Potato, Viruses, and Seed Certification in the USA to Provide Healthy Propagated Tubers

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ABSTRACT

Potatoes are vegetatively propagated and this can result in the dissemination of pathogens, and viruses in particular, in the tubers. Viruses infecting potato can be categorized by their mechanisms of transmission: aphid transmitted, mechanically transmitted, and soil-borne viruses. The most important viruses in North America include Potato leafroll virus, Potato virus Y, X, A, S, M, Tobacco rattle virus, and Potato mop top virus. The methods for chemical control of virus disease are greatly influenced by their mechanism of spread in the field. However, tubers play an important role in the spread of virus disease and this has led many regions to develop seed certification programs. The use of certified virus-free tubers by growers has been vital for control of disease worldwide. In addition, breeders have identified genetic resistance that can be introgressed into popular cultivated varieties and provides a method of control that is less costly than chemical application. In recent years there has been an emergence of viruses and recombinant virus strains that have posed new challenges to pathologists for seed certification and for breeders. Here we discuss the latest issues and challenges that viruses pose to potato production.

INTRODUCTION

Cultivated potato (Solanum tuberosum ssp. tuberosum) is the world’s third most important food crop and arguably is one of the most intensively managed, requiring irrigation, fertilization, and frequent pesticide applications to obtain competitive yields (Knutson et al. 1967). Since potatoes are vegetatively propagated, their production differs significantly from that of crops grown from true seed and this creates unique opportunities to propagate and spread many diseases, such as viruses (Khurana 2004). Commercial potato varieties are maintained in tissue culture, and the plantlets are transferred into hydroponic systems or greenhouses to obtain a first generation of potato tubers (Fig. 1) (Bohl et al. 2000). These “seed” tubers, which are destined only for planting, are multiplied for three or more years in the field before a final multiplication for consumption. Many vascular pathogens easily move into and survive in tubers (but not in true seeds) until the next growing season. Without proper management, seed potato stocks easily reach 100% disease incidence within a few years (Khurana 2004, Knutson et al. 1967). There are three major methods used in the US to control the spread of viruses. The first is chemical or cultural control of viruliferous vectors, which limits the spread of viruses from plant to plant. Second, the availability of certified seed that is free from virus prevents the introduction of viruses into production areas. Finally, incorporation of host resistance through breeding of new cultivars brings with it natural control mechanisms for several viruses and is particularly important in countries where seed potato certification programs are not effective.

There are several common aphid-transmitted potato viruses that seed potato certification programs have effectively controlled (not eradicated) (Khurana 2004), but new and emerging virus strains have undermined some of the efforts of these programs. The aphid-transmitted viruses have RNA genomes and belong to the genera Polerovirus, Potyvirus, and Carlavirus (Radcliffe and Ragsdale 2002). All potyviruses and carlaviruses are also mechanically transmissible. In the US, the Potato leaf roll virus (PLRV; a polerovirus) currently has a low incidence of occurrence. However, the incidence of Potato virus A (PVA; a potyvirus) may be increasing. The most common viruses in the US are Potato virus Y (PY; a potyvirus) and Potato virus S (PVS; a carlavirus). The recent spread of necrotic PVY strains has created new challenges for seed potato certification programs. In particular, PLRV and some strains of PVY cause potato tuber necrosis and this impedes market-
ability. Planting PVY infected seed tubers can limit marketable yield of some cultivars by ≤ 80% (Hane 1999). Once in the field, poty-, carla- and poleroviruses are spread either through feeding by insect vectors or through mechanical transmission. Aphid life cycles, control, and virus transmission in potato were extensively reviewed and will not be discussed here (Radcliffe and Ragsdale 2002). Only a few aphids colonize potato plants, including the green peach aphid (Myzus persicae), the potato aphid (Macrosiphum euphorbiae), and the glasshouse potato aphid (Aulacorthum solani) (Srinivasan et al. 2008). Systemic insecticides that are effective for controlling aphids and other pests include the organophosphate Monitor (methomidiphos) or the neonicotinoids, Admire (imidicloprid) and, more recently, Plati-
appears to be spreading (Kirk 2008, Tenorio et al. 2010). Tobacco rattle virus (TRV) occurs worldwide, but is only mechanically transmitted by seed potato certification in the US and Canada, and is rarely found in other countries with similarly effective certification programs. The distribution of soil-borne viruses on production in the US and Canada, is becoming concerned about the potential impact of these viruses on production in the US and Canada. Soil-borne diseases such as TRV and PMTV, are likely to be the biggest pathogen challenges for potato growers in the 21st century (Santala et al. 2010).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Virus</th>
<th>Source</th>
<th>Mechanism</th>
<th>Chromosome</th>
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<tr>
<td>Rs1^1</td>
<td>PVX</td>
<td>diploid P18</td>
<td>ER</td>
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<td>GP122 (RFLP/CAPS), STM003 (SSR)</td>
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<td>Hämäläinen et al. 1998; Sori et al. 1999</td>
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<td>ER</td>
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<td>ER</td>
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<td>38-530 (RFLP), CT220 (RFLP)</td>
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<td>S. chacoense</td>
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<td>C2_Atl42990 (COSII)</td>
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<td>S. tuberosum clone PA95A33-1</td>
<td>HR</td>
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<td>AAC-CGT-0347, ACG-CTG-0347, ACG-CTG-0347, ACG-CTG-0347, ACG-CTG-0347, ACG-CTG-0347, ACG-CTG-0378 (AFLP)</td>
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<td>GP230f2, GP2323f2 (COS)</td>
<td>Ross 1986; Marczewski et al. 2006</td>
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Abbreviations: RFLP=restriction fragment length polymorphism, CAPS=cleaved amplified polymorphic sequence, SSR=simple sequence repeat, RGL=genetic source (e.g. RGL=genome of potato), AFLP=amplified fragment length polymorphism, SCAR=sequence characterized amplified region.

