

Cultivar release publications often include the susceptibility to bruising (Novy et al. 2002; Novy et al. 2014; Novy et al. 2010; Novy et al. 2017). Spear et al. (2017) evaluated differences in blackspot and shatter bruise susceptibility among several cultivars used in the fresh pack industry. Cultivar resistance to physical damage has been measured using dynamic forces for two cultivars, Russet Burbank and Atlantic (Bajema et al. 1998a; Bajema and Hyde 1998; Bajema et al. 1998b; 1998c; 1998d). Many of these studies observed hydration, maturity, and nutrition affected mechanical failure properties that may influence bruising, however, correlations where not reported. Understanding these relationships would help to manage disease and shatter bruise for specific cultivars.

The mechanical failure properties of cultivars were evaluated by Castleberry and Jayanty (2017) using a static force to measure the varying hardness or pressure the tuber could resist based on hydration levels of tubers. The purpose of this study was to predict the likelihood of pressure bruise forming in storage. Approximately 55% of the variability in deformation could explain pressure flattening (Castleberry and Jayanty 2017). These mechanical failure properties provide important information to further study the physio-chemical properties of potatoes and how it may relate to shatter bruise susceptibility. Understanding the mechanical properties would lead to further questions regarding cell wall structure and how pectin plays a role in strengthening the cells and resisting shatter bruising.

Post-harvest fungicides can be applied as additional protection to potatoes going into storage when the risk of leak is high. There are several fungicides available for post-harvest use that are labeled for leak or other diseases, but control of the disease with the products can vary. Some of the common fungicides labeled for storage diseases are azoxystrobin mixed with fludioxonil, and difenoconazole (Gachango et al. 2012a; Gachango et al. 2012b), a mixture of hydrogen peroxide and peroxyacetic acid (Afek et al. 2001), phosphorous acid (Inglis et al. 2004; Miller et al. 2006), and *Pseudomonas syringae* (Al-Mughrabi et al. 2013). Novel biological control agents (BCA) have been developed by the National Center for Agricultural Utilization Research (NCAUR, USDA-ARS, Peoria, IL). Three strains of *Pseudomonas fluorescens* have been identified to reduce diseases caused by oomycetes such as *Phytophthora erythroseptica* (Schisler et al. 2009), and *Phytophthora infestans* (Slininger et al. 2007), but has not been tested against *Pythium ultimum*. *Fusarium sambucinum* is not an oomycete but BCAs have been shown to have effiacy aginst dry rot development in potatoes (Schisler et al. 1997; Slininger et al. 2001). These new BCAs are promising in controlling disease and decreasing the risk of fungicide resistance among pathogens due to having multiple modes of action for disease control; for example, BCAs can compete for nutrients (Elad and Chet 1987) and secrete anti-microbial metabolites that prevent the pathogen from infecting the host (Brodhagen et al. 2004). BCAs are not as efficient as conventional fungicides, but control has been observed when BCAs and conventional fungicides are mixed (Al-Mughrabi et al. 2013). The variability and lack of control by applied chemicals against oomycete diseases demonstrated a need to investigate cultural practices, biological agents, and/or fungicides to provide a complete integrated management program.

Shatter bruises are consequences of mechanical harvest and handling that can lead to greater weight loss and provide infection points for disease development from *P. ultimum*, *Fusarimum sambucinum*, and *Pectobacterium* species (Hesen 1960; Lulai and Corsini 1998; Taylor et al. 2004). As a result, tools have been developed to monitor for bruising. These tools have consisted of chemicals, instrumented spheres, and methods to identify and monitor bruises. In the past, shatter bruise could be detected with using a spray or dip application of catechol (Iritani 1968; Oleary and Iritani 1969), but the chemical has been identified as a possible carcinogen causing issues with safety and waste management. The apple industry has used iodine to determine harvest maturity; iodine reacts with starch and as apples mature starch is converted to sugar (Smith et al. 1979). This method could also be applied to the potato industry as starch is freed from cells when shatter bruises occur. Low concentration iodine solutions, used as anti-sceptics for livestock, are easily purchased from farm and ranch stores. These iodine solutions are safe to use, will stain shatter bruises blue, and there is no need to peel the potato. The use of iodine solutions can be a part of bruise prevention program to monitor shatter bruises during handling.

Instrumented spheres have become more popular with growers and the industry, but the data can be difficult to synthesize into a recommended action to reduce bruising. Instrumented spheres measure the acceleration a potato experiences during an impact or when the tuber hits an object (Zapp et al. 1990). There are a number of models on the market and each one is different in size, shape, make, and software. Comparing the results of one to another should not be done. Recommendations for maximum acceleration are made based on the cultivar Russet Burbank. Not all cultivars bruise the same, so by using recommendations meant for Russet Burbank operators may be allowing too high of impacts to occur if the cultivar being harvested is more susceptible to bruising. The result may be an increase in the risk of disease or weight loss in storage. Quantifying shatter bruise and blackspot bruise can help validate instrumented sphere data and set appropriate maximum acceleration thresholds to limit bruise. Chapter 4 is an extension bulletin to help the industry use the methods and tools for safe, quick, and validated information for bruise prevention among the different cultivars produced in the Pacific Northwest.

In order to successfully inoculate potato tubers with *P. ultimum*, a consistent and realistic method was needed to be developed. Previously, inoculation methods for *Pythium*

ultimum have included cutting out plugs of tubers and placing *P. ultimum* on agar into the wound (Salas et al. 2003) or abrasively wounding potatoes and soaking potatoes in a liquid batch of *P. ultimum* (Gachango et al. 2012b; Wharton et al. 2011). *Pythium ultimum* usually infects wounds sustained at harvest; therefore, a procedure was developed to mimic natural infection and studies were conducted to develop a protocol and is reported in Appendix A. The studies evaluated bruising methods, when to apply the inoculum, the impact on passive airflow, the necessary inoculum level, proper evaluation times, and when to evaluate for symptoms. Mimicking the natural harvest conditions made it possible to test cultural controls such as temperature and airflow in storage (Appendix B). Shatter bruise is also necessary for leak to infect tubers and was quantified in chapter 2 based on preliminary data (Appendix C). Questions have also arisen on the effectiveness of the foliar fungicide phosphorous acid against leak and a preliminary study is reported in Appendix D. The preliminary studies described in the Appendix A through D helped establish the research of the dissertation.

A strong integrated management program for leak should focus on all three parts of the disease triangle: host, pathogen, and environment. This dissertation studied each of the three factors to provide tools and recommendations for managing leak in potatoes. The first chapter focused on the effects harvest temperature and the ability to cool potatoes in storage would have on leak susceptibility among cultivars. Modifying the temperature changed the environment to be less favorable for the pathogen to infect potatoes. Chapter 2 focused on the host and how shatter bruise and tuber textural properties may impact disease development. Findings from this study have the potential to identify cultivars that are more resistant to shatter bruise and leak. By applying post-harvest fungicides, biological agents or conventional chemistries, *P. ultimum* cannot divide or compete for nutrients and disease may be reduced as discussed in Chapter 3. In the final chapter (Chapter 4), the tools developed from science-based research can produce reliable information to make decisions resulting in fewer defects and losses. This dissertation furthers research and understanding of the management of leak and areas to be exploited for future control.

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Chapter 1 : POTATO CULTIVAR SUSCEPTIBILITY TO PYTHIUM LEAK AS INFLUENCED BY HARVEST AND EARLY STORAGE TEMPERATURES

Hollingshead, A.K., N.L. Olsen, M. Thornton, J.S. Miller, and A.H.M. Lin. 2020. Potato cultivar susceptibility to Pythium leak as influenced by harvest and early storage temperatures. *American Journal of Potato Research*. doi:10.1007/s12230-020-09769-1.

ABSTRACT

Leak is caused primarily by the fungus-like organism *Pythium ultimum,* which can cause severe crop loss in storage. Previous research has shown temperature as a major contributing factor to the incidence of leak in stored potatoes. The hypothesis that pulp and early storage temperatures affect cultivar susceptibility of leak has not been studied. The objective of this study was to determine the leak susceptibility of multiple russet-skinned potato cultivars and understand how leak susceptibility is affected by pulp and early storage temperatures. To accomplish the objective, Russet Norkotah selections (CO-3, TXNS-112, TXNS-278, TXNS-296, and Standard) were bruised and inoculated with a dilute spray solution of oospores of *P. ultimum* and stored at 21.1℃for four days to determine susceptibility between selections. To understand how pulp temperature affects cultivar susceptibility, pulp temperatures of Bannock Russet, Clearwater Russet, Russet Burbank, Ranger Russet, Teton Russet, and Umatilla Russet tubers were equilibrated to 12.8, 15.5, 18.3, and 21.1℃ bruised, inoculated, and maintained at the same temperatures for four days. To address the effect of pulp and early storage temperature, cultivars Bannock Russet, Russet Norkotah CO-3, Russet Burbank, and Umatilla Russet tubers at tuber pulp temperatures of 15.5 or 21.1℃, were bruised, inoculated, and then held for 4 days at early storage temperatures of 12.8, 15.5, 18.3, and 21.1℃ to simulate the ability to alter pulp temperatures with storage conditions. Selections of Russet Norkotah were similar in leak incidence; however Standard Russet Norkotah was significantly more susceptible. The impact of pulp temperature at bruising and inoculation showed all cultivars were significantly less susceptible to leak (19 to 63% incidence) at 12.8℃ than at 21.1°C (72 to 93% incidence). When pulp temperatures were 15.5 or 21.1°C at bruising and inoculation and then exposed to early storage temperatures of 12.8, 15.5, 18.3, and 21.1°C, leak incidence

was 11, 34, 59, and 74%, respectively; indicating the overriding impact that immediately cooling potatoes, opposed to initial tuber pulp temperatures, has on leak development. Cultivar susceptibility of leak was affected by temperature. Results highlight the importance of growing cultivars that are less susceptible to leak and to harvest susceptible cultivars when pulp temperatures are below 15.5℃ or cool tubers below 15.5℃ when adequate refrigeration or cooling air are available to rapidly modify temperatures in storage.

Keywords: *Pythium ultimum*, Cultural management, Bruise, Refrigeration

INTRODUCTION

Storage of potatoes (*Solanum tuberosum* L.) is required to maintain the quality of a crop for year-round industry use (Voss 2016). The presence of disease can impact the duration potato tubers can be stored. Pythium leak is a common disease in potato producing areas (Salas and Secor 2001) with *Pythium ultimum* Trow var. *ultimum* (Barr et al. 1996; Porter et al. 2009) being the primary pathogen causing the disease in cooler climates (Powelson and Rowe 2008). Severe crop losses have been reported due to tuber decay in storage (Jones 1935; Powelson and Rowe 2008). Infected tubers become soft and tissue appears smoky grey or brown in color. When the lesion is pressed, dark liquid will leak or drip out of the tissue (Hawkins 1916; Jones 1935; Salas and Secor 2001). Leak is not likely to spread between tubers in storage because *P. ultimum* rarely produces zoospores, the motile asexual spores of oomycetes (Barr et al. 1996; Salas and Secor 2001; Strand 2006). However, infected and decaying tubers can promote other disease development due to the increase of available moisture leaked from damaged tubers. The moistened environment fosters other diseases, such as bacterial soft rot caused by *Pectobacterium* spp., which can infect healthy tubers in storage (Charkowski 2015; Lambert et al. 2005; Powelson and Rowe 2008).

Pythium ultimum needs an entry or opening point on the tuber to infect, which is most often the result of wounding (Blodgett and Ray 1945; Hawkins 1916; Salas and Secor 2001; Secor and Gudmestad 1999; Taylor et al. 2004). Mechanical damage at harvest is the most common source of wounding (Hesen 1960; Hudson and Orr 1977), resulting in shatter bruises and entry points for *P. ultimum* infection and subsequent storage decay (Blodgett and Ray 1945; Hudson and Orr 1977; Taylor et al. 2004). Potatoes without wounds are less likely to develop leak (Taylor et al. 2004). Leak has also been observed in tubers coming out of the field possibly caused by enlarged lenticels, insect damage, or other types of damage.

Chemical fungicides can be applied in the field to protect tubers from leak and other diseases. Mefenoxam (R enantiomer of metalaxyl) is a systemic fungicide used against oomycetes such as *P. ultimum* (Salas and Secor 2001), *Phytophthora infestans* (Bruck et al. 1980), and *Phytophthora erythroseptica* (Peters et al. 2001). Mefenoxam can be applied as an in-furrow or foliar application to control leak, but effectiveness against leak has been inconsistent (James and Stevenson 1999; Kirk et al. 2001b; Mulrooney 1982, 1998; Platt et al. 2003; Taylor et al. 2008; Taylor et al. 2004). The inconsistency of mefenoxam may be due to

variable soil moisture (Kirk et al. 2001b), the lack of active ingredient in tuber tissue (Barak et al. 1984; Bruin et al. 1982), or the presence of resistant isolates (Porter et al. 2009). Because leak is difficult to control using field-applied fungicides, recommendations for management focus on the integration of cultural practices. Those practices include planting resistant cultivars, harvesting when tuber pulp temperatures are below 21°C, minimizing shatter bruise, and promoting good skin set (Taylor et al. 2004).

Susceptibility of infection by *P. ultimum* differs among cultivars. Cultivars such as Snowden, FL-1625, and FL1867 showed lower leak incidence compared to Russet Burbank, Russet Norkotah, and Ranger Russet (Salas et al. 2003). Taylor et al. (2008) observed similar results, Russet Norkotah and Russet Burbank are more susceptible to leak than Snowden. Some clones with wild germplasm were observed to be resistant to leak but resistance was not found in the most commonly grown cultivars (Thompson et al. 2007). Hawkins and Harvey (1919) observed cultivar susceptibility to leak was associated with susceptibility to puncture damage. They theorized shatter bruise susceptibility may correspond to leak susceptibility. Cultivar screening for leak and other disease resistance caused by oomycetes can help in management decisions to decrease the risk of disease (Yellareddygari et al. 2019).

In conjunction with minimizing mechanical damage and shatter bruise, harvesting tubers at relatively low pulp temperatures is another method to manage leak (Goss and Jensen 1944; Hawkins 1916; Jones 1935). Pulp temperature is the internal temperature of a potato, which is recommended to be below 21°C during harvest (Gudmestad et al. 2007; Salas and Secor 2001). As temperatures drop below 20°C, Pythium leak infection diminishes, and *in vitro* growth is slowed (Goss and Jensen 1944; Hawkins 1916; Jones 1935; Triki et al. 2001).

Weather conditions can be variable at harvest and high pulp temperatures are not always avoidable. Higher temperatures at harvest translate into a greater need to remove field heat from potatoes once in storage. Higher temperatures also indicate the potential inability to utilize outside air to cool potatoes. Refrigeration can be used to cool potatoes and remove field heat and heat of respiration, which may help in reducing leak infection. The initial removal of field heat should decrease pulp temperatures to 10 to 15.5°C to limit disease and yet maintain temperatures that promote wound healing (ASABE Standards 2018; Kays and Paull 2004; Kleinkopf 1995).

The risk of leak development is not well understood for many of the russet-skinned

cultivars grown in the Pacific Northwest of the United States. Additionally, previously cited studies related to leak development at different temperatures did not include the possibility of cooling potatoes in storage to manage leak development. Understanding the susceptibility of commonly grown potato cultivars to leak and how temperature affects disease susceptibility would help in disease management and increase the overall quality of potatoes out of storage. The objective of this study was to determine how harvest pulp temperature and early storage temperature affect leak susceptibility in multiple potato cultivars.

MATERIALS AND METHODS

Isolate Maintenance and Preparation

A culture of *Pythium ultimum* var. *ultimum*, identified as isolate 09 MN 10–5, was obtained from Dr. Neil Gudmestad's lab at North Dakota State University (Fargo, ND) and used throughout all experiments. It was maintained on P_5ARP media (Jeffers and Martin 1986) and grown in VWR Diurnal Growth Chamber, model 2015 set to 21.1 ± 0.2 °C (VWR International, Cornelius, OR). *Pythium ultimum* was grown in a clarified tomato juice broth, using Campbell's V8 juice and calcium carbonate (0.01 g mL^{-1} of juice). The mixture was centrifuged for five min at 10,000 rpm (Sorvall RC-6 plus, Thermo Scientific). One-hundred mL of clarified V8 juice was added to 900 mL of de-ionized water. The broth was sterilized then cooled for use or stored at 4°C. Fifty plugs of *P. ultimum* were added to 1 L of broth and allowed to grow for 10 to 14 days at 21.1°C and shaken at 120 ± 1 rpm (New Brunswick Innova 2000, Eppendorf, Hamburg, Germany). After the pathogen grew for 10 to 14 days, mycelial mats were strained from the broth and placed in a Waring commercial blender (model 91–21.15, Torrington, CT) with 200 mL of deionized water, then homogenized for three minutes to then be adjusted to the intended inoculum concentration for trials.

Potato Cultivar Production and Storage

Six cultivars (Bannock Russet, Clearwater Russet, Ranger Russet, Russet Burbank, Teton Russet, and Umatilla Russet) were grown at the University of Idaho Kimberly Research and Extension Center (KREC; Kimberly, ID) in 2016. Cultivars were grown according to recommendations for south central Idaho (Stark and Love 2003). Russet Norkotah (selection CO-3) tubers were obtained from a commercial grower located in Rupert, Idaho. In 2017, Russet Norkotah selections (CO-3, TXNS-112, TXNS-278, TXNS-296, and Standard) were

grown at KREC following recommendations for south central Idaho. Cultivars and Russet Norkotah selections grown at KREC were sorted into storage bins at harvest and cured at 12.7 ± 0.15°C with 95% relative humidity (RH) for 17 days. The temperature was then ramped down to a holding temperature of 5.6°C (95% RH) at a rate of 0.3°C per day. Stored potatoes were used in the following studies. All studies included unwashed tubers sized to 170 to 340 g, which were kept at 95% RH before and after inoculation for all studies. Tubers ($n = 20$) were bruised for 90 s and inoculum was applied after 60 s of bruising in a cement mixer. Tubers were examined for leak incidence and severity four days after inoculation in each study.

Leak Susceptibility of Russet Norkotah Selections

Russet Norkotah selections (CO-3, TXNS-112, TXNS-278, TXNS-296, and Standard) were inoculated with *P. ultimum* to determine differences in selection susceptibility to *Pythium* infection. Pulp temperatures were maintained at 21.1°C before and after inoculation. This study consisted of two randomized complete block trials with four blocks (20 tubers for each treatment in a block). Inoculum was applied at a rate of 6.6 mL kg^{-1} using an air-assisted sprayer. The oospore concentration of the first trial was adjusted to $3.1x10^5$ oospores mL^{-1} and $2.5x10^5$ oospores mL^{-1} for the second trial.

Leak Susceptibility of Russet Cultivars Influenced by Maintained Pulp Temperature

This study was to test how susceptibility of leak among russet cultivars was influenced when pulp temperatures from harvest are maintained into storage. Bannock Russet, Clearwater Russet, Ranger Russet, Russet Burbank, Teton Russet, and Umatilla Russet potatoes were warmed to pulp temperature treatments of 12.8, 15.5, 18.3, and 21.1 ± 0.5°C in growth chambers (VWR International, Cornelius, Oregon, and Percival Scientific, Perry, IA). Inoculum ($2.5x10^5$ oospores mL^{-1}) was applied with an air-assisted sprayer at a rate of 2 mL $tuber^{-1}$. After inoculation, tubers were maintained at the same pulp temperature treatments. The experimental design was a 4x6 factorial randomized split-plot design repeated four times with one block.

Effect of Pulp Temperature, Early Storage Temperature and Cultivar on Leak Susceptibility

To complement the previous study, the question on leak susceptibility among cultivars as influenced by pulp and early storage temperatures was studied. This study used four russet cultivars (Bannock Russet, Russet Burbank, Umatilla Russet and Russet Norkotah CO-3), with

pulp temperatures of 15.5 and 21.1 ± 0.1℃. Inoculum was applied to wounded tubers as described previously at a concentration of $2.5x10^5$ oospores mL^{-1} using an air-assisted sprayer at a rate of 2 mL $tuber^{-1}$. Tubers were then moved to growth chambers set to 12.8, 15.5, 18.3, and 21.1 ± 0.5°C with 95% RH for four days to mimic early storage temperatures. The experimental design was a 2x4x4 factorial randomized split-plot design repeated four times with only one block each time.

Disease Evaluation

Four days after inoculation, tubers were evaluated for disease incidence and severity by cutting in half longitudinally. Percent severity was determined by visually estimating the lesion area of the halved tuber. Cultivar susceptibility to leak was defined by resistant (0 to 25% incidence), moderately resistant (26 to 50% incidence), moderately susceptible (51 to 75% incidence), and susceptible (76 to 100% incidence) at different pulp temperatures and early storage temperatures (adopted from Taylor et al. 2008 and Thompson et al. 2007).

Statistical Analysis

The incidence response of *Pythium* infected tubers was used to test probabilities of leak infection for different cultivars and selections inoculated and stored at set temperatures with logistic regression and analysis of deviance ($\alpha = 0.05$) for binomial data. Estimated marginal probabilities of leak incidence were compared using Tukey HSD adjustment ($\alpha = 0.05$) and reported as incidence (Piepho 2004; Searle et al. 1980). Weighted severity, referred to as severity, was analyzed using the mixed effects linear regression and analysis of deviance, and only included diseased tubers. Estimated marginal means of leak severity were compared using Tukey HSD adjustment ($\alpha = 0.05$). The logit transformation of leak incidence and the logit transformation of weighted leak severity were tested for association using Pearson's correlation (Salas et al. 2003). All functions and packages were performed using the R-package in RStudio (Bates et al. 2015; Lenth 2019; R Core Team 2013).

RESULTS

Leak Susceptibility of Russet Norkotah Selections

Five selections of Russet Norkotah were evaluated in two repeated trials for susceptibility to leak. Combining all Russet Norkotah selections, leak severity and leak incidence were 44% and 58%, respectively in the first trial and 36% and 26%, respectively in

the second trial. The two repeated trials were significantly different ($P = 0.044$), however comparisons between Russet Norkotah selections were similar, and therefore the trials were combined (Table 1.1). Selections of Russet Norkotah were significantly different in leak severity ($P < 0.0001$) and incidence ($P = 0.0003$). Standard Russet Norkotah had significantly greater severity (52%) and incidence (58%) compared to other selections of Russet Norkotah, which ranged from 34 to 41% for leak severity and 35 to 40% for leak incidence.

