

Distribution and diversity of *Potato
mop-top virus* in Sweden

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Cover: Spraing disease on potato tubers
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Abstract

During the last decades, spraing disease caused by *Potato mop-top virus* (PMTV) has become a severe problem in the potato production throughout the Nordic countries. In this thesis, it was shown that PMTV is widely distributed throughout Sweden, Norway and Denmark, and has now become prevalent in the potato-production areas within these countries. In Sweden, PMTV was found to be present further north than observed in an earlier study. Compared to the Nordic countries, the occurrence of PMTV in Poland, the Baltic countries and northwestern Russia is still very limited. Molecular characterization and phylogenetic relationship analyses were carried out for 43 new PMTV sequences originating from potato tubers and field soil from Sweden, Denmark and the USA. The results showed a limited diversity of PMTV based on RNA-CP and RNA-TGB. No strict geographical grouping was obtained for isolates from Europe, Asia and North America indicating that PMTV has spread through infected seed tubers. Weeds, as alternative hosts of plants viruses and their vectors, are an important factor in the epidemiology of plant virus diseases. PMTV was detected in the roots of the weed *Chenopodium album*, which had been naturally infected with the virus in the field. *C. album* may together with weeds of the family Solanaceae be an important factor for maintaining PMTV in a field. Sequence analysis revealed a close relationship between the PMTV isolates from *C. album* and other characterised isolates suggesting that the same genotypes of PMTV are infecting potato and *C. album*.

The presence in Sweden of three other potato-infecting viruses associated with necrotic symptoms in tubers was demonstrated: *Tobacco rattle virus* (TRV), *Tobacco necrosis virus* (TNV) and *Potato virus Y* (PVY). TRV has become prevalent in potato fields located from southern and up to the central part of Sweden. Analyses of tubers with typical necrotic symptoms of the ABC disease confirmed infection with TNV. In addition, two different necrotic strains of PVY, PVY^{NTN} and PVY^{NW}, were detected in symptomatic potatoes. The presence of TNV and PVY^{NTN}/PVY^{NW} in potato plants was to our knowledge confirmed for the first time in Sweden.

Keywords: ABC disease, *Chenopodium album*, genetic variability, phylogeny, *Potato mop-top virus*, *Potato virus Y*, *Tobacco necrosis virus*, *Tobacco rattle virus*, spraing disease, weed

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Für Steffen

"Nature does nothing in vain".

Aristotle (384 BC – 322 BC)

Contents

List of Publications	7
Abbreviations	9
1 Introduction	11
1.1 Potato	11
1.1.1 Taxonomy	11
1.1.2 History	12
1.1.3 Potato production and seed classification in Sweden	13
1.1.4 Diseases in potato	14
1.2 <i>Potato mop-top virus</i>	15
1.2.1 Distribution	15
1.2.2 Symptoms	15
1.2.3 Transmission	17
1.2.4 Host	18
1.2.5 Genome	19
1.2.6 Diversity	21
1.3 Other viruses causing necrotic symptoms in potatoes	21
1.4 Control of PMTV	22
2 Aims of the study	25
3 Results and discussion	27
3.1 Distribution of PMTV in the Baltic Sea region (paper I+II)	27
3.2 Incidence of symptomless potato tubers infected with PMTV (paper I+II)	28
3.3 Incidence of PMTV and TRV in tubers with spraing (paper I+II)	28
3.4 Presence of TRV and TNV in Sweden (paper I)	29
3.5 Detection of necrotic strains of PVY in potato (unpublished)	30
3.6 Diversity and evolution of PMTV (paper III)	31
3.7 Detection of PMTV in the weed <i>C. album</i> (paper IV)	33
3.8 Genetic diversity of PMTV isolates collected from <i>C. album</i> (paper IV)	34
4 Conclusion	35
5 Future perspectives	37
References	39