# Table 1 Virus resistance genes in potato.

Recent reports of virus diseases causing substantial yield losses and diminishing tuber quality have led breeders to increase their focus on incorporation of virus resistant germplasm into their programs (Barker and Dale 2006, David et al. 2010, Gieck et al. 2007, Karasev et al. 2008). Potato breeders have difficulty assessing genetic virus resistance in field plots because some common potato viruses, such as PVM, TRV, and PVX may cause mild, transient, or no foliar symptoms, although the same viruses have significant effects on tuber yield and quality. Breeding for resistance traits in potato is further complicated because cultivated potato (Solanum tuberosum L.) is a tetraploid that acts as a self-compatible species. The effects of virus resistance on yield and tuber quality are complex and depend on the specific virus strain and cultivar combination. It is important to understand the mechanisms of virus resistance in potato in order to develop effective breeding strategies.
Tacke et al. 1996; (Ares et al. 1998, Barker et al. 1998, Bukovinszki et al. 2007, Ehrenfeld et al. 2004, Gargouri-Bouzid et al. 2006, Kawchuk et al. 1991, Melander et al. 2001, Tacke et al. 1996, Vazquez Rove et al. 2001). In fact, potatoes resistant to PVY and PLRV have been commercialized in North America (Coffin et al. 1997, Lawson et al. 2001). Unfortunately, they were not accepted by the processing industry or international trading partners and are no longer available for use in resistant potato cultivars. Some potato viruses are important biotechnological tools; both PVX and TRV are commonly used as vectors for virus induced gene silencing and methods for using this technology are summarized nicely by (Lu et al. 2003). Technological advances have also improved our capacity to detect and diagnose potato viruses. ELISA is commonly used because of its simplicity and relatively low cost. However, multiplex PCR-based assays are now available for several potato viruses (Agindotan et al. 2007, Mortimer-Jones et al. 2009, Ryazantsev and Zavriev 2009). In addition, macroarrays detecting the most important potato viruses have been developed (Agindotan and Perry 2007, Agindotan and Perry 2008). Immunoassay strips, which allow quick and accurate diagnosis in the field, are increasing in popularity among inspectors and plant breeders. Even though there have been substantial advances in virus detection, indicator plants are still sometimes used when potato germplasm is moved across borders to inhibit the spread of uncharacterized viruses.

In this review, we have combined discussion of recent research related to the control of major potato viruses, all of which are positive-sense RNA viruses, including the biology of the viruses, their vectors, and the steps being taken to curtail their spread through certification and breeding of resistant varieties.

### Aphid-transmitted potyviruses: Potato virus Y (PVY) and Potato virus A (PVA)

PVY and PVA are members of the genus *Potyvirus*, one of the two largest genera of plant-infecting viruses. Potyviruses are flexuous rods and have monopartite genomes that are just under 10-kilobases long. Potyviruses encode one polyprotein that is cleaved into 10 proteins (Urcuqui-Inchima et al. 2001) as well as a recently discovered protein embedded within the genome (Chung et al. 2008). PVY and PVA infect mainly *Solanaceae*, including potato, tomato, pepper, tobacco and eggplant. PVY is more widespread and causes greater losses than PVA, so this review will focus on PVY. Infection with PVY can reduce potato yields by over 60% (Whitworth et al. 2003, Tribodet et al. 2006) and planting seed potatoes infected with PVY can reduce yields up to 80% (Bantarri et al. 1993, Hane 1999).

There are multiple strains of PVY that cause foliar symptoms ranging from mild mosaics to necrosis. The PVYO strain is the common strain that causes mosaic symptoms in most hosts but can cause foliar necrosis on some potato varieties. For example, when the potato variety ‘Goldrush’ is infected with PVYO, the leaves show a bright yellow necrotic strain, the common strain that causes mosaic symptoms in most hosts but can cause foliar necrosis on some potato varieties. For example, when the potato variety ‘Goldrush’ is infected with PVYO, the leaves show a bright yellow necrosis. This necrosis, referred to as potato tuber necrotic ringspot (PTNRS), diminishes tuber marketability.

The PVYO genome has a high degree of genetic variability and there are frequent reports of recombination among PVY strains (Baldauf et al. 2006, Inoue-Nagata et al. 2001, Singh et al. 2003). Several PVY recombinants have been documented since the 1980s including PVYO-Wi, PVYO-NE, and PVYO-NE (Ha et al. 2009, Ogawa et al. 2008). Each of these arose from various recombination events between PVYO and PVYN, but the exact mechanisms of recombination are not always practical or achievable for several reasons. The presence of multiple PVY strains within lots of potato and even in the same plant, as well as their frequent recombination, creates challenges for plant pathologists to accurately identify PVY strains and predict their impact on production. In addition, there is no single method currently available that distinguishes all strains. Serological techniques can distinguish between PVYN and PVYN, but do not differentiate N from NTN strains nor O from N:O strains (Karasev et al. 2010). Given that the necrotic strains PVY and PVYN cause veinal necrosis on tobacco plants (Tribodet et al. 2005), a tobacco bioassay remains the best method to identify necrotic isolates, but cannot be performed on a large scale for disease diagnosis. Many primer sets used in RT-PCR assays do not detect all possible strains, and the cost of these assays is prohibitive for use in seed potato certification (Nie and Singh 2002). Thus in principle, accurate diagnosis involves a combination of tobacco bioassay, serological detection, and RT-PCR tests. Considering the costs and extensive assays needed to identify virus strains, it is impractical for growers with large production systems and potato certification agencies to focus on the accurate diagnosis of a particular PVY strain or the management of individual PVY strains.