Leak Susceptibility of Russet Cultivars Influenced by Maintained Pulp Temperature

Leak incidence and severity of six russet cultivars with different pulp temperatures were examined and the correlation between leak as influenced by cultivar and pulp temperature were analyzed (Table 1.2). The six selected cultivars were significantly ($P < 0.0001$) different in leak incidence. Bannock Russet, Clearwater Russet, and Teton Russet had a higher leak incidence, with a range of 76 to 81%, compared to Ranger Russet, Russet Burbank, and Umatilla Russet (51 to 62%). Pulp temperature was highly significant ($P < 0.0001$) with leak incidence increasing from 35 to 86% when pulp temperatures increased from 12.8 to 21.1°C. The interaction between cultivar and tuber pulp temperature was not significant ($P = 0.44$), which indicates leak incidence increased in a similar manner in all cultivars in response to higher temperatures.

The severity of leak was significantly ($P < 0.0001$) influenced by pulp temperature, but there was no significant ($P = 0.78$) cultivar effect on severity (Figure 1.1). Leak severity was 8, 20, 41, and 57% when pulp temperatures were 12.8, 15.5, 18.3, and 21.1°C, respectively. The severity of leak varied by cultivar and pulp temperature as indicated by a significant interaction ($P < 0.0001$). At 12.8°C, there were no differences in leak severity among cultivars (Figure 1.1). However, when pulp temperature was increased to 15.5°C, Bannock Russet had a severity of 33%, which was significantly higher than the other cultivars, which ranged from 14 to 20% (Figure 1.1). When pulp temperature further increased to 18.3°C, leak severity for Bannock Russet was similar to Clearwater Russet and Russet Burbank, which was significantly higher than Ranger Russet, Teton Russet, and Umatilla Russet. At 21.1°C, Bannock Russet, had the highest leak severity of 82% while Umatilla Russet had the lowest severity of 23%; the remaining cultivars were not significantly different in leak severity, which ranged from 55 to 63%. It is worth noting that Umatilla Russet, which unlike other cultivars,

did not significantly increase in leak severity with increasing pulp temperatures (Figure 1.1).

Leak incidence and weighted leak severity were strongly correlated ($r = 0.76$), therefore were combined to serve as indices to present the interaction of disease and host at different temperatures (Taylor et al. 2008; Figure 1.2). This index is defined as the susceptibility of leak by incidence. As the pulp temperatures increased from 12.8 to 21.1°C, cultivar susceptibility to leak increased from moderately resistant to susceptible (Figure 1.2). At 18.3°C Bannock Russet, Clearwater Russet, and Teton Russet were susceptible and Ranger Russet, Russet Burbank, and Umatilla Russet were moderately susceptible to leak. The effect of pulp temperature on cultivar susceptibility provides evidence that pulp temperatures should be below 15.5°C to minimize the risk of leak.

Leak Susceptibility Among Cultivars When Pulp Temperatures are Adjusted by Early Storage Temperature

This study was to understand how leak susceptibility among cultivars would be influenced when pulp temperatures are adjusted due to early storage temperatures. Pulp temperature as the main effect was not significant in influencing leak incidence ($P = 0.08$). The interaction of harvest pulp temperature and cultivar on leak incidence was also not significant ($P = 0.07$). However, at a $\alpha = 0.1$ pulp temperature and the interaction would be considered significant. The trend for leak incidence in Russet Norkotah CO-3 increased when harvest pulp temperature increased from 15.5 to 21.1°C, while it decreased in the other three cultivars resulting in the interaction at $\alpha = 0.1$ (Table 1.3).

Leak incidence was significantly ($P < 0.0001$) affected by cultivar. Leak incidence was significantly lower for Russet Norkotah CO-3 (20%) compared to other cultivars (Table 1.3). The highest incidence of leak was observed in Bannock Russet (63%) followed by Umatilla Russet (49%) and Russet Burbank (38%). Early storage temperature had a significant ($P < 0.001$) effect on the incidence of leak. Leak incidence was 11% at 12.8°C and increased to 74% at 21.1°C early storage temperature (Table 1.3).

Leak severity was not affected ($P = 0.27$) by harvest pulp temperature. Early storage temperature significantly ($P < 0.001$) impacted the severity of leak (Figure 1.3). Leak severity was 8, 12, 28, and 42% at early storage temperatures of 12.8, 15.5, 18.3 and 21.1°C, respectively. Cultivar was not a significant ($P = 0.98$) factor, but the interaction between cultivar and early storage temperature was significant ($P < 0.0001$). Cultivar leak severity

significantly increased as early storage temperatures increased. Incidence of leak was very low in Russet Norkotah CO-3 with an early storage temperature of 12.8°C and only two infected tubers, thus causing the confidence interval of leak severity to be large and not significantly different from other temperatures or cultivars (Figure 1.3). Leak severity of Umatilla Russet did not increase in severity as early storage temperature increased. The association between leak incidence and leak severity was strongly correlated ($r = 0.77$).

The relative susceptibilities show that all cultivars were resistant to leak at 12.8°C, but relative susceptibilities increased as early storage temperature increased (Figure 1.4). Bannock Russet changed from resistant at 12.8°C to moderately susceptible to leak at 15.5°C. Russet Burbank and Umatilla Russet were moderately susceptible when early storage temperatures reached 18.3°C. Russet Norkotah CO-3 was considered moderately resistant to leak when early storage temperature was 21.1°C (Figure 1.4).

DISCUSSION

Potato cultivars grown in the United States have previously been reported to show variability in susceptibility to leak (Salas et al. 2003; Taylor et al. 2008; Thompson et al. 2007). This study observed differences in leak incidence and severity among russet cultivars grown in Idaho and pulp and early storage temperatures affected the degree of susceptibility. The degree of susceptibility can be managed by harvesting and storing cultivars in conditions where tubers are less susceptible to leak. For example, Bannock Russet, Clearwater Russet, and Teton Russet were moderately resistant to leak when pulp temperatures were maintained or cooled quickly to 12.8°C. Although, Russet Norkotah was moderately resistant to leak and could be harvested at warmer pulp temperatures of 21.1°C (Figure 1.2 and Figure 1.4).

Previous research reported Russet Norkotah was highly susceptible to leak when a single plug of *P. ultimum* was applied to an area where periderm was removed from the potato (Salas et al. 2003; Taylor et al. 2008; Taylor et al. 2004; Thompson et al. 2007). In our study, the observed cultivar differences to leak susceptibility and severity could be related to differences in susceptibility to shatter bruise. Hawkins and Harvey (1919) observed some potato cultivars were more resistant to wounding which corresponded to a lower susceptibility to leak. Salas et al. (2003) stated the relationship between leak incidence and bruise should be further investigated. Though shatter bruise was not directly measured in this study, Russet

Norkotah has been observed to be less susceptible to shatter bruise compared to other cultivars (Mosley et al. 2000; Novy et al. 2014; Spear et al. 2017). Screening newly developed cultivars for disease susceptibility to field-borne pathogens encourages better management decisions (Yellareddygari et al. 2019), however, susceptibility to bruising needs to be further investigated as a method to decrease the amount of leak and other diseases in new cultivars.

Weighted severity was analyzed to provide an idea of how quickly leak may develop in an infected tuber with multiple entry points. Cultivar and temperature were significant factors for leak severity, but Umatilla Russet had lower severity than the other cultivars. Lesions formed in Umatilla Russet did not expand as quickly as lesions in the other cultivars and may indicate different mechanisms or genes that relate to disease progression once infection occurs. This relationship was observed by Thompson et al. (2007) with the cultivars Etb 6–5-2 and Snowden being classified as resistant and moderately resistant, respectively. Etb 6–5-2 and Snowden showed similar leak incidence but lesions in Snowden were more developed. The same study by Thompson et al. (2007) also concluded several cultivars had a much higher incidence than Etb 6–5-2, but lesion severity was similar to Etb 6–5-2. A possible reason for these observations in the lack of correspondence between leak severity and incidence could be physiological differences in cell wall structure between cultivars.

With the vast number of cell wall components to be analyzed, the genome and secretome of *P. ultimum* determines the mechanism of *P. ultimum* entry into the host. Previous research results can help identify the cell wall components that may contribute to disease and bruise resistance. For example, Levesque et al. (2010) observed *P. ultimum* fails to produce many of the same enzymes produced by other oomycetes (i.e. *Phytophthora*). Based on the genome of *P. ultimum*, cellulases or other enzymes that breakdown the primary and secondary cell wall components are few. These hypotheses were validated by Zerillo et al. (2013), who observed *P. ultimum* struggled to grow on medias containing cellulose, xylan, and rhamnose, galactose, xylose, and other saccharides. Based on these results, Zerillo et al. (2013) hypothesized *P. ultimum* will only produce enough enzymes to degrade cell walls to reach starch and simple sugars. This hypothesis was also observed when carrots were inoculated with *P. ultimum*; xylanase, pectin methylesterase, and pectate lyase were not detected (Campion et al. 1997). The lack of certain enzymes produced by *P. ultimum* may result

in greater disease resistance in certain potato cultivars that produce more xylose, rhamnose, or galactose side groups.

A primary goal of storage management is to lower tuber pulp temperatures to a temperature which promotes wound healing but prevents disease development. This is done by either using refrigeration or bringing cool outside air into the storage facility so tubers reach a temperature of 10 to 15.5°C (ASABE Standards 2018; Kays and Paull 2004; Kleinkopf 1995). The recommendation to prevent leak is to stop harvesting when pulp temperatures are above 21°C (Salas and Secor 2001; Salas et al. 2003; Taylor et al. 2004). The need to remove field heat in storage when pulp temperatures are close to 21°C becomes a problem if adequate refrigeration or cooling air is not available. Our results show leak development and susceptibility to leak increases when pulp temperatures are maintained above 15.5°C or if temperatures increase because of the heat of respiration or lack of cooling (Table 1.1 and Figure 1.2). Harvesting and storing potatoes with pulp temperatures 15.5°C and below will decrease the need to remove field heat and minimize leak development in storage. The availability of refrigeration or cooling air can significantly reduce the development of leak when high pulp temperature (21.1°C) tubers are stored at relatively low temperatures (12.8 or 15.5°C). This reduction in disease could be the result of two possible mechanisms. First, the pathogen was exposed to lower air temperatures around the tuber shortly after inoculation. Second, the pulp temperatures of the tubers were reduced quickly (approximately 24 h). It is also possible these two reasons work together to reduce the overall infection of leak. Both possibilities affect *P. ultimium* similarly by slowing or stopping the physical growth of the pathogen (Goss and Jensen 1944; Hawkins 1916; Jones 1935; Triki et al. 2001). If the capacity to quickly reduce tuber pulp temperature is available, then harvesting at 21.1°C does not necessarily mean a high level of leak will develop. However, harvesting with pulp temperatures of 18.3 or 21.1°C will affect the rate of cooling potatoes in storage, which may make it infeasible to arrest the development of leak prior to the start of infection.

Growth of *P. ultimum* and decay of tubers has been observed to be greater at temperatures of 20 to 35°C compared to temperatures below 20°C (Goss and Jensen 1944; Hawkins 1916; Jones 1935; Triki et al. 2001). The greatest incidence of leak in our study was observed when tubers were maintained or warmed up to temperatures of 21.1°C. Temperatures greater than 21.1°C were not tested. Tuber pulp temperatures and early storage

temperatures of 18.3°C also showed high leak incidence in all cultivars, confirming pulp and early storage temperatures of 15.5°C and below are unfavorable to leak. Pulp temperatures below 18.3°C are also recommended to limit bruise and allow for removal of field heat in current storage designs (Knowles and Plissey 2008).

Temperature plays an important role in disease suppression for other diseases of potatoes as well. *Pectobacterium atroseptica* has been found to cause less disease in stored potatoes at temperatures of 10°C compared to 16°C (De Boer and Kelman 1978; Hauben et al. 1998). Development of pink rot and late blight in stored potatoes has also been reported to be lessened by storage temperatures below 15°C (Clayson and Miller 2007; Kirk et al. 2001a; Lennard 1980; Salas et al. 2000). Lower storage temperatures have been observed to decrease decay from *Botrytis cinerea*, *Neonectria galligena*, and *Phytophthora cactorum* among apple and pear cultivars as well (Berrie et al. 2011; Grove and Boal 1991).

In conclusion, these data show russet cultivars and cultivar selection vary in susceptibility to leak; this susceptibility to leak is also influenced by the pulp temperature of tubers during harvest when cooling air or refrigeration is not available. In addition, refrigeration or cooling air, when available, can influence leak susceptibility. Effective control of leak can be achieved when tubers are harvested or cooled to 12.8 to 15.5°C. Bannock Russet and Clearwater Russet are more susceptible to leak; therefore, it is recommended to harvest tubers with pulp temperatures of 12.8°C or use cooling to quickly decrease pulp temperatures. Ranger Russet, Russet Burbank, and Umatilla Russet are less susceptible to leak; thus, have a lower risk to leak even if tubers are harvested with pulp temperatures of 15.5°C. Russet Norkotah was moderately resistant to leak at 21.1°C and may have greater range in harvest and early storage temperatures for leak management. Knowing the susceptibility of each cultivar is useful in managing leak because some cultivars have more flexibility and can be harvested at a broader range of temperatures or require less cooling. Further research in managing storage ventilated air temperature for effectively removing field heat will be essential to minimize leak development. Understanding the mechanisms of leak susceptibility, along with bruise susceptibility and other diseases will be valuable to breeders for screening new cultivars for development and the industry to manage the quality of potatoes.

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TABLES

Table 1.1: Leak severity (%) and incidence (%) of five Russet Norkotah selections

Russet Norkotah selection	Leak severity (%)[1]	Leak incidence (%)[1]
CO-3	34.0 a	39 a
TXNS-112	36.0 a	35 a
TXNS-278	37.0 a	35 a
TXNS-296	41.0 a	40 a
Standard	52.3 b	58 b

[1] Values in a column followed by the same letter are not significantly different at α =0.05.

Table 1.2: Leak incidence (%) for russet potato cultivars as influenced by harvest pulp temperatures

	Pulp temperatures (C)				
	12.8	15.5	18.3	21.1	Cultivar main effect[1]
Cultivar	Leak incidence (%)				
Bannock Russet	63	75	86	92	81 a
Clearwater Russet	40	75	88	92	78 a
Ranger Russet	23	42	74	72	53 b
Russet Burbank	19	53	60	75	51 b
Teton Russet	46	68	82	93	76 a
Umatilla Russet	28	60	68	84	62 b
Pulp Temperature main effect[1]	35 a	63 b	78 c	86 d	

[1] Values in a column or row followed by the same letter are not significantly different at α =0.05

Table 1.3: Leak incidence (%) for each russet potato cultivar as influenced by pulp and early storage temperatures

Cultivar	Pulp temperature (C)	Early storage temperature (C) 12.8	15.5	18.3	21.1	Pulp temperature by cultivar[1]	Cultivar main effect[2]
		Leak incidence (%)					
Bannock Russet	15.5	27	59	80	89	67 e	63 d
	21.1	21	51	74	85	59 de	
Russet Burbank	15.5	12	34	59	74	42 bc	38 b
	21.1	9	27	51	67	35 b	
Russet Norkotah CO-3	15.5	4	13	30	45	18 a	20 a
	21.1	5	18	38	54	23 a	
Umatilla Russet	15.5	17	44	69	81	52 cd	49 c
	21.1	13	37	62	76	45 bc	
Early storage temperature main effect[2]		11 a	34 b	59 c	74 d		

[1] Values in a column or row with differing letters signify statistical significance (α=0.10).

[2] Values in a column or row with differing letters signify statistical significance (α=0.05).

FIGURES

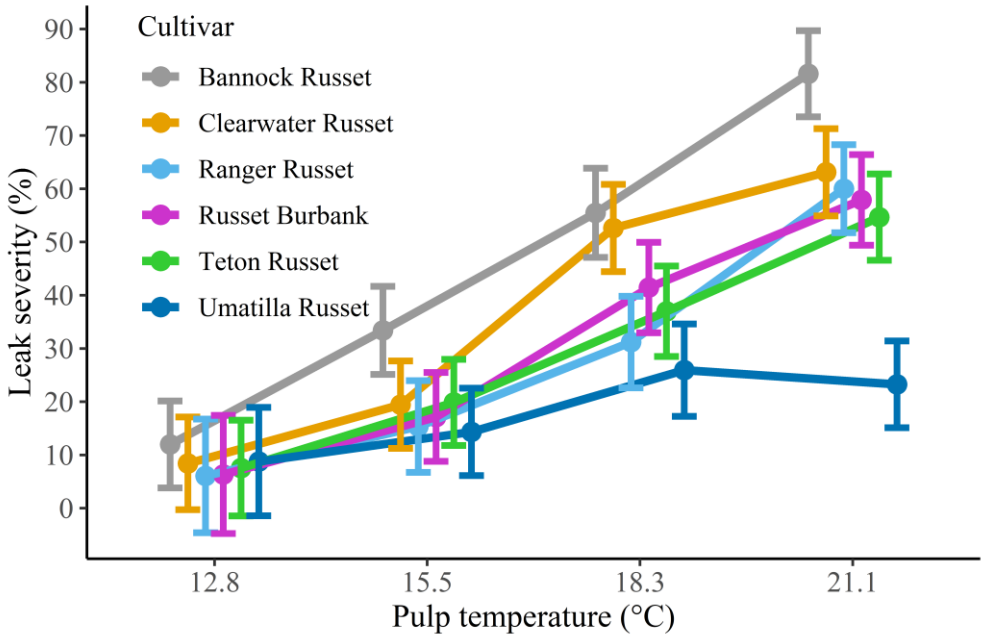

Figure 1.1: Leak severity (%) of russet potato cultivars as influenced by pulp temperatures. Error bars represent 95% confidence interval

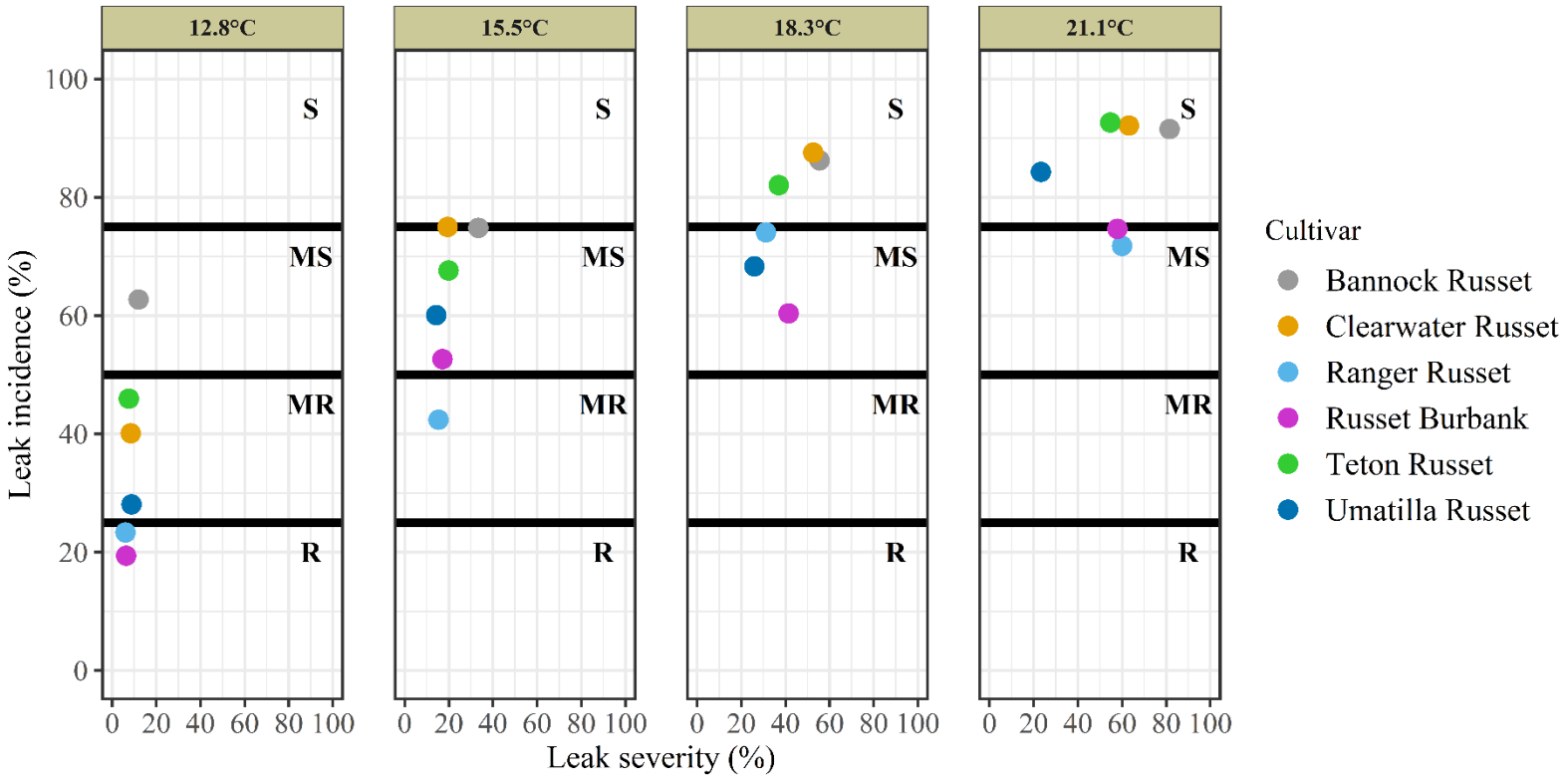

Figure 1.2: Relationship of leak incidence (%) to leak severity (%) in russet potato cultivars as influenced by pulp temperature. Leak susceptibility of cultivars at each pulp temperature were categorized: R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible (adapted from Taylor et al. 2008 and Thompson et al. 2007)

