STUDIES ON THE MANAGEMENT OF *POTATO MOP-TOP VIRUS*-INDUCED TUBER NECROSIS

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Studies on the management of *Potato mop-top virus*-induced tuber necrosis

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Tuber necrosis caused by the *Potato Mop-top Virus* (PMTV) has become one of the most important tuber necrosis diseases in the United States. PMTV is transmitted by the powdery scab pathogen (*Spongospora subterranea* f.sp *subterranea* (Sss)) and no effective methods of control are currently available for these pathogens. Potato cultivars have been reported to exhibit natural variability in their reaction to PMTV infection, making cultivar selection a viable management option. This dissertation focuses on finding short to long term strategies for managing PMTV tuber necrosis. In the first study, potato cultivars were assessed for sensitivity to PMTV-induced tuber necrosis in three separate field trials. Results of tuber assessments demonstrated that sensitivity to PMTV-induced tuber necrosis among cultivars follows a continuum of tolerant to sensitive. In the second study, advanced breeding selections of potato were evaluated for sensitivity to PMTV-induced tuber necrosis. The results revealed high variability in PMTV-induced tuber necrosis incidence and severity among selections and identified 17 of them to be tolerant, nine – moderately tolerant, eight - moderately sensitive, while six were found to be sensitive. Results of the field trials show that russet-skinned cultivars and selections are less sensitive to PMTV tuber necrosis than red-, yellow- and white-skinned types. In the third study, a growth chamber experiment was conducted to investigate the potential of using moisture regime to manage PMTV tuber necrosis. The results showed significant differences in PMTV tuber necrosis and powdery scab infection among moisture regimes. Maintaining soil moisture level at 90% field capacity throughout the season resulted in significantly higher incidence and severity of PMTV tuber necrosis and powdery scab infection than keeping soil at 60% field capacity. The results also show that the potato plant may be susceptible to PMTV-induced tuber necrosis and powdery scab infection throughout the season. The results of these investigations

offer potato growers the option to plant less sensitive cultivars in areas where PMTV and powdery scab exist. This information can be utilized in future breeding efforts to obtain resistant potato cultivars. Useful information on using soil moisture regime as a management strategy for PMTV tuber necrosis has been provided.

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DEDICATION

Dedicated to Kwaku Owusu and Adom Delasi Domfeh – we were in this together!

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CHAPTER ONE. LITERATURE REVIEW

The potato crop

The potato (*Solanum tuberosum* L.) is the world's fourth most important food crop in terms of cultivation and consumption, exceeded only by rice, wheat and corn (Stevenson *et al*., 2001). The potato provides an inexpensive source of calories for consumers (USDA, 2009) and serves as a source of primary income worldwide (Ovchinnikova *et al.*, 2011). In recent years, potato production has become increasingly important in developing countries and in 2005, the developing world's potato production exceeded that of the developed world for the first time (FAO, 2008). Leading potato producing countries include China, India, United States, Russia, Germany, Ukraine, Bangladesh, Netherlands, Poland and France (FAOSTAT, 2014).

The origin of potato can be traced to South America. While one hypothesis proposes lowland Chile as the origin, another points to the Andean region from western Venezuela to northern Argentina (Ovchinnikova *et al*., 2011). Potato is believed to have been introduced to Spain in the 16th century (Harding, 1993), from where it spread to other European countries and later to North America (Laufer and Martin, 1938; Ochoa, 2001). Potato is a dicotyledonous herbaceous plant propagated primarily by vegetative means from seed pieces. The highest tuber production is obtained under cool temperatures, sufficient nitrogen and intermediate photoperiods (Martin *et al*., 2006).

Diseases of potato

Potato is a host of many pathogens like viruses and viriods, fungi, oomycetes, bacteria and nematodes. These pathogens can cause significant reduction in tuber yield and/or quality, resulting in huge losses to potato growers. Viruses and virus-like pathogens constitute an important limiting factor in potato production, with over 40 of them reported to affect the crop

(Valkonen, 2007). Most plant viruses are transmitted to their host by a vector (Gutierrez *et al.*, 2013; Singh *et al*., 2008). Three routes of transmission of plant viruses and viriods to their hosts have been recognized: aphids and other insects (e.g. *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV*)*) (Delgado-Sanchez and Grogan, 1970; Harrison, 1984), soil-borne (e.g. *Potato mop-top virus* (PMTV), *Tobacco rattle virus* (TRV) and tuber-borne (e.g. *Potato spindle tuber viroid* (PSTVd)) (Calvert, 1968; de Hoop *et al*., 2008). More than 50 soil-borne viruses are transmitted by nematodes, fungi or fungal-like organisms (Verchot-Lubicz, 2003). Virus-transmitting nematodes constitute less than 1% of plant parasitic nematodes (Verchot-Lubicz, 2003). Tobraviruses and nepoviruses are two groups of viruses which rely on plant-parasitic nematodes as vectors of transmission (Taylor and Brown, 1997). *Trichodorus* and *Paratrichodorus spp*. are known to transmit tobraviruses of which one, TRV, affects potato. Fungi and fungal-like organisms which transmit viruses belong to the order Plasmodiophorales or Chytridiales. Of the 35 species in the order Plasmodiophorales, only three - *Polymyxa graminis*, *Polymyxa betae* and *Spongospora subterranea* are known to vector viruses (Verchot-Lubicz, 2003; Ward and Adams, 1998). Three species of the genus Spongospora have been identified: *S. subterranea* (f. sp. *subterranea* and f. sp. *nasturtii*), *S. cotulae* and *S. campanulae* (Karling, 1968). *Spongospora subterranea* f. sp. *subterranea* and *Spongospora subterranea* f.sp. *nasturtii* are known to be plant pathogens and virus vectors (Merz *et al*., 2005). *Spongospora subterranea* f.sp. s*ubterranea* (Sss) is the vector of PMTV (Arif *et al*., 1995) and also causes powdery scab in potato.

Potato mop-top virus (PMTV)

Distribution

PMTV is believed to have originated from the Andean region of South America (Hinostroza and French, 1972; Salazar and Jones, 1975; Tenorio *et al*., 2006) and now occurs in many parts of the world where potato is produced (Santala *et al*., 2010). In South America, PMTV has been found in Peru, Bolivia, Venezuela and Colombia (Gil *et al*., 2011; Hinostroza and French, 1972; Jones, 1975; Osorio-Giraldo *et al*., 2013; Salazar and Jones, 1975; Tenorio *et al*., 2006). PMTV has also been recorded in Costa Rica in Central America (Montero-Astúa *et al*., 2008; Vásquez *et al*., 2006). In Europe, PMTV occurs in Scotland, Northern Ireland, Netherlands, Czech Republic, Switzerland, Latvia and Poland (Budziszewska *et al*., 2010; Calvert and Harrison, 1966; Latvala-Kilby *et al*., 2009; Novak *et al*., 1983; Schwärzel, 2002; van Hoof and Rozendaal, 1969). PMTV has caused serious economic loses in the Nordic countries Denmark, Finland, Norway and Sweden in the past three to four decades (Buundgard *et al*., 2007; Germundsson *et al*., 2000; Kurppa, 1989; Latvala-Kilby *et al*., 2009; Molgaard and Nielsen, 1996; Nielsen & Mølgaard, 1997; Ryden *et al*., 1986; Sandgren, 1995). In Asia, PMTV has been reported in Japan, China and Pakistan (Arif *et al*., 2013; Hu *et al*., 2013; Imoto *et al*., 1986; Maoka *et al*., 2011; Nakayama *et al*., 2010). In North America, PMTV has been reported in USA and Canada (Xu *et al*., 2004). In the United States, PMTV was first reported in Maine in 2003 (Lambert *et al*., 2003), North Dakota in 2010 (David *et al*., 2010), Washington State in 2011 (Crosslin, 2011), Idaho in 2013 (Whitworth and Crosslin, 2013), Colorado and New Mexico in 2015 (Mallik and Gudmestad, 2015).

Symptoms and effects of PMTV

PMTV symptoms are variable, differing with cultivar, weather and season (Cooper and Harrison, 1973; Harrison, 1974). Typical symptoms of PMTV include slightly raised lines and rings on the tuber surface and/or brown arcs and lines in the flesh of tubers of sensitive cultivars (Calvert and Harrison, 1966; Harrison and Jones, 1970; Kurppa, 1989) (Fig. 1.1). These are the most common symptoms of primary infection and are similar to those induced by the nematode-transmitted *Tobacco rattle virus* (TRV, genus Tobravirus; Virgaviridae) (Adams *et al*., 2009) and potato tuber necrotic strain of *Potato virus Y* (PVY^{NTN}) (Jeffries, 1998). Other viruses causing necrosis in potato tubers include *Potato leaf roll virus*, *Alfalfa mosaic virus* and *Tomato spotted wilt virus*. In secondary PMTV infections, tubers may show reticulate or deep cracks, blotchy surface markings or distortions (Calvert and Harrison, 1966; Harrison and Jones, 1970; Tenorio *et al*., 2006). The appearance of external and internal PMTV symptoms on tubers is seldom observed at harvest. However, significant increase in symptom appearance during storage has been reported, particularly under conditions of fluctuating storage temperature (Domfeh *et al*., 2015; Kurppa, 1989; Molgaard and Nielsen; 1996, Sandgren, 1995).

Symptoms may also develop on the foliage and stems of a plant if infected seed is planted (Calvert, 1968; Harrison, 1974). Foliar symptoms include yellow blotch pattern (aucuba pattern) in the lower leaves; V-shaped yellow patterns (chevron patterns); internode shortening (mop-top); "thistle leaf" patterns around the veins and deformed leaves (Calvert, 1968). The development of symptoms on stems is rarely observed (Calvert, 1968; Torrance e*t al*., 1992).

PMTV infection has been reported to cause some yield losses (Calvert, 1968; Sandgren *et al*., 2002), however, qualitative losses are more important (Germundsson *et al*., 2002). PMTV-induced tuber necrosis causes severe quality reduction (Kurppa, 1989; Nielsen and Mølgaard,

1997), this renders affected tubers unsuitable for the French fry and chip (crisp) industries and are rejected by supermarkets and the food industry (Santala *et al*., 2010). Potato tuber yield reduction of 26 to 37% has been reported (Calvert, 1968; Kurppa, 1989). In another study, no tuber yield reduction was found despite the presence of severe PMTV tuber symptoms in cultivar Saturna (Nielsen and Mølgaard, 1997).

Fig. 1.1. Symptoms of PMTV tuber necrosis in potato tubers.

Transmission and host range

Natural transmission of PMTV occurs by the vector Sss, an obligate plasmodiophorid fungal-like organism (Margulis and Schwartz, 2000). It has been demonstrated that virus-free Sss can obtain PMTV from manually inoculated potato plants and transfer the virus to bait plants (Arif *et al*., 1995). Sss has thus been proven to be the vector of PMTV and it remains the only known vector (Jones and Harrison, 1969; Arif *et al*., 1995; Kirk, 2008). The zoospores of Sss transmit PMTV to potato plants when they penetrate the root tissue, stolons, young shoots and tubers (Hims and Preece, 1975) after acquiring the virus from an infected source through mechanisms which are not clearly understood (Germundsson *et al*., 2002). PMTV has been reported to remain infective in a field after 18 years without potatoes (Calvert, 1968), surviving in the resting spores of Sss, which themselves are also long-lived. By planting potato seed tubers

harboring virus-carrying Sss, PMTV can be spread into new fields (Jones and Harrison, 1969). PMTV infection is partially systemic and only a proportion of the progeny tubers from an infected plant carry the infection (Carnegie *et al*., 2010; Davey *et al*., 2014; Kirk, 2008). This can lead to the elimination of the virus from potato crops after a few generations when the tubers are planted in soil free of PMTV and Sss (Calvert, 1968; Davey *et al*., 2014; Kirk, 2008; Torrance *et al*., 1999). PMTV was found to be widespread throughout the potato growing regions of the United States and Canada (Xu *et al*., 2004) and this likely could explain why the virus has been detected in many states in the past decade or so. Generally, PMTV and Sss require similar environmental conditions for infection. Low temperature and high soil moisture enhance germination and motility of Sss zoospores and these favor infection (Kirk, 2008; Merz, 2008; Sandgren *et al*., 2002). The success of PMTV transmission by Sss has been reported to be greatest at 12 to 20ºC while little or no infection occurs above 24ºC (Carnegie *et al*., 2010).

Unlike its vector, PMTV has a narrow host range, infecting members of only three families namely, Solanaceae, Tetragoniaceae and Chenopodiaceae (Andersen *et al*., 2002; Brunt *et al*., 1990; Jones, 1981). PMTV infects species including *Chenopodium amaranticolor*, *Chenopodium album*, *Nicotiana benthamiana*, *Nicotiana debneyi*, *Nicotiana tabacum*, *Solanum nigrum* and *Tetragonia tetragonioides* (Jones and Harrison, 1969; Harrison and Jones, 1970; Jones and Harrison, 1972; Arif *et al*., 1995; Andersen *et al*., 2002).

PMTV genome

PMTV is the type member of the genus *Pomovirus* (King *et al*., 2012; Torrance and Mayo, 1997). The particles of PMTV are tubular, rigid and rod-shaped with a tripartite, positive-sense, single-stranded RNA genome (Scott *et al*., 1994; Torrance *et al*., 1999). RNA-Rep (6.5 kb) (Savenkov *et al*., 1999) comprises about half of the PMTV genome (Torrance *et al*., 1992)

and it is thought to be responsible for virus replication (Savenkov *et al*., 1999). RNA-Rep encodes proteins believed to be components of the viral RNA-dependent RNA polymerase (Savenkov *et al*., 1999). RNA-CP (3 kb) (Scott *et al*., 1994) encodes two proteins, the capsid protein and a read-through protein thought to be associated with vector transmission (Germundsson *et al*., 2002; Kashiwazaki *et al*., 1995, McGeachy and Barker, 2000). RNA-TGB (2.5 kb) (Scott *et al*., 1994) encodes the triple gene block proteins (TGB), three overlapping proteins utilized by many plant viruses for cell-to-cell movement within the host plant (Haupt *et al*., 2005). RNA-TGB also encodes a cysteine rich protein whose functions are unknown (Lukhovitskaya *et al*., 2005).

Long-distance movement of PMTV within the host plant can occur without the coat protein (Torrance *et al*., 2009). The occurrence of natural deletions in contiguous segments of PMTV RNA-CP sequence has been reported (Torrance *et al*., 1999). Such deletions occur in the read-through domain of the capsid protein and may interfere with vector transmission (Reavy *et al*., 1998; Sandgren *et al*., 2001; Tamada *et al*., 1996). Sequence deletions have been reported to occur in several other plant viruses and this has been attributed to errors in RNA replication (King *et al*., 1987). The distribution of PMTV in its host plant is known to be erratic (Torrance *et al*., 1992). PMTV is also gradually self-eliminating from potato stocks in the absence of re-infection (Calvert, 1968; Cooper *et al*., 1976). The spontaneous sequence deletion which occurs in the genome of this virus may explain the self-eliminating phenomenon (Torrance *et al*., 1999). The RNA segments were formerly referred to as: RNA 1 (now RNA-Rep), RNA 2 or RNA 3, depending on the isolate (now RNA-CP) and RNA 2 or RNA 3, depending on the isolate (now RNA-TGB) (Adams *et al*., 2012).

PMTV isolates

Characterized PMTV isolates include PMTV-T, type isolate of PMTV (Harrison and Jones, 1970), PMTV-S from Scotland (Arif *et al*., 1995) and PMTV-SW from Sweden (Sandgren *et al*., 2001). Other isolates have been reported from Denmark, Czech Republic Peru and USA (Čeřovská *et al*., 2007; Mayo *et al*., 1996; Nielsen and Nicolaisen, 2003; Pečenková *et al*., 2004, Ramesh *et al.*, 2014). In terms of symptom severity in indicator plants, several authors have reported differences among isolates (Nielsen and Nicolaisen, 2003). A high degree of sequence identity has been found among PMTV isolates from Europe, North & South America and Asia (Budziszewska *et al*., 2010; Čeřovská *et al*., 2003; Čeřovská *et al*., 2007; Crosslin, 2011; David *et al*., 2010, Hu *et al*., 2013; Lambert *et al*., 2003; Mallik and Gudmestad, 2015; Mayo *et al*., 1996; Ramesh *et al*., 2014; Tenorio *et al*., 2006; Whitworth & Crosslin, 2013, Xu *et al*., 2004). The limited genetic variability in the CP genes in PMTV from different parts of the world has allowed reliable detection of the virus with currently used monoclonal antibodies and PCR (Santala *et al*., 2010). Analyses of genetic variability within isolates across different geographic areas, taken together, reveal two distinguishable variants of RNA-CP and RNA-TGB sequences that occur in different combinations and as mixed infections (Nielsen and Nicolaisen, 2003; Latvala-Kilby *et al*., 2009).

PMTV detection

Symptoms of PMTV can sometimes be observed through visual inspection of plants and tubers. The occurrence of symptomless infection in some cultivars, coupled with the fact that other pathogens (e.g. TRV and the necrotic strain of Potato virus Y (PVY^{NTN})) and some physiological disorders can induce symptoms similar to those of PMTV, make it important that the presence of PMTV is confirmed with molecular or serological methods. Virus-specific and

sensitive techniques such as reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, immunocapture RT-PCR (IC-RT-PCR), qualitative amplification based specific hybridization (FLASH-PCR), RT-PCR-microplate hybridization (RT-PCR-MPH) and enzyme-linked immunosorbent assay (ELISA) have been utilized for the detection of PMTV in potato (Arif *et al*., 1994; Germundson *et al*., 2002; Latvala-Kilby *et al*., 2009; Nakayama *et al*., 2010; Sandgren *et al*., 2001; Sokemen *et al*., 1998). Primers used in RT-PCR for PMTV detection have been designed to target the highly conserved regions of RNA-CP and RNA-TGB (Mayo *et al*., 1996; Reavy *et al*., 1998; Sokemen *et al*., 1998). The use of soil-bait tests in which bait plants such as *Nicotiana debneyi*, *N. benthamiana* and *Solanum lycopersicum* are planted in infested soil, followed by detection of PMTV from the roots of the bait plants by RT-PCR has been reported (Arif *et al*., 1994; Arif *et al*., 2014; Davey, 2009; Nakayama *et al*., 2010; Sandgren, 1995). Sprouts from PMTV-infected tubers kept in the dark contain high concentrations of PMTV and have been used successfully in PMTV detection (Latvala-Kilby *et al*., 2009).

Management of Sss and powdery scab

Available management options for Sss and powdery scab are limited and do not offer much help with PMTV control (Santala *et al*., 2010). The resting spores of Sss are known to survive for many years in the soil and crop rotation may not be effective as a management strategy (Jones and Harrison, 1969, 1972). Seed chemical treatment and soil fumigation are some of the strategies attempted for the control of Sss. However, most of these chemical compounds are either not suitable for use due to phytotoxicity problems and detrimental effects on the environment or do not provide sufficient control. Mercury-containing chemicals provided adequate control but were banned in the 1980s (Merz and Falloon, 2009). Since then, the most promising chemical which gave the best, though incomplete control as seed treatment is

fluazinam (Falloon *et al*., 1996). The use of fluazinam has since been discontinued due to phytotoxicity concerns (Merz and Falloon, 2009). Avoidance, i.e., by planting only disease-free seed in disease-free soil, is perhaps the best method of control. The success of avoidance as a control strategy will require implementation of effective and internationally standardized certification rules (Merz and Falloon, 2009). Successful control may also be achieved by planting slightly later in the year when the temperature has increased and by reducing irrigation, especially during tuber initiation, when infection is most likely to occur (Fallon, 2008). Some cultivars are more susceptible to powdery scab than others (Hughes, 1980; Karling, 1968; Torres *et al*., 1995; Wastie, 1991), making cultivar selection a viable control option. In the long-term, the most cost effective and environmentally acceptable way of controlling powdery scab will be the development of resistant cultivars. It would be expected that resistance to Sss should result in reduction of PMTV infection rates in the field. However, experimental evidence indicates otherwise (Santala *et al*., 2010). This can be explained by the absence of correlation between the incidence of powdery scab and PMTV infection in tubers (Cooper *et al*., 1976; Kirk, 2008; Montero-Astúa *et al*., 2008; Sandgren *et al*., 2002; Tenorio *et al*., 2006). The low or complete absence of correlation between powdery scab incidence on tubers and PMTV infection has been attributed to differences in the optimal environmental conditions required by Sss and PMTV (Carnegie *et al*., 2010; Carnegie *et al*., 2012) and that Sss infection of other parts of the potato plant such as roots and stolons also cause PMTV transmission (Carnegie *et al*., 2012; Davey *et al*., 2014; Jones and Harrison, 1969; Santala *et al*., 2010). The possibility exists that a potato cultivar with high tuber resistance to Sss, may have low root resistance (Merz, 2008) and as such, only cultivars with immunity (no infection at all) to Sss in tubers, roots and stolons may prevent PMTV infection (Santala *et al*., 2010).

PMTV management

As with powdery scab, there is also no chemical treatment available for the management of PMTV. Some control of PMTV may be achieved by rogueing plants expressing symptoms; however, the absence of symptoms in some PMTV infections limits the success of this strategy (Davey, 2009). Other recommendations include avoiding potato production in soils infested with Sss or avoiding planting infected or contaminated seed. Even though there are no sources of resistance or tolerance to PMTV deliberately used in breeding programs (Barker *et al*., 1998; Santala *et al*., 2010), cultivars and other potato germplasm exhibit natural variability in their sensitivity to PMTV infection (Calvert, 1968; Carnegie *et al*., 2009; Kurppa, 1989; Latvala-Kilby *et al*., 2009; Nielsen and Engsbro, 1992; Nielsen and Molgaard, 1997; Sandgren, 1995; Sandgren *et al*., 2002; Tenorio *et al*., 2006). Cultivar selection can, therefore, help mitigate economic losses as a consequence of PMTV-induced tuber necrosis. In the long-term, the most sustainable management strategy will be to breed cultivars resistant to PMTV infection. Detection of PMTV resistance sources in the germplasm of *S. tuberosum* has proven difficult (Santala *et al*., 2010). A breeding line, NY99, which combines low PMTV incidence of infection in tubers with low incidence of tuber necrosis in infected tubers, has been identified (Sandgren *et al*., 2002). In addition, two clones belonging to the *S. tuberosum* Group Phureja, held at The James Hutton Institute, Scotland, have shown excellent field resistance to PMTV and powdery scab after two seasons of testing (Lahuf *et al*., 2014). These promising materials can be used in potato breeding to introduce resistance to commercial cultivars.