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Santala J., Samuilova O., Hannukkala A., Latvala S., Kortemaa H., **Beuch U.**, Kvarnheden A., Persson P., Topp K., Ørstad K., Spetz C., Nielsen S.L., Kirk H.G., Budziszewska M., Wieczorek P., Obrepalska-Stepłowska A., Pospieszny H., Kryszczuk A., Sztangret-Wiśniewska J., Yin Z., Chrzanowska M., Zimnoch-Guzowska E., Jackeviciene E., Taluntyte L., Pupola N., Mihailova J., Lielmane I., Järvekülg, L., Kotkas K., Rogozina E., Sozonov A., Tikhonovich I., Horn P., Broer I., Kuusiene S., Staniulis J., Uth J.G., Adam G., Valkonen, J. P. T (2010). Detection, distribution and control of *Potato mop-top virus*, a soil-borne virus, in northern Europe. *Annals of Applied Biology* 157(2), 163-178.
- II **Beuch U.**, Persson P., Edin E., Kvarnheden A. (2013). Necrotic diseases caused by viruses in Swedish potato tubers. *Plant Pathology*, in press.
- III **Beuch U.**, Berlin, S., Nicolaisen M., Nielsen S.L., Åkerblom J., Crosslin J., Hamm P.B, Santala J., Valkonen J.P.T., Kvarnheden A. Diversity and evolution of *Potato mop-top virus*. (Manuscript)
- IV **Beuch U.**, Mueller K., Kvarnheden A. Detection of *Potato mop-top virus* in the weed *Chenopodium album*. (Manuscript)

Papers I and II are reproduced with the permission of the publisher.

The contribution of Ulrike Beuch to the papers included in this thesis was as follows:

- I Participated in planning the experiments and carried out a part of the experimental work.
- II Participated in planning the experiments and carried out most of the experimental work. Summarized the results and wrote the manuscript.
- III Participated in planning the experiments and carried out most of the experimental work. Analysed and summarized results for the diversity study and wrote the first draft of the manuscript.
- IV Planned the experiments and participated in the sampling and in the experimental work. Analysed and summarized all the results and wrote the manuscript.

Abbreviations

AA	Amino Acid
BBNV	<i>Broad bean necrosis virus</i>
BNYVV	<i>Beet necrotic yellow vein virus</i>
BSBV	<i>Beet soil-borne virus</i>
BVQ	<i>Beet virus Q</i>
CP	Coat protein
Hel	Helicase
kDa	Kilo dalton (atomic mass unit)
MetT	Methyltransferase
PLRV	<i>Potato leaf roll virus</i>
PMTV	<i>Potato mop-top virus</i>
Pol	Polymerase
PTNRD	Potato tuber necrotic ringspot disease
PVY	<i>Potato virus Y</i>
RNA	Ribonucleic acid
RT	Readthrough
SBWMV	<i>Soil-borne wheat mosaic virus</i>
Sss	<i>Spongospora subterranea</i> f.sp. <i>subterranea</i>
TGB	Triple gene block
TNV	<i>Tobacco necrosis virus</i>
TRV	<i>Tobacco rattle virus</i>
UTR	Untranslated region
vRNP	Viral ribonucleoprotein

1 Introduction

1.1 Potato

Today, agriculture feeds more than 6 billion people worldwide. During the last decades, crop production and yields have expanded excessively due to irrigation, fertilization, progress in plant breeding, prophylactic measures, genetic engineering, and plant protection approaches such as fungicides and pesticides. Worldwide, the cultivated potato (*Solanum tuberosum*) is one of the most important food crops grown and consumed after rice, wheat and maize; with a production of 373 million tons in 2011 (faostat.fao.org). In many developing countries and in the industrialized world, potato is a basic food and source of primary income (Ovchinnikova *et al.*, 2011). Because of its fundamental importance as a staple food of humans, United Nations Food and Agriculture Organization (FAO) declared 2008 as the International Year of the potato.

1.1.1 Taxonomy

Taxonomically, potato is a member of the family Solanaceae – or “nightshade”-family of flowering plants; one of the largest and most diverse families with about 90 genera and more than 3000 species (Knapp *et al.*, 2004). Potato belongs to the genus *Solanum*, which is with around 1,500 species the largest genus within the family Solanaceae, and the genus also includes tomato (*S. lycopersicum*), eggplant (*S. melongena*) and woody nightshade (*S. dulcamara*) (Knapp, 2008). *S. tuberosum* is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum*, adapted to lower altitudes and longer day length, is the cultivated potato widely used in, for example, North America and Europe. The subspecies *andigena*, which is restricted to Central and South America, prefers cultivation at high altitudes and short days for tuberization (Roa *et al.*, 2010; Gopal *et al.*, 2013).