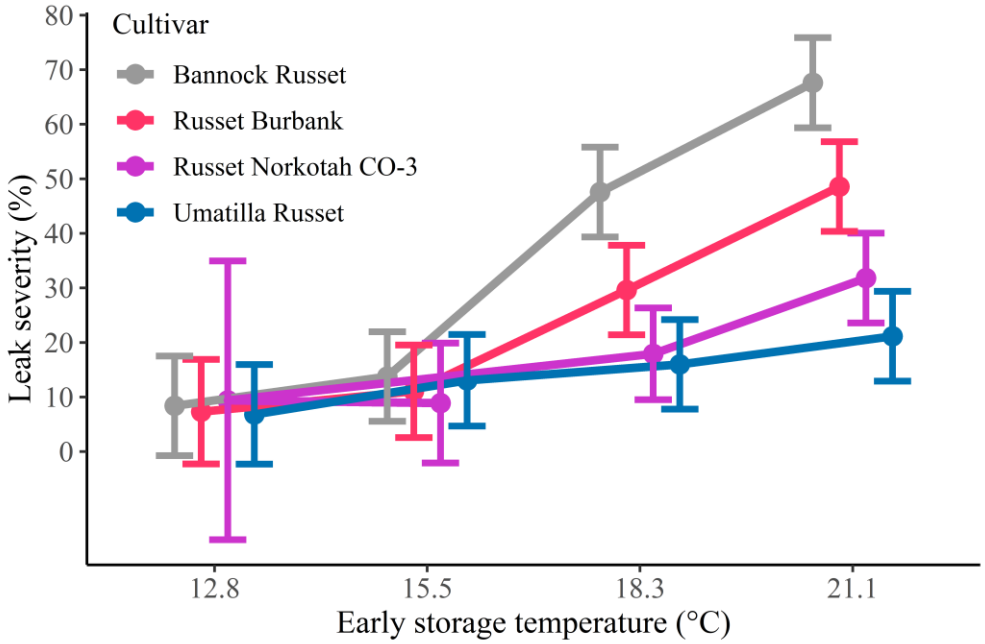

Figure 1.3: Leak severity (%) of russet potato cultivars as influenced by early storage temperature. Error bars represent 95% confidence intervals

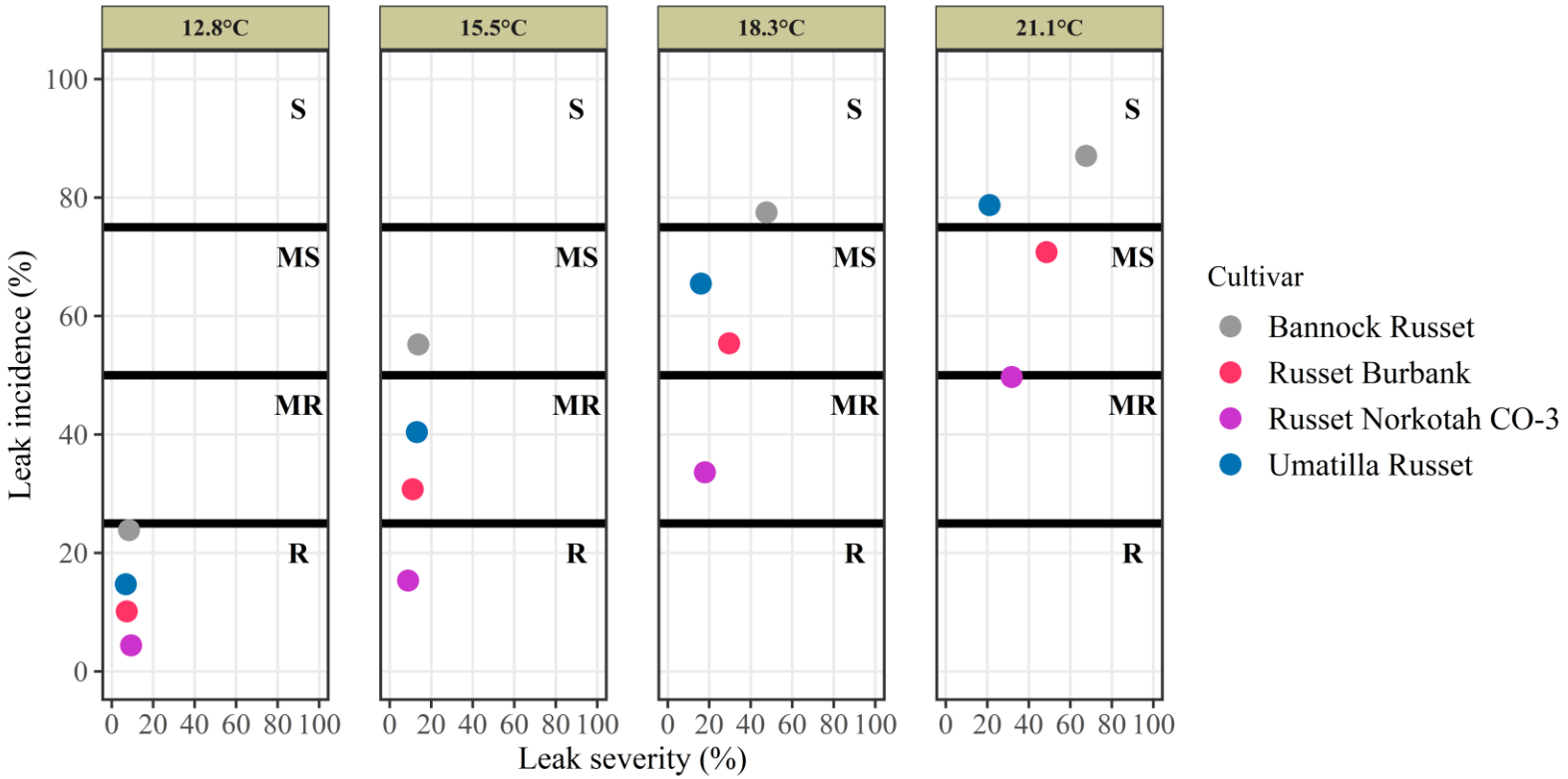

Figure 1.4: Leak incidence (%) to leak severity (%) in russet potato cultivars as influenced by early storage temperature. Leak susceptibility of cultivars at each early storage temperature were categorized: R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible (adapted from Taylor et al. 2008 and Thompson et al. 2007)

Chapter 2 : PYTHIUM LEAK SUSCEPTIBILITY INFLUENCED BY SHATTER BRUISE AND MECHANICAL FAILURE PROPERTIES OF POTATO (*SOLANUM TUBEROSUM* L.) TUBERS

ABSTRACT

The potato disease leak is caused by *Pythium ultimum* and infection is highly influenced by tuber temperature. *Pythium ultimum* infects a tuber via wounds or shatter bruises, which occur during mechanical harvesting and handling. Leak susceptibility may be related to shatter bruise potential and tuber mechanical properties may impact those susceptibilities. The objectives of this study were to (1) examine if the propensity to shatter bruise explains cultivar susceptibility to leak and (2) to investigate if mechanical failure properties identify shatter bruise and leak susceptibility among russet cultivars. Cultivars Bannock Russet, Clearwater Russet, Payette Russet, Russet Burbank, Ranger Russet, and Teton Russet were tested by warming tubers to 12.8 and 21.1°C. Subsamples were bruised and inoculated with *P. ultimum* and punctured with a texture analyzer. To measure shatter bruise area, a bruised subsample was selected and soaked in an iodine solution. Stained shatter bruises were removed, scanned, and processed with FIJI image analysis software. A texture analyzer measured the mechanical failure properties of load, deformation, work, and rigidity. Leak incidence was greater at 21.1°C (82%) than at 12.8°C (20%). Leak incidence was greater in Bannock Russet, Clearwater Russet, and Payette Russet compared to Ranger Russet, Russet Burbank, and Teton Russet at both temperatures. Shatter bruise area and number were not correlated to leak incidence. The mechanical failure property load was significantly correlated (r=-0.34 at 12.8 and -0.26 at 21.1°C) with leak incidence. Ranger Russet had significantly less shatter bruise area (0.43%) and number (6) compared to other cultivars. The lower shatter bruise area and number of Ranger Russet corresponded to stronger tubers requiring greater loads (38.6 N) and more work to break the periderm (54.0 mJ), while the cells were more rigid (16.1 N mm^{-1}) than other cultivars. Temperature and cultivar were the primary factors in the development of leak, although shatter bruise is an important factor in leak susceptibility. Tissue mechanical properties measured with a texture analyzer can be a tool to quantify a cultivars susceptibility to shatter bruise.

Key Words: *Pythium ultimum*, periderm, iodine, texture analyzer

INTRODUCTION

Quality of potatoes (*Solanum tuberosum* L.) is affected by disease and physiological disorders, which influence the duration potato tubers can be stored. Pythium leak is a common disease in potato producing areas with *Pythium ultimum* Trow var. *ultimum* being the primary pathogen causing the disease in cooler climates (Powelson and Rowe 2008). Severe crop losses have been reported due to tuber decay in storage (Jones 1935). When a potato is cut, the affected tissue appears smoky grey or brown in color (Salas and Secor 2001). Water leaking from infected tubers further promotes secondary disease development, caused by *Pectobacterium* species (Charkowski 2015).

Shatter bruises are cracks or cuts in the periderm extending into the flesh (Baritelle et al. 2000) and caused by physical impacts with equipment, foreign material, or other potatoes. After experiencing accelerations from physical impacts, the point of contact may develop either blackspot or shatter bruises, both influenced by tuber mechanical properties. Mathew and Hyde (1997) reported the primary defect of cultivar Russet Burbank tubers was blackspot bruise when dropped from heights of 50 to 200 mm, but the damage transitioned to predominantly shatter bruises when drop heights exceeded 200 mm. Some cultivars are more susceptible to shatter bruise than others and exhibit varying responses to impact forces (Bajema et al. 1998a; Spear et al. 2017).

Variability in shatter bruise susceptibility among potato cultivars is partially explained by the tuber mechanical properties under dynamic loads. Research showed that Russet Burbank withstood greater impact pressure and required more energy to break apart cells than the cultivar Atlantic (Bajema et al. 1998a; 1998b; Baritelle and Hyde 1999). Bajema et al. (1998a) noted differences in tissue failure properties may relate to Atlantic tubers being more susceptible to bruise than Russet Burbank tubers.

The ability of potato cultivars to resist impact force may also be related to the risk of shatter bruise development. Static loads applied to the periderm can be used to evaluate the load resistance before the periderm breaks (Hawkins and Harvey 1919). A similar method, such as applying a cylindrical probe to puncture the periderm, has been used to measure the resistance to damage among tomato cultivars (Desmet et al. 2002). In potatoes, Bordoloi et al. (2012) found cultivars varied in the physical load required to fracture or cause cells to break. Static loads have also been used to predict potato cultivar susceptibility to pressure bruise

using compression tests (Castleberry and Jayanty 2017). Cultivar differences were observed in the ability for cells to resist forces that may cause pressure flattening and/or pressure bruise (Castleberry and Jayanty 2017). Observations made by Hawkins and Harvey (1919) indicated cultivars showing greater resistance to puncture were also more resistant to the development of leak. Mechanical failure properties may correspond with the differences in leak susceptibilities observed in previous research (Hollingshead et al. 2020; Salas et al. 2003). Cell microstructures affect the mechanical properties of cells, but these properties have not yet been correlated with shatter bruise and leak disease development. Since *P. ultimum* needs a wound such as a shatter bruise to infect the tuber, assessing for shatter bruise susceptibility may be beneficial for growers and in cultivar development. It is hypothesized there is an association between load resistance of the periderm, shatter bruise susceptibility, and leak development.

The overall objective of this study was to evaluate relationships between leak, shatter bruise, and tuber mechanical properties among russet cultivars. The mechanical properties of tubers may also provide additional insight into why cultivars differ in disease and bruise susceptibility, which could be used to predict disease and shatter bruise at harvest. The specific objectives of the study were to (1) examine if the propensity to shatter bruise explains cultivar susceptibility to leak and (2) investigate if mechanical failure properties identify shatter bruise and leak susceptibility among russet cultivars.

MATERIALS AND METHODS

Potato cultivars, cultivation, and storage

Potato cultivars Bannock Russet, Clearwater Russet, Payette Russet, Ranger Russet, Russet Burbank, and Teton Russet were grown at the University of Idaho Kimberly Research and Extension Center (KREC) during the 2017 (year 1) and 2018 (year 2) growing seasons. Cultivars were grown and harvested according to recommendations for south central Idaho (Stark and Love 2003). Cultivars were stored and cured at 12.7 ± 0.15°C with 95% relative humidity (RH) for seventeen days. The temperature was then ramped down to a holding temperature of 5.6°C (95% RH) at a rate of 0.3°C day^{-1}. Tubers were treated with isopropyl (3-chlorophenyl) carbamate (chlorpropham; Aceto Agricultural Chemicals Corporation; 22 ppm) for sprout control 60 days after harvest. All trials included unwashed tubers ranging

from 170 to 340 g. For each cultivar, tubers were evaluated for leak incidence and severity, shatter bruise number and area, and mechanical failure properties. To establish the tuber pulp temperature treatments, tubers were warmed to 12.8 and 21.1 ±0.5°C (95% RH) in storage bins located at KREC three days prior to inoculation, bruising, and texture analysis. Experiments were repeated twice each year containing four replicates. The experiments took place approximately 50 to 70 days after harvest in year 1 and 115 to 165 days (total of four experiments).

Inoculation preparation

Culture of *Pythium ultimum* Trow var. *ultimum*, identified as isolate 09 MN 10-5, was obtained from Dr. Neil Gudmestad's lab at North Dakota State University and used throughout all experiments. It had been maintained on P_5ARP media (Jeffers and Martin 1986) and grown in VWR Diurnal Growth Chamber, model 2015 set to 21.5 ± 0.2C (VWR International, Cornelius, Oregon). *Pythium ultimum* was grown in a clarified tomato juice broth, using Campbell's V8 juice and calcium carbonate (0.1 g mL^{-1} of juice). The mixture of V8 juice was centrifuged for 5 minutes at 10,000 rpm (Sorvall RC-6 plus, Thermo Scientific) and 100 mL of supernatant was added to 900 mL of de-ionized water to make the broth, which was sterilized and cooled for use or stored in a refrigerator. Fifty plugs of *P. ultimum* was added to 1L of broth and allowed to grow for 12 to 14 days. Inoculum was prepared by straining oomycete mats from the broth and placing them in a blender with 200 mL of deionized water, then homogenized for three minutes. Oospore concentrations were adjusted to 2.5 x 10^5 oospores mL^{-1} and 1.0 x 10^5 oospores mL^{-1} in year 1 and year 2, respectively, and applied at 6.6 mL kg^{-1} using an air assisted sprayer.

Bruise and leak inoculation methods

Tubers with pulp temperature treatments of 12.8 and 21.1°C were bruised and inoculated, and then returned to the original storage bins to maintain pulp temperatures and 95% RH for four days until evaluated for disease. Potato tubers were bruised in a cement mixer for 90 seconds and inoculated with *P. ultimum* in the last 30 seconds of the cycle (n=20 tubers, 4 replicates, repeated twice each year). This trial was designed as a 2x6 factorial completely randomized split plot design with temperature as the main plot effect and cultivar as the sub-plot effect. Tubers were evaluated four days after inoculation by cutting

longitudinally in half to estimate percent severity by lesion area of the halved tuber. Only infected tubers were analyzed for a weighted severity (referred to as severity).

Shatter bruise area estimation of russet cultivars

Shatter bruise was quantified by counting the number of bruises on each tuber and by measuring the area of shatter bruise compared to the surface area of the whole tuber (n=5 tubers, 4 replicates, repeated twice each year) for each cultivar and pulp temperature treatment. Five tubers per replicate were randomly sub-sampled from 20 tubers (year 1) or 25 tubers per replicate (year 2) from the bruised pulp temperature treatments of 12.8°C and 21.1°C. The bruised subsamples were then soaked in 14% iodine solution containing 5% polyethoxylated nonylphenol iodine complex (Controlled Iodine Spray, Durvet, Inc., Blue Springs, MO) for two hours. Tubers were dried, weighed and the visually dyed portion of tubers were cut from the tuber and scanned with an Epson Perfection V39 scanner (Epson America, Inc., Long Beach, CA) into digital images uploaded to FIJI (Schindelin et al. 2012). The shatter bruise area was measured using FIJI (scale 238.0336 pixels cm^{-1}). The total surface area of a tuber was estimated based on the weight of the tuber using the regression equation developed by Edwards (1999). Percent bruise area was calculated by dividing the bruise area by tuber surface area. The number of bruises per tuber were visually counted from the scanned images of cut wounds.

Analysis of tuber mechanical failure properties

Compression tests were performed on whole tubers with the periderm intact (n=5 tubers, 4 replicates, repeated twice each year) for each cultivar and pulp temperature treatments of 12.8 and 21.1°C using a CT3 Texture Analyzer (AMETEK Brookfield, Middleboro, MA, USA). A 4 mm probe (T44) at a speed of 0.5 mm s^{-1} was inserted into the center of the face of the potato. The load to break the periderm, tissue deformation at the point of periderm breaking, work to break the skin, and rigidity of the tuber was measured using the TexturePro CT V1.8 software (AMETEK Brookfield, Middleboro, MA, USA). Figure 1 depicts a load-deformation curve and how the parameters were obtained. The load (newtons, N) indicates the maximum force needed to break the periderm. Deformation (mm) was measured as the distance the probe was inserted into the tuber to attain the maximum load. Work (mJ) was the area under the curve from when the trigger point was reached to the point

of load. Rigidity (N mm^{-1}) is the least-squares-fit slope of all points between trigger load and load.

Data analysis

Analysis of the data was performed using R (version 1.2.1335, R Foundation for Statistical Computing, Austria). Generalized linear mixed-effects model (GLMM) and binomial link function in the 'nlme' package was used to analyze the leak incidence for all cultivars inoculated and stored at set temperatures. Leak severity at 12.8°C was transformed (Ln) and analyzed separate of 21.1°C to meet assumptions of normality and homogeneity. Percent bruise area was transformed (Ln+1) to meet the assumptions of normality and homogeneity. Transformed leak severity, transformed bruise area, number of bruises, load, work, rigidity, deformation, and leak severity were analyzed using mixed-effects linear models (LMM) in the 'nlme' package (Bates et al. 2015). The estimated marginal means of cultivar and temperature means of response were analyzed with Tukey HSD adjustment (α=0.05) with 'emmeans' package (Lenth 2019). Correlations between variables were performed using Pearson's correlation, however correlations with leak incidence were done using Spearman's Rank correlation with the 'agricolae' package (Mendiburu 2019; Salas et al. 2003). Year and temperature were significant factors for leak development, shatter bruise, and mechanical failure properties; however, cultivar responses did not change over the two years or temperatures. For this reason, the interactions involving cultivar, year and temperature were combined to observe the main effects for shatter bruise and mechanical properties.

RESULTS

Leak incidence and severity of russet cultivars

The interaction between cultivar and temperature on leak incidence was significant ($p=0.003$) and therefore temperature response was separated (Table 2.1). Leak incidence in year 1 was greater (overall mean 59%) than in year 2 (overall mean 43%). The difference between year 1 and year 2 did not affect the cultivar response. Tubers inoculated and maintained at 12.8°C had significantly ($p <0.0001$) lower leak incidence (20%) than tubers inoculated and maintained at 21.1°C (82%). Leak incidence ranged from 7 to 41% with Russet Burbank having the lowest disease incidence and Clearwater Russet having the highest incidence at 12.8°C pulp temperatures (Table 2.1). At 21.1°C leak incidence ranged from 63

to 93%. The leak incidence for Russet Burbank (63%) was not significantly different than Teton Russet (69%) but was significantly lower than the other cultivars. Clearwater Russet and Payette Russet had significantly higher leak incidence (93%) compared to the other cultivars.

Leak severity means at 12.8°C and 21.1°C were analyzed separately because data at 12.8°C were not normally distributed and transformed. The main effects of cultivar and year at 12.8 and 21.1°C pulp temperatures were analyzed for leak severity. At pulp temperatures of 12.8°C the mean leak severities were 6 and 2% for year 1 and year 2, respectively ($p<0.0001$). Potatoes at pulp temperature of 21.1°C had mean leak severity of 48 and 45% for year 1 and year 2, respectively ($p=0.007$). At 12.8°C cultivar did not significantly influence ($p=0.41$) leak severity, which averaged between 5 and 7% for all cultivars (Table 2.1). Because leak severity did not vary by cultivar at 12.8°C no correlations with shatter bruise and mechanical failure properties at that temperature were presented (Figure 2.2a).

However, at 21.1°C leak severity was significantly ($p<0.0001$) affected by cultivar. Leak severity was lowest in Russet Burbank (39%) and Teton Russet (34%) tubers, while the severity in Bannock Russet (58%) was significantly higher than all the other cultivars. The moderate correlation ($r=0.45$) between leak incidence and leak severity was significant ($p<0.0001$) at 21.1°C pulp temperatures (Figure 2.2b).

Shatter bruise surface area and number for russet cultivars

Cultivars significantly ($p<0.0001$) differed for both percent shatter bruise area and number of shatter bruises per tuber (Table 2.2). Shatter bruise area and number were significantly ($p<0.0001$ and $p=0.01$, respectively) influenced by year. Year 1 had lower shatter bruise area (0.45%) and number (9) than year 2 (1.09% and 10, respectively). The effect of temperature on percent shatter bruise area was not significant ($p=0.054$). The mean number of shatter bruises per tuber was significantly ($p=0.018$) lower at 12.8°C (9) compared to 21.1°C (10). Ranger Russet had a significantly lower shatter bruise area (0.43%) compared to the other cultivars. Clearwater Russet shatter bruise area (0.61%) was not significantly different from Russet Burbank (0.77%) or Teton Russet (0.78%). Although, Clearwater Russet had significantly lower shatter bruise area than Bannock Russet (0.86%) and Payette Russet (0.84%). A significantly lower number of shatter bruises was observed for Ranger

Russet (6 bruises per tuber) compared to the other cultivars, which ranged from 9 to 11 shatter bruises.