High levels of resistance in potato to PMTV in transformed cultivars expressing PMTV-derived genes has been reported (Arif *et al*., 1999; Barker *et al*., 1998; Germundson *et al*., 2002; Melander *et al*., 2001; Reavy *et al*., 1995; Reavy *et al*., 1997). The advantages of transgenic

resistance to plant viruses using pathogen-derived sequences are well established and there are many examples where this approach has been successfully applied against potato viruses (reviewed by Acosta *et al.*, 1995). However, their use is subject to acceptance by consumers and the potato industry.

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CHAPTER TWO. SENSITIVITY OF POTATO CULTIVARS TO *POTATO MOP-TOP VIRUS*-INDUCED TUBER NECROSIS [1]

Abstract

Potato (*Solanum tuberosum*) cultivars representing four market classes were assessed for sensitivity to *Potato mop-top virus* (PMTV)-induced tuber necrosis in three separate trials in a field in North Dakota known to be infested with PMTV. Reverse transcription polymerase chain reaction (RT-PCR) confirmed the presence of PMTV in randomly selected samples. Results of tuber necrosis assessments conducted during storage demonstrated that sensitivity to PMTV-induced tuber necrosis among cultivars follows a continuum of tolerant to sensitive. As a group, the russet-skinned cultivars had a lower incidence of tuber necrosis than red-, yellow- and white-skinned cultivars. The incidence and severity of PMTV-induced tuber necrosis in trial one were significantly correlated with those parameters in trial two across years. Significant correlations also existed between the incidence of powdery scab on tubers and the incidence of PMTV-induced tuber necrosis in trial one across years. A significant correlation was also found between root gall numbers and powdery scab incidence and severity on tubers as well as PMTV-induced tuber necrosis incidence in trial two. The results of this study provide growers with disease management options by avoiding cultivars highly sensitive to PMTV-induced tuber necrosis development and potentially replacing them with tolerant cultivars in the same market class. It is apparent from these studies that field assessments can be used for the development of PMTV resistant germplasm for use in future breeding strategies.

[1] The material in this chapter was co-authored by Owusu Domfeh, F.G. Bittara and N.C. Gudmestad. Owusu Domfeh had primary responsibility for designing the experiments, collecting data and performing statistical analyses of the data used in this chapter. Owusu Domfeh wrote the manuscript and performed all corrections suggested by the reviewers and Senior Editor of the Plant Disease Journal.

Introduction

The *Potato mop-top virus* (PMTV) causes serious economic losses in potato (*Solanum tuberosum*) production in affected countries (Latvala-Kilby *et al*., 2009; Nielsen and Molgaard, 1997; Sandgren *et al*., 2002, Santala *et al*., 2010). PMTV, the type member of the genus *Pomovirus* (King *et al*., 2012; Torrance and Mayo, 1997), is a rod-shaped, tripartite, single-stranded RNA virus (Latvala-Kilby *et al*., 2009; Savenkov *et al*., 2003; Savenkov *et al*., 1999; Scott *et al*., 1994; Torrance *et al*., 1999). PMTV has been reported in northern and central Europe, Asia, North and South America (Arif *et al*., 2014; Calvert and Harrison, 1966; Hu *et al*., 2013; Imoto *et al*., 1986; Jones, 1975; Jones, 1988; Kurppa, 1989; Lambert *et al*., 2003; Nakayama *et al*., 2010; Nielsen and Engsbro, 1992; Nielsen and Molgaard, 1997; Salazar and Jones, 1975; Xu *et al*., 2004). In the United States, PMTV was first reported in Maine in 2003 (Lambert *et al*., 2003), North Dakota in 2010 (David *et al*., 2010), Washington State in 2011 (Crosslin, 2011), Idaho in 2013 (Whitworth and Crosslin, 2013) and Colorado in 2014 (N.C Gudmestad, *unpublished*).

PMTV is both seed- and soil-borne. PMTV is vectored by *Spongospora subterranea* f. sp. *subterranea* (Sss) (Arif *et al*., 1995; Calvert and Harrison, 1966), the causal agent of powdery scab in potato. PMTV has been reported to remain infective in a field after 18 years without potatoes (Calvert, 1968), surviving in the resting spores of Sss, which themselves are also long-lived. This clearly indicates that when PMTV is present in a field, it cannot be eliminated through normal or even prolonged periods of crop rotation. PMTV infection is partially systemic and only a proportion of the progeny tubers from an infected plant carry the infection (Carnegie *et al*., 2010a; Davey *et al*., 2014; Kirk, 2008). This can lead to the elimination of the virus from potato crops after a few generations when the tubers are planted in soil free of PMTV and Sss

(Calvert, 1968; Davey *et al*., 2014; Kirk, 2008; Torrance *et al*., 1999). Unlike its vector, PMTV has a narrow host range, infecting members of only three families namely, Solanaceae, Tetragoniaceae and Chenopodiaceae (Andersen *et al*., 2002; Brunt *et al*., 1990; Jones, 1981). Humid soil and cool weather are conducive to PMTV as these conditions favor its vector (Cooper and Harrison, 1973; Harrison and Jones, 1970; van de Graaf *et al*., 2005; Wale, 2000). The success of PMTV transmission by Sss has been reported to be greatest at 12 to 20ºC while little or no infection occurs above 24ºC (Carnegie *et al*., 2010b).

Cultivar and environmental conditions greatly influence symptoms induced by PMTV (Cooper and Harrison, 1973; Harrison, 1974). Symptoms of primary infection include rust-colored lines, arcs and rings on the surface of the tubers or internal brown arcs and flecks in the tuber flesh of sensitive cultivars (Calvert and Harrison, 1966; Harrison and Jones, 1970; Kurppa, 1989). In secondary infections, tubers may show reticulate or deep cracks, blotchy surface markings or distortions (Calvert and Harrison, 1966; Harrison and Jones, 1970). The symptoms described above are similar to those induced by *Tobacco rattle virus* (TRV), both of which render affected tubers unmarketable.

The leaves of plants grown from infected tubers may show different shades of yellow markings which can easily be confused with leaf symptoms induced by *Alfalfa mosaic virus* and *Potato aucuba mosaic virus* (Calvert and Harrison, 1966). PMTV may cause shortening of internodes, which results in a dwarfed appearance (mop-top) (Calvert, 1968). Virus-specific methods such as reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, immunocapture RT-PCR (IC-RT-PCR), qualitative amplification based specific hybridization (FLASH-PCR), RT-PCR-microplate hybridization (RT-PCR-MPH) and enzyme-linked immunosorbent assay (ELISA) have been used for the detection of PMTV in potato (Arif *et al*.,

1994; Germundsson *et al*., 2002; Latvala-Kilby *et al*., 2009; Nakayama *et al*., 2010; Ryazantsev and Zavriev, 2009; Sandgren *et al*., 2001; Sokemen *et al*., 1998; Torrance *et al*., 1993). In the absence of suitable chemical treatments or other methods for effective control of Sss or PMTV, the most efficient measure for the prevention of this disease is avoidance; that is, to plant clean seed and avoid soils infested with virus-carrying Sss (Merz *et al*., 2005). However, ensuring that only disease-free potato seeds are planted is difficult to achieve since PMTV is currently not tested or evaluated under seed certification regulations in the USA (Epp, 2014). Thus, genetic resistance remains the best option for the management of this disease once it has been introduced into a field or onto a farm. Field trials have been conducted to assess the sensitivity of potato cultivars to PMTV-induced tuber necrosis in Europe (Calvert, 1968; Carnegie *et al*., 2009; Kurppa, 1989; Nielsen and Engsbro, 1992; Nielsen and Molgaard, 1997; Sandgren, 1995; Sandgren *et al*., 2002). Despite PMTV becoming more important in the United States, there has not been a detailed evaluation of potato cultivars for sensitivity to PMTV-induced tuber necrosis. The primary objective of this study was to screen some commonly grown USA potato cultivars for their reaction to tuber necrosis caused by PMTV to determine if variability in symptom expression in tubers exists. To accomplish this, three cultivar trials were conducted in 2011, 2012 and 2013 in a potato field in North Dakota known to be infested with PMTV-carrying Sss.

Materials and methods

Cultivar trial one

Fourteen potato cultivars consisting of seven russet-, three white-, three red- and one yellow-skinned market types (Table 2.1) were evaluated for sensitivity to PMTV-induced tuber necrosis in a randomized complete block design with four replications in 2011 and 2012. Each replication consisted of 15 seed tubers per cultivar planted 0.3 m apart with potato spacers (cv.

Russet Burbank) planted between cultivars. The potato spacers between replications were used to provide continuous ground cover to decrease variability in soil moisture and soil temperature which can affect Sss infection (van de Graaf *et al*., 2005; Wale, 2000).

Cultivar trial two

Twenty-four cultivars of russet-, white-, red- and yellow-skinned market types were planted in 2011 (Table 2.1). In 2012, due to unavailability of seed, cvs. Puren and Patagonia were not evaluated but five additional cultivars – Dakota Crisp, Dakota Pearl, Colorado Rose, Alturas and Atlantic were included. In both years, a randomized complete block design with three replications was used. Each replication consisted of 5 seed tubers per cultivar planted in 2011 and 10 seed tubers in 2012 in rows 0.3 m apart. To ensure ground cover, seed tuber spacers (cv. Russet Burbank) were planted between cultivars.

Cultivar trial three

Five white-skinned potato cultivars in addition to a tolerant internal control cultivar Ivory Crisp, previously included in trial one and trial two (Table 2.1), were evaluated for sensitivity to PMTV-induced tuber necrosis in a randomized complete block design with three replications in 2013. Each replication consisted of 10 seed tubers per cultivar planted 0.3 m apart. Seed tuber spacers (cv. Russet Burbank) were planted between cultivars to ensure ground cover.

Table 2.1. Potato cultivars evaluated in this study.

Trial 1*			Trial 2			
Cultivar	**Skin color**	**Year of release**	**2011 Cultivars**	**2012 Cultivars**	**Skin color**	**Year of release**
Russet Burbank	Russet	1914	Nicolet	Nicolet	White	2012
Russet Norkotah	Russet	1987	Alpine Russet	Alpine Russet	Russet	2008
Ranger Russet	Russet	1991	Dakota Jewel	Dakota Jewel	Red	2004
Umatilla Russet	Russet	1998	Yagana	Yagana	Yellow	1983
Alpine Russet	Russet	2008	Kennebec	Kennebec	White	1948
Bannock Russet	Russet	1999	Dark Red Norland	Dark Red Norland	Red	1957
Dakota Trailblazer	Russet	2009	Puren		Yellow	1993
Ivory Crisp	White	2002	Snowden	Snowden	White	1990
Shepody	White	1980	Viking	Viking	Red	1963
Kennebec	White	1948	Red Pontiac	Red Pontiac	Red	1945
Yukon Gold	Yellow	1980	Yukon Gold	Yukon Gold	Yellow	1980
Red LaSoda	Red	1953	Red Norland	Red Norland	Red	1964
Red Pontiac	Red	1945	Lamoka	Lamoka	White	2011
Red Norland	Red	1964	Red LaSoda	Red LaSoda	Red	1953
			Patagonia		Red	2009
Trial 3 2013			Ivory Crisp	Ivory Crisp	White	2002
Nicolet	White	2012	Ranger Russet	Ranger Russet	Russet	1991
Snowden	White	1990	Bannock Russet	Bannock Russet	Russet	1999
Dakota Crisp	White	2005	Russet Burbank	Russet Burbank	Russet	1914
Atlantic	White	1976	Karu	Karu	Red	2002
Ivory Crisp	White	2002	Umatilla Russet	Umatilla Russet	Russet	1998
Lamoka	White	2011	Russet Norkotah	Russet Norkotah	Russet	1987
			Shepody	Shepody	White	1980
			Dakota Trailblazer	Dakota Trailblazer	Russet	2009
				Dakota Crisp	White	2005
				Dakota Pearl	White	1999
				Colorado Rose	Red	2000
				Alturas	Russet	2002
				Atlantic	White	1976

*The same 14 potato cultivars were planted in 2011 and 2012 for trial one.

Seed tubers used in all trials were obtained from seed potato farms which were free of PMTV as revealed by recent surveys. Each tuber was carefully examined during hand-cutting to prepare seed for planting and none of them had Sss lesions or internal symptoms of tuber necrosis. All trials were conducted on a sandy loam soil. In 2011, the average air and soil temperatures of the experimental site during the growing season as recorded by the North Dakota Agricultural Weather Network (NDAWN) were 18°C and 19°C, respectively. The amount of rainfall during the growing season totaled 355.6 mm while sprinkler irrigation amounted to 95.3

mm. In 2012, the average air and soil temperatures were 15.6°C and 17°C, respectively. A total of 518.2 mm of sprinkler irrigation was applied while rainfall amounted to 262.4 mm. In 2013, the average air and soil temperatures were 18.9°C and 19°C, respectively. The amount of sprinkler irrigation applied was 355.6 mm while rainfall amounted to 176 mm. The amount of nitrogen applied was 110 kg/ha in 2011, 319 kg/ha in 2012 and 333 kg/ha in 2013. Each year, the herbicides pendimethalin and rimsulfuron were applied at the rate of 2.8 l/ha and 105g/ha respectively. To control leafhoppers, green peach aphid and Colorado potato beetles, insecticides such as thiamethoxam, imidachloprid, abamectin and esfenvalerate were applied at rates recommended by manufacturers. Fungicides including chlorothalonil, fluopyram/pyrimethanil, boscalid and azoxystrobin were applied to control early and late blight as appropriate for an irrigated commercial potato crop in the Upper Great Plains of the USA.

Root gall evaluation

Five plants were carefully removed 90 days after planting. Roots were gently shaken to release attached soil and galls were evaluated under a magnifying glass. Galls on roots were counted and expressed as number of galls per plant in accordance with the method used by Hernandez Maldonado *et al*. (2013). Data on root galls were collected for trials one and two in 2012 only.

Post-harvest tuber sampling

After harvest, tubers were cured at a temperature of 10°C for three weeks and stored at 8 - 10°C thereafter. The tubers were evaluated three times for trial one and two times for trial two during storage for powdery scab infection on tubers and PMTV-induced tuber necrosis. For trial one, evaluations were conducted 37, 117 and 204 days post-harvest (DPH) in 2011 and 56, 167 and 214 DPH in 2012, respectively. For trial two, evaluations were done 70 and 136; 126 and

190 DPH in 2011 and 2012, respectively. Tubers were evaluated twice in trial three for PMTV-induced tuber necrosis at 63 and 133 DPH. For each trial, a sample consisting of 100 tubers per cultivar and replicate was taken at random and one-third (trial one) or half (trials two and three) of the tubers were graded at each evaluation. All harvested tubers were used when less than 100 tubers were available. In trial one, a total of 15,636 and 26,181 tubers were examined in 2011 and 2012, respectively. The total number of tubers examined in trial two was 1,975 and 3,834 in 2011 and 2012 respectively, while 1,254 tubers were examined in trial three. This brings the total number of tubers evaluated for PMTV-induced tuber necrosis in this study to 48,880. PMTV incidence and severity index were determined using previously published protocols (Nielsen and Molgaard, 1997). Washed tubers were cut lengthwise into 1cm thick slices with a SafeHands^{TM} Professional Mandolin slicer (Jaccard Corporation, NY). PMTV incidence was calculated as the number of tubers showing symptoms of PMTV-induced necrosis per the total number of tubers examined for each sample. The number of slices per tuber with internal necrosis was determined (a). The slice with the most severe necrosis was then covered with a mask of 1 cm broad strips and the number of squares with necrosis was recorded (b). An index of PMTV severity was calculated by multiplying the two measurements (a * b) and expressing the values between 0 and 1, where 0 indicates no necrosis and 1 indicates the presence of necrosis throughout the tuber. Cultivars were ranked based on the overall incidence means of tuber necrosis according to the following categorization: tolerant - < 5%; moderately tolerant - >5% to 10%; moderately sensitive – >10% to 15%; sensitive – >15%. The overall incidence was calculated by adding the incidence of each cultivar for 2011 to that of 2012. Only cultivars planted in 2011 and 2012 were included in the classification.

Powdery scab on tubers was visually assessed and severity scored by comparing the area of tuber covered by disease with a modified graphic scale (Falloon *et al*., 1995). An average percentage of the sample disease severity was calculated. Symptoms were confirmed by observation of cystosori under the microscope (400x) when required. Incidence was obtained by calculating the percentage of symptomatic tubers from the total number of tubers in the sample.

Detection of PMTV and TRV

Necrotic tissues were taken from slices of potato tubers with a sharp scalpel sterilized in 75% ethanol and flamed until red-hot between samples. The tissues were crushed in liquid nitrogen and stored at -80 ºC until used for RNA extraction. Total RNA was extracted using TRIzol® reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions, with the exception that 0.8 ml was added to each tube for tissue homogenization instead of 1 ml. The RNA pellets were air-dried for 5-10 minutes and thereafter, the pellets were dissolved in 100 µl of RNase-free water. Detection of PMTV in tubers was done by reverse transcription-PCR according to a previously published protocol (Nakayama *et al*., 2010) with the only modification being the use of 0.2 µl of random primers (500µg/ml) and 3.3 µl of RNase free water instead of 1 µl and 2.5 µl, respectively. To further demonstrate that tuber necrosis was caused by PMTV, RNA from tuber extractions were also tested for *Tobacco rattle virus* (TRV) using RT-PCR according to Robinson (Robinson, 1992). For trials one and two, a total of 150 and 200 randomly selected symptomatic tubers were tested for PMTV and TRV in 2011 and 2012, respectively, while 50 tubers were tested for trial three.

Statistical analysis

Statistical analyses of the experimental data were carried out using the Statistical Analysis Software (SAS) version 9.3. Separate analyses of variance (ANOVA) were conducted

on the percentage data for incidence and severity of powdery scab and PMTV-induced tuber necrosis for each year due to non-homogeneity of variance between years (Levene's test: $P < 0.000$) (Millikin and Johnson, 1992). In each trial, combined ANOVA analyses were carried out for data evaluated two or three times during storage as variances across evaluation periods were homogeneous (Millikin and Johnson, 1992). Residual plots of the percentage data sets (PMTV-induced tuber necrosis and powdery scab on tubers) as well as root gall data for trial two revealed that ANOVA could be performed without prior transformation as major assumptions were satisfied. Root gall number for trial one was square root transformed prior to ANOVA. Treatments were compared using the Fisher's Protected Least Significance Difference (LSD) test at $P \leq 0.05$. The Pearson's Correlation Coefficient was calculated to demonstrate the degree of association between parameters. The calculation of correlations between trials involved the 14 cultivars common to trials one and two over a two year period.

Results

Susceptibility of potato cultivars to powdery scab infection

In trials one and two, cultivars differed significantly ($P < 0.0001$) in susceptibility to root gall formation caused by Sss. In trial one, the mean number of galls per plant ranged from 0.8 in cv. Russet Norkotah to 110.3 in cv. Kennebec (Table 2.2). Cultivars Kennebec, Red Pontiac, Umatilla Russet, Red Norland, Ivory Crisp and Shepody were among the most susceptible, while cvs. Russet Norkotah, Bannock Russet and Yukon Gold were among the least affected. In trial two, the mean number of galls per plant ranged from 3.2 in cv. Dakota Jewel to 149.6 in Lamoka (Table 2.2). Root gall formation was most severe in cvs. Lamoka, Kennebec, Red Pontiac, Snowden and Shepody, while cvs. Dakota Jewel, Russet Norkotah, Karu, Dakota Trailblazer and Dakota Crisp were among the least affected.

Table 2.2. Mean number of *Spongospora* galls per plant on potato roots 90 days after planting in trials one and two. Data on root galls were collected in 2012 only.

Cultivar	**Mean number of [x,z] root galls**		**Cultivar**	**Mean number of [x] root galls**	
Trial 1			Trial 2		
Kennebec	110.3	a	Lamoka	149.6	a
Red Pontiac	65.0	b	Kennebec	115.3	ab
Umatilla Russet	46.3	bc	Red Pontiac	77.8	bc
Red Norland	38.0	bcd	Snowden	76.0	bc
Ivory Crisp	36.6	cde	Shepody	64.2	bcd
Shepody	26.1	def	Nicolet	43.7	cd
Russet Burbank	14.8	efg	Red LaSoda	32.6	cd
Red LaSoda	12.5	fgh	Umatilla Russet	26.3	cd
Alpine Russet	10.0	fghi	Yagana	25.1	cd
Ranger Russet	3.4	ghij	Alpine Russet	22.4	cd
Dakota Trailblazer	2.5	ghij	Dakota Pearl	16.2	cd
Yukon Gold	2.3	hij	Russet Burbank	12.9	cd
Bannock Russet	1.7	ij	Colorado Rose	12.2	cd
Russet Norkotah	0.8	j	Ivory Crisp	12.1	cd
			Viking	11.9	cd
			Dark Red Norland	10.0	d
			Alturas	9.5	d
			Atlantic	9.1	d
			Yukon Gold	8.5	d
			Bannock Russet	8.1	d
			Red Norland	6.3	d
			Ranger Russet	5.8	d
			Dakota Crisp	5.6	d
			Dakota Trailblazer	4.1	d
			Karu	3.5	d
			Russet Norkotah	3.4	d
			Dakota Jewel	3.2	d
LSD$_{(P = 0.05)}$	**25.8**			**66.0**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0.05).
[z] Root gall numbers were square root transformed prior to analysis. Reported means are based on the untransformed data.

In trials one and two, powdery scab lesion incidence and severity on tubers differed significantly ($P < 0.0001$) in 2011 and 2012. In trial one, the incidence of powdery scab lesions on tubers across cultivars ranged from zero to 47.5%, while severity ranged from zero to 2.9% in 2011 (Table 2.3). In 2012, powdery scab lesion incidence on tubers ranged from zero to 57%, while severity ranged from zero to 2.7% (Table 2.3). Powdery scab incidence and severity were highest in cvs. Shepody, Red LaSoda, Kennebec and Ivory Crisp across years. As expected,

powdery scab incidence and severity were lowest in the russet-skinned cultivars across years. Cultivars Russet Norkotah and Bannock Russet did not have powdery scab lesions on tubers in 2011 as was observed in cvs. Alpine Russet and Umatilla Russet in 2012.

Table 2.3. Mean incidence and severity of powdery scab caused by *Spongospora subterranea* f. sp. *subterranea* in 14 potato cultivars (trial one) planted in 2011 and 2012 and evaluated three times during a seven-month storage period.