Potyviruses, such as PVY and PVA, are spread in a non-persistent manner by numerous aphid species and there are chemical and cultural control methods used to limit insects as a means to limit virus infection. Since virus acquisition and spread take only seconds, systemic insecticides are not effective for control (Parker et al. 2006). Insecticides effectively control potato colonizing aphids (Parker et al. 2006), thus the most agronomically important aphid vectors for potato potyviruses are species that transiently associate with plants as they forage on potato in search for suitable host plants. The most important aphid species for PVY and PVA transmission vary among locations and years since the aphid species moving through a crop is affected by weather patterns, environmental conditions, and neighboring crops (Raech 1986). Low temperatures affect aphid survival and therefore cold winters can impact the spread of aphid populations and onset of viral diseases in the spring. However, climate warming is affecting aphid populations. The warmer winter temperatures favor aphid populations, but the hotter summers may be threatening. Thus, as seasonal climates change, we expect to see changes in the patterns of vector and disease spread (Hazell et al. 2010a, 2010b). Aphid populations...
can also be affected by the landscape structure surrounding potato fields. Researchers have shown that the sizes of bordering grassy fields can affect predation of potato pests (Werling and Gratton 2010). Since some aphid species preferentially land on field edges, surrounding fields of susceptible potatoes with either resistant potato varieties or crops that are not hosts of PVY, such as winter wheat, can be effective. Furthermore, combining cultural practices to control aphids that limit virus spread such as the use of crop border methods effectively reduces the incidence of PVY (Boiteau et al. 2009). Video recordings of insect movements show that mineral oil impedes styleset penetration and is an effective antifeedant treatment reducing the proportion of aphids that can transmit PVY (Powell et al. 1998). Two other cultural control methods used by seed potato growers to control aphid-borne viruses include use of green sprouting (chitting) causing plants to emerge and mature earlier, thus benefiting from mature plant resistance, and early vine kill to avoid late season aphid flights (Sauce and Doring 2004). Purchasing certified seed is also an important method to control PVY and PVA. Farmers growing potatoes for fresh use or processing rely on certified seed potatoes and rarely use other chemical or cultural control methods to control PVY spread.

In the mid-1990’s, PVYN was reported in Canada and subsequently PVY incidence in seed potatoes has increased in North America over the past decade. The Canadian government tried to eradicate PVYN from seed producing areas in the 1990s through implementation of a necrotic potato virus management plan (USDA 2004). This plan was also infested with trade goals, since it slowed movement of seed potatoes from Canada into the US and other countries. Canadians soon found PVYN eradication to be impossible. Diagnostic, regulatory, and inspection problems surrounding the spread of PVYN in Canada led to a $75 million, 15-year lawsuit involving around 180 growers.

An important component of the rising incidence of PVY in the 1990s was the popularity of several new russet varieties in the US that were susceptible to PVY. These varieties showed mild or no foliar symptoms, but served as large reservoirs for PVY, increasing the risk of virus spread to other varieties as well as providing more opportunities for virus mutation and recombination. In addition, changes in aphid populations, including the invasion of soybean aphid into the Midwest, may have driven the increase in PVY incidence (Davis et al. 2005). Added factors driving PVY incidence such as changes in rotation crops planted by potato growers as well as climate change influence local aphid abundance where aphids now quickly reach northern seed potato growing regions.

In 2002, tuber necrotic strains of PVY were first reported in the Pacific Northwest of the US. This triggered a large survey of PVY strains in Canada and the US and a review of the necrotic potato virus management plan. The survey revealed that many PVY strains are present in the US (Piche et al. 2004). As previously mentioned, researchers found that a single immunoassay or RT-PCR test could classify a strain as a tuber necrotic strain. Therefore, rather than attempting to eradicate specific strains, potato growers are now using strategies to reduce levels of PVY inoculum in seed potato lots and the use of genetic plant resistance to PVY has become a higher priority. New protocols are being implemented by the seed potato certification programs in the US and Canada that are designed to reduce the spread of PVY across North America. These changes include widespread use of post-harvest testing and shipping point inspections to eliminate lots with high incidence of PVY, and especially tuber necrosis strains of PVY. Furthermore, the revised Canada-US-Management Plan for potato viruses that cause tuber necrosis was expanded to include TRV, PMTV, and Alfalfa mosaic virus. This management plan is a result of an intersection of agriculture, science, and politics, and will not eradicate or even slow the spread of these viruses. Rather, it provides a mechanism to remove seed potato lots with high levels of tuber necrosis and a mechanism to preserve trade between the US and Canada.

While PVY is well controlled among commercial farms, it remains an important problem in seed production. PVY is the main reason for rejection of seed potato lots from certification and in some years, this virus can cause downgrading of more than half (and sometimes all) of the seed lots of susceptible varieties. This leads to shortages of certified seed potato lots, which can be事关 to trade, and affecting their contracts and long-term relationships with customers. When seed lots are downgraded, commercial farmers will plant non-certified seed, which leads to unpredictable yields and quality. This also affects their ability to buy crop insurance and may cause the grower to lose status as a reliable potato grower. Seed certification programs in some states will occasionally raise the tolerable PVY incidence rate if numerous seed lots have been downgraded, but this typically leads to increased levels of PVY since inoculum levels will be high in the following year.