There was an expected moderate correlation ($r=0.59$, $p<0.0001$) between the number of shatter bruises per tuber and shatter bruise area at 21.1°C (Figure 2.2b). Leak severity was weakly correlated ($r=0.32$, $p=0.005$) with shatter bruise number at 21.1°C, while leak incidence was not significantly correlated to shatter bruise number or area at either temperature.

Mechanical failure properties of russet cultivars

The data were pooled to analyze the main effects on load, deformation, work, and rigidity. The load needed to break the periderm was significantly ($p<0.0001$) affected by year (30.7 N in year 1 and 32.2 N in year 2), temperature (32.0 N at 12.8°C and 30.8 N at 21.1°C), and cultivar. Ranger Russet had significantly higher load values (38.6 N), therefore could withstand greater force before the periderm punctured compared to the other cultivars (Table 2.2). In contrast, Bannock Russet required less force (26.4 N) compared to the other cultivars, while Clearwater Russet, Payette Russet, Russet Burbank and Teton Russet were intermediate.

The periderm and underlying tissue deformed as a load was applied. This deformation was measured at the load before the periderm punctured. Deformation was significantly affected by cultivar ($p<0.0001$), temperature ($p=0.023$), and year ($p<0.0001$). Deformation was significantly lower at 21.1°C pulp temperature (2.50 mm) compared to 12.8°C (2.56 mm). Tissue deformation was also significantly different between years (2.12 mm, year 1 and 2.94 mm, year 2). Russet Burbank had the least deformation (2.31 mm) and Payette Russet had the most (2.70 mm, Table 2.2). Bannock Russet, Clearwater Russet, Ranger Russet, and Teton Russet were not statistically different from one another ranging between 2.52 and 2.58 mm of deformation.

The work or energy to reach load before the periderm and cells break was measured by the area under the curve (Figure 2.1). Temperature as a main effect was a significant ($p=0.008$) factor as the work to reach load was higher at 12.8°C (44.3 mJ) compared to 21.1°C (42.2 mJ). The significant ($p<0.0001$) differences between years indicated the work to reach peak force was greater in year 2 (49.0 mJ) than in year 1 (37.5 mJ). Work was also significantly ($p<0.0001$) affected by cultivar (Table 2.2). The skin and underlying cells

required significantly more work to break Ranger Russet (54.0 mJ) tubers compared to the other cultivars. Bannock Russet required significantly less work (36.8 mJ) than all other cultivars.

Rigidity is the least-squares-fit slope of all points between trigger load and load. Temperature did not impact rigidity ($p=0.47$). Tubers in year 1 (15.2 N mm^{-1}) had significantly ($p<0.0001$) greater rigidity than year 2 (12.0 N mm^{-1}). Cultivars were significantly ($p<0.0001$) different in rigidity (Table 2.2). Ranger Russet (16.1 N mm^{-1}) and Russet Burbank (15.6 N mm^{-1}) had significantly greater rigidity compared to the other cultivars and Bannock Russet (11.3 N mm^{-1}) had the lowest rigidity.

The correlation between load and deformation was not significant at 12.8 or 21.1°C pulp temperatures ($p=0.4$ and $p=0.68$, respectively), but load and deformation were correlated to work and rigidity at both temperatures (Figure 2.2). Rigidity and work were weakly correlated at 21.1°C ($r=-0.29$, $p=0.005$) but no correlation was shown at 12.8°C ($r=-0.19$, $p=0.06$). The strong correlations between mechanical failure properties were expected because load and deformation are elements of work and rigidity. Load was weakly correlated to leak incidence ($r=-0.34$, $p=0.0007$) at 12.8 and 21.1°C ($r=-0.26$, $p=0.01$). Leak severity was correlated ($r=-0.23$, $p=0.03$) with load at 21.1°C. Leak incidence was also significantly correlated with work ($r=-0.34$, $p=0.0007$) at 12.8°C. Shatter bruise area was moderately correlated to deformation ($r=0.39$, $p=0.0006$ at 12.8°C and 0.30, $p=0.009$ at 21.1°C) and rigidity ($r=-0.39$, $p=0.0007$ at 12.8°C and -0.37, $p=0.001$ at 21.1°C) but the correlation to load or work were not statistically significant to shatter bruise area ($p\geq0.13$). Shatter bruise number was weakly correlated with load ($r=-0.32$, $p=0.007$ at 12.8°C and $r=-0.37$, $p=0.002$ at 21.1°C) and work ($r=-0.25$, $p=0.03$ at 12.8°C and $r=-0.32$, $p=0.006$ at 21.1°C) but the correlations with deformation and rigidity were not statistically significant with shatter bruise number ($p\geq0.17$).

DISCUSSION

Shatter bruises are direct entry points for pathogens to infect and *Pythium ultimum* is an opportunistic pathogen that requires an entry point for infection of potatoes (Taylor et al. 2004). Shatter bruise is caused when potatoes experience physical impacts with other objects causing the cells and periderm to break. It results in quality concerns that influence economic returns and increase the risk of disease. Spear et al. (2017) observed Russet Burbank was

more susceptible to shatter bruise than Mountain Gem Russet, Teton Russet, and Russet Norkotah strains. Cultivars with greater propensity for shatter bruise could theoretically have a higher susceptibility to leak. However, leak incidence was not correlated to shatter bruise area and number. For example, shatter bruise area and number for Ranger Russet and Clearwater Russet were relatively low (Table 2.2); however, this did not correspond to lower leak incidence or severity (Table 2.1). Leak severity was positively correlated to shatter bruise number (Figure 2.2). The positive correlation between leak severity and shatter bruise number was expected due to increased entry and potential infection points allowing the pathogen to grow from multiple locations in the tissue.

There are two possible reasons for the lack of correlation between leak (incidence and severity) and shatter bruise (area and number). The first reason being potatoes bruised for the presented study mimicked typical conditions at harvest; therefore, tubers sustained multiple wounds. Taylor et al. (2004) observed only one wound was needed for leak to develop; therefore, the excessive wounds in this study (6 to 11 shatter bruises per tuber) may have overwhelmed the natural defenses of the potatoes causing more infection points than are needed for pathogen infection. Further studies using tubers with fewer shatter bruises, yet still mimicking the natural process, may provide a stronger relationship between shatter bruise and leak incidence. The second possible reason for the lack of correlation between leak incidence and shatter bruise is the mechanical failure properties of the tuber may affect how cells break and this may interact with the subsequent penetration of *P. ultimum* into cells and causing leak.

The mechanical failure properties were measured on whole potatoes with intact periderm to mimic the state of the tubers when impacted at harvest. Tubers were subjected to static forces in order to measure the force a tuber can resist before breaking the periderm (load), the depth the probe travels before breaking the periderm (deformation), the energy required to cause the periderm to break (work), and the cells resistance to deformation (rigidity). Hawkins and Harvey (1919) observed cultivars resisting greater loads before fracturing were more resistant to leak. The results of this study support those of Hawkins and Harvey (1919) as the cultivars Ranger Russet, Russet Burbank, and Teton Russet had lower leak incidence and resisted heavier loads than Bannock Russet, Clearwater Russet, and Payette Russet.

Mechanical properties may indicate the ease with which *P. ultimum* can disrupt cell walls. Studies have shown that *P. ultimum* does not produce cutinase (Baker and Bateman 1978); therefore, *P. ultimum* cannot gain entry to cutin-layered plant tissues. The cellulase produced by the pathogen is hypothesized to be for mycelial growth, not for pathogenicity (Campion et al. 1997; Lévesque et al. 2010). Therefore, *P. ultimum* needs an entry into a host, because it cannot break through the periderm on its own. Once *P. ultimum* gains access to the flesh of a potato, the mechanical failure properties and cell wall structure may determine the ability of *P. ultimum* to penetrate cell walls by disrupting pectin. *P. ultimum* produces several pectinases and can readily grow on agar amended with pectin, but there are some components to pectin such as arabinan and galactan which inhibit the growth of *P. ultimum* (Zerillo et al. 2013). It is hypothesized that tubers with more arabinan and galactan components may be more structurally sound and able to resist infection by *P. ultimum*. Further research of the cell wall components and their relation to leak development could improve methods for assessing and/or screening cultivars for leak susceptibility. If the cell walls resist greater loads and require more work to break, then the risk of leak incidence may decrease.

The response of leak development to the mechanical failure property of work may be more influential when the environment is unfavorable to *P. ultimum*. This is supported by the significant correlation between leak incidence and work at 12.8°C, but not at 21.1°C. Ideal temperatures for *P. ultimum* growth range between 20 and 30°C (Triki et al. 2001) and cultivar susceptibility to leak increased as the temperature rises from 13 to 21°C (Hollingshead et al. 2020). The data presented in this study reaffirmed leak susceptibility is greater at warmer temperatures. Temperature and tuber wetness have been the key in models predicting leak (Lui and Kushalappa 2003); however, the mechanical failure properties of load and work associated with tissue toughness may also be significant when assessing risk and susceptibility to leak. A recommendation for avoiding shatter bruise is to harvest when pulp temperatures are above 7.2°C (Knowles and Plissey 2008). Also, mechanical failure properties also start to stabilize from 10 to 20°C (Bajema et al. 1998a; 1998b). The results of this study support this previous recommendation and observation since shatter bruise (area and number) and mechanical failure properties were similar at 12.8 and 21.1°C.

Shatter bruise of potatoes is influenced by mechanical failure properties. Using tissue cores, Bajema et al. (1998a) observed differences in mechanical failure properties between

Russet Burbank and Atlantic. Russet Burbank withstood greater loads, had less deformation, required more work, and was more rigid. Therefore, it was concluded these properties may be a reason for Russet Burbank being less susceptible to bruise than Atlantic. Similarly, the cultivars in this study showed variation in resistance to mechanical failure properties. Ranger Russet tubers not only resisted the greatest loads before puncture but also required the greatest work. Ranger Russet tubers were also the most rigid compared to other cultivars, but deformation of Ranger Russet was similar to most of the other cultivars. There are some structural components of the cell wall that may explain why cultivars differ in the load required to puncture the periderm. Cell walls are inter-connected between cells and composed of cellulose, hemicellulose, and pectin. In potatoes, pectin is the most abundant and complex polysaccharide of these three (Hoff and Castro 1969) and aids in structural integrity, cell adhesion, and defense responses (Caffall and Mohnen 2009). There are sidechains of arabinan, galactan, and other groups adhering to cell walls and providing strength. When arabinan and galactan sidechains are not formed, the ability for those cells to adhere to one another diminishes (Øbro et al. 2004; Ulvskov et al. 2005). This could indicate cultivars with more arabinan and galactan sidechains may be more resistant to loads and require more work to break the periderm.

Another component that may influence cultivar mechanical properties is tuber turgidity (Falk et al. 1958). More hydrated tubers are considered more turgid and could resist deformation; therefore, if tubers are more turgid then deformation should be lower and rigidity higher. Bajema et al. (1998b) observed dehydrated cores resisted greater loads and deformed less, while the work to break the periderm increased, and cells were less rigid. Tubers were in storage longer in year 2 of this study than in year 1, which may have resulted in the observed increase in load, deformation, and work, and a decrease in rigidity. These mechanical failure property results suggested that the tubers in year 2 may have become more dehydrated with the additional time in storage (Kleinkopf and Olsen 2003) compared to tubers in year 1. Leak incidence was slightly lower in year 2 compared to year 1, which may be a result of a lower concentrate of inoculum being applied, the increase in the mechanical failure property load, or other unknown factors. But interestingly, shatter bruise area and number were higher in year 1. These results reinforce the lack of or low correlation between shatter bruise and leak incidence.

The correlations between mechanical failure properties with shatter bruise area and number showed load and work influenced shatter bruise number while deformation and rigidity influence shatter bruise area. The load and work explained the ability or inability for cell walls to adhere to one another; therefore, if a cultivar can resist greater loads and require more work to puncture or break the periderm then there would be less shatter bruise. This is evident by Ranger Russet resisting greater loads and requiring more work and exhibiting less shatter bruise. The results of this study showed that Ranger Russet was the only cultivar with less shatter bruise than Russet Burbank. Ranger Russet has been shown to be less susceptible to shatter bruise compared to various other cultivars (Novy et al. 2014; Novy et al. 2010). The turgidity of tubers would affect the deformation before the periderm breaks. With greater deformation more cells are likely impacted causing larger shatter bruises. The shatter bruise area of Bannock Russet and Payette Russet provided evidence of this relationship as both deformed more and were less rigid than other cultivars.

Monitoring shatter bruise is important to identify where shatter bruise occur during harvest operations. A new method of using iodine was adapted from the apple industry (Blanpied and Silsby 1992; Smith et al. 1979) to identify and measure shatter bruises in this study. The iodine stains exposed tissue blue as it reacts with starch. The blue-colored shatter bruises made it possible to quantify differences in shatter bruise area among russet cultivars using digital imaging. This method could be used to measure shatter bruise for research purposes or in the industry to evaluate conditions and equipment that may be causing tuber damage.

In this study, leak incidence was primarily influenced by temperature and cultivar. Shatter bruise is needed for *P. ultimum* to infect potatoes, but the incidence and severity of shatter bruise may have been too high to explain leak incidence. The mechanical failure properties identified differences among russet cultivars that may be a component in leak and shatter bruise susceptibility. More research is needed to understand fully how mechanical failure properties relate to leak and shatter bruise susceptibility by identifying differences among cultivars in cell wall components.

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TABLES

Table 2.1: Leak incidence and severity of russet cultivars at 12.8 and 21.1°C combined across experiments in year 1 and year 2

°C	Cultivar	Incidence (%)[a]	Severity (%)[ab]
12.8			
	Bannock Russet	25 c	6[cd]
	Clearwater Russet	41 d	6
	Payette Russet	24 c	5
	Ranger Russet	19 bc	5
	Russet Burbank	7 a	5
	Teton Russet	15 b	7
21.1			
	Bannock Russet	80 c	58 d[e]
	Clearwater Russet	93 d	51 c
	Payette Russet	93 d	45 b
	Ranger Russet	75 bc	50 bc
	Russet Burbank	63 a	39 a
	Teton Russet	69 ab	34 a

[a] Values with differing letters signify statistical significance (α=0.05) using Tukey HSD within a column and temperature.

[b] Leak infected tubers for each cultivar were analyzed only (weighted severity).

[c] Data at 12.8°C Ln transformation was performed to meet assumptions of normality.

[d] True means reported instead of estimated marginal means because of insignificance.

[e] Leak severity data at 21.1°C was normal and therefore not transformed.

Table 2.2: Shatter bruise development and mechanical failure properties of russet cultivars combined across 12.8 and 21.1°C pulp temperature treatments and experiments in year 1 and year 2

Cultivar	Shatter bruise area (%)[a]	Shatter bruise number[a]	Load (N)[a]	Deformation (mm)[a]	Work (mJ)[a]	Rigidity (N mm^{-1})[a]
Bannock Russet	0.86 c	11 b	26.4 a	2.58 bc	36.8 a	11.3 a
Clearwater Russet	0.61 b	9 b	28.8 b	2.56 b	40.8 b	12.6 b
Payette Russet	0.84 c	9 b	29.3 b	2.70 c	41.7 bc	12.3 b
Ranger Russet	0.43 a	6 a	38.6 d	2.53 b	54.0 d	16.1 d
Russet Burbank	0.77 bc	10 b	33.2 c	2.31 a	41.8 bc	15.6 d
Teton Russet	0.78 bc	10 b	32.3 c	2.52 b	44.4 c	13.8 c

[a] Values in columns with differing letters signify statistical significance (α=0.05) using Tukey HSD.

FIGURES

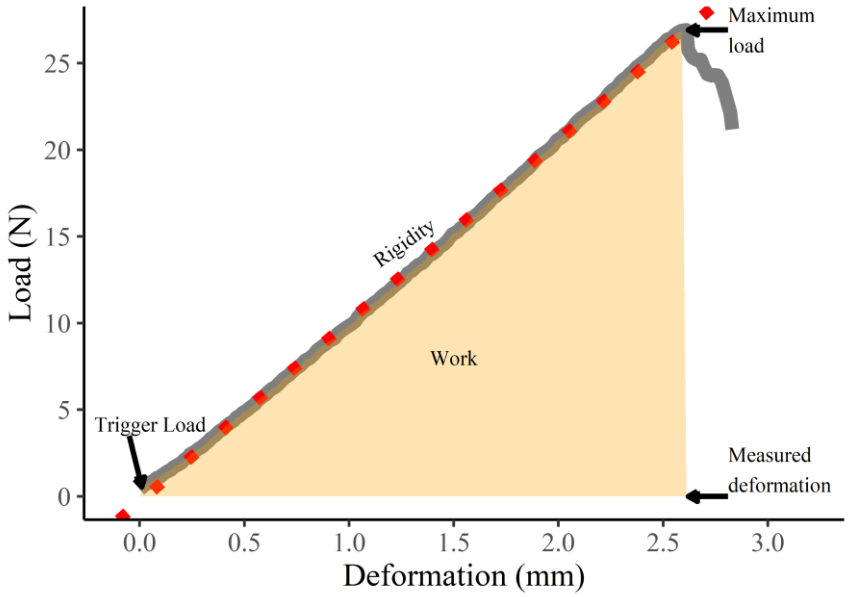

Figure 2.1: Texture profile plot starts measuring load at the trigger load and continues to a set depth (5 mm). Load is measured at the maximum load were the periderm breaks. Deformation is the depth of the probe when the periderm breaks. Work is the area (orange) under the curve. Rigidity (N mm^{-1}) is the least-squares-fit slope (red dotted line) of all points between maximum load and trigger load

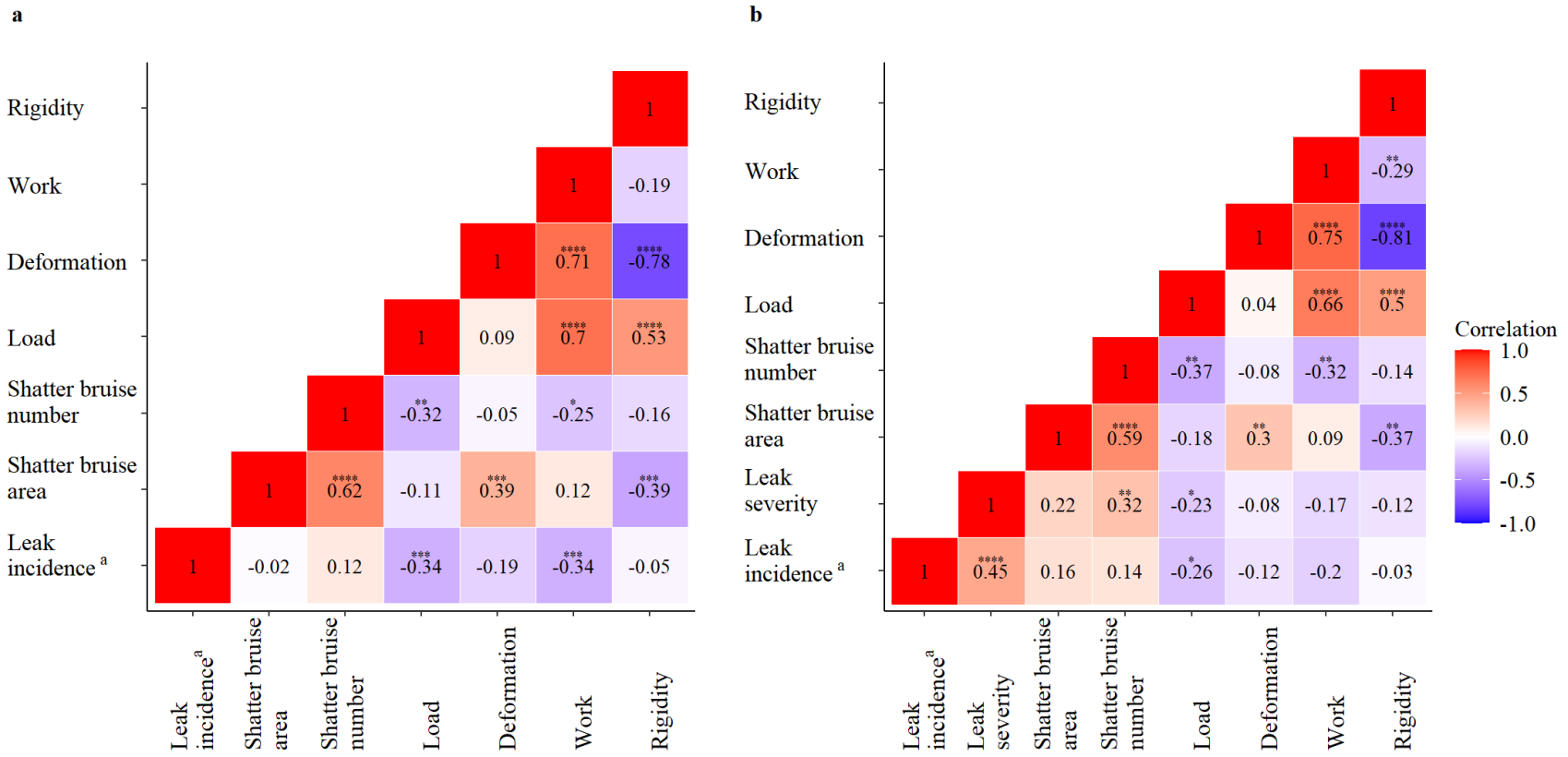

Figure 2.2: Pearson correlation coefficients (r) of each variable at 12.8°C (a) and 21.1°C (b) pulp temperature treatments over all cultivars. Asterisk(s) above coefficients denote statistical significance. *= 0.05 > p ≥ 0.01, **= 0.01 > p ≥ 0.001, ***= 0.001 > p ≥ 0.0001, ****= 0.0001 > p

[a]Spearman rank correlation was performed when correlating variables to leak incidence

Chapter 3 : EVALUATION OF BIOLOGICAL CONTROL AGENTS AND CONVENTIONAL PRODUCTS FOR POST-HARVEST APPLICATION ON POTATO (*SOLANUM TUBEROSUM* L.) TO MANAGE LEAK

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ABSTRACT

Biological control agents applied post-harvest may provide an effective way to manage leak of potatoes by competing with *Pythium ultimum*. The objective of this paper was to test the efficacy of various biological control agents and conventional post-harvest fungicides to manage leak. Two studies were performed with a *Pseudomonas fluorescens* triculture including desiccation tolerant variants of strains S11P12, P22Y05, and S22T04 in three formulations (a "fresh" triculture, dried on Kenite 700, or dried on Attapulgite clay) applied at a rate of 3.5 mL kg^{-1}. A third study contained treatments of the *P. fluorescens* "fresh" triculture, triculture dried on Kenite, triculture dried on Kenite blended with a fungicide containing a three-way mixture of azoxystrobin, fludioxonil, and difenoconazole (Azo+Flu+Dfz, 0.033 mL kg^{-1}), and triculture dried on Kenite rehydrated with nutrient broth Medium 1 blended with the same fungicide. Other treatments included *Pseudomonas syringae* (3.5 mL kg^{-1}) in Studies 1 and 2, while hydrogen peroxide and peroxyacetic acid (0.042 mL kg^{-1}), phosphorous acid (4.2 mL kg^{-1}), and Azo+Flu+Dfz (0.033 mL kg^{-1}) were used in all three studies. The three studies were each repeated twice using unwashed tubers (cv. 'Russet Burbank') that were wounded and inoculated with *P. ultimum*. After inoculation, tubers were treated, then stored at 21°C for four days prior to disease evaluation. In Study 1, none of the formulations of *P. fluorescens* triculture significantly controlled leak compared to the inoculated control; however, the "fresh" triculture formulation significantly decreased leak incidence by 24% in Study 2. In all three studies the three-way fungicide mixture Azo+Flu+Dfz resulted in lower leak incidence (1, 7, and 24%, in studies 1, 2, and 3 respectively) compared with the inoculated control (12, 37, and 50% leak incidence, in studies 1, 2, and 3 respectively). Study 3 showed the formulations of *P. fluorescens* strains were not

effective in leak control but when combined with Azo+Flu+Dfz leak incidence significantly decreased compared to the inoculated control. The lowest leak incidence was seen when the triculture component of the fungicide mix was rehydrated with Medium 1 in Study 3. The *P. fluorescens* triculture formulations mixed with azoxystrobin, fludioxonil, and difenoconazole and rehydrated with Medium 1 had similar bacterial counts as the "fresh" triculture and the triculture dried on Kenite, potentially indicating a greater activity level afforded by cell revival in dilute culture medium. *P. syringae*, phosphorous acid and hydrogen peroxide-peroxyacetic acid were ineffective in controlling leak in all three studies. The efficacy of the Azo+Flu+Dfz mixture appears promising for the management of leak in storage. The efficacy of *P. fluorescens* tricultures used alone was inconsistent in limiting leak incidence (only significant in 1 of 3 studies), and additional research and development may be warranted to realize its potential as biological control agent.