Cultivar	Powdery scab [x] incidence on tubers (%)		Powdery scab [x] severity on tubers (%)		Powdery scab [x] incidence on tubers (%)		Powdery scab [x] severity on tubers (%)	
	2011				2012			
Shepody	47.5	a	2.9	a	50.6	a	1.3	bc
Red LaSoda	43.1	ab	1.4	b	57.0	a	2.7	a
Kennebec	37.7	bc	1.5	b	55.7	a	1.5	b
Ivory Crisp	32.4	cd	0.7	c	27.8	b	0.4	bcd
Red Pontiac	29.1	d	0.8	c	17.1	bcd	0.2	cd
Red Norland	16.3	e	0.2	cd	21.9	bc	0.2	cd
Yukon Gold	14.4	e	0.1	d	8.6	cde	0.1	d
Ranger Russet	1.3	f	>0.0*	d	1.3	de	>0.0*	d
Russet Burbank	0.6	f	>0.0*	d	0.3	e	>0.0*	d
Alpine Russet	0.2	f	>0.0*	d	0.0	e	0.0	d
Umatilla Russet	0.2	f	>0.0*	d	0.0	e	0.0	d
Dakota Trailblazer	0.2	f	>0.0*	d	2.8	de	>0.0*	d
Russet Norkotah	0.0	f	0.0	d	0.9	e	>0.0*	d
Bannock Russet	0.0	f	0.0	d	0.9	e	>0.0*	d
LSD$_{(P = 0.05)}$	**7.8**		**0.6**		**15.9**		**1.1**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).
*Severity of powdery scab is not a true zero as there were a few tubers with powdery scab lesions.

In trial two, the incidence of powdery scab lesions on tubers ranged from zero in several russet-skinned cultivars to 50.8% in Kennebec while severity ranged from zero to 0.7% in 2011 (Table 2.4). Cultivars Kennebec, Shepody, Red LaSoda and Viking were among those with the highest incidence, while cvs. Alpine Russet, Ranger Russet, Bannock Russet, Russet Burbank, Russet Norkotah, Umatilla Russet and Dakota Trailblazer had no powdery scab lesions on tubers. In 2012, the incidence of powdery scab on tubers ranged from zero to 52.8%, while severity ranged from zero to 3.5% (Table 2.4). Cultivars Kennebec, Red LaSoda, Dakota Pearl,

Shepody and Red Pontiac were among the most susceptible to powdery scab lesion on tubers, while cvs. Bannock Russet, Russet Burbank, Umatilla Russet and Dakota Trailblazer did not have lesions.

Sensitivity of potato cultivars to PMTV-induced tuber necrosis

Cultivar trial one

PMTV-induced tuber necrosis incidence and severity differed significantly among cultivars ($P < 0.0001$) in both years. In 2011, incidence ranged from zero in cv. Bannock Russet to 5.2% in cv. Red Norland, while severity indices ranged from zero in cv. Bannock Russet to over 0.1 in cv. Red Norland (Table 2.5). In 2012, the incidence of PMTV-induced tuber necrosis ranged from over 0.3% in cv. Bannock Russet to 17% in cv. Red LaSoda, while severity indices ranged from 0.002 in cv. Bannock Russet to 0.14 in cv. Red Pontiac (Table 2.5). There was a highly significant correlation between PMTV-induced tuber necrosis incidence and severity among cultivars in 2011 ($r = 0.97$, $P = 0.001$) and in 2012 ($r = 0.66$, $P = 0.01$). The overall correlation between incidence and severity among cultivars across years was also significant ($r = 0.79$, $P = 0.001$). This means that cultivars with the highest incidence of PMTV-induced tuber necrosis also had the highest severity of the disease. Based on the two-year results of PMTV-induced tuber necrosis incidence, the cultivars can be ranked as follows – tolerant: Bannock Russet, Dakota Trailblazer, Yukon Gold, Ivory Crisp and Umatilla Russet (overall incidence of < 5%); moderately tolerant: Ranger Russet, Alpine Russet, Russet Burbank, Red Pontiac, Russet Norkotah and Shepody (overall incidence of >5% to 10%); moderately sensitive: Kennebec (13.9% overall incidence) and sensitive: Red Norland and Red LaSoda (overall incidence of 17.6% and 18.6% respectively) (Table 2.6).

Table 2.4. Mean incidence and severity of powdery scab caused by *Spongospora subterranea* f. sp. *subterranea* in potato cultivars (trial two) planted in 2011 and 2012 and evaluated twice during storage.

Cultivar	Powdery scab [x] incidence on tubers (%)		Powdery [x] scab severity on tubers (%)		Cultivar	Powdery [x] scab incidence on tubers (%)		Powdery [x] scab severity on tubers (%)	
	2011					2012			
Kennebec	50.8	a	0.7	a		52.8	a	3.5	a
Shepody	24.1	b	0.2	bcd		35.1	b	1.5	c
Red LaSoda	21.8	bc	0.2	bc		51.4	a	2.6	b
Viking	20.4	bcd	0.3	b		21.5	cd	0.3	ef
Lamoka	14.1	bcde	0.1	bcd		16.5	cdef	0.2	ef
Yukon Gold	13.0	bcdef	0.2	bcd		7.1	fgh	0.1	f
Ivory Crisp	13.0	bcdef	0.1	cd		27.3	bc	0.8	cde
Puren	9.4	cdefg	>0.0*	cd					
Dakota Jewel	9.0	defg	0.1	bcd		6.2	fgh	0.1	f
Red Pontiac	7.4	efg	0.1	cd		37.0	b	1.1	cd
Yagana	6.4	efg	>0.0*	cd		6.4	fgh	>0.0*	f
Dark Red Norland	4.3	efg	0.1	bcd		9.4	efgh	0.0	f
Nicolet	3.1	efg	>0.0*	d		16.7	cdef	0.2	ef
Red Norland	3.0	efg	>0.0*	d		20.7	cde	0.5	def
Karu	1.3	gf	>0.0*	d		3.6	gh	>0.0*	f
Patagonia	1.0	gf	>0.0*	d					
Snowden	0.6	gf	>0.0*	d		4.1	gh	>0.0*	f
Alpine Russet	0.0	g	0.0	d		0.7	h	>0.0*	f
Ranger Russet	0.0	g	0.0	d		0.6	h	>0.0*	f
Bannock Russet	0.0	g	0.0	d		0.0	h	0.0	f
Russet Burbank	0.0	g	0.0	d		0.0	h	0.0	f
Russet Norkotah	0.0	g	0.0	d		1.1	h	>0.0*	f
Umatilla Russet	0.0	g	0.0	d		0.0	h	0.0	f
Dakota Trailblazer	0.0	g	0.0	d		0.0	h	0.0	f
					Dakota Pearl	50.7	a	1.4	c
					Dakota Crisp	20.4	cde	0.4	def
					Colorado Rose	13.9	defg	0.3	ef
					Atlantic	5.6	fgh	>0.0*	f
					Alturas	1.0	h	>0.0*	f
LSD$_{(P = 0.05)}$	**12.5**		**0.2**			**12.1**		**0.7**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).

*Severity of powdery scab is not a true zero as there were a few tubers with powdery scab lesions.

In 2011 and 2012, PMTV-induced tuber necrosis incidence increased significantly during storage ($P < 0.0001$). A significant interaction ($P < 0.0001$) was found between period of evaluation and cultivar in PMTV-induced tuber necrosis incidence. Generally, more PMTV-induced tuber necrosis symptoms were found in the second and third evaluations than the first. The highest increase in incidence occurred in cvs. Kennebec, Red Norland, Red LaSoda, Red Pontiac and Shepody but remained unchanged in several cultivars.

Table 2.5. Mean incidence and severity index of *Potato mop-top virus* (PMTV)-induced tuber necrosis in 14 potato cultivars (trial one) planted in 2011 and 2012 and evaluated three times during a seven-month storage period.

Cultivar	**PMTV tuber [x] necrosis incidence (%)**		**PMTV tuber [x,y] necrosis severity index**		**PMTV tuber [x] necrosis incidence (%)**		**PMTV tuber [x,y] necrosis severity index**	
	2011				**2012**			
Red Norland	5.2	a	0.10	a	12.4	b	0.10	bd
Red LaSoda	1.7	b	0.03	b	17.0	a	0.11	abc
Ranger Russet	0.9	bc	>0.00*	bc	6.4	d	0.11	ab
Alpine Russet	0.5	bc	0.01	bc	5.5	de	0.11	abc
Kennebec	0.7	bc	>0.00*	c	13.2	b	0.05	de
Yukon Gold	0.7	bc	>0.00*	c	4.0	df	0.03	eg
Russet Burbank	0.4	bc	>0.00*	bc	4.7	df	0.05	def
Ivory Crisp	0.4	bc	0.01	bc	2.9	efg	0.05	def
Red Pontiac	0.4	bc	0.02	bc	9.4	c	0.14	a
Russet Norkotah	0.4	bc	>0.00*	c	5.7	d	0.06	cde
Dakota Trailblazer	0.2	c	>0.00*	bc	0.5	g	>0.00*	fg
Umatilla Russet	0.1	c	>0.00*	c	2.3	fg	0.02	eg
Shepody	0.1	c	>0.00*	bc	6.3	d	0.04	eg
Bannock Russet	0.0	c	0.00	c	0.3	g	>0.00*	g
LSD$_{(P = 0.05)}$	**1.41**		**0.03**		**2.68**		**0.05**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD ($P = 0{\cdot}05$).
[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber.
*Severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.

However, the severity of PMTV-induced tuber necrosis did not change during storage in 2011 ($P > 0.47$) but increased significantly ($P < 0.0001$) in 2012, with the highest increase occurring in cvs. Kennebec, Ranger Russet, Red LaSoda, Russet Norkotah, Red Norland and Russet Burbank. The presence of PMTV was readily confirmed in randomly selected symptomatic tubers by RT-PCR. TRV was not detected in any of tubers tested.

Table 2.6. Summary of sensitivity rankings of potato cultivars (trials one and two) based on *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence values summed for 2011 and 2012.

Trial	**Tolerant cultivars** (Overall incidence <5%)	**Moderately tolerant cultivars** (Overall incidence >5% to 10%)	**Moderately sensitive cultivars** (Overall incidence >10% to 15%)	**Sensitive cultivars** Overall incidence >15%)
Trial one	Bannock Russet, Dakota Trailblazer, Yukon Gold, Ivory Crisp and Umatilla Russet	Ranger Russet, Alpine Russet, Russet Burbank, Red Pontiac, Russet Norkotah and Shepody	Kennebec	Red Norland and Red LaSoda
Trial two	Bannock Russet, Dakota Trailblazer, Russet Norkotah, Umatilla Russet, Yukon Gold, Ivory Crisp, Russet Burbank, Karu and Shepody	Viking, Red Pontiac, Red Norland and Ranger Russet	Nicolet, Kennebec and Red LaSoda	Alpine Russet, Snowden, Lamoka, Dakota Jewel, Dark Red Norland and Yagana

Cultivar trial two

As with trial one, the incidence of PMTV-induced tuber necrosis was low among cultivars in 2011, therefore, differences in incidence and severity were not statistically significant among cultivars (Table 2.7). However, in 2012, differences among cultivars in PMTV-induced tuber incidence and severity were highly significant ($P < 0.0001$). PMTV-induced tuber necrosis incidence ranged from zero in cv. Bannock Russet to 29.9% in cv. Dakota Crisp, while severity indices ranged from zero in cv. Bannock Russet to 0.49% in cv. Lamoka (Table 2.7). Cultivars

such as Dakota Jewel, Dakota Crisp and Dark Red Norland had significantly higher PMTV-induced necrosis incidence than both Red Norland and Red LaSoda, while Bannock Russet, Umatilla Russet, Ivory Crisp, Russet Norkotah, Yukon Gold and Nicolet were among those least sensitive. There was no correlation between PMTV-induced tuber necrosis incidence and severity among cultivars in 2011 (r = 0.24, P = 0.20) and 2012 (r = 0.49, P = 0.10). However, the incidence of PMTV-induced tuber necrosis was significantly correlated with severity among cultivars across years (r = 0.53, P = 0.05). Based on the two-year results of PMTV tuber necrosis incidence, the cultivars can be ranked as follows – tolerant: Bannock Russet, Dakota Trailblazer, Russet Norkotah, Umatilla Russet, Yukon Gold, Ivory Crisp, Russet Burbank, Karu and Shepody (overall incidence of < 5%); moderately tolerant: Viking, Red Pontiac, Red Norland and Ranger Russet (overall incidence of >5% to 10%); moderately sensitive: Nicolet, Kennebec and Red LaSoda (overall incidence of >10% to 15%); sensitive: Alpine Russet, Snowden, Lamoka, Dakota Jewel, Dark Red Norland and Yagana (overall incidence of >15%) (Table 2.6).

In 2011, PMTV-induced tuber necrosis incidence increased significantly (P < 0.0001) during storage. As noted in cultivar trial one, a significant interaction (P < 0.0002) was found between time of evaluation and cultivar for PMTV tuber necrosis incidence. Generally, incidence was higher in the second evaluation than the first and cultivars such as Alpine Russet, Dakota Jewel, Kennebec, Nicolet, Puren and Yagana had the highest increase. The severity of PMTV tuber necrosis did not change during storage (P > 0.17). In 2012, changes in PMTV tuber necrosis incidence (P > 0.28) and severity (P > 0.67) during storage were not significant. However, a significant interaction was found between the time of evaluation and cultivar (P = 0.02) in PMTV tuber necrosis incidence. A large decrease in incidence (25.8%) was observed in cv. Dakota Jewel between the first and second evaluations but incidence increased in a number

of cultivars, such as Snowden, Red LaSoda, Alpine Russet, Red Norland and Dark Red Norland during the same period. Presence of PMTV was confirmed in randomly selected symptomatic tubers by RT-PCR and TRV was not detected in any of the tubers tested.

Cultivar trial three

Significant differences ($P < 0.0001$) in PMTV-induced tuber necrosis incidence and severity were found among cultivars. PMTV tuber necrosis incidence ranged from 1.6% in cv. Lamoka to 47% in cv. Nicolet (Table 2.8). Cultivars Dakota Crisp and Snowden had incidences of PMTV-induced tuber necrosis not significantly different from that of cv. Nicolet. The severity of PMTV-induced necrosis ranged from 0.007 in cv. Lamoka to 0.66 in cv. Nicolet. A very strong positive correlation ($r = 0.98$, $P = 0.001$) was found between PMTV-induced tuber necrosis incidence and severity. Changes in PMTV-induced tuber necrosis incidence ($P > 0.98$) and severity ($P > 0.99$) during storage were not statistically significant. PMTV was confirmed in randomly selected symptomatic tubers by RT-PCR but none tested positive for TRV.

Table 2.7. Mean incidence and severity of *Potato mop-top virus* (PMTV)-induced tuber necrosis in potato cultivars (trial two) planted in 2011 and 2012 and evaluated twice during storage.

Cultivar	PMTV tuber necrosis incidence (%)	PMTV [y] tuber necrosis severity index		PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity Index	
	2011			2012			
Nicolet	9.0	0.16		1.6	hi	>0.00*	g
Alpine Russet	8.9	0.02		8.6	defgh	0.10	defg
Dakota Jewel	8.3	0.16		24.3	ab	0.27	c
Yagana	5.1	0.11		15.7	cd	0.30	bc
Kennebec	3.3	>0.00*		10.8	cdefg	0.03	g
Dark Red Norland	3.1	0.04		25.0	ab	0.18	cde
Puren	3.0	0.07					
Snowden	2.2	0.06		13.9	cde	0.42	ab
Viking	2.1	0.18		6.3	efghi	0.08	efg
Red Pontiac	2.0	0.08		7.5	efghi	0.17	cdef
Yukon Gold	1.5	>0.00*		2.1	hi	>0.0*	g
Red Norland	1.4	0.06		7.0	efghi	0.03	g
Lamoka	1.0	0.05		18.8	bc	0.49	a
Red LaSoda	0.1	0.02		12.4	cdef	0.05	efg
Patagonia	0.1	0.12					
Ivory Crisp	>0.0*	>0.00*		1.5	hi	0.06	efg
Ranger Russet	0.0	0.00		5.8	efghi	0.05	efg
Bannock Russet	0.0	0.00		0.0	i	0.00	g
Russet Burbank	0.0	0.00		4.7	fghi	0.04	fg
Karu	0.0	0.00		3.6	ghi	0.04	efg
Umatilla Russet	0.0	0.00		0.5	hi	0.02	g
Russet Norkotah	0.0	0.00		0.9	hi	>0.00*	g
Shepody	0.0	0.00		3.6	ghi	0.06	efg
Dakota Trailblazer	0.0	0.00		0.8	hi	>0.00*	g
			Dakota Crisp	29.9	a	0.23	cd
			Dakota Pearl	11.5	cdefg	0.11	defg
			Colorado Rose	7.0	efghi	0.01	g
			Alturas	4.9	fghi	0.06	efg
			Atlantic	4.4	fghi	0.02	g
LSD$_{(P = 0.05)}$	**NS**	**NS**		**8.4**		**0.14**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).

[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber.

*Incidence or severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.

Table 2.8. Mean incidence and severity of *Potato mop-top virus* (PMTV)-induced tuber necrosis in six white-skinned potato cultivars (trial 3) planted in 2013 and evaluated two times during storage.

Cultivar	PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity index	
Nicolet	47.0	a	0.66	a
Snowden	42.0	a	0.41	a
Dakota Crisp	36.4	a	0.41	a
Atlantic	10.0	b	0.03	b
Ivory Crisp	9.4	b	0.13	b
Lamoka	1.6	b	>0.00*	b
LSD($P = 0.05$)	10.9		0.18	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).
[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber.
*Incidence or severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.

Comparison between PMTV-induced tuber necrosis sensitivity trials

A significant correlation was found between trials one and two in 2011 for PMTV-induced tuber necrosis severity (r = 0.60, P = 0.02) but not incidence (r = 0.09, P = 0.20), using the data from the 14 potato cultivars common between the two trials. However, highly significant and strong correlations were observed in incidence (r = 0.89, P = 0.001) and severity (r = 0.76, P = 0.001) between trials one and two in 2012. Overall, PMTV-induced tuber necrosis incidence and severity in trial one were significantly correlated with those parameters in trial two across years (r = 0.69, P = 0.01, respectively).

Relationship between powdery scab infection and PMTV-induced tuber necrosis

In trial one, root gall number was significantly correlated with powdery scab incidence (r = 0.51, P = 0.05) but not severity (r = 0.41, P = 0.20) on tubers. There was no correlation between root gall number and PMTV-induced tuber necrosis incidence (r = 0.45, P = 0.20) or

severity (r = 0.16, *P* = 0.20). A significant correlation (r = 0.62, *P* = 0.02) was found between powdery scab incidence on tubers and PMTV-induced tuber necrosis incidence across years. However, there was no correlation (r = 0.14, *P* = 0.20) between powdery scab severity on tubers and PMTV-induced tuber necrosis severity across years.

In trial two, root gall number was significantly correlated with incidence (r = 0.76, *P* = 0.01) and severity (0.81, *P* = 0.001) of powdery scab on tubers. Root gall number was also significantly correlated with PMTV-induced tuber necrosis incidence (r = 0.54, *P* = 0.05) but not severity (r = 0.45, *P* = 0.20). There was no correlation between powdery scab incidence and PMTV-induced tuber necrosis incidence (r = 0.48, *P* = 0.10) or severity (r = 0.17, *P* = 0.20) across years.

Discussion

This is the first study evaluating potato cultivars for sensitivity to PMTV-induced tuber necrosis conducted in the USA or North America although some North American cultivars were evaluated previously under South American conditions (Tenorio *et al*., 2006). This study was prompted by the increasing importance of the *Potato mop-top virus* in North America which poses a serious economic threat to growers. The results obtained in this study demonstrate the existence of a continuum of sensitivity to PMTV-induced tuber necrosis among commonly grown North American potato cultivars from tolerant to sensitive. Potato growers therefore, have the option of replacing cultivars highly sensitive to the development of PMTV-induced tuber necrosis with tolerant ones in the same market class in production areas where PMTV exists. The identification of cultivars tolerant to PMTV-induced tuber necrosis also provides breeders with useful information for future breeding strategies.

In our study, we made no attempt to ascertain whether the absence of tuber necrosis was due to resistance to PMTV infection or resistance to the expression of tuber necrosis. The results were based on disease response of plants. The occurrence of asymptomatic PMTV infections in tubers has been reported by many researchers (Carnegie *et al*., 2009; Latvala-Kilby *et al*., 2009; Santala *et al.*, 2010; Sokemen *et al*., 1998). The possibility of latent infections occurring means that incidence of PMTV infection among the cultivars could be higher than what has been reported based on tuber necrosis expression. Investigations to reveal the extent of symptomless infection was beyond the scope of this study and it is the focus of future research.

The overall significant correlations ($r = 0.69$, $P = 0.01$) in both incidence and severity of PMTV-induced tuber necrosis between trial one and trial two, demonstrate that levels of sensitivity among cultivars in 2011 and 2012 were consistent, an indication of the reliability and reproducibility of the results reported in this study. This evidence demonstrates that field trials can be used to effectively evaluate potato cultivars for sensitivity to PMTV tuber necrosis under North American conditions. The results also show some level of seasonal variation in PMTV tuber necrosis incidence across cultivars even though the ranking of cultivars within a particular season changed very little. Generally, the incidence of PMTV tuber necrosis increased from 2011 through 2013. Due to the low levels of PMTV tuber necrosis incidence observed in 2011, more water was applied via irrigation in 2012 and 2013 than 2011 in order to increase Sss infection. The low incidence in 2011 could, therefore, be due to the low frequency of irrigation applied in late summer which is the practice among some growers in an attempt to reduce pink rot (caused by *Phytophthora erythroseptica* Pethyb.) disease pressure. Seasonal variability in PMTV infection among cultivars has also been observed in previous studies (Carnegie *et al*., 2009; Sokemen *et al*., 1998). The occurrence of seasonal variation in PMTV tuber necrosis

emphasizes the importance of multi-year assessments for cultivar classification (Nielsen and Molgaard, 1997; Santala *et al*., 2010). Even though the cultivar classification reported in this manuscript is based on the results of trials conducted over two growing seasons, the data offer valuable information upon which future work in this area could be built.

Despite the fact that trial three of this study was conducted in only one year (2013), all the cultivars evaluated in this trial were evaluated at least once in trial two and the high PMTV tuber necrosis incidence observed in three out of the six cultivars tested in trial three made its inclusion in this report appropriate. Furthermore, the inclusion of trial three offered the opportunity to confirm the high incidence of tuber necrosis observed in cv. Dakota Crisp in 2012 since this cultivar was not evaluated in 2011.

PMTV tuber necrosis incidence (and severity to some extent), increased more in sensitive cultivars during storage. The most notable exception of a sensitive cultivar which had a large decrease in incidence between the first and second evaluations is Dakota Jewel in trial two (2012). A large decrease in PMTV tuber necrosis during storage was recorded in cv. Saturna (Nielsen and Engsbro, 1992; Nielsen and Molgaard, 1997), widely regarded to be one of the most sensitive cultivars to tuber necrosis in the Nordic countries (Kurppa, 1989; Sandgen, 1995; Santala *et al*., 2010). The reasons for the disappearance of tuber necrosis remain unknown but reabsorption of the brown color by the tuber has been implicated (Nielsen and Molgaard, 1997).