Botanically, the potato plant is an annual dicotyledonous herb that produces tubers: a swollen piece of underground stem thickened into a storage organ for starch (Haverkort *et al.*, 1990).

1.1.2 History

Two existing hypotheses propose the origin of the European potato: from lowland Chile or from the Andes in a region from western Venezuela to northern Argentina (Ovchinnikova *et al.*, 2011). More than 200 species of wild potato are found in South and Central America, whereas most of them are restricted to Peru (Hijmans & Spooner, 2001). The use of potato (*S. tuberosum* subsp. *andigena*) as a food source in South America traces back to several thousand years BC when Peruvian colonists started the cultivation of wild potato (Salaman, 1985). Archaeological documentation has reported the cultivation of potato from the Proto-Chimú and Proto-Nazca periods (200 AD) through to the Chimú period (800-1100 AD). During the Inca periods (1100-1500 AD), the potato became an important staple crop of the agricultural system with more than one hundred varieties (Salaman, 1985).

The first European contact with the potato was recorded in 1537 in the Magdalena valley, Colombia when Spanish invaders discovered the crop (Simmonds, 1976). In the early 16th century, the potato was probably introduced to Europe with Francisco Pizarro's return to Spain after his conquest of the Inca Empire. From Spain, potatoes quickly spread in Europe. In the beginning, the use of potato as a food source started slowly since Europeans were skeptical due to religious superstitions. The production was more used as feed for livestock before the potato became an important source for human diet.

Many believe that Jonas Alströmer (1685-1761) introduced the potato from France into Sweden in 1724, and established potato as an acceptable crop among Swedish farmers and consumers. However, Professor Olof Rudbeck (1630-1702) planted potatoes already in 1658 in the Hortus Botanicus at Uppsala University, as cited in his *Catalogus plantarum*. During the 1800s, potato became popular as a source for alcohol production and replaced cereals, which were more required for bread and other food products. The increased production of potato spirits escalated to enormous alcohol problems in the population but on the other hand increased the knowledge about potato as a food crop (potatisakademien.se). In 1753, the Swedish botanist and zoologist Carl Linnaeus (1707-1778) referred to cultivated potato as a single species, *S. tuberosum*, in his book *Species Plantarum*, a work which is now internationally accepted as the starting point of modern botanical nomenclature (Ovchinnikova

et al., 2011): “You say tomato, I say *Lycopersicon esculentum*. You say potato, I say *Solanum tuberosum*”.

1.1.3 Potato production and seed classification in Sweden

Today in Sweden, potato is mainly used for human consumption and for starch production. With 37.000 hectares of growing area, potato is the fifth largest cultivated crop in Swedish agriculture after wheat (424.000 ha), barley (343.00 ha), oats (190.00 ha) and rapeseed (96.000 ha) (scb.se). In 2012, the harvest of table potatoes and potatoes for starch production was estimated at 552 400 and 255 800 tonnes, respectively (scb.se). The potato cropping area is mainly located in the south with more than 50% of the annual production (Fig. 1).



Figure 1. Map of total table potato production in Sweden in 2012 according to the statistical survey conducted by the Swedish Board of Agriculture and Statistics Sweden (scb.se).

Today in Sweden, the most cultivated potato varieties are King Edward, Bintje and Asterix (scb.se). King Edward and Bintje are very old varieties,

introduced into Sweden during the 1940s, and they are preferred by consumers for their cooking and flavour characters. However, both varieties are known for their susceptibility to diseases, such as wart and late blight for cv. Bintje (Umarus, 1960; Leijerstam, 1986), and potato cyst nematode, late blight and spraing for King Edward (Whitehead *et al.*, 1980; Olofsson, 1991; Ryden *et al.*, 1994).

Seed potato production and import to Sweden are required to implement certain regulations by the Swedish seed certification program, which is a part of the Swedish Board of Agriculture. The Swedish regulations for seed potato follow the EC Council Directives 93/17/EEC, 2000/29/EEC and 2002/56/EC, which regulate the marketing of seed potatoes in the EU. All seed potatoes grown in Sweden originate from *in-vitro* microplants (Nuclear stock), which are extensively tested for a number of domestic and non-domestic pathogens (regulation: SJVFS 1995:90 & SJVFS 1995:94) to ensure a high level of plant health. Swedish seed potatoes are classified into several categories (SS, S1-3, SE1-2, E, A). Seed crops grown in the field are inspected during the growing season and there are strict levels for certain bacterial and virus diseases (SJVFS 1995:90, appendix 2). All seed potatoes imported to Sweden from the EU have to be certified according to the Swedish seed certification program.