Key words: *Pseudomonas fluorescens*, *Pythium ultimum*, Azoxystrobin, Fludioxonil, Difenoconazole, Phosphorous acid

INTRODUCTION

Microbial antagonists act as biological control agents (BCA) that compete with plant pathogens in natural agricultural ecosystems and may provide treatment options for reducing plant diseases and application rates of conventional chemical fungicides (Caulier et al. 2018; Schisler and Slininger 1994; Slininger et al. 2010). The National Center for Agricultural Utilization Research (NCAUR, USDA-ARS, Peoria, IL) has developed BCA post-harvest applications using bacterial species and strains of *Pseudomonas*, *Enterobacter*, and *Pantoea*, which were isolated from potato (*Solanum tuberosum* L.) periderm samples from Wisconsin potato fields having low incidence of Fusarium dry rot disease (Schisler and Slininger 1994). Bacterial strains were isolated from wounded potatoes that were did not develop Fusarium dry rot after being inoculated with *Fusarium sambucinum* (Schisler and Slininger 1994). These bacterial strains have since been effective in limiting dry rot in post-harvest applications (Schisler et al. 2000). Disease suppression has been reported to be greater with applications of mixtures that included multiple strains of BCAs (Schisler et al. 1997; Slininger et al. 2001). The strategy of mixing multiple strains of BCAs has also been successfully used in other crops (Cruz et al. 2006; de Boer et al. 1999; Duffy et al. 1996; Guetsky et al. 2001; Janisiewicz 1996; Leeman et al. 1996). Slininger et al. (2010) further showed the co-cultivation of three *Pseudomonas fluorescens* strains was more consistent than pure strains and blends of pure cultures in the suppression of post-harvest potato dry rot, late blight, pink rot, and sprouting.

Microbial antagonists can compete with soil-borne pathogens by sequestering nutrients or by producing antimicrobial compounds that are detrimental to plant pathogens and therefore can assist in post-harvest disease control (Brodhagen et al. 2004; Elad and Chet 1987; Haas and Défago 2005; Howell and Stipanovic 1980; Howie and Suslow 1991; Kloepper et al. 1980; Kraus and Loper 1992; Nelson et al. 1986; van Dijk and Nelson 2000). Three specific *P. fluorescens* strains (Schisler and Slininger 1994) have been developed by adaptive laboratory evolution to be resistant to desiccation in order to improve the stability of dried formulations, and these formulations have been tested as co-cultures for effectiveness against potato storage diseases (Slininger et al. 2018). Oomycetes successfully controlled by the desiccant resistant *P. fluorescens* strains include *Phytophthora infestans* (cause of late blight) and *Phytophthora erythroseptica* (cause of pink rot, Schisler et al. 2009; Slininger et

al. 2007; Slininger et al. 2010), but *Pythium ultimum* Trow var. *ultimum* (Barr et al. 1996) the causal agent of leak in potatoes, has not been tested. Other studies have shown BCA as seed treatments to be effective in controlling *Pythium* species in various crops (Becker and Cook 1988; Elad and Chet 1987; Howell and Stipanovic 1980; Weller and Cook 1986) and thus may be effective in controlling leak in stored potatoes.

Pythium ultimum infects potato tubers through wounds or openings and develops a dark lesion that profusely leaks water causing the disease leak (Powelson and Rowe 2008; Salas and Secor 2001). Leak can directly cause potatoes to decay (Jones 1935; Powelson and Rowe 2008) and indirectly provide ideal environments for other pathogens like *Pectobacterium* species to take advantage of the free moisture and anaerobic conditions (Charkowski 2015; Lambert et al. 2005). Mefenoxam (R-enantiomer of metalaxyl) is the primary chemical used to control leak, but effectiveness against leak has been inconsistent (Bosca et al. 2006; Gachango et al. 2012a; James and Stevenson 1999; Mulrooney 1982; 1998; Platt 2003; Taylor et al. 2004). Managing leak is difficult during the growing season because of variable soil moisture (Peters et al. 2001), low levels of mefenoxam in tubers (Barak et al. 1984; Bruin et al. 1982), and the presence of resistant isolates to mefenoxam (Porter et al. 2009; Taylor et al. 2008). In general, there are three types of post-harvest products that could be applied to potatoes for potential leak control: BCAs, conventional fungicides, and disinfectants.

Besides *P. fluorescens*, *Pseudomonas syringae*, another BCA, produces several antimicrobial compounds (Smilanick et al. 1996) that have inhibited pathogen-causing diseases in apple, citrus, and cucumber (Bull et al. 1998; Kawasaki et al. 2016; Zhou et al. 2001). Mavrodi et al. (2012) identified a strain of *P. syringae* having activity against *P. ultimum* on wheat roots. However, this species of *Pseudomonas* has not been tested against *P. ultimum* on stored potatoes. Studies performed on potatoes have shown post-harvest applications with *P. syringae* to significantly reduced the incidence of Fusarium dry rot and silver scurf compared to the inoculated control (Al-Mughrabi et al. 2013) but needs to be evaluated for efficacy against *P. ultimum*.

There are a limited number of conventional post-harvest fungicides labelled to manage potato storage diseases. Phosphorous acid-based fungicides are registered systemic fungicides for potato diseases. Pink rot and tuber late blight are well managed with phosphorous acid

foliar applications (Inglis et al. 2004; Johnson et al. 2004) and post-harvest tuber applications (Gachango et al. 2012a; Gachango et al. 2012b; Johnson 2008; Miller et al. 2006). Gachango et al. (2012a and 2012b) observed phosphorous acid activity against leak when applied on foliage and to tubers post-harvest. However, Johnson et al. (2004) did not show an effect on leak incidence or severity when phosphorous acid was applied to potato foliage. Tubers treated with a post-harvest application of the fungicide azoxystrobin showed a 25% reduction in leak incidence compared to the inoculated control (Gachango et al. 2012a). A three-way mixture of azoxystrobin, fludioxonil, and difenoconazole (Azo+Flu+Dfz) is registered for post-harvest use on potatoes to control Fusarium dry rot and silver scurf and although Gachango et al. (2012b) has shown Azo+Flu+Dfz to have activity against leak, it is not currently labelled for this disease.

Disinfectants have also been used to manage potato storage diseases with inconsistent results. For example, a mixture of hydrogen peroxide and peroxyacetic acid reduced the incidence of silver scurf by 36% compared with the non-treated control (Afek et al. 2001), however Miller et al. (2011) observed mixed results by location and year. The control of tuber late blight has also been inconsistent (Gachango et al. 2012a; Johnson 2008; Miller et al. 2006). These inconsistencies may be attributed to different application methods and the high potential for inactivation of disinfectants due to high organic matter on the surface of the potato tubers (Olsen et al. 2003).

Leak management has traditionally relied on cultural practices to limit wounding and to harvest when tuber pulp temperatures are below 15°C (Hollingshead et al. 2020). Post-harvest applications of conventional fungicides and BCAs could decrease disease development when used in conjunction with cultural practices and reduce the risk of resistance to conventional fungicides. Because BCAs are living organisms, handling precautions are necessary, and activity can be less predictable. Therefore, the objective of this paper was to test the efficacy of *P. fluorescens* triculture BCAs in different formulations, another post-harvest BCA product *Pseudomonas syringae*, and conventional chemical fungicides against Pythium leak of potatoes.

MATERIALS AND METHODS

Potato production and storage

Three separate studies (each repeated twice) were performed. For Studies 1 and 2, cv. Russet Burbank potatoes were grown at the University of Idaho Kimberly Research and Extension Center (KREC, Kimberly, ID) in 2017 according to recommendations for south central Idaho (Stark and Love 2003). Tubers were cured at 12.7 ± 0.15°C with 95% RH for 17 days. The temperature was then ramped down to a holding temperature of 5.6°C (95% RH) at a rate of 0.3°C per day. In 2018, for Study 3 Russet Burbank potatoes were obtained from a commercial storage held at 8.9°C. In all studies unwashed tubers (170 to 340 g) were warmed and held at 21°C (95% RH) prior to inoculation. The treatment of BCAs, fungicides, and disinfectants as outlined with bacterial counts, rates and source are further explained and listed in Table 1.

P. fluorescens strains and triculture production

Disease suppressive *P. fluorescens* biovar 5 strains S11P12 (NRRL B-21133) and P22Y05 (NRRL B-21053) and *P. fluorescens* biovar 1 strain S22T04 (NRRL B-21102) were originally isolated by Schisler and Slininger (1994). Their respective desiccation tolerant derivatives P12-42d-D9 (NRRL B-67669), Y05-214d-4 (NRRL B-67675) and T04-126d-F4 (NRRL B-67670) were obtained by adaptive laboratory evolution (Slininger et al. 2018), and stored lyophilized in the ARS Patent Culture Collection (NCAUR, USDA, Peoria, IL). Stock cultures of these bacteria in 10% glycerol were prepared and stored at -80°C. Glycerol stocks were streaked onto 1/5 strength trypticase soy broth agar plates (1/5 TSA; Difco Laboratories, Detroit, MI), incubated two to three days at 25°C, checked for purity and refrigerated up to one week as a source of preculture inoculum. Purity streaks were sub-cultured as bar streaks onto 1/5 TSA 24 h at 25°C before every experiment.

Liquid precultures of each bacterial triculture were grown on semi-defined complete liquid (SDCL) medium according to Slininger et al. (2010). The 24 h unwashed cells were harvested, sampled to determine optical density, and used to inoculate tricultures containing the three desiccation tolerant variants. BCA production cultures (75 mL each) were inoculated to a total initial optical density of 0.1 (620 nm, 1 cm pathlength) where desiccation tolerant variant strains had the initial optical density ratio P12-42d-D9: T04-126d-F4: Y05-214d-4 of 1:4:1 in the triculture. The production cultures were shaken at 250 rpm at 25°C until harvested at 28 h. At harvest, the optical density and viable cell counts of tricultures were assessed.

Colony forming units (cfu) of each strain population in the tricultures were determined using dilution plating onto selective media as described previously (Slininger et al. 2010).

Preparation of P. fluorescens triculture treatments

To prepare "fresh" triculture treatments, 200 mL of BCA production culture was centrifuged to pellet the cells, supernatant removed, and the pellet resuspended in 100 mL of sterile pH 7 buffer. The cell suspension was refrigerated (4 to 6°C) until treatment application 6 days after production culture harvest.

To prepare each dried BCA treatment, 200 mL of BCA production culture was centrifuged (7000 rpm) to pellet cells, pelleted cells were resuspended in 30 mL pH 7 buffer (Schisler et al. 2016) containing 26.67 g L^{-1} fructose and maintained on ice. The 30 mL BCA cell suspension was added with 10 mL chilled distilled water to 20 g of Kenite 700, and the mixture kept on ice. Similarly, a 30 mL BCA cell suspension was added to 20 g of Attapulgite 325L (Georgia) clay, and the mixture kept on ice. The BCA-carrier mixtures were stirred until a uniform product resulted. Moist BCA-carrier mixtures were transferred to 12.7 x 19.1 cm aluminum trays. The trays were placed in a humidity chamber maintaining a constant airflow (6 psi) and 60% relative humidity (RH, Jackson and Payne 2007), allowing the contents of the trays to dry slowly overnight to a 3 to 5% target moisture range determined using an Omnimark moisture balance (typically 3% for Kenite and 5% for Attapulgite, on a percent wet weight basis). Each dry BCA-carrier powder treatment was scraped from the drying tray. Replicate BCA-carrier treatments were pooled, mixed thoroughly and refrigerated until application.

Post-harvest treatments

Fresh variant triculture (BCA 1) product for spray application was prepared with 42.5 mL of shaken double strength cell concentrate mixed with 42.5 mL distilled water containing 8 g L^{-1} fructose osmo-protectant. This mix provided 4 g L^{-1} fructose in the spray, which was equivalent to the fructose content of the dried triculture product sprays. The ready to spray product was stored in the refrigerator for no more than 3 h until applied to potatoes.

Dry triculture products were rehydrated to obtain 10^9 cfu mL^{-1} in the ready to spray treatment. Dry variant triculture on Kenite 700 was standardly rehydrated (BCA 2) as follows: 57.5 mL distilled water was added to 8.5 g of dry product with vigorous shaking for 1 min; after the product settled for 1 min, it was shaken 10 sec and allowed to settle for 2 min. Then

the top 42.5 mL was decanted, and 42.5 mL of cold distilled water was added to obtain an 85 mL cell suspension. The variant triculture dried onto Attapulgite clay with standard rehydration (BCA 3) was similarly prepared.

When the reconstituted dried variant triculture was applied in combination with a three-way mixture of Azo+Flu+Dfz (Stadium^{R}, Syngenta Crop Protection, LLC, Greensboro, NC), 42.5 mL of double strength Azo+Flu+Dfz solution (0.0185 mL mL^{-1}) was added instead of water to the 42.5 mL cell suspension prepared from dried variant triculture on Kenite, so the spray would deliver the BCA along with Azo+Flu+Dfz at an application rate of 0.033 mL kg^{-1} when BCA was applied at a rate of 3.5 mL kg^{-1} (BCA 2 + Azo+Flu+Dfz).

Rehydrating BCA 2 in low level nutrient medium (Medium 1) instead of water, to activate cell metabolism, was performed to observe any benefits of an extended rehydration of the dried variant triculture with Azo+Flu+Dfz added (BCA 2-M1 + Azo+Flu+Dfz). This treatment was similarly prepared as the treatments that were rehydrated under the standard method, except 57.5 mL of Medium 1 was used in place of water to reconstitute the dried variant triculture. The top 42.5 mL was decanted, shaken at 25°C for 16 to 17 h, and then refrigerated until applied. The Medium 1 rehydrated variant triculture suspension was then diluted in half with 42.5 mL of cold double strength Azo+Flu+Dfz solution and applied to potatoes. BCAs were applied at a rate of 3.5 mL kg^{-1} (Table 1).

Treatments of Azo+Flu+Dfz (0.033 mL kg^{-1}), hydrogen peroxide (27%) and peroxyacetic acid (2%, HPPA, 0.042 mL kg^{-1}, StorOx^{R} 2.0, Biosafe Systems LLC., East Hartford, CT), *P. syringae* strain ESC-10 (0.006 g kg^{-1}, Bio-Save^{R} 10 LP, Jet Harvest Solutions, Longwood, FL), and phosphorous acid (4.2 mL kg^{-1}, Phostrol^{R}, Nufarm Americas Inc, Alsip, IL) were applied to inoculated Russet Burbank potatoes in the two repeated trials of the three studies. Treatments were compared to an inoculated control of *P. ultimum*, treated with distilled water and a non-inoculated control (Table 1).

P. ultimum isolate maintenance and preparation

Culture of *P. ultimum*, identified as isolate 09 MN 10-5, was obtained from Dr. Neil Gudmestad's lab at North Dakota State University (Fargo, ND) and used throughout all experiments. It was maintained on P_5ARP media (Jeffers and Martin 1986) and grown in VWR Diurnal Growth Chamber, model 2015 set to 21.1 ± 0.2°C (VWR International, Cornelius, OR). V8 liquid broth was prepared by mixing Campbell's V8 juice and calcium

carbonate (0.1 g mL^{-1} of juice) which was centrifuged for 5 min at 10,000 rpm (Sorvall RC-6 plus, Thermo Scientific). One-hundred ml of clarified V8 juice was added to 900 ml of de-ionized water. The broth was autoclaved then cooled to room temperature for use. Fifty plugs of *P. ultimum* were added to 1 L of broth, shaken (120 ±1 rpm, New Brunswick Innova 2000, Eppendorf, Hamburg, Germany) and maintained at 21°C. After the culture grew for 10 to 14 days, mycelial mats were strained from the broth and placed in a Waring commercial blender (model 91-21.15, Torrington, CT) with 200 mL of de-ionized water, then homogenized for three minutes. The adjusted oospore concentrations were 1.0×10^5 oospores mL^{-1} for Studies 1 and 2 in 2017 and 2.5×10^5 oospores mL^{-1} for Study 3 in 2018.

Inoculation of tubers with P. ultimum and application of treatments

For each trial, 80 Russet Burbank potatoes (20 tubers $replicate^{-1}$ $treatment^{-1}$ $trial^{-1}$) were bruised by tumbling for 90 seconds in a cement mixer. Inoculum was applied after the first 60 seconds allowing for inoculated tubers to tumble for 30 seconds. For Study 1 the inoculum was applied using an air-assisted sprayer at a rate of 7.1 mL kg^{-1}. The inoculum for Studies 2 and 3 were applied at a rate of 6.6 mL kg^{-1}. Inoculated tubers were covered in plastic to maintain high humidity for approximately 30 min and then treated with the test treatments using an air assisted sprayer at the rates found in Table 1. Tubers were stored in enclosed boxes within a larger environmentally controlled bin set at 21°C (95% RH). Four days later tubers were sliced open longitudinally to be evaluated for incidence and severity of leak development. A weighted severity was calculated as the average percentage of the cut area showing disease symptoms using only infected tubers in the calculation.

Data Analysis

Effects of treatments on leak incidence were compared by mixed effects generalized linear model and mixed effects linear model on weighted leak severity. The estimated marginal means of leak incidence and leak severity were compared (α=0.05) by Tukey HSD adjustment in the R statistical package using Rstudio (Team R 2016) and reported in results. Packages used in the analysis include emmeans (Lenth 2019) and lme4 (Bates et al. 2015).

RESULTS

In Study 1, repeated trials were significantly different for leak incidence (p=0.0002) but not for weighted severity (p=0.43). The first trial had significantly lower leak (5%)

compared to the second trial (10%) but the treatment by trial interaction was not significant; therefore, the trials were combined with the results reported in Table 2. The bacterial counts for the 'fresh' triculture formulation (BCA 1) and the triculture dried on Kenite (BCA 2) appeared to be higher than the triculture dried on Attapulgite (BCA3) and *P. syringae* (Table 1). Azo+Flu+Dfz was the only treatment that significantly reduced leak incidence (1%) compared to the inoculated control (12%, Table 2). Leak incidence for the treatments BCA 1, BCA 3, *P. syringae,* and HPPA were not significantly different than the treatment Azo+Flu+Dfz. Leak incidence in tubers treated with phosphorous acid (18%) and BCA 2 (13%) were not significantly different from the inoculated control (12%). The weighted severity of treatments BCA 1 (29%) and phosphorous acid (31%) were significantly less than treatment BCA 2 (49%); no other treatments were significantly different in weighted severity. The low incidence in the non-inoculated control and Azo+Flu+Dfz treatments resulted in not being able to use the weighted severity in the analysis.

In Study 2, repeated trials significantly impacted leak incidence (p=0.048) but not for weighted severity (p=0.36). The first trial had a leak incidence of 14% compared to the leak incidence of 31% for trial 2 but the treatment by trial interaction was not significant; therefore, the trials were combined and reported in Table 2. The bacterial counts for the "fresh" triculture formulation (BCA 1) and the triculture dried on Kenite (BCA 2) appeared to be higher than the triculture dried on Attapulgite (BCA 3) and *P. syringae* (Table 1). Leak incidence was not significantly different between treatments of Azo+Flu+Dfz (7%) and BCA 1 (13%) and both treatments were significantly lower than the inoculated control (37%). None of the other treatments were significantly different from the inoculated control as leak incidence ranged from 21 to 37% (Table 2). The inoculated control treatment had the highest weighted severity (69%) but was only significantly different from Azo+Flu+Dfz (45%) and BCA 1 (49%) treatments.