Since PMTV is vectored by Sss, susceptibility of cultivars to powdery scab at root and tuber phases was also evaluated. The results demonstrated significant variability in susceptibility to root gall formation (Merz *et al*., 2004) and powdery scab on tubers among cultivars. Generally, the russet-skinned cultivars were less susceptible than the red-, white- and yellow

skinned cultivars to powdery scab infection on tubers (Nitzan *et al*., 2008), but susceptibility to root gall formation did not appear to be associated with skin color. The absence of overall correlation (r = 0.48, *P* = 0.2) between incidence of powdery scab on tubers and incidence of PMTV-induced tuber necrosis in trial two is consistent with previously published results (Cooper *et al*., 1976; Kirk, 2008; Montero-Astua *et al*., 2008; Nielsen and Nicolaisen, 2000; Sandgren *et al*., 2002; Tenorio *et al*., 2006). However, in trial one, the strong overall correlation (r = 0.62, *P* = 0.02) found between incidence of powdery scab on tubers and PMTV tuber necrosis incidence contradict the reports cited above. A study conducted in Scotland to investigate the transmission of PMTV from infected seed tubers to daughter plants found statistically significant correlations (r = 0.48; r = 0.61 in 2004 and 2005, respectively) between the incidence of PMTV and powdery scab for those crops in which both PMTV and powdery scab were present (Davey *et al*., 2014). The low or complete absence of correlation between powdery scab on tubers and PMTV infection has been attributed to differences in the optimal environmental conditions required by Sss and PMTV (Carnegie *et al*., 2010b; Carnegie *et al*., 2012) and that Sss infection of other parts of the potato plant such as roots and stolons also cause PMTV transmission (Carnegie *et al*., 2012; Davey *et al*., 2014; Jones and Harrison, 1969; Santala *et al*., 2010). However, it is likely in the present study that the strong correlation between powdery scab on tubers and PMTV-induced tuber necrosis is due to the fact that 50% of the cultivars evaluated were russet-skinned and not very sensitive to either disease.

The results reported in the present study indicate statistically significant correlations between root gall formation and powdery scab on tubers in trials one and two, as well as PMTV tuber necrosis in trial two. The strongest correlation occurred between root gall formation and powdery scab incidence and severity (r = 0.76, *P* = 0.01; r = 0.81; *P* = 0.001, respectively) in

trial two. While the low correlation found between root gall formation and powdery scab, as well PMTV necrosis supports the findings of other workers (Carnegie *et al*., 2010b; van de Graaf *et al*., 2007), the very strong association between powdery scab and root gall numbers in trial two contradict the report of van de Graaf *et al*. (2007) who noted that the optimum temperature range required for Sss infection of roots is higher than the 12-15ºC required for tuber phase infection. The work of van de Graaf *et al*. (2007) and Carnegie *et al*. (2010b) were carried out under constant (controlled) conditions, while our work was done in the field where temperature fluctuates day and night during the growing season and this could explain differences in results.

It is evident from the results that russet-skinned cultivars were the least susceptible to powdery scab lesions on tubers and this appeared to be associated with low sensitivity to PMTV-induced tuber necrosis. In contrast, many russet-skinned cultivars were susceptible to root gall formation. It is interesting to note that cultivars like Dakota Crisp, Dakota Jewel, Dark Red Norland and Red Norland which were sensitive to both PMTV-induced tuber necrosis and powdery scab infection on tubers had low levels of Sss root infection. These data, therefore, suggest that powdery scab infection on tubers is better linked with, and may be more important in leading to PMTV-induced necrosis expression in tubers than the presence of root galls. Here again, we did not investigate symptomless infection by Sss on roots or on tubers, but this will be the focus of future studies.

In this study, we found that among the commonly grown potato cultivars in USA, the russet-skinned cultivars, in comparison with red- , yellow- and white-skinned cultivars, are not only less susceptible to powdery scab infection on tubers, but are also less sensitive to PMTV-induced tuber necrosis. Differences in sensitivity to PMTV-induced tuber necrosis found among cultivars confirms that natural variability exists in North American potato cultivars which could

provide economic relief to potato producers affected by this disease. Potato growers now have the option to plant less sensitive cultivars in the same market class, especially in areas with history of Sss and PMTV. Cultivars found to be tolerant to the tuber necrosis phase of PMTV can also be utilized in future breeding efforts. The results of this and other studies indicate that soils in parts of USA are already infested with powdery scab and PMTV in some cases. It is important that a comprehensive survey be conducted to assess the extent of infestation of these two pathogens in USA. By using harmonized sampling and virus detection procedures, joint PMTV surveys involving 10 countries in Northern Europe were carried out from 2005 to 2008 (Santala *et al*., 2010). The experience gained through this project will be useful for planning the surveys in North America. Strict seed surveillance measures need to be put in place to help prevent infestation of disease-free soils. In addition, the evaluation of cultivars for sensitivity should be expanded to cover more cultivars and other germplasm including advanced breeding materials. Furthermore, the extent of latent infections in cultivars should be investigated through techniques such as RT-PCR or ELISA.

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CHAPTER THREE. SENSITIVITY TO TUBER NECROSIS CAUSED BY *POTATO MOP-TOP VIRUS* IN ADVANCED POTATO (*SOLANUM TUBEROSUM* L.) BREEDING SELECTIONS

Abstract

PMTV is transmitted by the powdery scab pathogen (*Spongospora subterranea* f.sp. *subterranea* (Sss)) and no effective methods of control are currently available. Potato cultivars have been reported to exhibit natural variability in their reaction to PMTV infection, making cultivar selection a viable management option to minimize economic impact of the disease. Advanced breeding selections of potato (*Solanum tuberosum* L.) from different market classes were evaluated for sensitivity to PMTV-induced tuber necrosis in a field in North Dakota, known to be infested with PMTV. Sixty-four selections made up of 14 russet-, 19 white-, 21 red- and 10 yellow-skinned market types were planted in 2011. In 2012, 23 russet-, 12 white- , 17 red- and 5 yellow-skinned breeding materials were assessed. Commercial cultivars, which range from sensitive to tolerant in their reaction to PMTV-induced tuber necrosis incidence, were included in each market class as internal controls. Results of tuber assessments revealed high variability in PMTV-induced tuber necrosis incidence and severity among advanced breeding selections. Based on PMTV-induced tuber necrosis incidence results over a two-year period across all market types, a total of 17 selections were found to be tolerant, nine – moderately tolerant, eight - moderately sensitive, while six were found to be sensitive. The russet-skinned types had lower tuber necrosis incidence than the red-, white- and yellow-skinned types. Among the red- and russet-skinned selections, PMTV tuber necrosis incidence for 2011 was significantly correlated with that of 2012. Increases in the incidence of PMTV tuber necrosis during storage was significantly influenced by selection type and skin-color. In future studies, it will be necessary to

determine if breeding selections which did not exhibit PMTV tuber necrosis are tolerant or resistant to the virus. Selections in which the absence of tuber necrosis correlate with no or low virus accumulation could be utilized as parents in breeding programs to introduce PMTV resistance into commercial potato cultivars. In the short term, tolerant selections with other desirable agronomic characteristics can be released as commercial cultivars and growers may utilize them, instead of sensitive cultivars which display tuber necrosis, as a means to limit the economic impact of PMTV-induced tuber necrosis.

Introduction

Potato mop top virus (PMTV), the type member of the genus *Pomovirus*, is seed- and soil-borne, and has straight tubular, rod-shaped particles (Torrance and Mayo, 1997; Sokemen *et al*., 1998). PMTV has a tripartite genome consisting of three single-stranded, positive sense RNA molecules (Scott *et al*., 1994; Torrance *et al*., 1999). PMTV is a serious pathogen of potato and can cause significant economic losses in sensitive potato cultivars (Harrison and Jones, 1970; Sandgren *et al*., 2002). PMTV has been is recognized as a threat to potato production in Northern Europe, Asia, South and North America (Calvert and Harrison, 1966; Crosslin, 2011; David *et al*., 2010; Harrison *et al*., 1997; Lambert *et al*., 2003; Latvala-Kilby *et al*., 2009; Mallik and Gudmestad, 2015; Wale, 2000; Whitworth and Crosslin, 2013; Xu *et al*., 2004). PMTV is believed to have originated from the Andean region of South America (Hinostroza and French, 1972; Salazar and Jones, 1975; Tenorio *et al*., 2006). PMTV is vectored by *Spongospora subterranea* f.sp. *subterranea* (Sss) (Arif *et al*., 1995; Calvert and Harrison, 1966; Harrison and Jones, 1970; Jones and Harrison, 1969), a fungal-like organism that causes powdery scab on potato. Cool temperature and high soil moisture enhance infection with Sss by favoring germination and movement of zoospores (Merz, 2008; Sandgren *et al.*, 2002). PMTV survives in

the resting cystosori of Sss, which can persist in the soil for many years (Jones and Harrison, 1972), making eradication of PMTV from an infested field difficult if not impossible. Field to field spread of PMTV occurs through infected seed and movement of virus-carrying cystosori of Sss attached to seed tubers or in adhering soil (Sandgren *et al*., 2002). Typical primary symptoms of PMTV infection include brown arcs and rings in the flesh of tubers (Calvert and Harrison, 1966; Harrison and Jones, 1971) and are sometimes visible on the tuber surface (Jefferies, 1998). Symptoms induced when infected tubers are planted, i.e. secondary infection, include misshapen or deep cracks on tubers (Calvert, 1968; Tenorio *et al*., 2006) and foliar symptoms such as mottling, shortening of internodes and yellow blotches or rings (Xu *et al*., 2004). Foliar symptoms are strongly affected by prevailing environmental conditions (Calvert, 1968; Carnegie *et al*., 2010). Tuber symptoms render affected tubers unsuitable for processing or consumption (Carnegie *et al*., 2012).

Genetic resistance remains the best option for the management of PMTV once it has been introduced into a field or onto a farm. Field trials have been conducted to assess the susceptibility/sensitivity of potato cultivars to PMTV-induced tuber necrosis in Europe (Calvert, 1968; Carnegie *et al*., 2009; Kurppa, 1989; Nielsen and Molgaard, 1997; Sandgren *et al*., 2002), North America (Domfeh *et al*., 2015) and in the Andean Region of South America (Tenorio *et al*., 2006). Results of these trials suggest that natural variability exists in potato germplasm in susceptibility/sensitivity to PMTV infection. With recent reports of PMTV occurring in many parts of USA, it has become important to screen a wide range of potato germplasm for their reaction to PMTV infection. The primary objective of this study was to screen some North and South American advanced potato breeding selections for their reaction to tuber necrosis caused

by PMTV. To accomplish this, a trial was conducted in 2011 and 2012 in a potato field in North Dakota known to be infested with PMTV-carrying Sss.

Materials and methods

Sixty-four advanced breeding selections made up of 14 russet-, 19 white-, 21 red- and 10 yellow-skinned market types were planted in 2011 (Table 3.1). In 2012, 23 russet-, 12 white- , 17 red- and 5 yellow-skinned breeding materials were planted (Table 3.2). Due to unavailability of seed of some breeding selections that were dropped from further evaluation, a number of clones across all skin-types planted in 2011 were not planted in 2012 but additional clones were included in 2012. A total of 92 potato genotypes were evaluated and of these, 11 were commercial cultivars included as internal controls. Fifty-one genotypes were tested in both years. The majority of the genotypes evaluated for PMTV tuber necrosis sensitivity were obtained from the NDSU potato breeding program from genetic material that the program evaluates on an annual basis. Clones beginning with ND, CO, W, NY, A, and O belong to the potato breeding programs of North Dakota, Colorado, Wisconsin, New York (Cornell), Idaho (USDA-ARS, Aberdeen) and Oregon, respectively. "AOND", "AND", and "ATND" clones were bred and selected through collaborations among the potato breeding programs of Idaho, Oregon, North Dakota and Texas. Clones that start with RC, RG, RA, R, RK, SPA and T (T10-12) originated from the INIA-Remehue National Potato Breeding Program of Chile. Internal controls, consisting of commercial cultivars with known reactions to PMTV induced tuber necrosis ranging from sensitive to tolerant (Domfeh *et al*. 2015) were used in all field trials. For the russet-skinned genotypes, cvs. Alpine Russet (sensitive), Ranger Russet (moderately tolerant) and Bannock Russet (tolerant) were included. Among the white-skinned genotypes, cvs. Lamoka (sensitive), Kennebec (moderately sensitive) and Ivory Crisp (tolerant) were used. With the red-

skinned clones, cvs. Dakota Jewel (sensitive), Red LaSoda (moderately sensitive) and Red Pontiac (moderately tolerant) were included. Cultivars Yagana (sensitive) and Yukon Gold (tolerant) served as internal controls for the yellow-skinned genotypes. In both years, a randomized complete block design with three replications was used. Each replication consisted of 5 seed pieces per selection planted in 2011 and 10 seed pieces in 2012 in rows 0.3 m apart. To ensure ground cover, seed tuber spacers (cv. Russet Burbank) were planted between cultivars. The trial was conducted on a sandy loam soil with approximately 3.0% organic matter.

Seed tubers used in this trial were obtained from seed potato farms which were free of PMTV as revealed by recent surveys (Gudmestad, unpublished). During the survey, soil was collected from seed farms and assayed using *Nicotiana debneyi* as bait plants which were subsequently tested by reverse transcription (RT)-PCR. Each seed tuber was also carefully examined during hand-cutting to prepare seed for planting and none of them had Sss lesions or internal symptoms of tuber necrosis. In 2011, the average air and soil temperatures of the experimental site during the growing season as recorded by the North Dakota Agricultural Weather Network (NDAWN) were 18°C and 19°C, respectively. The amount of rainfall during the growing season totaled 355.6 mm, while sprinkler irrigation amounted to 95.3 mm. In 2012, the average air and soil temperatures were 15.6°C and 17°C, respectively. A total of 518.2 mm of sprinkler irrigation was applied, while rainfall amounted to 262.4 mm. Each year, the herbicides pendimethalin and rimsulfuron were applied at the rate of 2.8 l/ha and 105 g/ha respectively. To control leafhoppers, green peach aphid and Colorado potato beetles, insecticides such as thiamethoxam, imidacloprid, abamectin and esfenvalerate were applied at rates recommended by manufacturers. Fungicides including chlorothalonil, fluopyram/pyrimethanil, boscalid and azoxystrobin were applied to control early and late blight as appropriate for an

irrigated commercial potato crop in the Upper Great Plains of the USA. The 2011 and 2012 field trials were planted on 24-25th May and 30th April; and harvested on 5th October and 5th September, respectively.

Table 3.1. Advanced breeding selections planted in field screening trial in 2011.

Red-skinned selections [z]	**Russet-skinned selections** [z]	**White-skinned selections** [z]	**Yellow-skinned selections** [z]
AND00272-1R	AND01804-3Russ	CO95051-7W	R 87009-28
ATND98459-1RY	ND049289-1Russ	MSL-292A	R89045-35
ND028842b-1RY	ND049423b-1Russ	ND060601CAB-2	R91007-5
ND050167C-3R	ND049546b-10Russ	ND060715B-15	RA148-48
ND060728-5R	ND050082Cb-2Russ	ND060835C-4	RA16-5
ND060733b-4RY	ND050105C-1Russ	ND060847CB-1	RA362-54
ND4659-5R	ND059769Ab-1Russ	ND6956b-13	RA517-123
ND8058-11R	ND060742C-1Russ	ND7519-1	RA519-50
ND8314-1R	ND060766b-4Russ	ND7550C-1	RA82-4
ND8555-8R	ND060796AB-1Russ	ND8304-2	RC06-109
R90070-8	ND6400C-1Russ	ND8307C-3	Yukon Gold
R90134-6	ND8068-5Russ	ND8331Cb-2	Yagana
R90160-5	ND8229-3	ND8331Cb-3	
R90213-6	ND8413-7Russ	ND8559-20	
R91129-11	Bannock Russet	NY 138	
RA20-6	Alpine Russet	NY 139	
RA89044-45	Ranger Russet	R65A-70	
RA90213-60		RA151-24	
RC72-35		W2717-5	
SPA161		Ivory Crisp	
T10-12		Kennebec	
Dakota Jewel		Lamoka	
Red Pontiac			
Red LaSoda			

[z] Commercial cultivars Dakota Jewel, Red Pontiac, Red LaSoda, Bannock Russet, Alpine Russet, Ranger Russet, Ivory Crisp, Kennebec, Lamoka, Yukon Gold and Yagana were included as internal controls. These cultivars range from tolerant to sensitive in their reaction to PMTV-induced tuber necrosis incidence (Domfeh *et al*., 2015).

Table 3.2. Advanced breeding materials planted in 2012.

Red-skinned selections [z]	Russet-skinned selections [z]	White-skinned selections [z]	Yellow-skinned selections [z]
AND00272-1R	AND00618-1RussY	ND060835C-4	R87009-28
ATND98459-1RY	AND01804-3Russ	ND6956b-13	R91007-5
ND060728-5R	AND97279-5Russ	ND7519-1	RA517-123
ND4659-5R	AND99362B-1Russ	ND7550C-1	RC06-109
ND8314-1R	AOND95292-3Russ	ND8229-3	RK24-48
ND8555-8R	ND039194AB-1Russ	ND8304-2	Yukon Gold
R90070-8	ND049289-1Russ	ND8305-1	Yagana
R90096-5	ND049381C-2Russ	ND8307C-3	
R91129-11	ND049423b-1Russ	ND8331Cb-2	
RA20-6	ND049517B-1Russ	ND8331Cb-3	
RA89044-45	ND049546b-10Russ	ND8559-20	
RA90213-60	ND050082Cb-2Russ	RA151-24	
RC72-35	ND050105C-1Russ	Ivory Crisp	
RC89-25	ND060735-3Russ	Kennebec	
RG47-3	ND060742C-1Russ	Lamoka	
SPA161	ND060761B-3Russ		
T10-12	ND060766b-4Russ		
Dakota Jewel	ND060770B-5Russ		
Red Pontiac	ND060796AB-1Russ		
Red LaSoda	ND070927-2Russ		
	ND6400C-1Russ		
	ND8068-5Russ		
	ND8413-7Russ		
	Bannock Russet		
	Alpine Russet		
	Ranger Russet		

[z] Commercial cultivars Dakota Jewel, Red Pontiac, Red LaSoda, Bannock Russet, Alpine Russet, Ranger Russet, Ivory Crisp, Kennebec, Lamoka, Yukon Gold and Yagana were included as internal controls. These cultivars range from tolerant to sensitive in their reaction to PMTV-induced tuber necrosis incidence (Domfeh *et al*., 2015).

Post-harvest tuber sampling

After harvest, tubers were cured at a temperature of 10°C for three weeks and stored at 8 - 10°C thereafter. The tubers were evaluated twice during storage for PMTV-induced tuber necrosis. Evaluations were performed at 70 and 136; 126 and 190 days post-harvest in 2011 and 2012, respectively. Samples consisting of 100 tubers per clone and replicates were taken at random and half of the tubers were graded at each evaluation date. All harvested tubers were used when less than 100 tubers were available. A total of 6,078 and 8,042 tubers were examined

in 2011 and 2012 respectively, bringing the total number of tubers evaluated in this study to 14,120.

PMTV incidence and severity index were determined using previously published protocols (Nielsen and Molgaard, 1997). Washed tubers were cut lengthwise into 1cm thick slices with a SafeHands^{TM} Professional Mandolin slicer (Jaccard Corporation, NY). PMTV incidence was calculated as the number of tubers showing symptoms of PMTV-induced tuber necrosis per the total number of tubers examined for each sample. The number of slices per tuber with internal necrosis was determined (a). The tuber slice with the most severe internal necrosis was covered with a clear transparency with 1 cm wide vertical and horizontal strips. The number of squares with necrosis was recorded (b). An index of PMTV severity was calculated by multiplying the two measurements (a * b) and expressing the values between 0 and 1, where 0 indicates no necrosis and 1 indicates the presence of necrosis throughout the tuber. Cultivars and advanced selections were ranked based on the overall incidence means of tuber necrosis according the following categorization: tolerant - < 5%; moderately tolerant - >5% to 10%; moderately sensitive – >10% to 15%; sensitive – >15%.

Detection of PMTV and TRV

Necrotic tissues were taken from slices of potato tubers with a sharp scalpel sterilized in 75% ethanol and flamed until red-hot between samples. The tissues were crushed in liquid nitrogen and stored at -80 ºC until used for RNA extraction. Total RNA was extracted using TRIzol® reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions, with the exception that 0.8 ml was added to each tube for tissue homogenization instead of 1 ml. The RNA pellets were air-dried for 5-10 minutes and thereafter, the pellets were dissolved in 100 µl of RNase-free water. Detection of PMTV in tubers was done by reverse

transcription (RT)-PCR according to a previously published protocol (Nakayama *et al*., 2010) with the only modification being the use of 0.2 µl of random primers (500µg/ml) and 3.3 µl of RNase free water instead of 1 µl and 2.5 µl, respectively. To further demonstrate that tuber necrosis was caused by PMTV, RNA from tuber extractions were also tested for *Tobacco rattle virus* (TRV) using RT-PCR (Robinson, 1992). A total of 150 randomly selected symptomatic tubers were tested for the presence of PMTV and TRV in 2011 and 2012.

Statistical analysis

Statistical analyses of the experimental data were carried out using the Statistical Analysis Software (SAS) version 9.3. Separate analyses of variance (ANOVA) were conducted on PMTV tuber necrosis incidence and severity data for each year due to non-homogeneity of variance between years (Levene's test: $P < 0.000$) (Millikin and Johnson, 1992). Preliminary analysis of the data revealed significant differences among skin-types in PMTV tuber necrosis incidence and severity. The data were subsequently partitioned into skin-types using the Proc Sort function in SAS prior to analysis. Combined ANOVA analyses were carried out on the data obtained after two evaluations, as variances across evaluation periods were homogeneous (Millikin and Johnson, 1992). Residual plots of the data sets revealed that ANOVA could be performed without prior transformation as major assumptions were satisfied. Treatments were compared using the Fisher's Protected Least Significance Difference (LSD) test at $P \leq 0.05$. The Pearson's Correlation Coefficient was calculated to demonstrate the degree of association between parameters. The calculation of correlations between years involved those selections planted in 2011 and 2012 (red-skinned – 17; russet-skinned – 15; white-skinned – 13, yellow-skinned – 6 and overall - 51).