Although genetic resistance remains the best long-term strategy for combating viral disease of potato, most of the potato acreage grown in North America is susceptible to PVY since other disease resistance and processing characteristics remain more important than resistance. It is difficult for growers and seed potato certification inspectors to see PVY symptoms on these varieties, which include mainly ‘Russet Norkotah’, ‘Silvertorn Russet’, and ‘Gem Russet’ (Hane 1999, Rykbost et al. 1999). Now potato breeders are learning about the genetic backgrounds of the varieties that they plant (Mollow and Thill 2004) and have increased efforts to release varieties that are resistant to PVY, or that at least show typical symptoms of this virus.

Therefore, breeding for resistance to this virus is especially important. Three sources of ER to PVY exist in potato: Rsio from S. stoloniferum Schlecht. et Behé, Rsio from S. chacoense Bitt. (Asama et al. 1982), and Rsst from S. tuberosum ssp. andigena Hawkes (Munoz et al. 1975). These three genes are considered different from one another since they map to different locations within their respective genomes. Resistance encoded by the Rsio gene has been incorporated into several cultivars developed through European breeding programs (Barker and Dale 2006, Ross 1986). Cleaved-amplified polymorphic sequence (CAPS) markers derived from the restriction fragment polymorphism loci GP122 were used to detect the Rsio gene in different germplasm sources (Valkonen et al. 2008). The Rsio gene was also introgressed from S. stoloniferum and both the Rsio and Rsio genes map to chromosome XII (Flis et al. 2005, Song et al. 2005, Valkonen et al. 2008). At least Rsio, Rsst, and Rsio genes can be seen in both (Flis et al. 2005, Song et al. 2005, Wittek et al. 2006); however, Valkonen et al. (2008) identified markers linked to both Rsio and Rsio, that have identical estimated genetic distances to the resistance gene, suggesting that these genes may be identical.

PVY resistance from S. chacoense has not been widely utilized in potato breeding although some cultivars with the Rsst gene have been developed (Hane 1999, Hatamura et al. 1995). Rsst maps to potato chromosome IX (Hosaka et al. 2001, Sato et al. 2006) and is located in a region with several NB-LRR genes with nucleotide similarity to the tomato Slv-5 gene conferring resistance to tomato spotted wilt virus (D. Halterman and X. Cai, unpublished data, Brommonschenkel et al. 2000).

Resistance derived from S. tuberosum ssp. andigena confers to accessions Eri to various PVY strains, including the recombinant strain PVY (Hembright and Valkonen 2001, Munoz et al. 1975, Whittworth et al. 2009). Markers associated with Rsst place the gene on chromosome XI (Hämäläinen et al. 1998). These markers include two sequence characterized amplified regions (SCAR) (Kasai et al. 2000) and a CAPS (Sorris et al. 1999). Interestingly, the latter marker shows homology to the kinase motif of previously cloned NB-LRR protein N, which confers resistance to Tobacco mosaic virus (TMV) in Nicotiana gluti-
PLRV infected fields also influence aphid immigration and emigration. In controlled experiments, researchers have shown that the influence of PLRV on aphid behavior depends on disease progression and plant age (Eigenbrode et al. 2002, Werner et al. 2009). Winged (alate) aphids spread the virus for long distances between fields, and non-winged (apterous) aphids are important in plant-to-plant spread within a field (Taliansky et al. 2003). Aphid feeding introduces PLRV into the phloem tissue where the virus multiplies, spreads, and initiates disease. If infected plants produce tubers, these tubers will grow into symptomatic plants that produce little, but that serve as inoculum sources in the following year.

Interestingly, isolates of PLRV show little sequence divergence, and no strains have been entered into the classification indexes. Studies comparing geographic isolates from several continents have found few differences with the published sequences, thus geography and aphid vectors do not function as evolutionary pressures for PLRV. Diagnosis is therefore straightforward using DAS-ELISA or RT-PCR (Du 2006).

The most widely applied methods to effectively control PLRV include the use of insecticides and seed potato certification. Insecticide application suppresses aphid populations in the field, thereby preventing inoculation and net necrosis in tubers (Roosen et al. 1997). The recent and widespread use of systemic insecticides has greatly reduced PLRV incidence in North America. Seed potato certification officials now report only a few plants infected with PLRV. For example, no seed potato lots have been rejected in Wisconsin due to PLRV incidence for at least the past decade. There are several cultural methods to control PLRV that are not widely used, mostly because of the high costs for implementing these methods in large production systems. These include heat treatment, or even cryogenic treatment of micro-plants, as a means to eliminate PLRV from tissue culture generated plants (Wang et al. 2006). Whitewash sprays and reflective materials are effective for controlling aphid-transmitted PLRV as well as PVY (Marco 1986) in certain small plots. For small plots, covering plants with a white net or spraying them with mineral oil or zinc containing Loven, Talbín, Dabak (Tapazol Co.), and Virol effectively reduces PLRV as well as PVY incidence. The whitewash sprays and netting increase leaf reflectivity making them less attractive to aphids (Marco 1986). Heat treatment of tubers can eliminate PLRV, but not PVY, PVA, PVS, PVX, or TRV (Kaiser 1980). Heat-treated tubers show better survival and progeny plants are virus-free. Some potato varieties grown in the US are susceptible to PLRV (Corsini and Brown 2001) and losses due to planting PLRV infected seed can reduce yields by up to 80% (Bannarri et al. 1993). The polygenic nature of most PLRV resistance sources in potato has made breeding for resistance difficult (Barker et al. 1994, Jansky 2000, Szewczynski et al. 1990). A major QTL (PLRV.1) for PLRV resistance was identified in diploid populations containing S. chacoense, S. sanguinea, and S. tuberosum germplasm and contributes 60% of the variance (Szewczynski et al. 2001). A second QTL (PLRV.4) was also identified on the same chromosome (Marczewski et al. 2004). There are some monogenic sources of resistance to PLRV (Barker and Solomon 1990, Brown and Thomas 1993). For example, breeding programs have recently included monogenic PLRV resistance derived from S. etuberosum Lindl., which is a non-tuberizing wild species of potato (Kelley et al. 2003). In susceptible potato varieties, there are reductions in seed yields, numbers of stems per plant, and in the size and number of marketable tubers (Hamm 1999, Harper et al. 1975).
Mechanistically transmitted potexvirus and carlaviruses: PVX, PVM and PVS