1.1.4 Diseases in potato

Potato can be affected by a wide range of pathogenic organisms, including bacteria, fungi, oomycetes, nematodes, viroids and viruses, which cause reductions in yield and quality. Apart from the oomycete *Phytophthora infestans*, the causal agent of late blight disease, viruses are the most important parasites in cultivated potatoes with over 40 viruses and virus-like pathogens (Ahmadvand *et al.*, 2012). Most plant viruses depend on a vector for transmission between plants (Gutierrez *et al.*, 2013). For potato viruses, two main routes of vector transmission occur: aphids (e.g., *Potato virus Y* (PVY), *Potato leaf roll virus* (PLRV)) (Delgado-Sanchez & Grogan, 1970; Harrison, 1984) and soil-borne (e.g., *Potato mop-top virus* (PMTV), *Tobacco rattle virus* (TRV)) (Calvert, 1968).

Over 50 viruses belonging to 12 genera have been identified as soil-borne viruses, transmitted either by nematodes, fungi or protists (Verchot-Lubicz, 2003). Virus-transmitting protists belong to the order *Plasmodiophorales* or *Chytridiales* and are zoosporic parasites of plant roots. Only three of approximately 35 species of the order *Plasmodiophorales* are viral vectors: *Polymyxa betae*, *Polymyxa graminis*, and *Spongospora subterranea* (Verchot-Lubicz, 2003).

Two subspecies of *S. subterranea* are known to be plant parasites and virus vectors: *S. subterranea* f.sp. *nasturtii* (Walsh & Clay, 1993) and *S. subterranea* f.sp. *subterranea*, the causal agent of powdery scab disease on potato and vector for PMTV (Arif *et al.*, 1995)

1.2 *Potato mop-top virus*

1.2.1 Distribution

PMTV occurs in many potato-production areas of the world. The centre of origin of PMTV is probably the Andean region of South America (Mayo *et al.*, 1996) where it has been found in Peru (Hinostroza & French, 1972; Salazar & Jones, 1975; Tenorio *et al.*, 2006), Bolivia (Jones, 1975), Venezuela (Ortega & Leopardi, 1989) and recently in Colombia (Gil *et al.*, 2011; Osorio-Giraldo *et al.*, 2013). PMTV has also been detected in Costa Rica, Central America (Vásquez *et al.*, 2006; Montero-Astúa *et al.*, 2008) and it has been found in many potato areas of North America, including both the USA and Canada (Lambert *et al.*, 2003; Xu *et al.*, 2004; David *et al.*, 2010; Crosslin, 2011; Whitworth & Crosslin, 2013)

In Asia, PMTV was first found in Hiroshima in Japan (Imoto *et al.*, 1981) and it is now widely distributed throughout the country (Nakayama *et al.*, 2010; Maoka *et al.*, 2011). Recently, the presence of PMTV has also been reported in China (Hu *et al.*, 2013).

In Europe, PMTV was first described from Scotland and Northern Ireland (Calvert & Harrison, 1966). In the following years, the virus was reported in several European countries including the Netherlands (van Hoof & Rozendaal, 1969), Ireland (Fuxe, 1980), Czech Republic (Novak *et al.*, 1983), Switzerland (Schwärzel, 2002) and recently in Latvia (Latvala-Kilby *et al.*, 2009) as well as Poland (Budziszewska *et al.*, 2010).

The Nordic countries Denmark, Finland, Norway and Sweden have been affected by PMTV for the last 30 to 40 years (Björnstad, 1969; Ryden *et al.*, 1986; Kurppa, 1989; Sandgren, 1995; Molgaard & Nielsen, 1996; Nielsen & Mølgaard, 1997; Germundsson *et al.*, 2000; Buundgard *et al.*, 2007; Latvala-Kilby *et al.*, 2009) and the virus can cause severe economical problems in the potato production within these countries.