Results

Tuber necrosis evaluated was determined to be caused by PMTV and not TRV based on RT-PCR results. Due to non-homogeneity of variance between years, separate analyses were done for each year and the results are presented accordingly. Data on PMTV-induced tuber necrosis incidence ($P < 0.005$) and severity ($P < 0.0001$) differed significantly across skin-types in 2011. Mean tuber necrosis incidence ranged from 2.3% in yellow-skinned selections to 7.1% in red-skinned selections (Table 3.3). The mean tuber necrosis incidence among white-skinned selections was not significantly different from those of red- and russet-skinned selections. Russet- and yellow-skinned selections had statistically similar mean tuber necrosis incidence (Table 3.3). PMTV tuber necrosis severity was significantly higher in the red-skinned selections than the other selections, all of which had similar severity indexes. In 2012, differences in tuber necrosis incidence ($P < 0.0398$) and severity ($P < 0.0004$) across skin-type were also statistically significant (Table 3.3). PMTV tuber necrosis incidence ranged from 4.7% in russet-skinned selections to 7.8% in red-skinned selections. PMTV tuber necrosis incidence among red-skinned selections was significantly higher than that of russet-skinned, but similar to those of white- and yellow-skinned selections (Table 3.3). PMTV tuber necrosis severity ranged from 0.04 in russet-skinned selections to 0.12 in white- and yellow-skinned selections (Table 3.3). Yellow- and white-skinned selections had significantly higher tuber necrosis severity than russet-skinned selections.

Table 3.3. Summary of *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence (%) and severity index of several advanced breeding selections across skin-types in 2011 and 2012. Tuber necrosis assessments were conducted twice during storage and analyses were performed on the combined data.

Skin color	PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity index		Skin color	PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity index	
	2011					2012			
Red	7.1	a	0.21	a	Red	7.8	a	0.09	ab
White	5.6	ab	0.11	b	Yellow	7.6	ab	0.12	a
Russet	2.5	bc	0.04	b	White	6.4	ab	0.12	a
Yellow	2.3	c	0.06	b	Russet	4.7	b	0.04	b
$LSD_{0.05}$	**3.2**		**0.07**			**2.9**		**0.05**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).
[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).

PMTV-induced tuber necrosis incidence and severity differed significantly among red-skinned advanced breeding selections in 2011 ($P < 0.0039$ – incidence; $P < 0.0001$ - severity) and 2012 ($P < 0.0001$). In 2011, the incidence of PMTV tuber necrosis ranged from zero in two selections (RA20-6 and RA89044-45) to 29.9% in selection SPA161, while severity ranged from zero in selection RA20-6 and RA89044-45 to 1.0 in selection ND8314-1R (Table 3.4). None of the selections had significantly lower PMTV tuber incidence than the moderately tolerant cv. Red Pontiac internal control. Two selections, SPA161 and ND8314-1R had significantly higher tuber incidence and severity than the sensitive standard, Dakota Jewel (Table 3.4). In 2012, incidence ranged from zero in two selections (R90096-5 and T10-12) to 30.4% in selection ND060728-5R, while severity ranged from zero in selections R90096-5 and T10-12 to 0.36 in selection SPA161 (Table 3.4). None of the selections had significantly lower tuber necrosis incidence than the moderately tolerant cv. Red Pontiac and none proved significantly more sensitive than the sensitive cv. Dakota Jewel (Table 3.4). PMTV tuber necrosis incidence ($r = 0.57$, $P < 0.02$) and severity ($r = 0.48$, $P < 0.05$) data in 2011 were significantly correlated with

those parameters in 2012. Highly significant correlations were found between incidence and severity among red-skinned selections in 2011 (r = 0.96, $P > 0.001$) and in 2012 (r = 0.94, $P < 0.001$). Based on the two-year PMTV tuber necrosis incidence data, the red-skinned selections and internal control cultivars can be classified as follows – tolerant: RC72-35, RA20-6, RA89044-45, ND4659-5R, R90070-8 and T10-12 (overall incidence <5%); moderately tolerant: R91129-11, ND8555-8R and Red Pontiac (overall incidence >5% to 10%); moderately sensitive: ATND98459-1RY, RA90213-60, AND00272-1R and Red LaSoda (overall incidence >10% to 15%) and sensitive: SPA161, ND8314-1R, ND060728-5R and Dakota Jewel (overall incidence >15%).

Differences in PMTV-induced tuber necrosis incidence among the russet-skinned selections were not statistically significant but severity differed significantly ($P < 0.0006$) in 2011. Tuber necrosis incidence ranged from zero in cv. Bannock Russet and five selections to 9.6% in ND060742C-1Russ, while severity ranged from zero in cv. Bannock Russet and five selections to 0.39 in ND060742C-1Russ (Table 3.5). PMTV-induced tuber necrosis incidence ($P < 0.0001$) and severity ($P = 0.0005$) differed significantly in 2012. Tuber necrosis incidence ranged from zero in five selections and cv. Bannock Russet to 19% in AND97279-5Russ, while severity ranged from zero in five selections and cv. Bannock Russet to 0.25 in ND060742C-1Russ (Table 3.5). A total of 19 selections had tuber necrosis incidence statistically similar to that of the tolerant cv. Bannock Russet, while AND97279-5Russ had significantly higher incidence than the sensitive cv. Alpine Russet (Table 3.5). Significant correlations were found in tuber necrosis incidence (r = 0.53, $P < 0.05$) and severity (r = 0.91, $P < 0.001$) between the 2011 and 2012 data. Significant correlations were also found between incidence and severity in 2011 (r = 0.70, $P < 0.01$) and in 2012 (r = 0.74, $P < 0.01$).

Table 3.4. *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence (%) and severity index of several advanced breeding selections with red skin-type planted in 2011 and 2012. Tuber necrosis assessments were conducted twice during storage and analyses were performed on the combined data.

Advanced breeding selection [z]	PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity index		Advanced breeding selection [z]	PMTV tuber necrosis incidence (%) [x]		PMTV tuber necrosis severity index [x,y]	
2011					**2012**				
SPA161	29.9	a	0.80	ab	ND060728-5R	30.4	a	0.32	a
ND8314-1R	26.0	ab	1.00	a	SPA161	24.4	ab	0.36	a
ND060728-5R	23.5	abc	0.61	bc	Dakota Jewel	24.3	ab	0.27	ab
R90134-6	14.4	abcd	0.30	def	RC89-25	19.0	bc	0.15	bcd
ND050167C-3R	12.5	abcd	0.36	de	Red LaSoda	12.4	cd	0.05	cdef
AND00272-1R	11.6	bcd	0.23	defg	ATND98459-1RY	10.1	cde	0.14	bcde
R90213-6	8.3	cd	0.32	def	Red Pontiac	7.5	def	0.17	bc
Dakota Jewel	8.3	cd	0.16	efgh	ND8555-8R	4.8	def	0.08	cdef
ND8058-11R	8.3	cd	0.41	cd	RC72-35	4.2	def	0.01	def
RA90213-60	7.5	cd	0.31	def	RA90213-60	3.8	def	0.06	cdef
T10-12	4.6	d	0.02	h	ND8314-1R	3.7	def	0.03	def
ND028842b-1RY	4.3	d	0.21	defgh	R91129-11	3.4	def	0.06	cdef
ND060733b-4RY	4.3	d	0.14	efgh	ND4659-5R	2.2	ef	0.03	def
ND8555-8R	2.5	d	0.05	gh	RA89044-45	1.7	ef	0.02	def
R91129-11	2.1	d	0.02	h	RA20-6	1.5	ef	0.01	ef
Red Pontiac	2.0	d	0.08	fgh	RG47-3	0.9	ef	>0.00*	ef
R90160-5	1.4	d	0.01	h	AND00272-1R	0.8	f	0.01	ef
R90070-8	1.1	d	0.02	h	R90070-8	0.5	f	>0.00*	f
ND4659-5R	0.9	d	0.01	h	R90096-5	0.0	f	0.00	f
Red LaSoda	0.1	d	0.02	h	T10-12	0.0	f	0.00	f
ATND98459-1RY	>0.0*	d	0.02	h					
RC72-35	>0.0*	d	0.01	h					
RA20-6	0.0	d	0.00	h					
RA89044-45	0.0	d	0.00	h					
$LSD_{0.05}$	**16.3**		**0.24**			**9.3**		**0.14**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).

[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).

*Incidence or severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.

[z] Commercial cultivars Dakota Jewel, Red LaSoda and Red Pontiac were included as internal controls.

Table 3.5. *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence (%) and severity index of several advanced breeding selections with russet skin-type planted in 2011 and 2012. Tuber necrosis assessments were conducted twice during storage and analyses were performed on the combined data.

Advanced breeding selection [z]	PMTV tuber necrosis incidence (%)	PMTV tuber necrosis severity index [x,y]		Advanced breeding selection [z]	PMTV tuber [x] necrosis incidence (%)		PMTV tuber necrosis severity index [x,y]	
	2011				**2012**			
ND060742C-1Russ	9.6	0.39	a	AND97279-5Russ	19.0	a	0.20	ab
Alpine Russet	8.9	0.02	b	ND060735-3Russ	13.7	ab	0.08	c
ND6400C-1Russ	7.5	0.10	b	ND060742C-1Russ	12.9	abc	0.25	a
ND049289-1Russ	4.2	0.03	b	ND070927-2Russ	9.0	bcd	0.02	c
ND8229-3	3.4	0.09	b	Alpine Russet	8.6	bcde	0.10	bc
ND050082Cb-2Russ	3.0	0.03	b	ND050105C-1Russ	8.6	bcde	0.02	c
ND050105C-1Russ	2.8	0.06	b	ND8413-7Russ	6.6	bcdef	0.03	c
ND059769Ab-1Russ	2.4	0.09	b	AND99362B-1Russ	6.5	bcdef	0.11	bc
ND049423b-1Russ	1.1	0.01	b	ND049381C-2Russ	6.4	bcdef	0.10	bc
ND8068-5Russ	0.0	0.00	b	Ranger Russet	5.8	cdef	0.05	c
Ranger Russet	0.0	0.00	b	ND060796AB-1Russ	5.8	cdef	0.05	c
ND060796AB-1Russ	0.0	0.00	b	ND039194AB-1Russ	5.1	def	0.02	c
AND01804-3Russ	0.0	0.00	b	ND049517B-1Russ	4.3	def	0.03	c
ND049546b-10Russ	0.0	0.00	b	ND049289-1Russ	3.0	def	0.00	c
ND060766b-4Russ	0.0	0.00	b	ND050082Cb-2Russ	2.8	def	0.00	c
ND8413-7Russ	0.0	0.00	b	ND049423b-1Russ	1.5	ef	0.00	c
Bannock Russet	0.0	0.00	b	ND8068-5Russ	1.5	ef	0.01	c
				AOND95292-3Russ	0.6	f	>0.00*	c
				ND060761B-3Russ	0.6	f	>0.00*	c
				AND01804-3Russ	0.5	f	>0.00*	c
				Bannock Russet	0.0	f	0.00	c
				AND00618-1RussY	0.0	f	0.00	c
				ND060766b-4Russ	0.0	f	0.00	c
				ND6400C-1Russ	0.0	f	0.00	c
				ND060770B-5Russ	0.0	f	0.00	c
				ND049546B-10Russ	0.0	f	0.00	c
$LSD_{0.05}$	**NS**	**0.15**			**7.3**		**0.11**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).

[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).

*Incidence or severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.

[z] Commercial cultivars Alpine Russet, Bannock Russet and Ranger Russet were included as internal controls.

Based on the two-year PMTV tuber necrosis incidence data, the russet-skinned selections and internal control cultivars can be classified as follows – tolerant: ND060766b-4Russ, ND049546b-10Russ, AND01804-3Russ, ND8068-5Russ, ND049423b-1Russ and Bannock Russet (overall incidence <5%); moderately tolerant: ND050082Cb-2Russ, ND060796AB-1Russ, ND8413-7Russ, ND049289-1Russ, ND6400C-1Russ and Ranger Russet (overall incidence >5% to 10%); moderately sensitive: ND050105C-1Russ (overall incidence >10% to 15%) and sensitive: ND060742C-1Russ and Alpine Russet (overall incidence >15%).

Differences in PMTV-induced tuber necrosis incidence among white-skinned selections were not statistically significant but severity differed significantly ($P < 0.0001$) in 2011. Tuber necrosis incidence ranged from zero in selection ND8331Cb-3 to 23.2% in ND060601CAB-2, while severity ranged from zero in ND8331Cb-3 to 0.64 in ND060601CAB-2 (Table 3.6). In 2012, both incidence and severity differed significantly ($P < 0.0001$) among the selections. Tuber necrosis incidence ranged from 0.5% in selection RA 151-24 to 18.8% in cv. Lamoka, while severity ranged from 0.01 in selection ND8307C-3 to 0.5 in selection ND7550C-1 (Table 3.6). None of the selections had significantly lower tuber necrosis incidence or severity than the tolerant cv. Ivory Crisp. There was no correlation in PMTV tuber necrosis incidence data between 2011 and 2012 ($r = 0.55$, $P < 0.10$). However, the tuber necrosis severity data in 2011 were significantly ($r = 0.62$, $P < 0.05$) correlated with those of 2012. A significant correlation was also found between incidence and severity in 2011 ($r = 0.85$, $P > 0.01$) and in 2012 ($r = 0.66$, $P < 0.02$). Based on the two-year PMTV tuber necrosis incidence data, the white-skinned selections and internal control cultivars can be classified as follows – tolerant: ND8331Cb-3, ND8331Cb-2, ND7519-1, ND8559-20, ND8307C-3 and Ivory Crisp (overall incidence <5%); moderately tolerant: RA151-24 (overall incidence >5% to 10%); moderately sensitive:

ND060835C-4, ND6956b-13 and Kennebec (overall incidence >10% to 15%) and sensitive: ND7550C-1, ND8304-2 and Lamoka (overall incidence >15%).

Table 3.6. *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence (%) and severity index of several advanced breeding selections with white skin-type planted in 2011 and 2012. Tuber necrosis assessments were conducted twice during storage and analyses were performed on the combined data.

Advanced breeding selection [z]	PMTV tuber necrosis incidence (%)	PMTV tuber [x,y] necrosis severity index		Advanced breeding selection [z]	PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity index	
	2011				2012			
ND060601CAB-2	23.2	0.64	a	Lamoka	18.8	a	0.49	a
ND060715B-15	14.7	0.19	bcd	ND8304-2	17.7	ab	0.13	b
ND8304-2	14.1	0.20	bcd	ND7550C-1	11.7	ab	0.50	a
ND7550C-1	11.9	0.28	b	Kennebec	10.8	bc	0.03	b
ND060847CB-1	11.8	0.12	bcd	ND8229-3	10.0	cd	0.16	b
MSL-292A	8.4	0.16	bcd	ND6956b-13	8.2	cde	0.05	b
RA151-24	5.4	0.05	cd	ND060835C-4	5.3	cdef	0.17	b
ND060835C-4	5.4	0.19	bcd	ND8305-1	3.2	def	0.03	b
ND6956b-13	5.2	0.02	d	ND8307C-3	2.5	ef	0.01	b
CO95051-7W	4.2	0.05	cd	ND8331Cb-2	1.9	ef	0.02	b
Kennebec	3.3	0.01	d	ND8559-20	1.8	ef	0.02	b
R65A-70	2.9	0.26	bc	Ivory Crisp	1.4	ef	0.05	b
W2717-5	2.7	0.03	cd	ND8331Cb-3	1.0	ef	0.08	b
ND7519-1	2.4	0.04	cd	ND7519-1	1.0	ef	0.09	b
ND8307C-3	2.2	0.03	cd	RA151-24	0.5	f	0.02	b
ND8559-20	2.2	0.01	d					
NY-138	1.7	>0.00*	d					
Lamoka	1.0	0.05	bcd					
NY-139	>0.0*	0.01	d					
Ivory Crisp	>0.0*	>0.00*	d					
ND8331Cb-2	>0.0*	>0.00*	d					
ND8331Cb-3	0.0	0.00	d					
$LSD_{0.05}$	**NS**	**0.23**			**7.5**		**0.17**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).

[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).

*Severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.

[z] Commercial cultivars Ivory Crisp, Lamoka and Kennebec were included as internal controls.

Differences in PMTV-induced tuber necrosis incidence among yellow-skinned selections were not statistically significant but severity differed significantly ($P < 0.0088$) in 2011. Tuber necrosis incidence ranged from zero in three selections to 9.7% in selection RA82-4, while severity ranged from zero to 0.32 in RA82-4 (Table 3.7). In 2012, incidence ($P < 0.0002$) and severity ($P < 0.0389$) differed significantly among yellow-skinned selections. Tuber necrosis incidence ranged from 1.7% in RK24-48 to 15.7% in cv. Yagana (Table 3.7). None of the selections had significantly lower tuber necrosis incidence or severity than cv. the tolerant cv. Yukon Gold or higher incidence/severity than the sensitive cv. Yagana. There was no correlation in the tuber necrosis incidence or severity data between 2011 and 2012. Significant correlation was found between tuber necrosis incidence and severity in 2011 ($r = 0.87$, $P < 0.05$). Based on the two-year PMTV tuber necrosis incidence data, the yellow-skinned selections and internal controls can be classified as follows – tolerant: R91007-5 and Yukon Gold (overall incidence <5%); moderately tolerant: RC06-109 (overall incidence >5% to 10%); moderately sensitive: R87009-28 and RA517-123 (overall incidence >10% to 15%) and sensitive: Yagana (overall incidence >15%) (Table 3.8).

Table 3.7. *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence (%) and severity index of several advanced breeding selections with yellow skin-type planted in 2011 and 2012. Tuber necrosis assessments were conducted twice during storage and analyses were performed on the combined data.

Advanced breeding selection [z]	PMTV tuber necrosis incidence (%)	PMTV tuber [x,y] necrosis severity index		Advanced breeding selection [z]	PMTV tuber [x] necrosis incidence (%)		PMTV tuber [x,y] necrosis severity index	
2011				**2012**				
RA82-4	9.7	0.32	a	Yagana	15.7	a	0.30	a
RA362-54	5.4	0.07	b	RA517-123	12.4	ab	0.04	bc
Yagana	5.1	0.11	b	R87009-28	11.9	ab	0.23	abc
R87009-28	3.0	0.11	b	RC06-109	7.2	bc	0.24	abc
RA519-50	1.3	0.02	b	Yukon Gold	2.1	c	>0.00*	c
Yukon Gold	1.5	0.01	b	R91007-5	2.0	c	0.01	c
RA517-123	1.0	0.05	b	RK24-48	1.7	c	0.03	bc
R91007-5	0.9	0.03	b					
R89045-35	>0.0*	0.02	b					
RA148-48	0.0	0.00	b					
RA16-5	0.0	0.00	b					
RC06-109	0.0	0.00	b					
$LSD_{0.05}$	**NS**	**0.18**			**6.8**		**0.23**	

[x] Means with the same letter are not significantly different based on Fisher's protected LSD (P = 0·05).
[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).
*Severity of PMTV-induced tuber necrosis is not a true zero as there were a few tubers with internal necrosis.
[z] Commercial cultivars Yagana and Yukon Gold were included as internal controls.

The overall correlations in tuber necrosis incidence (r = 0.55, $P < 0.001$) and severity (r = 0.41, $P < 0.01$) data across skin-types between 2011 and 2012 were significant. Significant interactions were found in PMTV-induced tuber necrosis incidence between selection and period of evaluation ($P < 0.0001$) as well as between skin-color and evaluation period ($P < 0.0001$) in 2011. Across skin-type, the increase in incidence during storage was higher in the red- and white-skinned selections than the yellow- and russet-skinned types (Fig. 3.1). Generally, incidence was higher in the second evaluation than the first and selections such as SPA161 (red-skinned), ND8314-1 (red-skinned), ND060728-5R (red-skinned) and ND060601CAB-2 (white-skinned) had the highest increase (Figs. 3.2 – 3.5). The severity of PMTV tuber necrosis did not

change during storage. In 2012, changes in PMTV tuber necrosis incidence and severity during storage were not significant.

Table 3.8. Summary of sensitivity rankings of advanced breeding selections based on *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence values summed for 2011 and 2012.

Skin color [z]	**Tolerant selections** (Overall incidence <5%)	**Moderately tolerant selections** (Overall incidence >5% to 10%)	**Moderately sensitive selections** (Overall incidence >10% to 15%)	**Sensitive selections** (Overall incidence >15%)
Red	RC72-35 RA20-6 RA89044-45 ND4659-5R R90070-8 T10-12	R91129-11 ND8555-8R Red Pontiac	ATND98459-1RY RA90213-60 AND00272-1R Red LaSoda	SPA161 ND8314-1R ND060728-5R Dakota Jewel
Russet	ND060766b-4Russ ND049546b-10Russ AND01804-3Russ ND8068-5Russ ND049423b-1Russ Bannock Russet	ND050082Cb-2Russ ND060796AB-1Russ ND8413-7Russ ND049289-1Russ ND6400C-1Russ Ranger Russet	ND050105C-1Russ	ND060742C-1Russ Alpine Russet
White	ND8331Cb-3 ND8331Cb-2 ND7519-1 ND8559-20 ND8307C-3 Ivory Crisp	RA151-24	ND060835C-4 ND6956b-13 Kennebec	ND7550C-1 ND8304-2 Lamoka
Yellow	R91007-5 Yukon Gold	RC06-109	R87009-28 RA517-123	Yagana

[z] Commercial cultivars Dakota Jewel, Red Pontiac, Red LaSoda, Kennebec, Ivory Crisp, Lamoka, Bannock Russet, Ranger Russet, Alpine Russet, Yagana and Yukon Gold are internal controls.

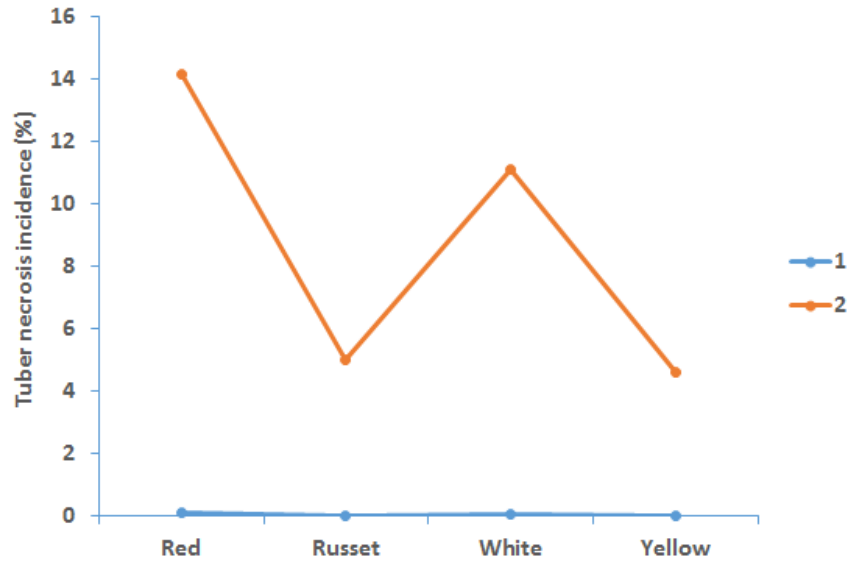

Fig. 3.1. PMTV-induced tuber necrosis incidence at two evaluation periods among red-, russet-, white- and yellow-skinned advanced breeding selections in 2011. Evaluations were performed at 70 and 136 days post-harvest. Tubers were stored at 8-10 °C.