PVX is the type member of the Potexvirus genus, in contrast, PVS and PVM are little studied members of the Carlavirus genus; all are members of the family Flexiviridae (Martelli et al. 2009). Potexviruses and carlaviruses have monopartite genomes of roughly 8.5 to 9.5 kilobases. Both PVX and PVS isolates of genes is similar with the viral replicase located near the 5’ end of the genome, followed by the movement and coat protein genes. Two important features unique to carlaviruses are the genes encoding papain-like cysteine protease and additional nucleic-acid-binding proteins which are located at the 3’ end of the carlavirus genome (Adams et al. 2004). The genomes of both viruses are packaged into flexuous rod shaped particles.

Both PVX and PVS occur wherever potatoes are grown, however the common strains rarely cause symptoms on potato (Pourrahim et al. 2007, Salazar 2006). There are numerous strains of PVX and these are classified into four groups according to their reactions with dominant resistance genes Nb and Ns that are directly involved in strain-specific recognition of PVX in cultivated potato varieties (Valkonen et al. 1994). Thus PVX strains are defined based on the classic gene-for-gene resistance response. Group 1 strains (Roth1, XS, P551, NL1 and Scot10) cause HR in the presence of Nb or Ns, group 2 strains (EX, DY, CP2 and WS2) cause HR in the presence of Nb, group 3 strains (UK3, S, X3, S6111, XA, CPG, KP, CP and CT23) cause HR with Ns, and group 4 strains (HB and CP4) react with neither Nb or Ns (Malcutti et al. 2000, Valkonen et al. 1994). The viral coat protein is the determinant for resistance mediated by Nb and Rx (Bendahmane et al. 1995, Santa Cruz and Baulcombe 1995) while the TGBp1 protein is responsible for Nb mediated resistance (Malcutti et al. 1999). Unlike the PVY strains, recombination is not the primary mechanism for evolution of PVX strains (Malcutti et al. 2000). While recombination can be used to explain strains that have acquired Nb and Ns virulence determinants, phylogenetic analysis indicates that the virulence determinant located in TGBp1 and CP coding sequences reflect multiple acquisitions or even losses (Malcutti et al. 2000). PVX is only transmitted mechanically and typically causes a mild mosaic disease on the foliage (Franc and Battarri 1984, Franc and Battarri 2001). When PVX and PVS occur in the same plant they act synergistically, and cause “rugous mosaic disease on the foliage (Franc and Banttari 1984, Franc and Battarri 1984). PVS is considered to be mainly transmitted mechanically (Franc and Banttari 2001, Lambert et al. 2007), but there are some strains that are transmitted non-persistently by aphids (Wardrop et al. 1989). The role and mechanisms for aphid transmission require further investigation. PVX symptoms range from mild to severe mosaic on the foliage, but to date no potato virus symptoms. PVX occasionally causes 20% yield reductions.

The widespread use of vegetative propagation through tissue culture (Fig. 1) has minimized the presence of PVX and PVS in North American potato production. Since PVX-free plantlets serve as the basis for potato production, and the few early generation seed potato farms in Canada and the US are free of PVX, this virus is hard to find in seed potatoes. However, PVX still occurs on operations that do not routinely plant certified seed. Potato varieties are initiated into tissue culture and produce plantlets that are first tested for common viruses, and then treated with heat and anti-viral chemicals to eliminate any viruses that may be present (Zapata et al. 1995). Heating micro-plants to 42°C alongside treatment with salicylic acid for 4 weeks leads to high survival of PVX-free plants (Lopez-Delgado et al. 2000). Compounds like cysteine protease and additional nucleic-acid-binding proteins are useful for combating both PVX and PVS (Conrad 1991). Of the common viruses, PVS is the most difficult to eradicate from potato tissue culture plantlets and the basis for this remains unknown. In fact, the primary method for PVSV control has been to ensure that potato tissue culture plantlets are PVS-free. Therefore, this is seen as the critical step for propagating PVX-free potatoes for seed certification. The titers of another carlavivirus, Potato virus M (PVM), decline over time in tissue culture plantlets and this virus is typically eliminated in the absence of heat or antiviral compounds. Thus, the difficulties of eradicating PVX cannot be generalized among all carlaviruses.

The Nb gene, derived from S. tuberosum ssp. andigena, confers hypersensitive resistance to PVX and sources of the Nb gene have been used in European breeding programs (Marczewski et al. 1998). Potato plants containing the Nb gene are symptomless after inoculation with the virus. A SCAR marker associated with Nb has been mapped to a region on chromosome VIII that does not correlate with resistance to any other pathogens and thereby extends the coverage of virus R genes within the potato genome (Gebhardt and Valkonen 2001, Marczewski et al. 2002, Szajko et al. 2008).