A higher concentration of *P. ultimum* oospores for inoculation was used in Study 3 compared to Studies 1 and 2, resulting in greater incidence of leak for all treatments (Table 3). The repeated trials did not significantly affect leak incidence (p=0.10); therefore, the trials were combined. The bacterial counts for "fresh" triculture (BCA 1), the triculture dried on Kenite (BCA 2), and the triculture dried on Kenite combined Azo+Flu+Dfz (BCA 2 + Azo+Flu+Dfz) ranged from 1.56 to 1.79 x 10^9cfu mL^{-1}; however, the bacterial counts were

slightly higher for BCA-M1 + Azo+Flu+Dfz (2.63 x 10^9 cfu mL^{-1}, Table 1). The addition of the fungicide did not appear to affect bacterial counts of the BCAs. The BCA treatments without Azo+Flu+Dfz were not effective in controlling leak incidence compared to the inoculated control; however, leak incidence was significantly reduced compared to the inoculated control by 26, 20 and 33% for treatments Azo+Flu+Dfz, BCA 2 + Azo+Flu+Dfz, and BCA 2-M1 + Azo+Flu+Dfz, respectively (Table 3). The leak incidence of tubers treated with BCA 2 + Azo+Flu+Dfz, and BCA 2-M1 + Azo+Flu+Dfz were not significantly different from that of Azo+Flu+Dfz alone. Treatment BCA 2 + Azo+Flu+Dfz did not significantly reduce leak incidence compared to BCA 1, BCA 2, or phosphorous acid. However, treatment BCA2-M1 + Azo+Flu+Dfz did significantly reduce leak incidence compared to BCA 1 and BCA 2. Leak incidence of phosphorous acid (47%) and hydrogen peroxide-peroxyacetic acid (50%) treatments were not significantly different from the inoculated control (50%, Table 3).

DISCUSSION

Pythium ultimum is an opportunistic pathogen, which has difficulty competing for resources against other microbes (Elad and Chet 1987; Tedla and Stanghellini 1992; van Dijk and Nelson 2000); thus, BCAs may be effective against *P. ultimum*. *Pseudomonas* species suppress *Pythium* species by producing siderophores and secondary metabolites (Howell and Stipanovic 1980; Howie and Suslow 1991; Nowakthompson et al. 1994; Sarniguet et al. 1995), and through direct competition for nutrients (Elad and Chet 1987; Kraus and Loper 1992). Though the direct mode of action has not been identified, mixtures of *P. fluorescens* strains have been observed to decrease dry rot by 70% (Schisler et al. 1997) and co-cultured *P. flourescens* have been shown to effectively inhibit sprouting, late blight, dry rot, and pink rot development in stored potatoes (Slininger et al. 2010). Furthermore, Burkhead et al. (1995) showed all *Pseudomonas* strains able to suppress dry rot were also positive for production of one or more antifungal metabolites in bioauthography screening results.

The *P. fluorescens* triculture in BCA 1 treatment significantly reduced leak incidence compared to the inoculated control in only one of the three studies, indicating efficacy in controlling leak of stored potatoes may be inconsistent. The inconsistent efficacy observed in this study may also be explained by the different levels of applied bacterial counts of BCAs. However, the levels of the triculture in BCA 3 for Studies 1 and 2 would indicate the

Attapulgite clay dried bacterial triculture does not recover as well as the "fresh" triculture or the triculture dried on Kenite diatomaceous earth. Recently, Schisler et al. (2019) hypothesized inconsistent BCA performance by a yeast antagonist may be attributable to nutrients provided by non-viable cells of the BCA counteracting biocontrol provided by live cells of the same yeast antagonist. Non-viable cells of the BCAs used in the present study could similarly contribute to their inconsistent biocontrol performance.

Infection of potatoes by *P. ultimum* develops in less than 24 h (Hollingshead 2020) and has been observed to infect seed of other crops in 4 to 24 h after germination (Lifshitz et al. 1986; Nelson 1987; 1988; Nelson et al. 1986; Osburn and Schroth 1988; Stanghellini 1974; Stanghellini and Hancock 1971a). This rapid infection rate by *P. ultimum* is influenced by the temperature of tubers and may provide an advantage to the pathogen over *P. fluorescens* (Stanghellini 1974; Stanghellini and Hancock 1971b; Triki et al. 2001). Secondary metabolites that inhibit *P. ultimum* growth may not be in high enough concentrations until three days after application (Sarniguet et al. 1995). However, Kraus and Loper (1992) found secondary metabolites of *P. fluorescens* were not the only mechanism for pathogen inhibition, and competition for nutrients was another important mechanism. Under the bioassay conditions applied, the active mechanisms of *P. ultimum* inhibition by the "fresh" cell preparation BCA 1 was strong enough to reduce leak incidence from 12% to 8% in Study 1 and from 37% to 13% in Study 2, the latter being a statistically significant reduction. However, not all BCA treatments were able to decrease leak incidence, indicating the need for additional technology development to improve consistency against fast-acting pathogens such as *Pythium ultimum*. Dried formulations BCA 2 and 3 were particularly problematic. Even though viable cell counts were sufficient to sometimes allow small reductions of leak incidence, a significant reduction across Studies 1-3 was not seen. Rehydration methodology for reviving dried cells to a more competitive active state is being pursued to improve the efficacy and consistency.

Study 3 utilized a higher pathogen challenge to address two additional strategies to improve leak control: the use of Medium 1 nutrient rehydration to more fully restore dried BCA cell activity prior to application and the use of dried BCA in mixture with chemical fungicides. This study found post-harvest applications of BCAs combined with Azo+Flu+Dfz did not decrease leak incidence compared to the Azo+Flu+Dfz treatment. Even though, in

study 3 the leak incidence of tubers treated with BCA 2-M1 + Azo+Flu+Dfz was not significantly lower than BCA 2 + Azo+Flu+Dfz nor Azo+Flu+Dfz, disease incidence was low and may indicate rehydration of the BCA with Medium 1 aids the recovery of viable cell counts and metabolic activity of BCA populations after dry storage. The rehydration with Medium 1 could result in greater revival of active BCAs that are more capable of competing with *P. ultimum* by sequestering nutrients and/or producing inhibitory secondary metabolites. The effects of Medium 1 on BCA populations and secondary metabolite production and the effectual control of leak warrants further investigation.

The Azo+Flu+Dfz treatment decreased leak incidence in each of the studies which is consistent with Gachango et al. (2012b). Gachango et al. (2012b) observed azoxystrobin to be effective in controlling leak; however, fludioxonil and difenoconazole have not been studied separately for activity against *Pythium* species (Bartlett et al. 2002; Gachango et al. 2012a; Gachango et al. 2012b). Azo+Flu+Dfz appears to be primarily responsible for decreasing leak incidence alone or mixed with BCA treatments; however, the BCA treatments may become more competitive with weakened *P. ultimum* when Azo+Flu+Dfz are mixed with biological antagonists. Therefore, a next step of this research is to test the efficacy of BCA treatments mixed with lower rates of Azo+Flu+Dfz to control leak development.

Pseudomonas syringae is another BCA of several potato diseases (Al-Mughrabi et al. 2013). However, when tested against *P. ultimum, P. syringae* strain 39A2 was not as effective as *P. fluorescens* (Mavrodi et al. 2012). *Pseudomonas syringae* may also need more time to produce secondary metabolites and compete with the rapid growth of *P. ultimum*. The average number of colonies of *P. syringae* was less than the number of *P. fluorescens* colonies (Table 1) and may have contributed to a lack of control in this study. Al-Mughrabi et al. (2013) observed 10^5 cfu of *P. syringae* was effective when mixed with thiabendazole against tubers inoculated with *Fusarium sambucinum* and *Helminthosporium solani*. *Pseudomonas syringae* concentrations may need to be increased or applied with a conventional fungicide such as Azo+Flu+Dfz to increase the control of leak.

Hydrogen peroxide plus peroxyacetic acid and phosphorous acid are registered for use in post-harvest applications for potatoes. However, neither of these post-harvest products demonstrated significant control of leak in stored potatoes in these studies. These results are in contrast to the findings of Gachango et al. (2012a), which showed hydrogen peroxide

significantly reduced leak incidence by 36%. In our study the tubers were not washed, which may result in hydrogen peroxide reacting with the soil on tuber surfaces before it interacts and kills propagules of *P. ultimum* (Feliziani et al. 2016).

Phosphorous acid is very effective against *Phytophthora erythroseptica* and *Phytophthora infestans* (Clayson and Miller 2007; Miller et al. 2006). However, foliar applications of phosphorous acid were not effective in protecting tubers going into storage from leak (Inglis et al. 2004; Johnson et al. 2004). Our study did not show efficacy against leak with a post-harvest application, in contrast to research reported by Gachango et al. (2012a; 2012b). The differences in results may be due to cultivar, if tubers were washed or not, or variation in the inoculation and application procedures.

Results of these studies underscore the lack of efficacy against leak for many of the post-harvest products. The BCA treatments of *P. fluorescens* were inconsistent in reducing leak when applied to inoculated potatoes and the treatments of *P. syringae*, HPPA, and phosphorous acid were ineffective in controlling leak. The mixture of Azo+Flu+Dfz was the only treatment that consistently reduced leak in these studies. When Azo+Flu+Dfz were combined with strains of *P. fluorescens* the incidence of leak was significantly lower than the inoculated control, but not significantly different from Azo+Flu+Dfz alone. There may be potential for *P. fluorescens* BCAs to reduce leak when rehydrated with a nutrient source that may improve BCA competitiveness, and this strategy warrants further study. Combining BCAs with conventional fungicides may allow for decreased rates of conventional fungicides and would provide another mode of action to broaden bioactivity and reduce the risk of pathogen resistance.

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TABLES

Table 3.1: Post-harvest treatments, labels, application rates, sources and bacterial counts for all studies

Treatment labels	Treatments	Total Bacterial counts (cfu's ml^{-1}) [ab]			Application rate	Source
		Study 1	Study 2	Study 3		
BCA 1	"Fresh" variant triculture	3.06 x 10^9	4.10 x 10^9	1.68 x 10^9	3.5 mL kg^{-1}	NCAUR, USDA-ARS, Peoria, IL (Slininger et al. 2010)
BCA 2	Dry variant triculture on Kenite	1.22 x 10^9	4.05 x 10^8	1.79 x 10^9		
BCA 3	Dry variant triculture on Attapulgite clay	5.27 x 10^5	1.10 x 10^6	NA		
BCA 2 + Azo+Flu+ Dfz	Dry variant triculture on Kenite + Azo+Flu+Dfz[c]	NA	NA	1.56 x 10^9	3.5 mL kg^{-1} + 0.033 mL kg^{-1}	NCAUR, USDA-ARS, Peoria, IL (Slininger et al. 2010) + Stadium[R], Syngenta Crop Protection, LLC, Greensboro, NC
BCA 2-M1 + Azo+Flu+ Dfz	Dry variant triculture on Kenite rehydrated with nutrient broth medium 1 + Azo+Flu+Dfz[c]	NA	NA	2.63 x 10^9		
P. syringae	*Pseudomonas syringae* strain ESC-10	1.00 x 10^8	8.90 x 10^7	NA	3.5 mL kg^{-1}	Bio-Save[R] 10 LP, Jet Harvest Solutions, Longwood, FL
HPPA	Hydrogen peroxide (27%) Peroxyacetic acid (2%)				0.042 mL kg^{-1}	StorOx[R] 2.0, Biosafe Systems LLC., East Hartford, CT
	Phosphorous acid				4.2 mL kg^{-1}	Phostrol[R], Nufarm Americas Inc, Alsip, IL
Azo+Flu+ Dfz[c]	Azoxystrobin (12.51%) Fludioxonil (12.51%) Difenoconazole (9.76%)				0.033 mL kg^{-1}	Stadium[R], Syngenta Crop Protection, LLC, Greensboro, NC
	Inoculated control (*Pythium ultimum*)					
	Non-inoculated control					

[a] Variant triculture treatments were not part of study if counts are NA
[b] Total average counts include bacterial variants S11P12, S22T04, and P22Y05
[c] Azo=Azoxystrobin, Flu=Fludioxonil, Dfz=Difenoconazole

Table 3.2: Means of leak incidence and weighted severity between post-harvest treatments in studies conducted in 2017

Treatment[a]	Study 1		Study 2	
	Incidence (%)[b]	Weighted severity (%)[b]	Incidence (%)[c]	Weighted severity (%)
BCA 1	8 ab	29 a	13 ab	49 a
BCA 2	13 b	48 b	23 bc	63 ab
BCA 3	9 ab	35 ab	31 c	63 ab
P. syringae	7 ab	39 ab	21 bc	61 ab
HPPA	11 ab	36 ab	22 bc	60 ab
Phosphorous acid	18 b	31 a	25 bc	59 ab
Azo+Flu+Dfz	1 a	23[c]	7 a	45 a
Inoculated control	12 b	37 ab	37 c	69 b
Non-inoculated control	2 a	33[c]	0[c]	NA[d]
p-value	<0.0001	0.013	<0.0001	0.0076

[a] Treatments consisted of variant tricultures formulations. BCA 1= "Fresh" variant triculture, BCA 2= Variant triculture dried on Kenite, BCA 3=Variant triculture dried on Attapulgite clay, HPPA=Hydrogen peroxide and peroxyacetic acid, Azo+Flu+Dfz=azoxystrobin, fludioxonil, and difenoconazole mixture

[b] Within columns, values with differing letters signify statistical significance (α=0.05)

[c] Omitted from analysis due to too few tubers infected

[d] No symptoms were observed to calculate weighted severity

Table 3.3: Means of leak incidence between post-harvest treatments in Study 3 conducted in 2018

Treatment[a]	Incidence (%)[b]	
BCA 1	42	cd
BCA 2	45	cd
BCA 2 + Azo+Flu+Dfz	30	bc
BCA 2-M1 + Azo+Flu+Dfz	17	b
HPPA	50	d
Phosphorous acid	47	cd
Azo+Flu+Dfz	24	b
Inoculated control	50	d
Non-inoculated control	1	a
p-value	<0.0001	

[a] Treatment abbreviation definitions: BCA 1= "Fresh" variant triculture, BCA 2= Variant triculture dried on Kenite, BCA 2 + Azo+Flu+Dfz= Variant triculture dried on Kenite with azoxystrobin, fludioxonil, and difenoconazole, BCA 2-M1 + Azo+Flu+Dfz= BCA 2 + Azo+Flu+Dfz but BCA 2 was rehydrated overnight with nutrient broth Medium 1 instead of distilled water, HPPA=Hydrogen peroxide and peroxyacetic acid, Azo+Flu+Dfz=azoxystrobin, fludioxonil, and difenoconazole mixture

[b] Values with differing letters signify statistical significance (α=0.05)

Chapter 4 : MONITORING TOOLS FOR A POTATO BRUISE PREVENTION PROGRAM

Hollingshead, A.K, R. Hendricks, N.L. Olsen, and M.T. Thornton. 2020. Monitoring tools for a potato bruise prevention program". University of Idaho Extension BUL 966.

INTRODUCTION

Bruising occurs whenever a potato impacts another surface, typically during windrowing, harvesting, conveying and throughout the handling and packing process (Brook 1993; Hyde et al. 1992). Bruises are more prevalent after potatoes fall from a significant height; hit the metal sidewalls of a conveyor; experience abrupt changes in direction; and/or collide against insufficiently padded equipment. In conjunction with force, acceleration, drop height, and impact surface, the degree of bruise development depends on tuber characteristics such as cultivar and pulp temperature. Consequently, knowing a cultivar's response to a physical impact and the risk for bruise development are beneficial in developing a bruise prevention program.

Shatter and blackspot bruises are two main defects that influence potato quality (Figure 4.1). Both result from an impact by an external force and can occur on the same potato. Shatter bruise occurs when the external impact force is great enough to cause the cells to rupture by physically breaking the skin, where it forms cracks or cuts (Baritelle et al. 2000). Though the force may not necessarily break the skin, if it damages the underlying cells, a blackspot bruise will start to develop within a few hours. The black discoloration forms when the damaged cells' phenolic compounds (e.g., tyrosine) are oxidized by the enzyme polyphenol oxidase into a dark pigment called melanin.

Both bruise defects are serious, particularly when they cause direct and indirect losses. Losses may be from direct waste, customer rejections, weight loss, and decay due to disease. In 1993, a 10% reduction in bruise was estimated to return an additional 74.7 million dollars to the US potato industry (Brook 1996). Adjusting only for inflation, that same 10% improvement would be equivalent to 134 million dollars in 2020. Regardless, any improvement on quality significantly enhances the potato's economic value.

Given the economic consequences of potato bruising, it is critical to quickly identify shatter and blackspot bruises and to pinpoint where impact forces occur in an operation. This bulletin provides crucial tools and detection methods that will help make effective management decisions to minimize bruising and thus mitigate quality issues.

SHATTER BRUISE MONITORING

Shatter bruise appears as cuts or cracks in the skin that reduce the visual appeal to a consumer. Water loss through these wounds is greater until healed. These cracks and cuts are also entry points for diseases such as Pythium leak, bacterial soft rot, and Fusarium dry rot.

Monitoring shatter bruise levels during handling operations provides opportunities to modify equipment and conditions and to identify lots or fields that have a greater degree of shatter bruise. A high level of shatter bruise at harvest will demand more intensive storage management that promotes wound healing, minimizes disease development, and avoids higher weight loss and pressure flattening and bruise.

A relatively simple way to detect shatter bruises is to use an iodine solution. Iodine interacts with starch to form a blue color. Any break in the skin of the potato, such as a shatter bruise, exposes the starch in the tissue to the iodine solution, which turns a blue or black color (Figure 4.2). This coloration makes it easy to determine the number and severity of the shatter bruises. Commercial iodine solutions (1%–2% titratable iodine) can be found at most farm/ranch stores in the livestock department. These solutions are considered safe and generally prevent infection of wounds on livestock. The procedure is simple but requires some initial setup.

IODINE STAINING PROCEDURE

PREPARATION

- Identify various handling operations where bruising may be a problem (windrower, sharp directional changes, harvester, truck to stinger, etc.).
- Sample 15–25 potatoes from each location. Keep them separated in mesh bags. **Label the bags by location**. Mesh bags keep samples separated and make it easier to wash off soil and iodine from the potatoes.
- Procure the following supplies:

- Two containers large enough to fit multiple samples of 15–25 potatoes that are submerged in liquid
- Iodine solution (label will say “1% or 2% titratable iodine”)
- Mesh bags to hold samples

PROTOCOL

1. Wash tubers to remove excess soil. Washing potatoes will help keep the water clean and allow bruises to color more readily.
2. Prepare the containers. Fill both containers with enough water to submerge the potatoes in the mesh bags (use one to submerge the potatoes in iodine and the other to wash/rinse them after the iodine bath).
3. Mix the iodine solution (Table 4.1). Combine the iodine with water to produce a specific ratio, which determines the length of time the tubers need to soak to reveal any shatter bruises. For example, a 1:1 (volume of iodine solution to water) ratio requires at least 20 minutes for shatter bruises to turn color. A ratio of 1:15 will need at least 60 minutes.

   Note: If reusing the iodine solution, additional time to discolor may be needed.
4. Soak potatoes in mesh bags for the appropriate time in the dilute iodine solution.
5. Wash or rinse potatoes in a second container filled with water.
6. Count the number of shatter bruises per tuber in each sample (potatoes do not need to be peeled to see the shatter bruises).
7. Wear gloves and dispose of the used iodine solution in the same manner as other pesticides.
8. If the iodine:water solution is fairly clean, simply cover, store, and reuse it. Solutions should not be kept for more than four days. However, the extremely dilute solutions (1:6.5 or less) will not keep for more than two days. Discard tubers after the evaluations are complete and do not allow them to enter the food system.

   Note: Efficacy of the iodine solution decreases the more it is used and if exposed to sunlight.

Although the iodine staining procedure helps to identify the level of shatter bruise that occurs during handling operations, an absence of shatter bruises does not mean physical impacts are not damaging potatoes. The impact may be causing blackspot bruise instead.

BLACKSPOT BRUISE MONITORING

Blackspot bruise forms when the damaged cells' phenolic compounds are oxidized by the enzyme polyphenol oxidase, which in turn produces quinones (pink color), and then transforms into dark pigments (melanin). To check for bruising, the potato must be peeled. The damaged cells appear as pink, red, brown, or black spots. Development of the black pigment is not immediate; however, it takes time to become visually apparent. The darkening is irreversible and considered a defect both for fresh and processing potatoes. Ideally, the sooner blackspot bruises are identified from impacts caused by conditions or equipment, the easier it will be to implement more effective changes or adjustments to reduce the potential for blackspot bruising. Peeling potatoes to identify the proportion of early blackspot bruise symptoms is therefore a useful tool to prevent further damage and adjust handling protocols for bruise control.

EARLY BLACKSPOT BRUISE MONITORING TECHNIQUE

This method allows early detection of bruise incidence but does not identify 100% of the bruises and scorable defects that will develop over a longer period of time.

1. Collect samples. Take tuber samples from multiple locations within the handling operation. Areas to consider: where tubers experience directional changes, drop from a conveyor, or move through specific pieces of equipment. Label the samples by location to keep track of the test areas.
    a. Sample size depends on the operation—if there are multiple opportunities for impact in the sampling area, take a larger sample. Each sample should range from 4.5 to 18.1 kg per location.
    b. Measure and record tuber pulp temperatures.
2. Hold tubers at warm temperatures.

   a. Place samples at room temperature (e.g., 21°C) for 3–5 hours. If it's necessary to test for the total incidence and severity of blackspot bruise, hold tubers for 24–48 hours before peeling.
   b. If you use a hot box (32–38°C), the duration of time until discoloration develops may be shorter but the severity of bruises may be slightly exaggerated at these higher temperatures.
3. Assess bruise.
   a. After tubers have been warmed for 3–5 hours, peel the skin and examine for discoloration.
   b. You may need to peel a few more layers of the potato to see the darkest discoloration.
   c. Discoloration will range from pink to dark brown the first few hours after impact (Figure 4.3). **Note**: Not all bruises will have formed at this time, especially mild bruises from lower impacts.
4. Examine all the peeled tubers and make note of the incidence and severity of the bruises.
5. Based on the level of bruising, make equipment adjustments to reduce impact forces at the sample location.