1.2.2 Symptoms

Primary infections with PMTV can induce slightly raised necrotic arcs and rings on the surface of potato tubers and/or internal brown arcs and rings in the

tuber flesh, described as spraing disease (Calvert & Harrison, 1966; Calvert, 1968; Harrison & Jones, 1971; Kurppa, 1989) (Fig. 2). In the Nordic countries, spraing disease outbreaks have resulted in severe quality problems in staple potatoes and potatoes produced for food industry such as French fries and chips (crisps) (Sandgren, 1995; Nielsen & Mølgaard, 1997; Latvala-Kilby *et al.*, 2009).

The development of internal and external spraing symptoms in susceptible cultivars is rarely observed at harvest, but usually appears during storage as a result of fluctuations in temperature. A significant increase in spraing symptoms after harvest by alternating the storage temperature between 13-18°C and 5-8°C at 1-2 week intervals has been reported by Mølgaard and Nielsen (1996), Sandgren (1995), Kurppa (1989) and Harrison and Jones (1971) showing the importance of the physiological stage of tubers and environmental conditions for symptom development.

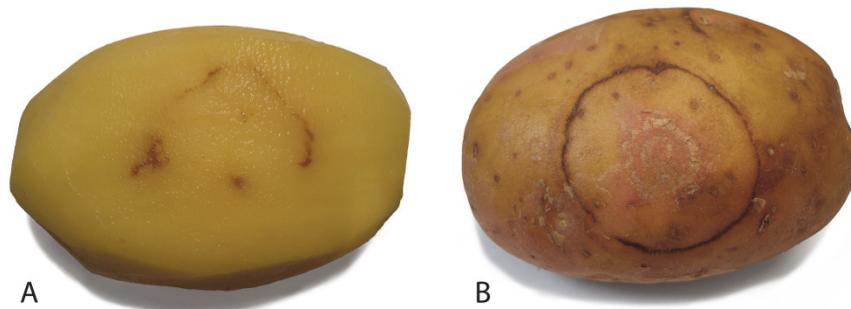


Figure 2. Internal (A) and external (B) spraing symptoms in/on potato caused by infection with *Potato mop-top virus* (PMTV).

However, the incidence of spraing symptoms can vary to a high degree from year to year within the same cultivar and field (Sandgren, 1995; Latvala-Kilby *et al.*, 2009). A high incidence of symptomless PMTV tuber infections has been observed in many studies (Sandgren, 1995; Sokmen *et al.*, 1998; Latvala-Kilby *et al.*, 2009) underlining that visual inspections are insufficient for virus recognition. The knowledge of the common occurrence of latently infected tubers has not always been implemented in seed certification programs or the breeding for resistance to PMTV.

Foliar symptoms in a potato crop appear when PMTV moves systemically from infected seed tubers to the stem and leaves of the potato plant (Calvert,

1968; Cooper & Harrison, 1973; Harrison, 1974). Characteristic foliar symptoms are yellow blotches or V-shaped patterns on leaves and extreme shortening of the internodes ('mop-top') which occurs on potato crops grown in Scotland, Norway and Peru (Tenorio *et al.*, 2006; Carnegie *et al.*, 2011). Foliar symptoms are very rarely seen in the Nordic countries, except Norway. A reason might be the marine and moist climate in Norway and Scotland that affects the physiological state of potato plants and may enhance systemic movement of PMTV (Carnegie *et al.*, 2011).

It is difficult to predict the outbreak of spraing disease because environmental conditions during the growing seasons have an important impact (Cooper & Harrison, 1973). PMTV is vectored by zoospores of *S. subterranea* and low temperature and high moisture enhance the mobility of the zoospores leading to increased infection of potato plants by both pathogens (Sandgren *et al.*, 2002; Merz, 2008).

1.2.3 Transmission

S. subterranea f.sp. *subterranea* (family Plasmodiophoridae; hereafter abbreviated as 'Sss') is the causal agent for powdery scab, an economically significant disease on potato tubers, and it occurs in potato-growing areas worldwide (Kirk, 2008; Merz, 2008; Gau *et al.*, 2013). The symptoms of powdery scab are described as tiny, hollow lesions filled with brown powder consisting of resting spores commonly found in spore balls (sporosori), each containing more than 100 spores (Harrison *et al.*, 1997) (Fig. 3). The resting spores of Sss are able to survive in soil for more than 15 years (Calvert, 1968). Under wet conditions, the resting spores germinate and release primary zoospores, which attach to and penetrate potato root tissue, stolons, young shoots and tubers (Hims & Preece, 1975). After penetration, the zoospore develops into a multinucleate plasmodium, which after increasing in size and cleaving into segments forms zoosporangia, each harbouring numerous secondary zoospores that can re-infect the roots and induce root galls (Hims & Preece, 1975; Merz, 2008) (Fig. 3).