Extreme resistance to PVX is conferred by Rx1 (Bendahmane et al. 1997, van der Voort et al. 1999), Rx1, and Rx2 (also known as Rx1 from S. acaule) (Ritter et al. 1991). While both Rx1 and Rx2 are found on chromosome XII, they are considered different genes (Bendahmane et al. 1997). The Rx2 gene is located on chromosome V (Ritter et al. 1991). Both Rx1 and Rx2 are linked to the Gpa2 and Gpa3 genes, respectively for resistance to Globodera pallida (van der Voort et al. 1999), suggesting an ancestral duplication of the loci on both chromosomes. To date, Rx1d and Rx2 are the only potato virus resistance genes that have been cloned (Bendahmane et al. 1999, Bendahmane et al. 2000). Both genes encode CC-NB-RR proteins that exhibit the same specificity for the PVX coat protein (Bendahmane et al. 1995, Querci et al. 1995). Further molecular experiments using Rx2 showed that it physically interacts with a Ran-GTPase-activating protein (RanGAP2), which is also required for FR1 and FR2 resistance to PVX (Sacco et al. 2007, Tameling and Baulcombe 2007).

Hypersensitive resistance to PVX has also been identified. The dominant Nb gene maps near the Rx2 gene on chromosome V (De Jong et al. 1997b) and recognizes the 25-kDa PVX movement protein to elicitor resistance (Malcutti et al. 1999). The Nxb gene derived from S. phureja is found on chromosome IX near the Ns-1 and Nxb genes for PVY resistance (Tomniska et al. 1997). PVX HR genes such as Nxb, Nxb, and Nbs are available in many cultivars.
of potato and the $N_{xh}$ gene has provided useful resistance for many years (Barker and Dale 2006).

**Soil-borne Tobaviruses and Pomoviruses: TRV and PMTV**

TRV is the type member of the Tobavirius genus and PMTV is the type member of the Pomovirius genus. Both viruses have a plus-strand genome, packaged into rod-shaped particles. TRV rarely causes foliar symptoms in potato, even in plants with severe tuber symptoms. Foliar symptoms of PMTV develop when plants are grown from infected tubers and only occur when plants are grown at temperatures below 20°C (Carnegie et al. 2010). The most common symptom of PMTV is the development of obscure patterns on the stems which consist of bright yellow blotches and ring or line patterns on lower or middle leaves. A less common secondary symptom consists of pale, V-shaped, chlorotic chevrons, usually on the leaflets of young upper leaves, and ultimately resulting in a distinct mosaic in the upper leaves. A third type of symptom consists of extreme shortening of internodes accompanied by crowding or bunching of foliage, described as a “mop-top”. Some of the smaller leaves may have wavy or rolled margins and the overall effect is a dwarfed and bunched growth habit.

TRV is spread by stubby root nematodes (*Trichodorus* and *Paratrichodorus* species), which are widespread (Van Hoof 1968). Both the virus and the vector have very wide host ranges, thus once a field is infested with viruliferous nematodes, it is impossible to eliminate the virus from the field. The virus is controlled by planting resistant cultivars and treating fields with nematicides prior to or at planting (Ingham et al. 2007, Ingham et al. 2000, Weingartner et al. 1983). Treatment after planting is not effective and will not greatly impact virus spread. The stubby root nematode is often overlooked during routine nematode sampling, due to both its shape, which results in it being discarded during typical nematode extraction procedures, and its migratory nature in the soil. These nematodes stay just above the water line in the soil, so may be below the depth of the sampling probe. Thus, unlike with other nematodes, growers typically do not have information on the incidence of stubby root nematodes in their fields.

PMTV is vectored by the pathogen that causes powdery scab disease in potatoes, *Spongospora subterranea* f. sp. *subterranea*, which is present throughout the world (Kirk 2008). Temperature affects the success of transmission with greatest success at 12 to 20°C and little or no infection above 25°C (Carnegie et al. 2010). PMTV is not transmissible by aphids or other vectors, but may be transmitted to some hosts by grafting or mechanical inoculations. PMTV is retained in *S. subterranea* spore balls, which are stable for many years in soil (Kirk 2008). The degree of transmission through seed tubers is variable, and in the absence of the vector, plants may become free of the virus after a few generations. However, since the vector is typically tuber-borne, this is not a common situation. TRV and PMTV are distinct together, not only because they are soil-borne, but also because both viruses cause severe necrosis in tubers, known as spraing or corky ringspot. Necrotic arcs or brown spots are evident in tuber flesh that dries into cork-like tissue (Fig. 2D). The yield losses caused by these viruses are generally minor, but quality losses due to spraing can cause rejection of entire fields from supermarkets or processors. There are extreme situations where potato plants are infected with TRV with no apparent foliar symptoms but severe tuber symptoms. Thus, growers are surprised by the near total losses at harvest of a crop that appeared healthy. For both TRV and PMTV, the storage temperature and its fluctuation are important factors contributing to symptom expression in tubers. More symptoms occur with wound healing at 18°C followed by storage at 8°C than when wound healing occurs at 25°C (Ryden et al. 1994; Molgaard and Nielsen 1996). A colder storage temperature, 4°C, is preferable for seed potatoes to inhibit development of PMTV related spraying symptoms (Sandgren 1995). Tuber maturity also affects symptom development, with mature tubers being more susceptible (Molgaard and Nielsen 1996). Therefore storage regimes are designed for diagnosis of spraying and repeated cycles of temperature shifts are used as a screening technique.