The early blackspot bruise monitoring technique was derived from a series of experiments utilizing multiple cultivars and years. The studies suggest discoloration, primarily pink discoloration, began to develop within the first hour after impact (Table 4.2). The change in bruise color from pink to brown primarily occurred 2–3 hours after impact, and pink discoloration declined rapidly after that time. The highest incidence and severity of blackspot bruise occurred at 24 hours; however, over 75% of the total bruises could generally be seen after 3–5 hours, depending upon force of impact and cultivar. For example, Russet Norkotah appeared slower while Ranger Russet was faster to develop blackspot bruises.

These studies highlight the utility of pink- or brown-/black-colored bruises as an early indicator of blackspot bruise incidence within an operation; but be aware that bruise development differs based on the cultivar. Also, lighter colors may indicate the freshest bruises, which can help in pinpointing the bruise source. Lastly, you can begin making bruise assessments within a few hours after sampling—it is not necessary to wait twenty-four hours.

Use your monitoring results to modify equipment, operation management, and/or other conditions to minimize blackspot bruise potential.

INSTRUMENTED SPHERE

An instrumented sphere is a logging device that measures and records the acceleration of an impact whose data can indicate which equipment, locations, or areas may increase the risk for bruising (Figure 4.4). The most problematic locations are where drop heights are large, padding is worn, and/or belt speeds are too fast (Figure 4.5).

The data that an instrumented sphere records includes

- Peak acceleration as g-force (G) (1 G = 9.8m/s^2). This data is measured by accelerometers and indicates the magnitude of an impact.
- Change in velocity. This measurement accounts for the impact surface and magnitude of the impact. **However, not all instrumented sphere models record change in velocity**.
- A time stamp. This log identifies when the impact took place. It helps to determine the locations that recorded damaging peak accelerations when testing multiple potential points of impact.

Peak acceleration and change in velocity are used to estimate a bruise threshold—defined as the force required to bruise a certain percentage of potatoes from one impact. For example, when Mathew and Hyde (1997) studied the bruising of potatoes that had been dropped 2.5 cm above a steel surface, the 10% bruise threshold was reached; however, the bruise threshold increased to 25.4 cm when the steel was coated with a cushioned material. The more rigid a surface, the lower the change in velocity, which increases the likelihood of bruising. If change in velocity is not recorded, then the threshold relies on the maximum acceleration that will cause a bruise. **Note**: the data from an instrumented sphere manufactured by one company cannot necessarily be interchangeable with the data from another instrumented sphere due to potential differences in technology, weight, and size, which affect both the recorded impact and change in velocity.

An individual instrumented sphere may or may not come with a preprogrammed bruise threshold. In general, typical thresholds range from 40-100 G but need to be determined for varying cultivars and operating systems. Cultivars may react differently to a

given impact and have a higher or lower bruise threshold. Bruise threshold can also be affected by pulp temperature and impact material. An instrumented sphere can be calibrated to determine the appropriate bruise threshold by testing the amount of bruise that occurs for each cultivar at different drop heights and pulp temperatures. Quantifying the amount of shatter and blackspot bruise, as described previously, and corresponding it to data recorded by the instrumented sphere will help to fine-tune bruise thresholds.

PROTOCOL TO ESTABLISH BRUISE THRESHOLDS

1. Drop potatoes from different heights onto various materials and record if blackspot and/or shatter bruise forms (pulp temperature should be noted as well). **Repeat for each cultivar.**
2. Drop an instrumented sphere at those same heights and onto different materials to obtain peak acceleration and change in velocity (if recorded).
3. Document the relationships that exist in the data.
    a. Plot the relationship between bruise incidence and drop height.
    b. Plot the relationship of peak acceleration and change in velocity (if available).
    c. Plot the relationship of drop height and peak acceleration.
4. Set an appropriate bruise threshold for an instrumented sphere.
    a. This may change based on impact surface, temperature, and cultivar.
    b. Use manufacturer information for guidance.
5. Run an instrumented sphere through each specific piece of handling equipment. Data is easier to interpret with 1 location of interest per run, but access to equipment may require multiple locations to be tested in each run (3 or less is ideal). Repeat runs at least 3–4 times to identify consistency in equipment. The results of the instrumented sphere may change from one run to the next as it falls onto potatoes, belts, or metal frames. Results consistently above the threshold indicate more potatoes are likely to bruise than when results vary or are below the identified threshold. However, adjustments may need to be made in both circumstances.
    a. **Note that an instrumented sphere sinks in water**. Do not place it in flumes or wash barrels.

   b. **It is important to have many observers watch an instrumented sphere because it can easily get lost**. Shorter runs make it easier to track.
6. Locations with peak accelerations above the set threshold have a high potential for causing bruises. Check data by quantifying the bruising from samples collected at locations having above-threshold impacts.

CONCLUSION

Monitoring tools such as the iodine solution, rapid bruise development, and/or the instrumented sphere provide targeted information regarding beneficial modifications to operations, equipment, and/or conditions. Having quick, reliable, and easy information that identifies where to make these changes will result in fewer quality defects and losses.

EQUIPMENT MODIFICATIONS AFTER EVALUATIONS

1. Results from bruise monitoring assessments will dictate if further steps need to be taken to minimize shatter and blackspot bruises. Modifications may include adjusting or consulting the following:
   a. Harvest/handling equipment
      i. lower drop heights
      ii. add/replace cushioning
      iii. run belts full of potatoes
      iv. ensure proper equipment operation
   b. Environmental conditions
      i. lower/raise potato pulp temperatures
      ii. ensure proper soil moisture
   c. For additional information to bruise management , refer to https://www.uidaho.edu/cals/potatoes/bruise-management
2. After making adjustments to problem locations, resample to ensure the adjustments lowered the bruise level.

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TABLES

Table 4.1: To make 38 L of a dilute iodine solution, mix various ratios of iodine to water. The table describes the volumes of iodine solution (1% titratable iodine) and water to make up 38 L. The time needed for coloration to occur is dependent upon the ratio. Total liters of the mix can be adjusted based on size of containers and number of samples

Ratio (iodine:water)	Iodine solution (L)	Water (L)	Time for tubers to color (minutes)
1:15	2.4	35.6	60
01:06.5	5	33	50
1:03	9.5	28.5	30
1:01	19	19	20

Table 4.2: Blackspot bruise incidence for three cultivars bruised at a 30.5 cm drop height with pulp temperatures of 12.8°C and evaluated at room temperature (21°C)

Cultivar[a]	Bruise coloration index[b]	Hour Evaluated					
		1	2	3	4	5	24
		Blackspot Incidence (%)					
Russet Burbank	Pink	54	62	46	14	6	6
	Total	54	65	73	77	80	95
Russet Norkotah	Pink	29	43	36	21	7	2
	Total	29	46	52	64	67	88
Ranger Russet	Pink	58	29	7	6	2	0
	Total	70	74	76	82	79	97

[a] Russet Burbank and Russet Norkotah were examined twice in 2018 and once in 2019. Ranger Russet was only examined in 2019. Trials in multiple years were combined.

[b]Pink incidence only includes bruises that were rated a 2 on the blackspot bruise color intensity scale (1 to 5): 1=none, 2=pink, 3=light brown, 4=dark brown, 5=black discoloration. Total incidence includes ratings of 2 to 5.

FIGURES

Figure 4.1: Potato tuber with shatter bruises (a). Blackspot bruises revealed after peeling a potato (b). Courtesy of Nora Olsen

Figure 4.2: The wounds on these potatoes are dark blue or black from soaking in a dilute iodine solution. Courtesy of Andrew Hollingshead

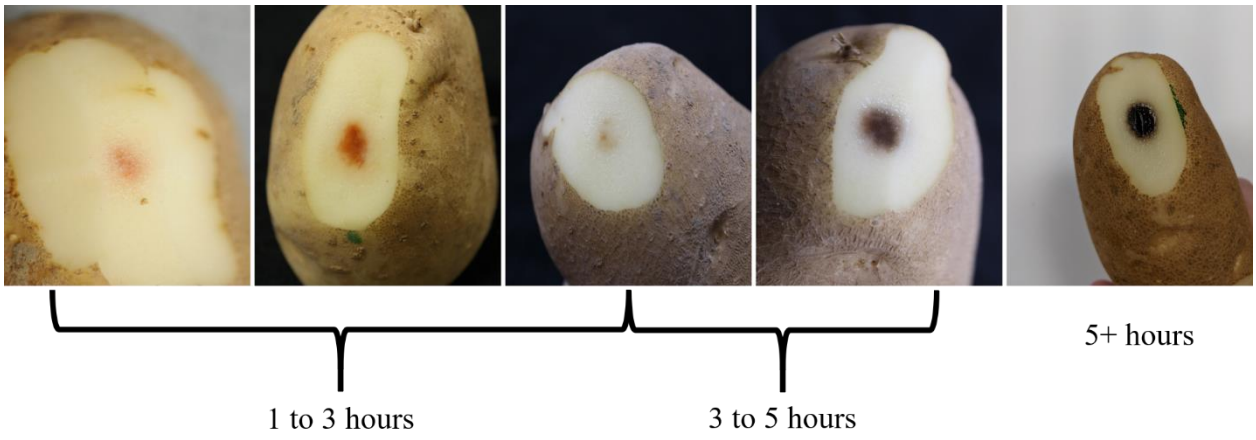

Figure 4.3: Range of bruise color seen within 5 hours in a Russet Burbank potato after an impact of 30.5 cm by a metal weight. Courtesy of Rabecka Hendricks

Figure 4.4: Example of an instrumented sphere [Techmark, Inc. Impact Recoding device (IRD)™] placed on top of soil to be harvested with potatoes to monitor the impacts during harvest. Courtesy of Rabecka Hendricks.

Figure 4.5: An IRD™ (red ball) recording impacts as potatoes are being conveyed in a fresh pack operation. Courtesy of Carlos Centeno

APPENDIX A: PROCEDURE FOR DEVELOPING INOCULUM PROTOCOL

Pythium ultimum can be a difficult pathogen to work with because it does not compete well with other pathogens, rarely produces motile zoospores, and instead oospores are the primary source of infection. Inoculation methods often used were artificial and did not reflect the typical course of inoculation and infection found during potato harvest. Inoculation methods were developed to mimic the environment and conditions in which disease develops during harvest and storage. To mimic this course, potatoes needed to be wounded, kept in a favorable environment for disease to develop, inoculated with the appropriate concentrations of oospores, and be easily and consistently repeatable. The inoculation procedure used in the studies was determined by first experimenting on how long to sufficiently wound in a cement mixer, when to spray the inoculum on wounded potatoes, how airflow influences leak development, determine the level of *P. ultimum* oospores to be applied to potatoes, and how soon after inoculation potatoes can be evaluated.

Pythium ultimum infects tubers through open wounds usually sustained at harvest, but previously published research indicated wounding potatoes by removing the periderm using a corkborer, abrasive pads, or wire brushes (Gachango et al. 2012; Salas et al. 2003; Taylor et al. 2004). Wounding during harvest involves potatoes impacting or falling onto potatoes or metal equipment, therefore a cement mixer was used to mimic the conditions during harvest that would cause shatter bruises for pathogen infection. Twenty Russet Norkotah tubers were bruised in a cement mixer for 3 or 5 minutes and $5x10^5$ and $1.3x10^6$ (2x2 factorial design) oospores mL^{-1} concentrations were sprayed onto potatoes at a rate of 2 mL $tuber^{-1}$ with only one replicate and performed once. The first results showed 100% disease incidence when bruising for 3 and 5 minutes with $5x10^5$ and $1.3x10^6$ oospores mL^{-1} concentrations (Table A.1). The pressure for leak was too high for the initial trial, so bruising time in the cement mixer was reduced to 1.5 and 3 minutes and inoculum was applied at of 2 mL $tuber^{-1}$ ($5x10^5$ oospores mL^{-1}). Inoculum was also applied at 30, 60, 80, or 90 s during the bruise cycle with two non-inoculated controls (2x4 factorial design). The trial was repeated two times with only one replicate. Treatments significantly affected leak incidence ($p=0.0008$). Bruising the potatoes for 1.5 min and applying inoculum with 80 s remaining in the bruise cycle had significantly less leak incidence than bruising for 3 min and applying inoculum with 60 s

remaining in the bruise cycle (Table A.2). A contrast showed bruising for 3 minutes resulted in significantly ($p=0.0009$) more leak incidence than bruising for 1.5 minutes. Bruising potatoes for 1.5 minutes allowed *P. ultimum* to infect but not overwhelm the tubers. The contrast between inoculating with 30 s remaining in the bruise cycle versus inoculating with 60 s or more was not significantly ($p=0.78$) different. This indicated that the total bruise time influenced leak incidence more than when inoculum was applied during the bruise cycle.

Tubers of cultivar Russet Norkotah with pulp temperatures of 15.5 and 21.1°C were bruised for 90 seconds and inoculated ($5x10^5$ oospores mL^{-1}) after 0, 30, 60, 75 seconds, and compared to a no bruise control. The experiment was 2x5 factorial completely randomized split-plot design repeated twice with three to four replicates (n=20 tubers $treatment^{-1}$ $replicate^{-1}$). Leak incidence was significantly affected by timing of inoculation ($p=0.0013$) and temperature ($p=0.0014$). Timing of inoculum treatments had varying leak incidence, but inoculum applied with 90 s left (22%) in the bruise cycle was significantly lower than applying with 60 or 15 s left (33 and 38 % respectively, Table A.3). Leak incidence was not significantly different when inoculum was applied with 30 seconds left in the 90 second bruise cycle and the other inoculation timings. This supports the data of Table A.2 that timing of inoculation does not affect leak incidence and the inoculum can be applied at any moment during the 90 second bruise cycle. It was chosen to inoculate with 30 s left in the bruise cycle to allow for sufficient bruising. Leak incidence was significantly lower at 15.5°C than 21.1°C. The no bruise control had zero leak incidence showing that leak needs a wound to infect potatoes.

Soil can adhere to the periderm of potatoes during harvest, which has the potential of carrying oospores. It was important to know if the soil adhering to the potatoes would alter leak incidence. Russet Norkotah potatoes (n=178 potatoes), half washed and the other half remained unwashed, were either inoculated ($1x10^5$ oospores mL^{-1}) or not inoculated. The leak incidence between inoculated washed tubers and unwashed tubers was 15.9% while there was no leak development in non-inoculated tubers. It was concluded that tubers did not need to be washed before inoculation.

High airflow in storages are recommended if crops are being stored with a known disease problem because excess moisture creates favorable environments for secondary pathogens. It was hypothesized that by sealing boxes and limiting airflow, disease incidence

would increase. Plastic boxes (53.3x38.7x31.1 cm) with lids were used to hold inoculated tubers during incubation and each box had holes (1 to 2 of 2.54 cm in diameter) cut out of the ends for airflow. These holes were covered with tape for 0, 1, or 4 days to reduce airflow. Russet Norkotah tubers maintained at 21.1°C were inoculated with $5x10^5$ or $1x10^6$ oospores $mL^{-}1$ and placed in these boxes. The treatments significantly ($p=0.007$) affected leak incidence however, the results showed inoculum level was more likely to cause differences in leak incidence compared to passive airflow (Table A.4).

Throughout all the studies *P. ultimum* Trow var. *ultimum* isolate 09-MN.10-5 was obtained from Dr. Neil Gudmestad from North Dakota State University. Oospore concentrations of $5x10^4$, $1x10^5$, $2.5x10^5$, and $5x10^5$ mL^{-1} and a non-inoculated control were tested on Standard Norkotah Russet tubers at 21°C. Oospore concentration significantly ($p<0.0001$) affected leak incidence. The mean leak incidence ranged from 34 to 61% (non-inoculated control not included, Figure A.1). To avoid overwhelming the potatoes with the pathogen, $2.5x10^5$ oospores mL^{-1} was the target concentration used for studies unless stated differently.

Leak develops quickly and can be evaluated within four days, but it was not known if it could be evaluated earlier. Evaluations were done 1, 2, 3, and 4 days after inoculation (DAI). Cultivar Bannock Russet at pulp temperatures of 21.1°C were used. The experiment was done three times with 4 replicates; each replicate contained 20 tubers. Days after inoculation were significantly different however, experiments 2 and 3 had much higher leak incidence than experiment 1. Experiment 1 was performed with tmore mature potatoes of a previous crop (2017-2018). While, experiments 2 and 3 were performed earlier in the season with the 2018-2019 crop. Leak incidence increased from 1 to 2 DAI in experiments 2 and 3 but was similar between 2 and 4 DAI. However, severity was difficult to see 1 DAI but increased significantly ($p<0.0001$) after 4 DAI (Table A.5, Figure A.2).

These preliminary studies helped to obtain a unique procedure for inoculating *P. ultimum* that made it possible to complete the many trials of this dissertation. The overall procedure is provided below.

PYTHIUM ULTIMUM INOCULATION PROCEEDURE

1. Grow *P. ultimum* on Clarified V8 or PDA.

2. Place 50 plugs with a #3 corkborer for each liter of clarified V8 broth
    a. Shake in the dark for 10 to 12 days at 21 C
3. Strain mycelium from V8 broth.
4. Blend mycelium for 3 minutes in a blender with 200 mL^{-1} deionized water.
5. Adjust inoculum to $2.5x10^5$ oospores mL^{-1} or desired level of inoculum.
6. Inoculum applied at 6.6 mL kg^{-1} of potatoes.
7. Incubate inoculated potatoes for 4 days at 21.1°C (95% RH) with passive airflow.
8. Evaluate after 4 days of incubation by cutting tubers in half longitudinally.

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TABLES

Table A.1: Russet Norkotah tubers maintained at 21.1°C inoculated 315 days after harvest with *Pythium ultimum* for 3 or 5 minutes. No replicates and was not repeated

Treatment	Bruise time (min)	Inoculum level (oospores ml^{-1})	Leak severity (%)	Leak incidence (%)	Soft rot severity (%)
1	5	$1.3*10^6$	34	100	30
2	5	$5*10^5$	43	100	28
3	3	$1.3*10^6$	44	100	30
4	3	$5*10^5$	39	95	27

Table A.2: Russet Norkotah tubers maintained at 21.1°C inoculated ($5x10^5$ oospores mL^{-1}) with *Pythium ultimum* for 1.5 or 3 minutes. Stored 4 days[a]

Treatment	Bruise time (min)	Time (s) remaining during bruising when inoculum was applied	Leak incidence (%)[b]
1	3	60	0[c]
2	3	60	99 b
3	3	90	95 ab
4	3	30	91 ab
5	1.5	60	81 ab
6	1.5	80	75 a
7	1.5	30	91 ab
8	1.5	60	0[c]

[a] Repeated twice with only 1 replicate (n=20 tubers trt-1 rep-1)

[b] Different letters denote statistical significance (α=0.05) within a column

[c] Treatment 1 and 8 were non-inoculated controls and were removed from analysis

Table A.3: Russet Norkotah tubers inoculated with *Pythium ultimum* ($5x10^5$ oospores mL^{-1}) at different times and pulp temperatures during a 90 second bruise cycle

Treatment	Time (s) remaining in during bruising when inoculum was applied	Leak incidence (%)
1	90	22 a[a]
2	60	33 b
3	30	30 ab
4	15	38 b
5	no bruise	0[b]
Temperature (°C)		
15.5		24 a
21.1		37 b

[a] Different letter denotes statistical significance (α=0.05) within a column

[b] Treatment 5 was a non-inoculated control and were removed from analysis

Table A.4: Russet Norkotah tubers maintained at 21.1°C were inoculated and sealed in boxes by covering holes with duct tape to not allow fresh air into the box[a]

Treatment	inoculum	Days sealed	Leak incidence (%)[b]
1	0	1	3[c]
2	$5*10^5$	1	86 ab
3	$5*10^5$	4	89 ab
4	$5*10^5$	0	79 a
5	$5*10^6$	1	91 ab
6	$5*10^6$	4	94 ab
7	$5*10^6$	0	96 b
8	0	1	13[c]

[a] Repeated four times with only 1 replicate (n=20 tubers trt-1 rep-1). ANOVA was used to compare means

[b] Different letters denote statistical significance (α=0.05) within a column

[c] Treatment 1 and 8 were non-inoculated controls and were removed from analysis

Table A.5: Bannock Russet tubers inoculated ($2.5x10^5$ oospores mL^{-1}) and evaluated on different days after inoculation (DAI). Repeated three times with 4 blocks each.