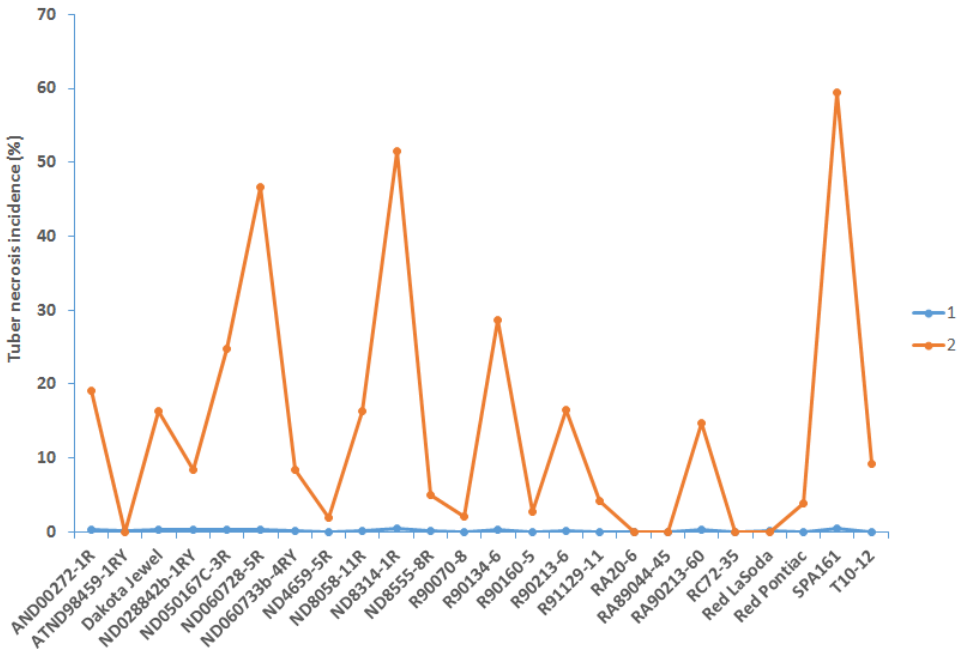

Fig. 3.2. PMTV tuber necrosis incidence among red-skinned selections at two evaluation periods in 2011. Evaluations were performed at 70 and 136 days post-harvest. Tubers were stored at 8-10 °C.

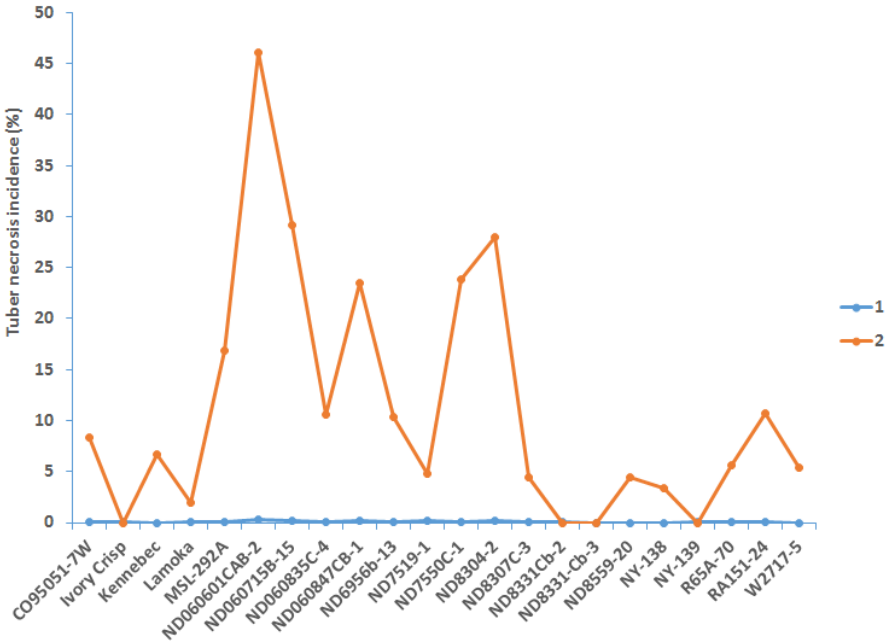

Fig. 3.3. PMTV tuber necrosis incidence among white-skinned selections at two evaluation periods in 2011. Evaluations were performed at 70 and 136 days post-harvest. Tubers were stored at 8-10 °C.

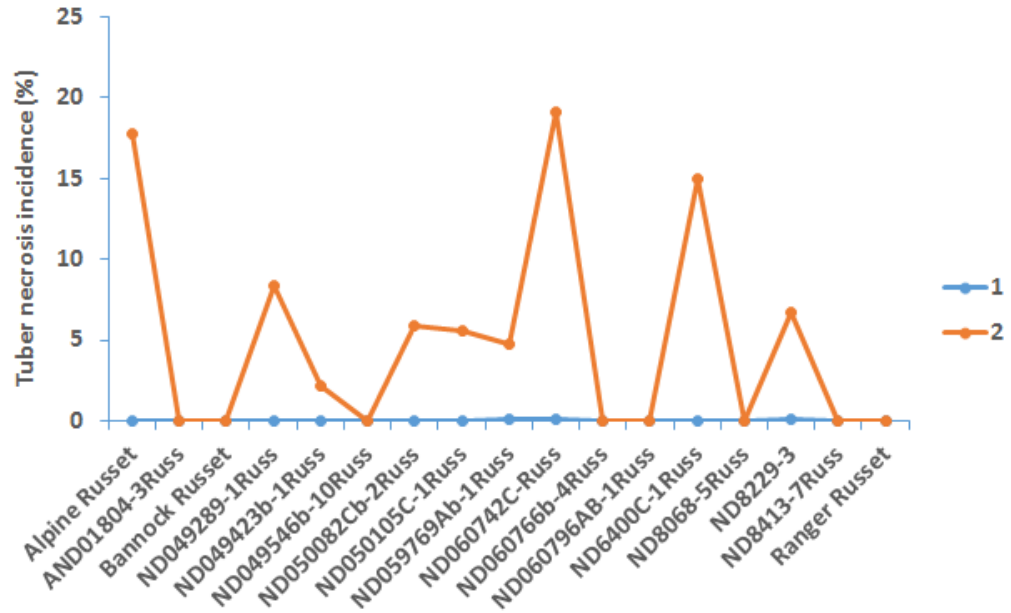

Fig. 3.4. PMTV tuber necrosis incidence among russet-skinned selections at two evaluation periods in 2011. Evaluations were performed at 70 and 136 days post-harvest. Tubers were stored at 8-10 °C.

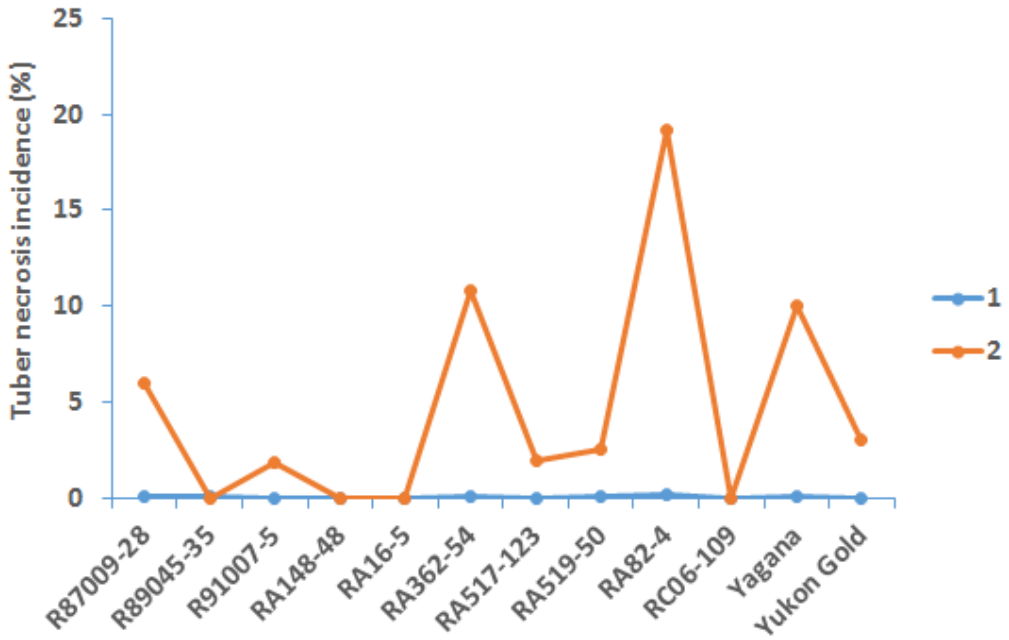

Fig. 3.5. PMTV tuber necrosis incidence among yellow-skinned selections at two evaluation periods in 2011. Evaluations were performed at 70 and 136 days post-harvest. Tubers were stored at 8-10 °C.

Discussion

This is the first study conducted in the Americas on the reaction of advanced potato selections to PMTV-induced tuber necrosis. This study demonstrated the existence of natural variability among advanced potato breeding selections in their reaction to PMTV-induced tuber necrosis. The advanced breeding materials were selected from a broad genetic base across North and South America, representing different market classes. This information may offer assistance to potato growers who encounter PMTV by identifying genetic material within a particular market type that is tolerant to the virus. The use of potato genotypes tolerant to PMTV that do not express the tuber necrosis phase of the virus can reduce the economic impact of the disease if and when the selections are released as commercial cultivars.

Russet-skinned selections had lower PMTV tuber necrosis incidence compared to the white-, red- and yellow-skinned types, which is in agreement with a previous study (Domfeh *et*

al., 2015). It is worth noting that none of the tubers of two russet-skinned selections, ND060766b-4Russ and ND049546b-10Russ had tuber necrosis over two seasons. High levels of tolerance to PMTV tuber necrosis in russet-skinned cultivars has been reported (Domfeh *et al*., 2015). Russet-skinned potato selections have been found to be resistant to the tuber phase of powdery scab (Miller, 2001; Nitzan *et al*., 2008; Perla *et al*., 2014). This could, in part, explain the tolerance to PMTV tuber necrosis, assuming some degree of correlation between powdery scab incidence on tubers and PMTV-induced tuber necrosis incidence existed in this study as reported previously (Davey *et al*., 2014; Domfeh *et al*., 2015). High physiological levels of the storage protein lipoxygenase (LOX; EC 1.13.11.12) found in russet-skinned tubers has been linked with resistance to powdery scab (Perla *et al*., 2014). Mechanisms such as accumulation of phytoalexins, hypersensitive response-mediated cell death and accumulation of suberin in the periderm of the tubers are believed to be activated at higher physiological levels of LOX protein (Perla *et al*., 2014). In contrast, tubers of two red-skinned selections, SPA 161 and ND060728-5R were the most sensitive to tuber necrosis in both years of the study. The high level of sensitivity to tuber necrosis among some red-skinned cultivars is also supported by results of a previous study (Domfeh *et al*., 2015).

In the study reported here, tubers were evaluated for symptom expression (disease response of plants), with those which reacted severely described as "sensitive" while those with little or no apparent effect are considered "tolerant" (Cooper and Jones, 1983). PMTV was readily confirmed by RT-PCR in randomly selected tubers but none were positive for TRV. The tuber necrosis symptoms observed (conspicuous brown-colored arcs, rings and lines) were, thus, likely caused by PMTV. In some cultivars (e.g. Cara and Saturna), a good correlation has been found between PMTV infection and the occurrence of tuber necrosis (Davey, 2009), indicating

that tuber necrosis may be a good indicator of PMTV infection (Cooper and Harrison, 1973; Jones and Harrison, 1972; Kurppa, 1989). In future studies, it would be worthwhile to determine if some of the selections tested here that did not show tuber necrosis are resistant to the virus or only tolerant to necrosis development.

Transmission of PMTV to potato plants is dependent on the successful infection of the Sss vector, which is heavily influenced by environmental conditions (Cooper and Harrison, 1973; Harrison, 1974; Sandgren *et al*., 2002; Sokemen *et al*., 1998). Relatively cool soil temperatures (12-15°C) and high soil moisture enhance infection with Sss by favoring germination and movement of zoospores (Merz, 2008; Sandgren *et al.,* 2002). The differences in environmental conditions between the two study years may have influenced the results of these trials. Significant differences in PMTV tuber necrosis incidence were detected only among the red-skinned selections in 2011, yet in 2012, significant differences were found among selections of all skin-types. In 2011, the average air and soil temperatures of the experimental site during the growing season were 18°C and 19°C, respectively. The total amount of moisture applied to the crop via rainfall and irrigation during the growing season totaled 450.9 mm. In 2012, the average air and soil temperatures were 15.6°C and 17°C, respectively and a total of 780.6 mm of sprinkler irrigation and rainfall was applied to the crop. The lower moisture level and higher average temperatures in 2011 may have contributed to the overall lower tuber necrosis incidence observed. The lower overall incidence in 2011 made it difficult to detect statistically significant differences except in the more sensitive red-skinned selections. The lower temperatures and higher moisture levels in 2012 gave higher tuber necrosis incidence levels which enabled the detection of significant differences among all skin-types. The inability to detect statistical differences in tuber necrosis incidence data among the white-skinned selections in 2011 could

partly be attributed to higher variability among replications. This explains why the mean tuber necrosis difference of 23 between white-skinned selection ND060601CAB-2 and ND8331Cb-3 is not statistically significant.

The overall significant correlation observed between the 2011 and 2012 PMTV tuber necrosis incidence and severity across skin-types indicate the reliability and reproducibility of the results and further demonstrate that field trials can be used to screen potato germplasm for their reaction to PMTV infection under North American conditions. Increase in PMTV tuber necrosis incidence during storage has been reported (Harrison and Jones, 1971; Kurppa, 1989; Molgaard and Nielsen, 1996; Nielsen and Engsbro, 1992; Ryden *et al*., 1989; Sandgren, 1995; Sandgren *et al*., 2002). Our results show that PMTV tuber necrosis incidence was significantly higher in the second evaluation than the first in 2011 and this occurred mainly in the tubers of sensitive red- and white-skinned selections. This evidence suggests that PMTV tuber necrosis incidence increased more in sensitive selections during storage and this is consistent with previously published reports (Domfeh *et al*., 2015; Harrison and Jones, 1971). The results also imply that conducting tuber necrosis assessment at harvest may underestimate the incidence of the disease.

In this study, we have demonstrated the existence of natural variability among advanced potato breeding selections in their reaction to PMTV-induced tuber necrosis. A total of 17 advanced breeding selections made up of six red-, five russet-, five white and one yellow-skinned types have been found to be tolerant to PMTV tuber necrosis. This information provides potential assistance to potato growers in areas where PMTV causes significant economic losses. In the short term, tolerant selections which have other desirable agronomic characteristics could be released as commercial cultivars for growers to plant. In the long term, tolerant selections

which combine absence of tuber necrosis with little or no accumulation of PMTV can be utilized in breeding programs to introduce resistance into commercial cultivars. The screening of potato germplasm for reaction to PMTV infection should be widened to include more genetically diverse material such as wild *Solanum* species.

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CHAPTER FOUR. MOISTURE MANAGEMENT AS A POTENTIAL DISEASE CONTROL STRATEGY FOR *POTATO MOP-TOP VIRUS*-INDUCED TUBER NECROSIS

Abstract

Potato mop-top virus (PMTV) is transmitted by *Spongospora subterranea* f.sp. *subterranea* (Sss), the causal agent of powdery scab in potato and has become one of the most important tuber necrosis viruses in the United States. The virus has been confirmed in six major potato-producing states in the USA since its identification in 2003. Currently, no control methods are available for PMTV or its vector. A growth chamber experiment was conducted to investigate the potential of using moisture regime adjustments to manage tuber necrosis caused by PMTV. Two commercial potato cultivars with varying levels of sensitivity to PMTV, Dakota Crisp and Ivory Crisp were grown in soil obtained from a PMTV infested field. Over the course of the plant growth cycle, plants of each cultivar were subjected to moisture regimes of wet throughout (WT), wet early/dry late (WEDL), dry early/wet late (DEWL) and dry throughout (DT). Soil moisture levels of 90 and 60% field capacity were considered wet and dry respectively, while early and late refer to first and last 50 days after planting, respectively. Results of visual assessment conducted three months after storage showed significant differences in root gall formation, powdery scab on tubers, and PMTV tuber necrosis among moisture regimes. Powdery scab incidence was significantly higher in the WT and DEWL regimes than WEDL and DT regimes. PMTV tuber necrosis incidence did not differ between the WT and DEWL or between DEWL and WEDL moisture regimes. PMTV tuber necrosis incidence was however, significantly higher in WT than WEDL and DT regimes, the latter being significantly lower than the other three regimes. PMTV tuber necrosis severity was significantly higher in the

WT than the other regimes which did not differ statistically among themselves. A significant interaction was found between cultivar and moisture regime on root gall formation, with the highest number of galls found on Ivory Crisp grown in the WT moisture regime. A significant correlation was found between powdery scab incidence on tubers and PMTV-induced tuber necrosis incidence. The results of this study demonstrate the potential of using irrigation management during the growing season to reduce the likelihood of powdery scab infection and PMTV-induced tuber necrosis development in potato.

Introduction

Potato mop-top virus (PMTV), classified as the type member of the genus Pomovirus (Pringle, 1999; Torrance and Mayo, 1997), causes necrotic symptoms in sensitive potato cultivars (Domfeh *et al*., 2015; Harrison and Jones, 1970; Latvala-Kilby *et al.*, 2009; Nielsen and Molgaard, 1997; Sandgren *et al*., 2002; Santala *et al*., 2010). Symptoms of PMTV include internal rust colored arcs, rings and lines (Calvert and Harrison, 1966; Harrison and Jones, 1970; Kurppa, 1989) which are sometimes visible superficially (Jefferies, 1998). PMTV can also induce cracking of tubers and a wide range of foliar symptoms including yellow blotches or rings, mottling and stunting in potato plants (Calvert and Harrison, 1966; Harrison and Jones, 1970; Kurppa, 1989).

PMTV is transmitted by the zoospores of the soil-borne biotrophic protozoan pathogen, *Spongospora subterranea* f.sp. *subterranea* (Sss) (Arif *et al*., 1995, Jones and Harrison, 1969) which also causes powdery scab in potato. Powdery scab is characterized by cankers and scabs on tubers (Hims and Preece, 1975) and make tubers appear unsightly, resulting in extensive economic losses (Wale, 2000). In addition, infected root systems may develop galls containing cystosori which can survive in the soil for many years (Jones and Harrison, 1969, 1972;

Andersen *et al*., 2002). PMTV resides in the cystosori and can also persist for several years (Calvert, 1968; Jones and Harrison, 1972), making it very difficult, if not impossible, to eliminate it from an infested field. Field to field spread of PMTV occurs through planting infected seed tubers, movement of infested soil and seed tubers or machinery with adhered viruliferous cystosori (Sandgren *et al*., 2002).

Cool temperature and high soil moisture favor Sss infection by providing favorable conditions for release and motility of zoospores (Jones and Harrison, 1969; Sandgren *et al*., 2002, Wale, 2000). Powdery scab infection is, consequently, most prevalent in countries with cool, wet climates. In some warmer countries, Sss and PMTV infection has been reported, mainly from cool areas with high altitude (Montero-Astua *et al*., 2008; Salazar and Jones, 1975). The upsurge in powdery scab outbreaks in the potato-growing industry in recent years has been linked with use of popular but susceptible cultivars, increased use of irrigation and the lack of an effective seed tuber inspection scheme (Harrison *et al.*, 1997; Taylor *et al*., 1986; Taylor and Fret, 1981; Wale, 2000; Qu *et al*., 2006). Environmental factors that favor PMTV infection are generally considered to be those required for Sss infection in potato. In Scotland, it has been reported that the occurrence of PMTV follows rainfall and when the annual precipitation falls below 760 mm, there is practically no PMTV infection (Cooper and Harrison, 1973). This is not true in all cases, because in Sweden, PMTV occurs in the eastern part of the potato growing area where precipitation is below 760 mm (Sandgren, 1995). In another study in Denmark, the incidence of PMTV tuber necrosis was shown to correlate best with precipitation at the period when stolon formation and tuber set occurs and when tubers are infected by Sss (Jones, 1988).

Conditions that replicate the cool, wet soils reported to favor development of powdery scab occur after irrigation (de Boer *et al*., 1985) and this may have consequences for PMTV

infection. It has been widely reported that the period at and shortly after tuber set is the most susceptible period for Sss development (Taylor and Fret, 1981; Taylor *et al*., 1986). Many authors have therefore suggested that powdery scab incidence and severity can be substantially reduced by delaying irrigation until tuber set or several weeks post tuber set (Taylor and Flett, 1981; de Boer *et al*., 1985; Adams *et al*., 1987; Burnett, 1991). Other reports on the other hand, suggest that wet conditions later in the growing season may still result in Sss infection (Forsund, 1971; Hims, 1976a, 1976b; Adams, 1975; Parker, 1984; Christ and Weidner, 1988).

The effect of different moisture regimes on powdery scab development has been extensively studied in controlled experiments, however, no such studies have been conducted to determine the influence of moisture regimes on PMTV tuber necrosis development. The objective of this study was to investigate how soil moisture regimes affect the development of PMTV-induced tuber necrosis. This was accomplished through a growth chamber experiment using Sss and PMTV infested field soil.

Materials and methods

Determination of soil properties

Soil was obtained from a field at Larimore, Grand Forks County of North Dakota, known to be infested with PMTV and Sss. The soil (sandy loam) was air-dried for three weeks and sieved to remove large lumps and plant debris thereafter. The field capacity of the soil was determined using two samples weighing 250g each (Cassel and Nielsen, 1999). The gravimetric water content of the soil was determined by weighing a sample of the soil, followed by oven drying at 105°C for 48 hours and re-weighing (Reynolds, 1970). The gravimetric water content of the air-dried soil was then determined by the equation:

$$\theta = \frac{\left(\text{Soil mass (wet)} - \text{Soil mass (dry)}\right)}{\text{Soil mass (dry)}}$$

The same quantity of soil (2,650 g) was used in each pot and the amount of water required to bring this soil to 60 or 90% field capacity was determined by exploring the relationship between density of water, gravimetric water content and field capacity.