TRV is frequently grouped with PMTV in European countries for regulatory efforts, as the tuber symptoms appear similar. PMTV was first reported in North America in shipments of potatoes that were being sent from the US to Canada and screened by the Canadian Food Inspection Agency in 2004 (Xu et al. 2004). Currently TRV and PMTV are covered by the necrotic virus management plan. In this plan, seed produced for commercial production can be shipped between the USA and Canada but federal inspectors must provide quality assurance that seed with less than 0.5% incidence of necrotic tuber Zoospores can be used as seed potatoes (USDA 2004). Federal inspectors provide recertification of material moving from a production State or Canada. The difficulty lies in assessing whether tuber necrosis is due to a viral pathogen, and this relies on improved testing methodologies and adequate training of inspectors to identify the causes of internal necrosis (USDA 2004). Unfortunately, the distribution of TRV was thought to be limited in the USA to a few production areas in the western part of the country, the southeastern USA, and sporadically throughout the rest of the USA and Canada (Gieck et al. 2007). However, it has recently been reported in new areas in North Dakota, Michigan, Minnesota, and Wisconsin, and it appears to be present on seed potato farms, which is facilitating the spread of this virus (Gudmestad et al. 2008; Crosslin et al. 2010; David et al. 2010). The recent finds were due to severe outbreaks that caused total crop losses, highlighting the importance of this emerging problem. Thus, we can expect TRV and PMTV to continue to spread across North America.

Detection of viruses in soil is complicated both by the soil matrix, the uneven distribution of viruliferous nematodes and *S. subterranea* in soil, and the migratory nature of the stubby root nematode that vectors TRV. The lack of foliar symptoms of TRV and during primary infection of PMTV makes it difficult to control this disease through seed certification inspections. In addition, TRV RNA-1 is sometimes transmitted without RNA-2, resulting in a type of infection termed ‘non-multiplying’ since TRV RNA-1 can replicate in plants but cannot be spread by nematodes in the absence of RNA-2. Non-multiplying infections cause problems for virus identification since serological assays, which rely on the detection of coat protein, fail to detect TRV in these infections. Traditional RTPCR assays are a more reliable method for detection of TRV (Crosslin et al. 1999, Xu and Nie 2006). In contrast, some researchers have reported that ELISA is preferable for PMTV detection (Sokmen et al. 1998). RT-PCR assays with fluorescent probes have increased the sensitivity of virus detection making accurate diagnosis easier (Mumford et al. 2000). Baiting methods, which trap the PMTV vector *S. subterranea*, combined with RT-PCR, have also proven effective in detection of this virus (Nakayama et al. 2010). RT-PCR can also successfully detect TRV in its nematode vectors (Boutsika et al. 2004; Riga et al. 2009).

Resistance to TRV corky ringspot (CRS) disease is available in some cultivated varieties of potato (Brown et al. 2009, Brown et al. 2000, Dale et al. 2004, Dale et al. 2000, Harrison 1968, Richardson 1970, Shumaker et al. 1984, Weingartner and McSorley 1994). Parental materials containing resistance to CRS are fairly common choices for breeding materials as about 20% of all varieties contain some level of resistance (Brown et al. 2009). Harrison (1968) first described CRS resistance in British varieties and ‘Bintje’. Since then, resistance has been identified in varieties from Europe and North America, including ‘Malta’ and ‘Bintje’ (Swiezynski et al. 1998), Poland, including ‘Jantar’ (Brown et al. 2000), and New Zealand, including ‘Fianna’ and ‘Karaka’ (Brown et al. 2009). Breeding for
dependence on imported seed-potatoes. This transition to producers to sell high quality seed-potatoes and reduce their ability to detect viruses in seed-potato production has enabled local producers to increase their profitability. A reported 12-year study conducted in Brazil showed that the availability and popularization of ELISA services to screen for PVY infection has been one of the main reasons for establishment of federal seed potato certification standards. In North America, seed potato certification is based mainly on visual inspection of growing plants since it has historically been effective and the benefit of large-scale laboratory testing for pathogens is not worth the added cost. Thus, varieties that did not show easily recognized symptoms of important pathogens were discouraged (de Souza-Dias and Betti 2003). However, the evolution of new PVY strains (discussed previously) that elicit few symptoms regardless of the host genotype makes roguing for infected plants difficult. Therefore, certification agencies are now forced to expend time and funds towards screening for PVY infection using biochemical methods, such as RT-PCR (Trank 1991).

In many countries, vegetative propagation and seed potato certification programs (Fig. 1) have reduced or eliminated many significant tuber-borne pathogens, such as PVX and PLRV (Gudmestad 1991, Trank 1991). For example, a reported 12-year study conducted in Brazil showed that the availability and popularization of ELISA services to detect viruses in seed-potato production has enabled local producers to sell high quality seed-potatoes and reduce their dependence on imported seed-potatoes. This transition to local production has improved the private sector economy, with limited host ranges, are only spread by potato colonizing aphids or mechanically, and do not survive in soil; thus, they are effectively dealt with by eliminating seed potato lots carrying these pathogens and by effective insecticides (Johnson 2008). In contrast, the recent emergence of soil-borne viruses has been a challenge for seed potato certification programs (Johnson 2008). TRV and PMTV are not yet present in all production fields, but are widespread enough that there is less interest by the potato industry to impose quarantines or enforce geographic information system mapping of contaminated fields. Therefore, we predict that these soil-borne viruses are likely to become significant problems over the next few decades. Generally, seed potatoes are divided into two main classes, one is considered suitable for planting on farms that raise seed potatoes and the other is suitable for planting on farms that raise potatoes for table stock or processing (Gagnon et al. 2007). PVY incidence in seed lots is one of the main characteristics used to determine which class a lot of seed potatoes falls into; it is the most common reason for down-grading seed potato lots in North America. For example, in Wisconsin, PVY is the only virus that has caused seed potatoes to be down-graded in class for at least the past decade (Genger and Charkowski 2007). The names used to describe the different classes of seed, subcategories in each class, and virus incidence thresholds for each seed class vary by seed potato certification agency. There is a zero tolerance for a virus in tissue culture and in greenhouse and this is insured by visual inspection and mandatory laboratory testing for growers of certified seed potatoes (Fig. 1). A virus incidence of 0.5 to 2% is allowed for lots destined for use on a seed potato farm. Seed potato lots with