Evaluation DAI	Leak incidence (%)[a]		
	experiment 1	experiment 2	experiment 3
1	21 a	80 a	76 a
2	15 a	96 b	92 b
3	42 b	100 b	88 ab
4	44 b	98 b	93 b
p-values			
DAI	<0.0001	0.002	0.01

[a] Different letters denote statistical significance (α=0.05) within a column

FIGURES

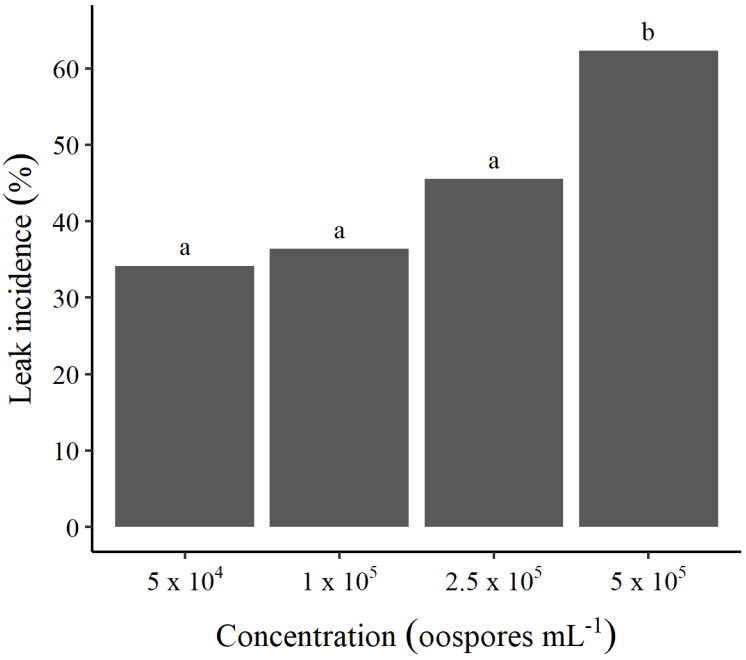

Figure A.1: Russet Norkotah potatoes treated with different concentrations of *Pythium ultimum*. Differing letters above bars denote significant differences (α=0.05)

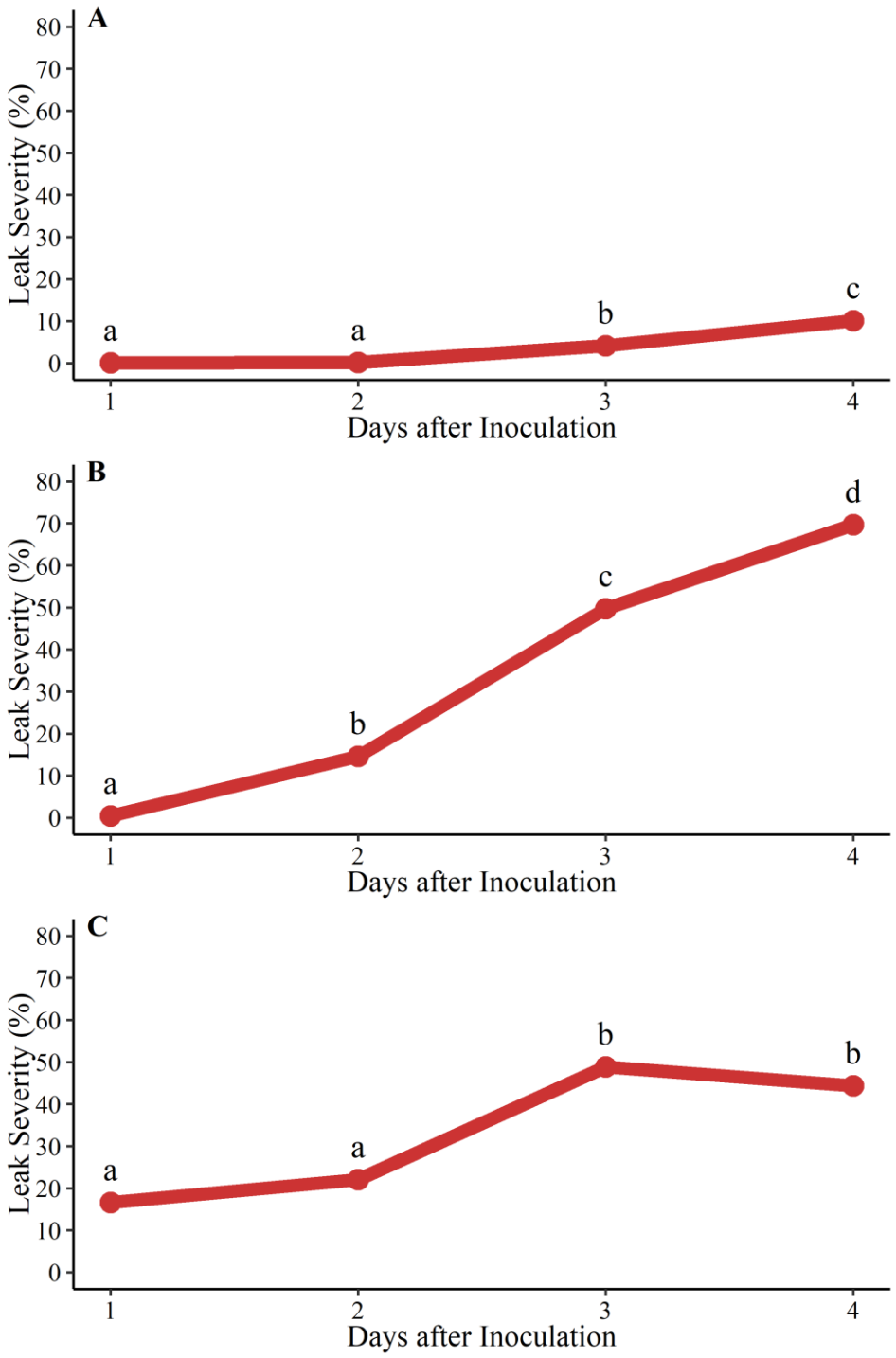

Figure A.2: Leak severity in cultivar Bannock Russet evaluated 1 to 4 days after inoculation with *Pythium ultimum* for (A) experiment 1, (B) experiment 2, (C) experiment 3. Letters above points denote significant differences within experiment (α=0.05).

APPENDIX B: SUPPLEMENTAL DATA FOR CHAPTER 1

Results of preliminary studies aided in showing how airflow and tuber pulp temperature affect leak incidence. Storages are typically designed to provide 10 to 25 CFM ton^{-1} which could dry potatoes and create less favorable environment for leak to develop. Treatments of passive airflow, standard airflow, 4x standard airflow and a non-inoculated control were applied to tubers after inoculation with $5x10^5$ oospores mL^{-1} on Russet Norkotah potatoes with pulp temperatures of 21.1°C. Excluding the non-inoculated control from analysis, airflow did not significantly ($p=0.34$) affect leak incidence (Table B.1). Testing the effect of tuber pulp temperature on leak incidence for cultivars of Russet Burbank and Russet Norkotah was done by equilibrating pulp temperatures to 12.8, 15.5, 18.3, and 21.1°C before inoculation and maintaining those pulp temperatures until evaluation 4 days later. Cultivar was significant ($p<0.0001$) with Russet Norkotah only having 16% leak incidence and Russet Burbank having 57%. Leak incidence was significantly ($p<0.0001$) influenced by pulp temperature between the two cultivars (Figure B.1).

TABLE

Table B.1: Russet Norkotah tubers exposed to different airflows after inoculation[a]

Treatment	Target CFM CWT^{-1}	Average CFM CWT^{-1}	Leak incidence (%)
4x standard airflow	2-5	3.69	57
Passive airflow	0	0.02	54
Standard airflow	0.5-1.25	0.86	49
Non-inoculated	0	0.02	4

[a]Repeated four times with 4 blocks (n=20 tubers trt^{-1} rep^{-1}). ANOVA was used to compare means. p-value=0.34

[b] Non-inoculated control was removed from analysis

FIGURE

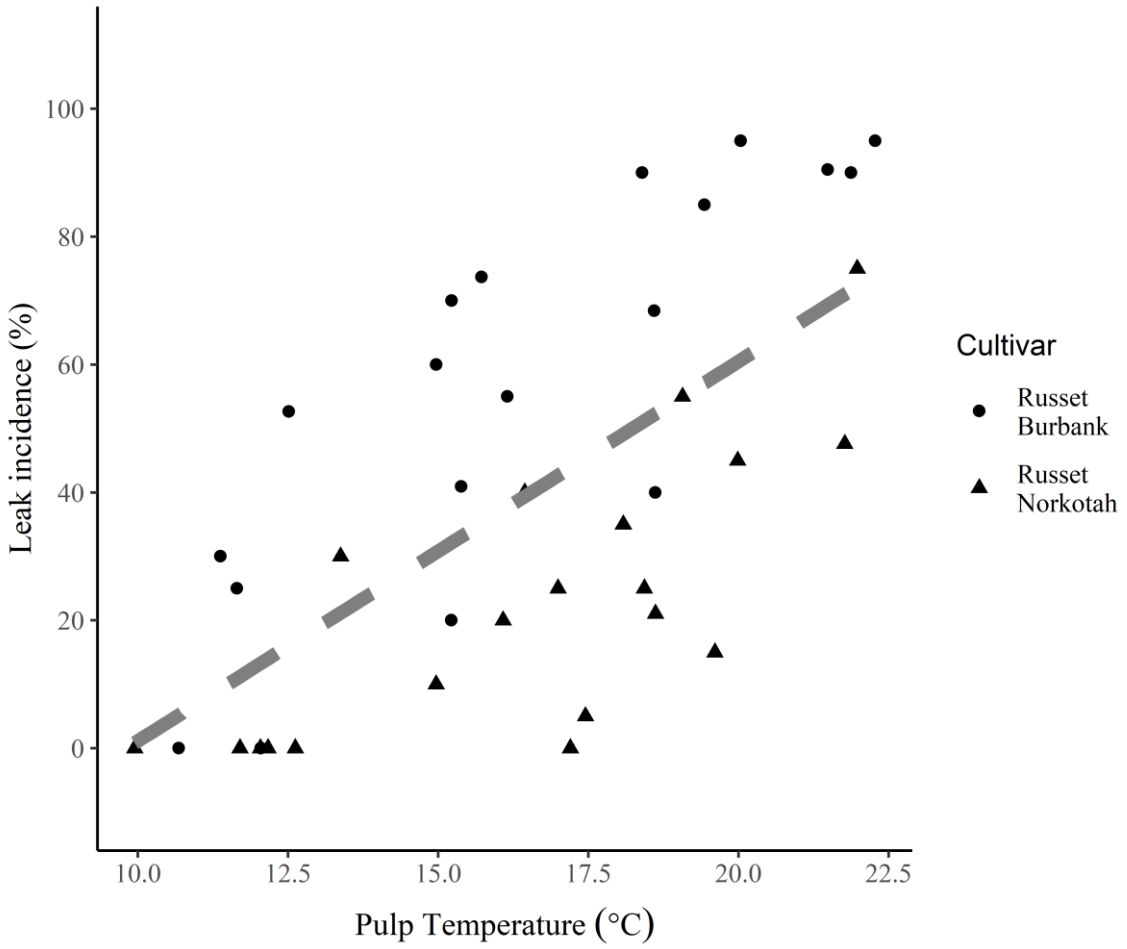

Figure B.1: Leak incidence in Russet Burbank and Russet Norkotah tubers from 10 to 22°C. Dashed line is the linear regression line for the combined data between Russet Burbank and Russet Norkotah

APPENDIX C: SUPPLEMENTAL DATA FOR CHAPTER 2

In Chapter 2 a regression developed by Edwards (1999) was used to estimate the surface area of a tuber based on tuber weight. Edwards used the cultivar Russet Burbank; however, it was unknown if the regression is appropriate for other russet cultivars. Ten tubers from each cultivar: Clearwater Russet, Payette Russet, Ranger Russet, Teton Russet, Standard Russet Norkotah, and Umatilla Russet were weighed and peeled. The peelings were then scanned into digital images using an Epson Perfection V39 scanner (Epson America, Inc., Long Beach, CA) into digital images. The image processing software, FIJI (Schindelin et al. 2012) measured the area of each peel for each tuber to then get a total surface area. Data was correlated in R (version 1.2.1335, R Foundation for Statistical Computing, Austria). The Pearson correlation coefficient was r=0.93 with and adjusted R^2 of 0.86 (Figure C.1). The estimated surface area based on the regression and the measured surface area were highly correlated indicating the regression could be used to estimate the tuber surface area of non-Russet Burbank russet cultivars and was used in chapter 2.

The data from chapter 2 observed various bruising between Bannock Russet, Clearwater Russet, Payette Russet, Ranger Russet, Russet Burbank, and Teton Russet. However, it was unclear if leak development would vary based on the amount of wounding tubers sustained. Two experiments occurred 205 and 265 days after harvest. Bannock Russet, Russet Norkotah, and Umatilla Russet were bruised for 30, 60, and 90 s. Inoculum (2.5×10^5 and 2.1×10^5) was applied with 30 s left in the bruise cycle (20 tubers $block^{-1}$ $tuber^{-1}$). The design was a 3x3 factorial design. Leak incidence was significantly affected by cultivar ($p=0.0002$) and time ($p<0.0001$). Leak incidence was not significantly affected by time ($p=0.57$). Russet Norkotah had significantly less leak incidence than Bannock Russet and Umatilla Russet (Table C.1) supporting the results of Chapter 1. Leak incidence also increased from an average of 17% when tubers were bruised for 30 s to 39% when bruised for 90 s. This shows that longer bruising times increased the amount of leak.

A follow up study was done with tubers harvested the following year to observe the number of shatter bruises after 30, 60, and 90 s of tumbling in a cement mixer. Two experiments occurring 42 and 72 days after harvest inoculated ($2.5x10^5$ oospores mL^{-1}, rate=6.6 mL^{-1} kg) Bannock Russet, Russet Burbank and Russet Norkotah tubers. Each block

contained 26 tubers tumbling time^{-1} cultivar^{-1}. Six tubers were placed in iodine solution for shatter bruises to stain blue and the other 20 tubers were maintained at 21.1°C to incubate for four days. After the tubers soaked in iodine solution for at least 2 hours they were removed, rinsed, dried, and weighed. The shatter bruises were then cut out of the tuber processed into digital images using an Epson Perfection V39 scanner. The image processing software, FIJI (Schindelin et al. 2012) measured the area of each shatter bruise. The area of shatter bruise was significantly affected by tumbling time ($p=0.0007$) and cultivar ($p=0.005$). Shatter bruise area increased from 0.44% to 0.71% from 30 s of tumbling to 90 s. Russet Norkotah had significantly less shatter bruise area than Bannock Russet and Russet Burbank (Table C.2). The means of leak incidence and percent shatter bruise for each replicate, cultivar and tumbling time were analyzed in a linear regression model using R. Percent shatter bruise did not significantly ($p=0.08$) affect leak incidence but cultivar was significant ($p<0.0001$). The cultivars significantly differed in leak incidence from one another: Russet Norkotah (29%), Russet Burbank (67%), and Bannock Russet (81%). Shatter bruise number was significantly affected by cultivar ($p=0.0001$) and time ($p=0.0001$). The interaction between cultivar and time was not significant ($p=0.27$). The shatter bruise number was significantly lower for Russet Norkotah than Russet Burbank and Bannock Russet (Table C.2). The linear regression showed leak incidence was significantly affected by cultivar ($p<0.0001$) and shatter bruise number ($p<0.004$). this data indicates as shatter bruise number increases by 1 there is an increase of 1.9% leak incidence among cultivars between 0 and 20 shatter bruises (Figure C.2).

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TABLES

Table C.1: Leak incidence (%) for each bruise treatment and russet cultivar

Leak incidence (%) for bruise cycle time (s)				
Cultivar	30s	60s	90s	Mean leak incidence (%) for cultivar[a]
	Leak incidence (%)			
Bannock Russet	27	33	53	38 a
Russet Norkotah	5	13	21	13 b
Umatilla Russet	18	34	42	32 b
Mean leak incidence for each bruise cycle[b]	17 a	27 b	39 c	

[a] Different letters denote statistical significance (α=0.05) within a column

[b] Different letters denote statistical significance (α=0.05) within a row

Table C.2: Shatter bruise area (%) for each bruise treatment and russet cultivar

Cultivar	Bruise cycle time (s)			Mean shatter bruise area (%) for cultivar[a]	
	30s	60s	90s		
	Shatter bruise area (%)				
Russet Norkotah	0.31	0.38	0.48	0.39	a
Russet Burbank	0.51	0.72	0.77	0.66	b
Bannock Russet	0.53	0.88	0.94	0.76	b
Mean shatter bruise area (%) for bruise cycle time (s)[b]	0.44 a	0.63 b	0.71 b		

[a] Different letters denote statistical significance (α=0.05) within a column

[b] Different letters denote statistical significance (α=0.05) within a row

FIGURES

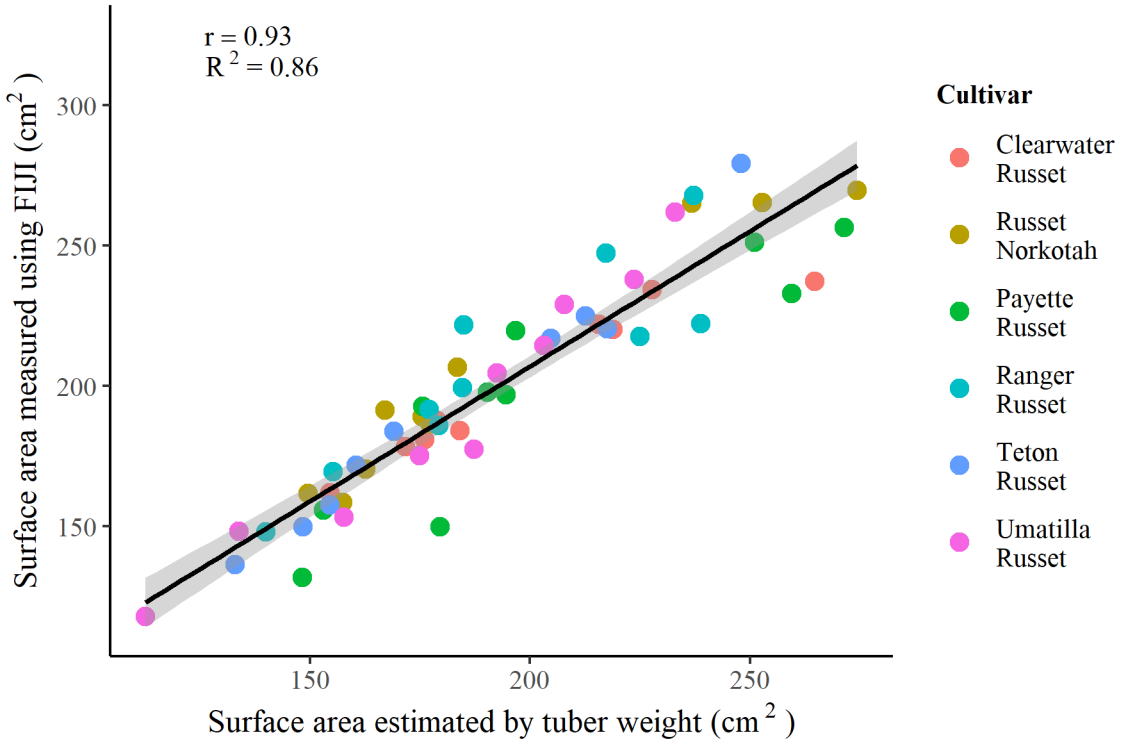

Figure C.1: Measured surface area to estimated surface area based on the regression developed by Edwards (1999) using the weight of the tuber

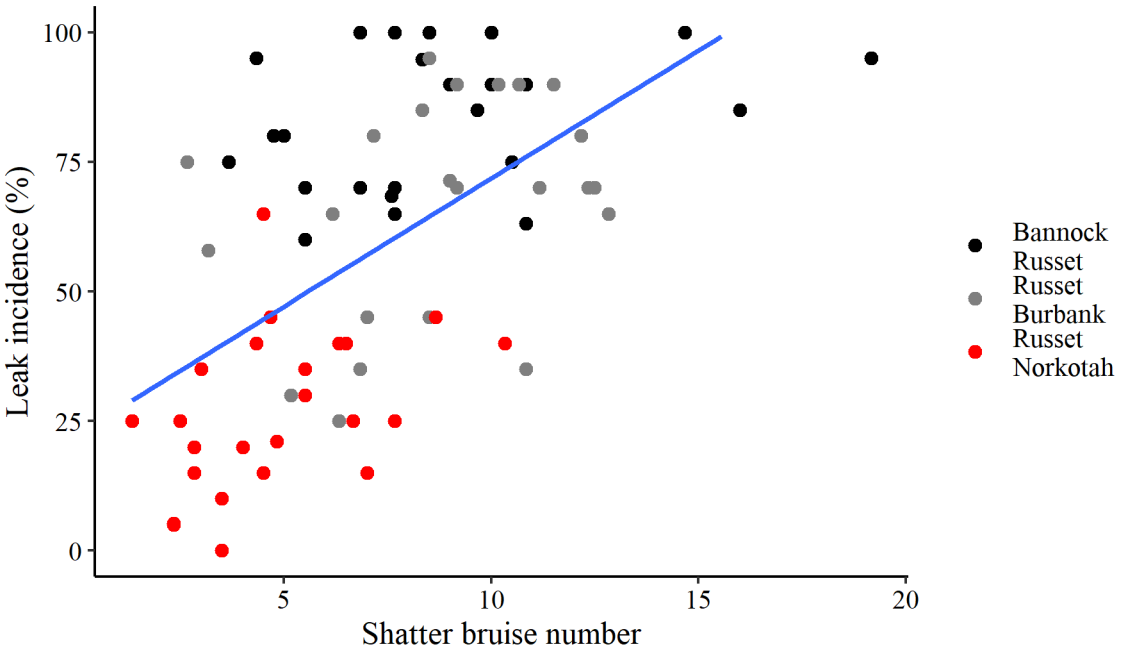

Figure C.2: Linear regression of leak incidence by shatter bruise number and cultivar. R^2=0.7

APPENDIX D: SUPPLEMENTAL DATA FOR CHAPTER 3

There have been questions regarding leak control with foliar applications of phosphorous acid. The efficacy of phosphorous acid was tested by applying a water control, phosphorous acid treatment to plants early, standard, and late (Miller Research study, Table D.1). Tubers were harvested then wounded inoculated with *P. ultimum* ($5x10^5$ oospores mL^{-1}) in storage (no fungicide applied in storage). The treatments did not significantly ($p=0.56$) affect leak incidence compared to the non-treated control and was not repeated a second year (Table D.2). This supported the findings by Johnson et al. (2004) leak was not affected by foliar applications of phosphorous acid. This also corresponds to the results of chapter 2 of this dissertation as phosphorous acid was not effective in controlling leak when applied post-harvest.

REFERENCE

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TABLES

Table D.1: Treatments of phosphorous acid (117 L ha^{-1}, Resist 57™, Actagro, LLC) were applied according to plant growth stages

Treatment	Full Emergence	Dime-Sized Tubers	Row Closure (RC)	RC+14 days	RC+28 days
Water treatment	No				
Early	Phosphorous acid applied			No	
Standard	No	Phosphorous acid applied			No
Late	No		Phosphorous acid applied		

Table D.2: Leak incidence and severity of Russet Norkotah in post-harvest inoculations after foliar applications of phosphorous acid at different timings (Table D.1). No differences were observed in leak incidence ($p=0.56$, $\alpha=0.05$)

Treatment	Incidence (%)	Severity (%)
Water treatment	33	12
Early	38	17
Standard	35	13
Late	39	13

APPENDIX E: PERMISSION TO REPRINT CHAPTER 1

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