Growth chamber experiment

The treatments consisted of four moisture regimes implemented over the course of the plant growing cycle. Each treatment was replicated eight times in two potato cultivars arranged in a completely randomized design with one plant per replication. The experiment was performed twice. The moisture regimes were: wet early/dry late (WEDL); dry early/wet late (DEWL); wet throughout (WT) and dry throughout (DT). Dry treatments were maintained at 60% field capacity while wet treatments were kept at 90% field capacity moisture levels. The WEDL treatment received water at 90% field capacity up to 50 days post-planting and 60% field capacity for the remaining 50 days. The DEWL treatment was kept at 60% field capacity for the first 50 days post-planting and at 90% field capacity for a further 50 days. The WT and DT treatments were kept at 90% and 60% field capacity, respectively, throughout the duration of the experiment. Potato cultivars Ivory Crisp and Dakota Crisp, tolerant to and sensitive to PMTV-induced tuber necrosis, respectively (Domfeh *et al.*, 2015) were subjected to each moisture regime.

Potato seed tubers were planted in pots filled with 1,325 g of soil (half of 2,650g) and watered with 156 ml or 253 ml of water (i.e., half of 312 or 505 ml) predetermined to bring moisture level to 60 or 90% field capacity, respectively. Following this, the remaining amount of

soil (1,325g) was added and watered with the remaining half of water to bring moisture level to 60 or 90% field capacity. This was done to ensure that water got to the seed tubers to facilitate germination, which was necessary, particularly, in the case of dry treatments. The potato seed tubers were planted in plastic azalea pots which were 22 cm wide around the top and 17 cm deep (Fig. 4.1 A).

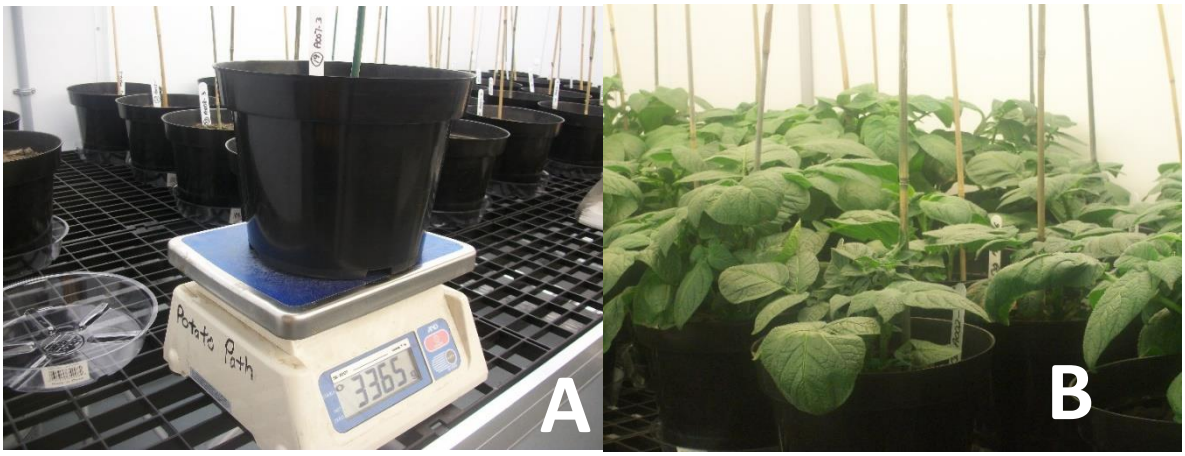

Fig. 4.1. A pot being weighed after planting (A) and potato seedlings at 6 weeks old growing in the growth chamber (B).

Water was applied twice daily throughout the experiment. During the first three weeks, each pot was weighed and the amount of water lost was found by subtracting the current weight from the initial weight (taken at planting). Water was then added accordingly to bring the moisture levels to 60 or 90% field capacity. Subsequently, determination of the amount of water to apply was done with a moisture sensor (HydroSense, Campbell Scientific, Australia) (Fig. 4.2) since weight measurements were no longer appropriate as the potato seed pieces began to germinate and gain weight. The two probes of the moisture sensor (12 cm long and spaced 3.2 cm apart) were carefully inserted into at least three different locations in each pot and the average percent volumetric water content was calculated. This reading was then subtracted from

the initial reading (percent volumetric water content taken at planting) and the amount of water required was calculated through proportions.

Throughout the experiment, day and night temperatures were maintained at 16°C (16 hours) and 12°C (8 hours), respectively. Granules of Osmocote® Plus 15-9-12 (3-4 months), applied at the rate of 5g per pot provided fertilization.

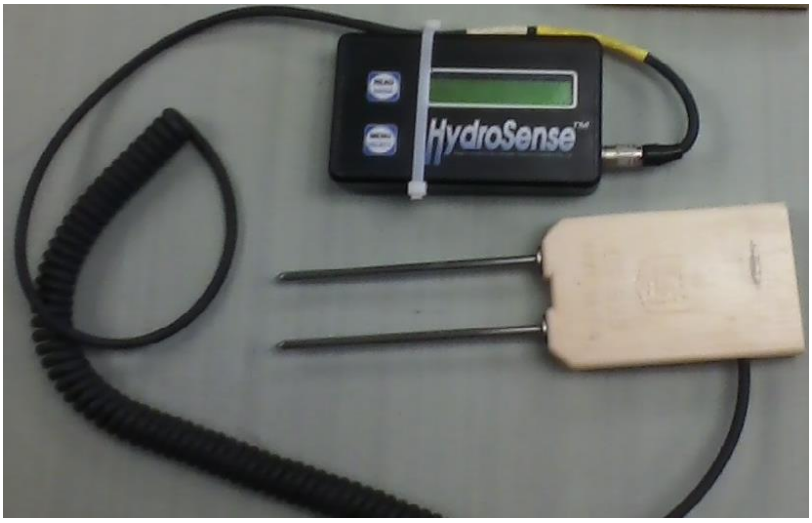

Fig. 4.2. HydroSense moisture sensor for measuring volumetric water content.

Root gall evaluation

All plants were carefully removed from soil at the end of the experiment (100 days after planting). Roots were gently shaken and washed under gently running tap water to release attached soil. Galls were evaluated under a magnifying glass. Galls on roots were counted and expressed as number of galls per plant (Hernandez Maldonado *et al*., 2013).

Post-harvest tuber evaluation

After harvest, tubers were cured at a temperature of 10°C for three weeks and stored at 8 - 10°C thereafter. The tubers were evaluated three months after harvesting for powdery scab infection on tubers and PMTV-induced tuber necrosis. The total number of tubers examined was

416 in the first trial and 546 in the second. PMTV incidence and severity index were determined using previously published protocols (Nielsen and Molgaard, 1997). Washed tubers were cut lengthwise into 1cm thick slices with a SafeHands Professional Mandolin slicer (Jaccard Corporation, NY). PMTV incidence was calculated as the number of tubers showing symptoms of PMTV-induced tuber necrosis per the total number of tubers examined for each sample. The number of slices per tuber with internal necrosis was determined (a). The tuber slice with the most severe internal necrosis was covered with a clear transparency with 1 cm wide vertical and horizontal strips. The number of squares with necrosis was recorded (b). An index of PMTV severity was calculated by multiplying the two measurements (a * b) and expressing the values between 0 and 1, where 0 indicates no necrosis and 1 indicates the presence of necrosis throughout the tuber. Powdery scab on tubers was visually assessed and severity scored by comparing the area of tuber covered by disease with a modified graphic scale (Fallon *et al*., 1995). An average percentage of the sample disease severity was calculated. Symptoms were confirmed by observation of cystosori under the microscope (400x) when required. Incidence was obtained by calculating the percentage of symptomatic tubers from the total number of tubers in the sample.

Confirmation of PMTV by RT-PCR

Necrotic tissues were taken from slices of potato tubers with a sharp scalpel sterilized in 75% ethanol and flamed until red-hot between samples. The tissues were crushed in liquid nitrogen and stored at -80 ºC until used for RNA extraction. Total RNA was extracted using TRIzol® reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions, with the exception that 0.8 ml was added to each tube for tissue homogenization instead of 1 ml. The RNA pellets were air-dried for 5-10 minutes and thereafter, the pellets were

dissolved in 100 µl of RNase-free water. Detection of PMTV in tubers was done by reverse transcription-PCR according to a previously published protocol (Nakayama *et al*., 2010) with the only modification being the use of 0.2 µl of random primers (500µg/ml) and 3.3 µl of RNase free water instead of 1 µl and 2.5 µl respectively. A total of 51 and 10 symptomatic tubers of Dakota Crisp and Ivory Crisp, respectively, were tested for the presence of PMTV in the first trial. For the second trial, 35 and 13 symptomatic tubers of Dakota Crisp and Ivory Crisp, respectively were tested.

Statistical analysis

Statistical analyses of the experimental data were carried out using the Statistical Analysis Software (SAS) version 9.3. Combined ANOVA analyses were carried out for the two experiments as variance homogeneity existed in all parameters investigated (Millikin and Johnson, 1992). Residual plots of the data sets revealed that ANOVA could be performed without prior transformation as major assumptions were satisfied. Treatments were compared using the Fisher's Protected Least Significance Difference (LSD) test at $P \leq 0.05$. The Pearson's correlation coefficient was calculated on moisture regimes summed across cultivar levels for comparisons (n = 8, 6 df).

Results

PMTV was readily confirmed in symptomatic tubers of both cultivars by RT-PCR. The data for trials one and two were combined for all parameters before analysis and the results are presented accordingly.

Sss root gall formation

A significant interaction ($P < 0.0007$) was found between moisture regime and cultivar in Sss gall formation on roots of plants. There was no significant difference among the four moisture regimes in cv. Dakota Crisp (Fig. 4.3). However, in cv. Ivory Crisp, the number of root galls formed in the WT regime was significantly higher than those formed in the rest of the moisture regimes (Fig. 4.3). There was no significant difference in root gall formation between WEDL and DEWL moisture regimes but significantly higher number of galls were formed in the WEDL regime than the DT regime. This indicates that the effect of moisture regime on the formation of root galls was cultivar dependent. The results show that Ivory Crisp is more susceptible to root gall formation than Dakota Crisp and this difference is most evident when soil moisture content is high. There was a very strong positive correlation between root gall data for trial one and trial two ($r = 0.96$, $P < 0.001$).

Powdery scab infection on tubers

The incidence ($P < 0.0012$) and severity ($P < 0.0049$) of powdery scab infection on tubers differed significantly among moisture regimes. The mean incidence of powdery scab lesions on tubers ranged from 15% in the DT moisture regime to 32% in the WT regime (Table 4.1). The mean powdery scab incidence in the DEWL regime was not statistically different from that of the WT regime but both regimes had significantly higher incidence than WEDL and DT regimes. The mean powdery scab severity was highest in DEWL (0.7%) and lowest in the DT moisture regime (0.2%) (Table 4.1). No significant difference was found in incidence or severity of powdery scab between cvs. Ivory Crisp and Dakota Crisp (Table 4.2). A significant ($r = 0.86$; $P < 0.01$) positive correlation was found between powdery scab incidence and severity.

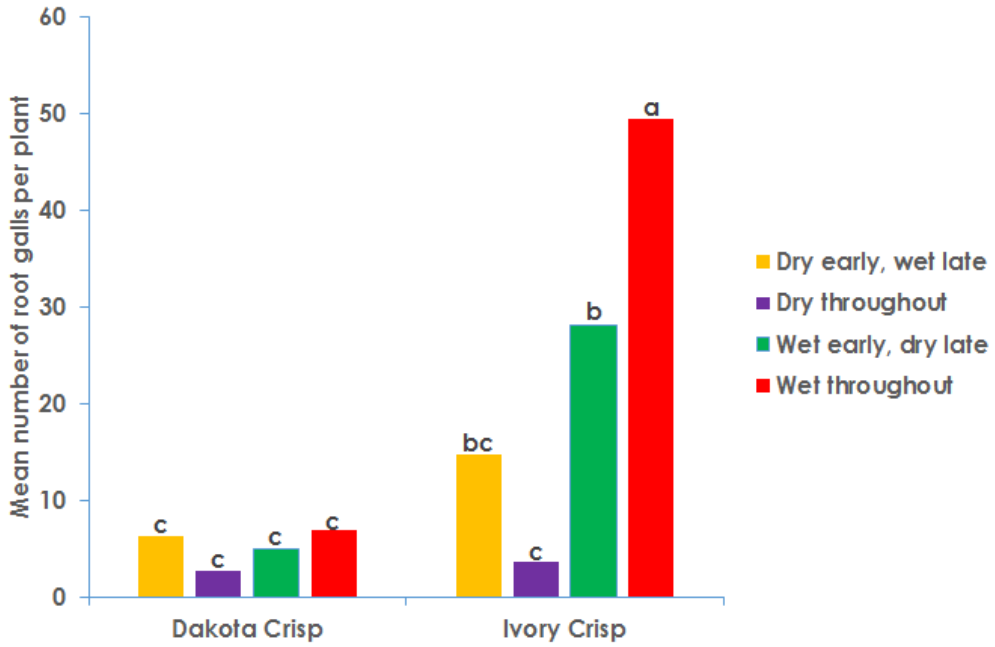

Fig. 4.3. Mean number of *Spongospora subterranea* f.sp. *subterranea* galls on roots across soil moisture regimes in cvs. Dakota Crisp and Ivory Crisp. The data represent the interaction between moisture regime and cultivar on root gall formation based on data combined after two trials. Means with the same letter are not significantly different based on Fisher's protected LSD ($P < 0.05$)

Table 4.1. Mean powdery scab incidence and severity among four moisture regimes across two potato cultivars.

Moisture regime [1]	**Powdery scab incidence (%)**[x]	**Powdery scabseverity (%)**[x]
WT	31.9 a	0.6 ab
DEWL	28.5 a	0.7 a
WEDL	18.6 b	0.4 bc
DT	15.3 b	0.2 c
$LSD_{0.05}$	**9.32**	**0.35**

[1]WT = wet throughout; DEWL = dry early/wet late; WEDL = wet early/dry late; DT = dry throughout.

[x] Means with the same letter are not significantly different based on Fisher's Protected LSD ($P = 0.05$).

Table 4.2. Mean powdery scab incidence and severity in two potato cultivars across all moisture levels.

Cultivars	**Powdery scab incidence (%)**	**Powdery scab severity (%) ***
Dakota Crisp	25.0	0.6
Ivory Crisp	22.2	0.4
$LSD_{0.05}$	**NS**	**NS**

***** NS = no significance.

Potato mop-top virus-induced tuber necrosis

PMTV tuber necrosis incidence ($P < 0.0008$) and severity ($P < 0.0009$) differed significantly among moisture regimes. The range of PMTV tuber necrosis incidence was from 4.1% in DT regime to 18.0% in WT regime (Table 4.3). PMTV tuber necrosis incidence was significantly higher in WT, DEWL and WEDL moisture regimes than that of DT regime. Significantly higher PMTV tuber necrosis incidence was found in the WT regime than WEDL regime but both of these regimes did not differ significantly from the DEWL regime in tuber necrosis incidence. PMTV tuber severity index ranged from 0.04 in DT to 0.15 in WT moisture regime (Table 4.3). PMTV severity index did not differ statistically among the DEWL, WEDL and DT regimes. Cultivars Ivory Crisp and Dakota Crisp differed significantly in tuber necrosis incidence ($P < 0.0001$), but not in severity. A mean PMTV tuber necrosis incidence of 17.0% was found in cv. Dakota Crisp, while Ivory Crisp had 5.8% across all moisture regimes (Table 4.4). A very strong positive correlation was found between PMTV tuber necrosis incidence and severity index ($r = 0.95$; $P < 0.001$). PMTV incidence ($r = 0.74$; $P < 0.05$) and severity ($r = 0.88$; $P < 0.01$) data for the first trial were significantly correlated with those of the second trial.

Table 4.3. Mean PMTV tuber necrosis incidence and severity index among four moisture regimes across two potato cultivars.

Moisture regime[1]	**PMTV tuber necrosis** [x] **incidence (%)**	**PMTV tuber necrosis** [x, y] **severity index**
WT	18.0 a	0.15 a
DEWL	12.6 ab	0.06 b
WEDL	10.9 b	0.08 b
DT	4.1 c	0.04 b
$LSD_{0.05}$	**6.6**	**0.06**

[1] WT = wet throughout; DEWL = dry early/wet late; WEDL = wet early/dry late; DT = dry throughout.
[x] Means with the same letter are not significantly different based on Fisher's Protected LSD (*P* = 0.05).
[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).

Table 4.4. Mean PMTV incidence and severity index of two potato cultivars across all moisture regimes.

Cultivar	**PMTV incidence (%)** [x]	**PMTV severity index *,** [y]
Dakota Crisp	17.0 a	0.14 a
Ivory Crisp	5.8 b	0.03 b
$LSD_{0.05}$	**4.7**	**0.04**

[x] Means with the same letter are not significantly different based on Fisher's Protected LSD (*P* = 0.05).
* NS = no significance
[y] Index is given as a value between 0 and 1, where 0 indicates no tuber necrosis and 1 presence of necrosis through the tuber (Nielsen and Molgaard, 1997).

Relationship between Sss root galls, powdery scab and PMTV tuber necrosis

There was no correlation between root gall formation and powdery scab on tubers. Similarly, no correlation was found between root gall formation and PMTV tuber necrosis. The correlation between PMTV tuber necrosis severity and powdery scab severity on tubers was also not significant ($r = 0.42$, $P < 0.20$). However, a significant correlation was found between powdery scab incidence on tubers and PMTV tuber necrosis incidence ($r = 0.7$, $P < 0.05$).

Discussion

The potential of using soil moisture management as a strategy to mitigate the economic consequences of PMTV- induced tuber necrosis was investigated in this study. To the best of our knowledge, this is the first report establishing the direct relationship between soil moisture regime and the development of PMTV tuber necrosis in a controlled experiment. The only previous reports linking soil moisture content and PMTV tuber necrosis were based on field observations in Europe (Cooper and Harrison, 1973; Carnegie *et al*., 2012; Davey *et al*., 2008; Jones, 1988).

The results of this study show that maintaining soil moisture level at 90% field capacity (wet) throughout the growing season results in high PMTV tuber necrosis incidence, while keeping moisture level at 60% field capacity (dry) leads to low PMTV incidence. The results also indicate that keeping the soil dry early in the growing season and wet later and vice versa may result in a similar PMTV tuber necrosis incidence. However, the WEDL regime resulted in significantly lower tuber necrosis incidence in comparison with the WT regime. In Scotland, observations were made on more than 130 fields of seed potatoes in different parts of that country in 1971 and 1972 to investigate if PMTV tuber necrosis development is influenced by wet cool weather as reported for Sss infection (Cooper and Harrison, 1973). The results showed

that PMTV tuber necrosis incidence increased as potential water deficit decreased, and subsequent correlation analysis revealed that PMTV tuber necrosis was more prevalent as rainfall increased (Cooper and Harrison, 1973). The authors showed that practically no PMTV tuber necrosis occurred when annual precipitation was below 760 mm and added that tuber necrosis incidence may increase significantly with rainfall of over 1140 mm/year, irrespective of soil type. Even though our experimental design differs from that of Cooper and Harrison (1973), results of both studies are similar and imply that high soil moisture levels lead to high incidence of PMTV tuber necrosis incidence. In another study in Denmark, the incidence of PMTV tuber necrosis was shown to correlate best with precipitation at the period when stolon formation and tuber set occurs (Jones, 1988). This implies that PMTV tuber necrosis incidence would be highest when soil is wet in the first half of the season as stolon formation and tuber set mostly occur at this time. Our results suggest that high PMTV tuber necrosis incidence may occur when soil is wet late in the growing season as well and clearly demonstrate that the potato plant may be susceptible to PMTV tuber necrosis throughout the growing season. As multiplication of Sss zoosporangia occur through the season, the build-up of PMTV inoculum is expected, and with new stolons and tubers being formed even towards the end of the growing season, the high incidence in the DEWL regime is not surprising.

Unlike PMTV tuber necrosis, the effect of soil moisture regime on Sss infection has been extensively studied (Adams *et al*, 1987; Burnette, 1991; Diriwächter and Parbery, 1991; Hughes, 1980; Taylor and Fret, 1981; Taylor *et al*., 1986; Nachmias and Krikun, 1988; van der Graaf *et al*., 2005, 2007). In our study, root gall formation was significantly greater in plants grown in soil kept wet throughout the growing season than those subjected to fluctuating wet and dry regimes. A similar outcome was found with the incidence of powdery scab on tubers, however,

the difference between the WT and DEWL regimes was not significant. In a similar study, plants grown in constant dampness had greater tuber and root gall incidence than those subjected to fluctuating regimes of wetness, though the effect on root gall formation was not significant (van der Graaf *et al*., 2007). There are contrasting reports about the importance of constant versus fluctuating soil moisture conditions in powdery scab development. While some authors found powdery scab incidence and severity to be significantly higher under the condition of constant dampness than fluctuating wet and dry conditions (Adams *et al*, 1987; van der Graaf *et al*., 2005, 2007), others concluded that fluctuating conditions are more conducive (Burnette, 1991; Hims, 1976a). Under conditions of constant high soil moisture, oxygen levels may be depleted while carbon dioxide levels increase, which has been reported to slow down tuber development and prolong the period of susceptibility to infection by Sss, thereby favoring disease development (Diriwächter and Parbery, 1991).

In our study, powdery scab incidence and severity were significantly higher in DEWL than WEDL moisture regimes. This result does not agree with many previous reports which identified the period at and shortly after tuber set as the most susceptible period for disease development (Adams *et al*., 1987; Burnett, 1991; de Boer *et al*., 1985; Hughes, 1980; Taylor and Flett, 1981). The previous reports show that powdery scab severity is associated with wet soil during the first half of the growing season and conclude that withholding irrigation just before and after tuber initiation may decrease disease severity. Our finding supports the work of other researchers who found that powdery scab infection may still result from wet conditions later in the growing season (Forsund, 1971; Hims, 1976a, 1976b; Adams, 1975; Parker, 1984; Christ and Weidner, 1988; Diriwächter and Parbery, 1991). High levels of powdery scab have been reported to occur if soil is maintained at field capacity for 20-40 days, having been dry prior to tuber

initiation (Hims, 1976a). In Australia, it was reported that for two consecutive seasons of field trials, delaying irrigation until tuber set did not decrease powdery scab severity (Taylor *et al*., 1986). Uneven tuber set, which was evident at the time our experiment was harvested, coupled with the proliferation of lenticels on mature tubers by high moisture levels later in the season ensured that tubers remained susceptible for a longer period and this partly explains our results.