The role of seed potato certification in potato virus control

The history and progress of seed potato certification has been reviewed several times over the past decades (Callison et al. 1982, Gudmestad 1991, Leach 1938, Slack 1993, Trank 1991). Seed potato certification programs were first established in Europe and the idea was imported into North America in 1913. At the time, variety purity and the use of fewer well-characterized varieties were considered the primary goals, although third party inspection of seed potato quality and disease thresholds was also seen as important. Attempts, generally led by the customers of seed potato growers, have been made to federalize seed potato certification in the US since the 1920s. As a result, state-based potato grower associations were formed prior to 1913 in many regions, and these associations were responsible for setting up seed certification schemes. Currently, each state has different regulations and the certification agencies are managed by state departments of agriculture, universities, or grower groups, depending on the state. Today, federal standards are in place, but the agencies remain local. Along with the desire to ease export of seed potatoes, the effort to control necrotic strains of PVY was one of the main reasons for establishment of federal seed potato certification standards (Trank 1991). In North America, seed potato certification is based mainly on visual inspection of growing plants since it has historically been effective and the benefit of large-scale laboratory testing for pathogens is not worth the added cost. Thus, varieties that did not show easily recognized symptoms of important pathogens were discouraged (de Souza-Dias and Betti 2003). However, the evolution of new PVY strains (discussed previously) that elicit few symptoms regardless of the host genotype makes roguing for infected plants difficult. Therefore, certification agencies are now forced to expend time and funds towards screening for PVY infection using biochemical methods, such as RT-PCR (Trank 1991).

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Fig. 2 Images of PVY- and TRV-infected potato plants. (A) Three branches of ‘Red Nordland’ variety shows a typical healthy set of leaves at the top. (B) The ‘Dark Red Nordland’ shows mild PVY symptoms. The top of the plant shows symptoms and the bottom is healthy. (C) Typical PVY-infected ‘Goldrush Russet’ variety shows necrosis on the foliage. The top has mosaic symptoms. (D) TRV-infected potato tubers. The sprouting is rarely found in arcs and concentric circles as often reported. The inset shows tuber distortions that are common among TRV-infected potatoes.

local production has improved the private sector economy, have limited host ranges, are only spread by potato colonizing aphids or mechanically, and do not survive in soil; thus, they are effectively dealt with by eliminating seed potato lots carrying these pathogens and by effective insecticides (Johnson 2008). In contrast, the recent emergence of soil-borne viruses has been a challenge for seed potato certification programs (Johnson 2008). TRV and PMTV are not yet present in all production fields, but are widespread enough that there is less interest by the potato industry to impose quarantines or enforce geographic information system mapping of contaminated fields. Therefore, we predict that these soil-borne viruses are likely to become significant problems over the next few decades. Generally, seed potatoes are divided into two main classes, one is considered suitable for planting on farms that raise seed potatoes and the other is suitable for planting on farms that raise potatoes for table stock or processing (Gagnon et al. 2007). PVY incidence in seed lots is one of the main characteristics used to determine which class a lot of seed potatoes falls into; it is the most common reason for down-grading seed potato lots in North America. For example, in Wisconsin, PVY is the only virus that has caused seed potatoes to be down-graded in class for at least the past decade (Genger and Charkowski 2007). The names used to describe the different classes of seed, subcategories in each class, and virus incidence thresholds for each seed class vary by seed potato certification agency. There is a zero tolerance for a virus in tissue culture and in greenhouses and this is insured by visual inspection and mandatory laboratory testing for growers of certified seed potatoes (Fig. 1). A virus incidence of 0.5 to 2% is allowed for lots destined for use on a seed potato farm. Seed potato lots with
virus incidence of more than 5 to 10% are considered to have too high of an incidence for use as planting stock on any farm (Genger and Charkowski 2007). Currently, these percentages are based mainly on visual inspection, not laboratory testing. The cost of extensive laboratory testing, while providing more accurate data, is prohibitively expensive.

CONCLUSIONS

Potato viruses have been intensively studied due to the significance of the host as a food crop and the effect that the diseases can have on yield and marketability. Great efforts have been made to integrate genetic resistance into cultivated varieties and stop virus spread through the use of certified seed. The biology and transmission of the majority of the major viruses continues to be well understood and detection methods, while sometimes expensive, are efficient at detecting even minute amounts of the pathogen. While detection, certification, and breeding efforts have limited the spread of viral diseases in potato in many cases, new strains of existing viruses and new viruses altogether continue to cause problems in developed agricultural systems. In these cases, controlling the insect, nematode, or protozoan vectors becomes critical. In developing countries, the adoption of reliable seed certification systems can have a dramatic effect on potato yield. In both cases, a better understanding of host resistance mechanisms and the incorporation of resistance into cultivated varieties using traditional breeding or biotechnological approaches provides the best long-term strategy for combating viral diseases.

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