In our study, no correlation was found between the occurrence of galls on roots and powdery scab on tubers (Christ, 2001; van de Graaf *et al*., 2007), nor between root gall formation and PMTV tuber necrosis. Our results also show that cv. Ivory Crisp is susceptible to root galling but tolerant to PMTV tuber necrosis, while the direct opposite is true for cv. Dakota Crisp. This further supports the lack of correlation between root galling and PMTV tuber necrosis. The correlation found between powdery scab incidence on tubers and PMTV tuber necrosis in this study is inconsistent with many published reports (Cooper *et al*., 1976; Kirk, 2008; Montero-Astua *et al*., 2008; Nielsen and Nicolaisen, 2000; Sandgren *et al*., 2002; Tenorio *et al*., 2006). However, our result is consistent with at least two reports (Davey *et al*., 2014; Domfeh *et al*., 2015). In a study conducted in Scotland to investigate the transmission of PMTV from infected seed tubers to daughter plants, statistically significant correlations ($r = 0.48$; $r = 0.61$ in 2004 and 2005, respectively) were found between the incidence of PMTV tuber necrosis and powdery scab formation on tubers (Davey *et al*., 2014). In another study, a strong overall positive correlation ($r = 0.62$, $P = 0.02$) was found between incidence of powdery scab on tubers and PMTV tuber necrosis incidence (Domfeh *et al*., 2015). In this report, it was hypothesized that the correlation between powdery scab incidence and PMTV tuber necrosis incidence could be due to the fact that 50% of the cultivars used in that trial were russet-skinned which are tolerant to both diseases and hence the correlation may have been an artifact created by choice of cultivars. However,

results presented in the current study, and that reported elsewhere (Davey *et al*., 2014), suggest that the correlation may have been real. These results suggest that Sss infection of potato tubers, which may lead to the direct injection of PMTV into the tubers, has a greater chance of inducing tuber necrosis than infection via roots (Domfeh *et al*., 2015).

The results of this study show that soil moisture plays a significant role in the development of PMTV tuber necrosis and that an appropriate use of irrigation may reduce disease pressure. With the practice of irrigation increasing in potato production in USA and other countries where PMTV and Sss exist, it is important to know which periods during the growing season potato plants are most susceptible and possibly avoid irrigation during those periods. For commercial application of the results presented here, it would be important to consider whether gains made through disease suppression by withholding irrigation outweighs yield loss that would be caused by water stress. Further field studies are necessary to determine the merits of using irrigation management to mitigate PMTV tuber necrosis while also maintaining tuber quality.

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GENERAL CONCLUSIONS

Potato mop-top virus (PMTV; genus Pomovirus; family Virgaviridae) can cause serious economic losses in sensitive potato cultivars by inducing necrotic symptoms in the flesh of tubers (Harrison & Reavy, 2002; Adams *et al*., 2009). PMTV is transmitted by the soil-borne *Spongospora subterranea* f.sp. *subterranea* (Sss), a fungal-like organism that causes powdery scab on potato. PMTV is widely distributed in the potato growing areas of South and North America, parts of Asia and northern Europe. In the United States, PMTV was first discovered in 2003 in Maine and since then, the virus has been confirmed in potato tubers from North Dakota, Washington State, Idaho, Colorado and New Mexico (Lambert *et al*., 2003; David *et al*., 2010; Crosslin, 2011; Whitworth and Crosslin, 2013; Mallik and Gudmestad, 2015), making PMTV an important emerging tuber necrosis disease in the USA. There are no commercial cultivars that are resistant to PMTV or its vector and no other suitable control options are available. Published reports mainly from Europe, suggest that potato cultivars differ in their susceptibility/sensitivity to PMTV infection.

The first two research chapters of this dissertation focus on using field trials to screen a large number of commercial potato cultivars and advanced breeding selections/clones obtained from different potato breeding programs across North and South America for their sensitivity to PMTV-induced tuber necrosis. In the last chapter, the potential of using soil moisture regime for the management of PMTV tuber necrosis was investigated. In these studies, randomly selected symptomatic tubers were tested for the presence of PMTV and *Tobacco rattle virus* (TRV) by reverse transmission polymerase reaction (RT-PCR) and the results confirmed that the tuber necrosis observed was caused by PMTV and not TRV.

In the first research chapter, the existence of natural variability in sensitivity to PMTV-induced tuber necrosis was demonstrated among the commonly grown potato cultivars in North America. Based on visual assessments of PMTV-induced tuber necrosis conducted on tubers obtained after two seasons, cultivars were categorized into groups of tolerant, moderately tolerant, moderately sensitive and sensitive. The russet-skinned cultivars were found to be less sensitive to PMTV-induced tuber necrosis than the red-, white- and yellow-skinned cultivars. It was found that PMTV tuber necrosis incidence increases significantly in storage and hence conducting visual assessment at harvest may lead to underestimation of the actual incidence levels.

In research chapter two, results of visual assessments after two growing seasons also revealed significant variability in PMTV-induced tuber necrosis incidence and severity among advanced breeding selections. A total of 17 selections were found to be tolerant, nine – moderately tolerant, eight - moderately sensitive while six were found to be sensitive. Like the first study, russet-skinned selections had lower tuber necrosis incidence than the red-, white- and yellow-skinned types. Evidence was found that the type of selection and skin-color significantly influenced development of PMTV tuber necrosis during storage, with the highest increases occurring in sensitive red- and white-skinned selections.

Significant correlations were found in PMTV tuber necrosis incidence and severity between trials and across years in both studies, demonstrating the reliability and reproducibility of the results of these field trials and also clearly indicate that potato germplasm can be successfully screened for their sensitivity to PMTV tuber necrosis under North American conditions. The lower PMTV tuber necrosis incidence obtained in 2011, which corresponded with lower soil moisture levels and higher average temperatures in the two studies, further

confirm the already established fact that environmental conditions heavily impact PMTV infection. The results of these studies are of immense commercial value, in that, potato growers in areas where PMTV exists, have the option of growing less sensitive cultivars as a means to limit the economic impact of PMTV-induced tuber necrosis. In the long term, tolerant cultivars/selections can be utilized as parents in potato breeding programs to obtain resistant materials.

In the third research chapter, results of visual assessments conducted after three months of storage showed significant differences in root gall formation, powdery scab on tubers, and PMTV tuber necrosis among soil moisture regimes. Evidence was found that indicates that maintaining soil moisture level at 90% field capacity (wet) throughout the growing season results in high PMTV tuber necrosis, root gall formation and powdery scab incidence on tubers, while keeping moisture level at 60% field capacity (dry) leads to low incidence in all three parameters. The results also indicate that keeping the soil dry early in the growing season and wet later and vice versa, may result in a similar PMTV tuber necrosis or root gall incidence. Powdery scab incidence however, was significantly higher in the DEWL than the WEDL regime. Contrary to reports which suggest that the potato plant is most susceptible to powdery scab and PMTV infection during the first half of the growing season and that wet conditions early in the growing may lead to higher disease, our results agree with those who have indicated that wet conditions during the latter part of the growing season can also result in high disease pressure. Our results show that cv. Ivory Crisp is susceptible to root galling but less sensitive to PMTV tuber necrosis, while the opposite is true for Dakota Crisp, demonstrating the lack of correlation between the two parameters. The results of this study provide further evidence that soil moisture content plays an important role in the development of PMTV tuber necrosis and Sss infection and that

appropriate management of irrigation during the growing season may lessen the likelihood of powdery scab infection and PMTV-induced tuber necrosis development in potato.

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APPENDIX A

Table A.1. Analysis of variance for number of *Spongospora* galls for trials one and two in 2012.

	Trial one				Trial two			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	3	2015.18	671.73	2.07 ^{NS}	2	5992.63	2996.31	1.85 ^{NS}
Cultivar	13	50866.69	3912.82	12.07***	26	106751.09	4105.81	2.53*
Error	39	12644.13	324.21		52	84361.64	1622.34	
Corrected Total	55	65526.00			80	197105.36		

P. * = 0.05; *** = 0.0001; NS = no significance

Table A.2. Analysis of variance for incidence and severity of powdery scab in 14 potato cultivars planted in 2011 (trial one).

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	3	1780.42	593.47	4.79*	3	5.06	1.69	2.79 ^{NS}
Cultivar	13	25018.58	1924.51	15.54***	13	34.17	2.63	4.35**
Error	39	4829.87	123.84		39	23.54	0.60	
Corrected Total	55	31628.87			55	62.76		

P: * = 0.05; ** = 0.001; *** = 0.0001; NS = no significance

Table A.3. Analysis of variance for incidence and severity of powdery scab in 14 potato cultivars planted in 2012 (trial one).

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	3	252.80	84.27	2.81^{NS}	3	0.91	0.30	2.01^{NS}
Cultivar	13	17422.54	1340.20	44.66***	13	37.68	2.90	19.21***
Error	39	1170.23	30.01		39	5.88	0.15	
Corrected Total	55	18845.57			55	44.48		

P: **** = 0.0001; NS = no significance

Table A.4. Analysis of variance for incidence and severity of powdery scab in 24 potato cultivars planted in 2011 (trial two).

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	241.13	120.57	2.09 ^{NS}	2	0.06	0.03	1.92 ^{NS}
Cultivar	23	9557.10	415.53	7.19***	23	1.71	0.07	4.7***
Error	46	2656.89	57.76		46	0.73	0.02	
Corrected Total	71	12455.12			71	2.49		

P: **** = 0.0001; NS = no significance

Table A.5. Analysis of variance for incidence and severity of powdery scab in 27 potato cultivars planted in 2012 (trial two).

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	11.79	5.90	0.11 ^{NS}	2	0.04	0.02	0.09 ^{NS}
Cultivar	26	22362.96	860.11	15.69***	26	57.89	2.23	10.99***
Error	52	2851.07	54.83		52	10.53	0.20	
Corrected Total	80	25225.82			80	68.46		

P: **** = 0.0001; NS = no significance

Table A.6. Analysis of variance for incidence and severity index of *Potato mop-top virus* (PMTV)-induced tuber necrosis in 14 potato cultivars planted in 2011 (trial one).

	Incidence				Severity			
Source	DF	ANOVA SS	Mean Square	F Value	DF	ANOVA SS	Mean Square	F Value
Rep	3	101.37	33.79	3.08*	3	0.05	0.015	3.36*
Cultivar	13	1648.02	126.77	11.56***	13	0.70	0.053	11.79***
SD	2	356.03	178.02	16.24***	2	0.01	0.004	0.82 ^{NS}
SD*cultivar	26	946.97	36.42	3.32***	26	0.09	0.004	0.78 ^{NS}
Error	888	9736.61	10.96		888	4.03	0.005	
Corrected Total	932	12788.99			932	4.87		

P. * = 0.05; *** = 0.0001; NS = no significance; SD = Sampling date

Table A.7. Analysis of variance for incidence and severity index of *Potato mop-top virus* (PMTV)-induced tuber necrosis in 14 potato cultivars planted in 2012 (trial one).

	Incidence				Severity			
Source	DF	ANOVA SS	Mean Square	F Value	DF	ANOVA SS	Mean Square	F Value
Rep	3	211.88	70.63	2.45*	3	0.003	0.001	0.12 ^{NS}
Cultivar	13	15046.88	1157.45	40.11***	13	1.230	0.095	10.57***
SD	2	5545.81	2772.90	96.08***	2	0.329	0.164	18.37***
SD*cultivar	26	2942.02	113.15	3.92***	26	0.247	0.009	1.06 ^{NS}
Error	627	18095.03	28.86		627	5.612	0.009	
Corrected Total	671	41841.62			671	7.420		

P. * = 0.05; *** = 0.0001; NS = no significance; SD = Sampling date

Table A.8. Analysis of variance for incidence and severity index of *Potato mop-top virus* (PMTV)-induced tuber necrosis in 24 potato cultivars planted in 2011 (trial two).

	Incidence				Severity			
Source	DF	ANOVA SS	Mean Square	F Value	DF	ANOVA SS	Mean Square	F Value
Rep	2	31.65	15.82	0.91 NS	2	0.067	0.033	2.27 ^{NS}
Cultivar	23	1166.98	50.74	2.92***	23	0.464	0.020	1.36 ^{NS}
SD	1	618.06	618.06	35.53***	1	0.035	0.035	2.35 ^{NS}
SD*cultivar	23	1144.95	49.78	2.86**	23	0.459	0.020	1.35 ^{NS}
Error	94	1635.38	17.40		94	1.388	0.015	
Corrected Total	143	4597.02			143	2.413		

P: ** = 0.001; *** = 0.0001; NS = no significance; SD = Sampling date

Table A.9. Analysis of variance for incidence and severity index of *Potato mop-top virus* (PMTV)-induced tuber necrosis in 27 potato cultivars planted in 2012 (trial two).

	Incidence				Severity			
Source	DF	ANOVA SS	Mean Square	F Value	DF	ANOVA SS	Mean Square	F Value
Rep	2	207.35	103.67	2.28 ^{NS}	2	0.016	0.008	0.41 ^{NS}
Cultivar	26	10169.63	391.14	8.62***	26	2.670	0.103	5.2***
SD	1	55.32	55.32	1.22 ^{NS}	1	0.004	0.004	0.18 ^{NS}
SD*cultivar	26	2216.54	85.25	1.88*	26	0.361	0.014	0.7 ^{NS}
Error	106	4811.27	45.39		106	2.095	0.020	
Corrected Total	161	17460.12			161	5.146		

P: * = 0.05; *** = 0.0001; NS = no significance; SD = Sampling date

Table A.10. Analysis of variance for incidence and severity of *Potato mop-top virus* (PMTV)-induced tuber necrosis in six white-skinned potato cultivars planted in 2013 (trial 3).

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	0.65	0.33	0.32694 ^{NS}	2	0.002	0.001	0.05 NS
Clone	5	11472.49	2294.50	26.86***	5	1.621	0.324	13.68***
Error	28	2391.64	85.42		28	0.664	0.024	
Corrected Total	35	13864.78			35	2.288		

P: *** = 0.0001; NS = no significance

APPENDIX B

Table B.1. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with red skin-type planted in 2011.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	822.59	411.29	2.02 ^{NS}	23	10.03	0.44	9.88***
Selection	23	10133.06	440.57	2.16*	2	0.90	0.45	10.23***
Error	118	24024.11	203.59		118	5.21	0.04	
Corrected Total	143	34979.76			143	16.14		

P: * = 0.05; *** = 0.0001; NS = no significance

Table B.2. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with red skin-type planted in 2012.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	204.15	102.08	1.54 ^{NS}	2	0.024	0.012	0.79 ^{NS}
Selection	19	9990.79	525.83	7.93***	19	1.443	0.076	5.1***
Error	98	6496.32	66.29		98	1.460	0.015	
Corrected Total	119	16691.27			119	2.927		

P: *** = 0.0001; NS = no significance

Table B.3. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with russet skin-type planted in 2011.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	104.76	52.38	1.15 ^{NS}	2	0.104	0.052	2.91 ^{NS}
Selection	16	1035.11	64.69	1.42 ^{NS}	16	0.855	0.053	3 **
Error	83	3770.22	45.42		83	1.477	0.018	
Corrected Total	101	4910.09			101	2.436		

P: ** = 0.001; NS = no significance

Table B.4. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with russet skin-type planted in 2012.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	144.37	72.19	1.74 ^{NS}	2	0.007	0.004	0.36 ^{NS}
Selection	25	3751.01	150.04	3.63 ***	25	0.630	0.025	2.51**
Error	128	5295.77	41.37		128	1.282	0.010	
Corrected Total	155	9191.15			155	1.919		

P: ** = 0.001; **** = 0.0001; NS = no significance

Table B.5. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with white skin-type planted in 2011.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	1101.44	550.72	3.8*	2	0.22	0.11	2.78 NS
Selection	21	4608.50	219.45	1.51 NS	21	2.81	0.13	3.3***
Error	108	15666.98	145.06		108	4.38	0.04	
Corrected Total	131	21376.91			131	7.41		

P: * = 0.05; **** = 0.0001; NS = no significance

Table B.6. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with white skin-type planted in 2012.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	5.97	2.98	0.07 ^{NS}	2	0.022	0.011	0.5 ^{NS}
Selection	14	3194.79	228.20	5.41***	14	2.140	0.153	6.99***
Error	73	3079.22	42.18		73	1.596	0.022	
Corrected Total	89	6279.98			89	3.758		

P: **** = 0.0001; NS = no significance

Table B.7. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with yellow skin-type planted in 2011.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	61.81	30.90	0.79 ^{NS}	2	0.052	0.026	1.47 ^{NS}
Selection	12	590.20	49.18	1.26 ^{NS}	12	0.539	0.045	2.56*
Error	57	2218.04	38.91		57	0.999		
Corrected Total	71	2871.60			71	1.590		

P: * = 0.05; NS = no significance

Table B.8. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections with yellow skin-type planted in 2012.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	33.21	16.61	0.5 NS	2	0.012	0.006	0.16 NS
Selection	6	1224.60	204.10	6.15**	6	0.590	0.098	2.54 *
Error	33	1094.88	33.18		33	1.274	0.039	
Corrected Total	41	2352.69			41	1.876		

P: * = 0.05; ** = 0.001; NS = no significance

Table B.9. Analysis of variance for *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence and severity index of several advanced breeding selections across skin-types in 2011.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	1488.83	744.41	5.28*	2	0.912	0.456	7.59**
Skin-type	3	1843.11	614.37	4.35*	3	2.108	0.703	11.7***
Error	444	62649.54	141.10		444	26.66	0.060	
Corrected Total	449	65981.48			449	29.68		

P: * = 0.05; ** = 0.001; *** = 0.0001; NS = No significance

Table B.10. *Potato mop-top virus* (PMTV)-induced tuber necrosis incidence (%) and severity index of several advanced breeding selections across skin-types in 2012.

	Incidence				Severity			
Source	DF	Type III SS	Mean Square	F Value	DF	Type III SS	Mean Square	F Value
Rep	2	188.61	94.30	1.1 ^{NS}	2	0.008	0.004	0.16 ^{NS}
Skin-type	3	717.20	239.07	2.8*	3	0.488	0.163	6.24**
Error	402	34326.47	85.39		402	10.472	0.026	
Corrected Total	407	35232.29			407	10.968		

P: * = 0.05; ** = 0.001; NS = No significance

APPENDIX C

Table C.1. Analysis of variance for *Spongospora subterranea* f.sp. *subterranea* galls on roots across moisture regimes.

Source	DF	ANOVA SS	Mean Square	F Value
Moisture regime	3	10747.65	3582.55	8.1***
Experiment	1	126.01	126.01	0.28 ^{NS}
Cultivar	1	11193.82	11193.82	25.32***
Moisture reg.*Exp.	3	140.09	46.70	0.11 ^{NS}
Moisture reg.*cultivar	3	8002.90	2667.63	6.03**
Exp.*cultivar	1	4.88	4.88	0.01 ^{NS}
Error	115	50847.34	442.15	
Corrected Total	127	81062.68		

P: * = 0.05; ** = 0.001; NS = No significance

Table C.2. Analysis of variance for powdery scab incidence and severity among four moisture regimes summed across two potato cultivars.

		Incidence				Severity		
Source	DF	Anova SS	Mean Square	F Value	DF	Anova SS	Mean Square	F Value
Moisture regime	3	5966.71	1988.90	5.62**	3	6.63	2.21	4.53*
Experiment	1	1701.19	1701.19	4.81*	1	0.64	0.64	1.31 ^{NS}
Cultivar	1	255.04	255.04	0.72 ^{NS}	1	0.89	0.89	1.82 ^{NS}
Moisture reg.*Exp.	3	1411.26	470.42	1.33 ^{NS}	3	2.13	0.71	1.45 ^{NS}
Moisture reg.*cultivar	3	1886.19	628.73	1.78 ^{NS}	3	1.63	0.54	1.11 ^{NS}
Exp.*cultivar	1	1960.01	1960.01	5.54*	1	0.70	0.70	1.42 ^{NS}
Error	115	40715.31	354.05		115	56.19	0.49	
Corrected Total	127	53895.71			127	68.79		

P: * = 0.05; ** = 0.001; NS = No significance

Table C.3. Analysis of variance for PMTV tuber necrosis incidence and severity index among four moisture regimes summed across two potato cultivars.

Source	DF	Anova SS	Mean Square	F Value	DF	Anova SS	Mean Square	F Value
Moisture regime	3	3159.86	1053.29	5.94**	3	0.2261	0.0754	5.91**
Experiment	1	45.16	45.16	0.25 ^{NS}	1	0.0001	0.0001	0.00 ^{NS}
Cultivar	1	4060.24	4060.24	22.88***	1	0.3938	0.3938	30.86***
Moisture reg.*Exp.	3	202.53	67.51	0.38 ^{NS}	3	0.0140	0.0047	0.37 ^{NS}
Moisture reg.*cultivar	3	451.49	150.50	0.85 ^{NS}	3	0.0474	0.0158	1.24 ^{NS}
Exp.*cultivar	1	181.05	181.05	1.02 ^{NS}	1	0.0001	0.0001	0.01 ^{NS}
Error	115	20407.10	177.45		115	1.4676	0.0128	
Corrected Total	127	28507.43			127	2.1491		

P: * = 0.05; ** = 0.001; *** = 0.0001; NS = No significance

APPENDIX D

Table D.1. Protocol of total RNA extraction.

Reagent	Amount (µl)
TRIZOL	0.8
Chloroform	200
Isopropyl alcohol	500
75% ethanol	1000
RNase-free water	100

Table D.2. Reverse transcription-polymerase chain reaction (RT-PCR) for PMTV detection.

Reagents	Amount (µl)
RT-PCR	3.3
Water	2.0
MLV buffer	2.0
dNTP	0.2
Random Hexamer	0.5
MLV transcriptase	
PCR	
Water	16.8
Gotaq buffer	2.5
$MgCl_2$	2.0
dNTP	0.5
PMTV 9P	0.5
PMTV 9M	0.5
Gotaq polymerase	0.2

Table D.3. Reverse transcription-polymerase chain reaction (RT-PCR) for TRV detection.

Reagent	Amount (µl)
Step one	
Water	4
TRV A	2
RT-PCR	
Water	2
Dntp	2
MLV Buffer	4
MLV transcriptase	1
PCR	
Water	14.05
Gotaq buffer	2.5
$MgCl_2$	1.75
dNTP	0.5
Forward primer	0.5
Reverse primer	0.5
Gotaq polymerase	0.2

Table D.4. Primer pairs used in RT-PCR amplification of PMTV and TRV.

PMTV	
PMTV-9P: 5′-GCTTGATCCAGAAGTCATTAAGG-3′	Nakayama *et al.* (2010)
PMTV-9M: 5′-CCTGGAAGCACCAATACTTAACG-3′	
TRV	
C819: 5′-CTATGCACCAGCCCAGCGTAACC-3′	Robinson (1992)
H360: 5′-CATGAAGGCTGCCGTGAGGAAGT-3′	