Sss is the only known vector for PMTV and its zoospores are responsible for transmission of PMTV to potato plants (Jones & Harrison, 1969; Arif *et al.*, 1995; Kirk, 2008). When zoospores of Sss develop in virus-infected host tissues, the virions of PMTV enter the zoospores. Infection with PMTV begins when these zoospores, which are carrying the virions, penetrate new host cells (Hims & Preece, 1975). However, the exact mechanism of virus acquisition by Sss is still not known (Kirk, 2008). PMTV is able to remain infective in resting spores in soil for more than 15 years (Calvert, 1968; Jones & Harrison, 1969;

Jones & Harrison, 1972) which indicates that the virus cannot be removed by crop rotation periods (Kirk, 2008).

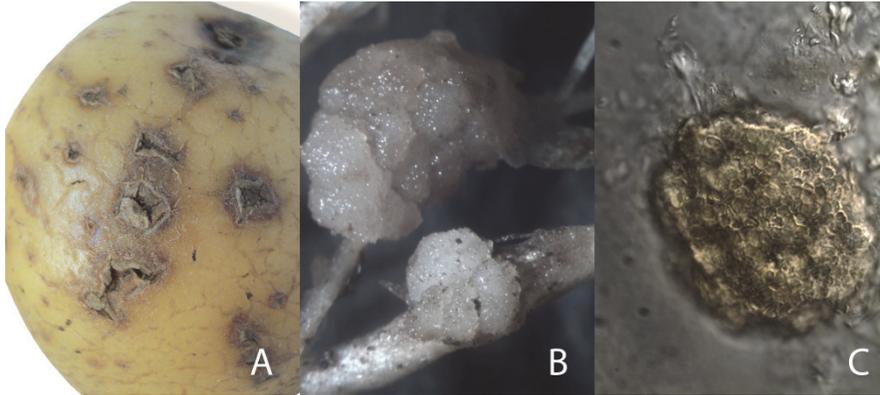


Figure 3. (A) Powdery scab lesions on the tuber surface caused by *S. subterranea* f. sp. *subterranea*, (B) Development of galls on the roots of potato, (C) Spore balls (sporosori) of *S. subterranea* f. sp. *subterranea*, each containing more than 100 resting spores. (Pictures B and C provided by Arne Schwelm)

When PMTV enters the host, it spreads irregularly and slowly within the plant. Not all stems and daughter tubers will be infected with the virus (Torrance *et al.*, 1999; Kirk, 2008; Davey *et al.*, 2013). This suggests that PMTV may self-eliminate from the potato crop over a number of generations in the absence of its vector (Calvert, 1968; Torrance *et al.*, 1999; Davey *et al.*, 2013).

1.2.4 Host

The main host for PMTV is potato (*S. tuberosum*), although under experimental conditions, PMTV is also able to infect weed plants of the families Chenopodiaceae and Solanaceae including the species *Chenopodium amaranticolor*, *Chenopodium album*, *Chenopodium quinoa*, *Datura stramonium*, *Nicotiana benthamiana*, *Nicotiana debneyi*, *Nicotiana tabacum*, *Solanum nigrum* and *Tetragonia tetragonioides* (Jones & Harrison, 1969; Harrison & Jones, 1970; Jones & Harrison, 1972; Arif *et al.*, 1995; Andersen *et al.*, 2002).

Plants of the family Chenopodiaceae develop local lesions in the inoculated leaves and are often used as test plants in virus diagnostics (Calvert & Harrison, 1966; Harrison & Jones, 1970). Solanaceous hosts become infected systemically and different species of *Nicotiana* are usually used for

propagation of PMTV in infected leaves and as bait plants to isolate PMTV from soil containing viruliferous zoospores of Sss (Sandgren, 1995; Nielsen & Mølgaard, 1997).

An experimental study with 17 weed species common in Danish potato fields showed that only *S. nigrum* became infected with PMTV by viruliferous zoospores of Sss in a closed hydroponic system (Andersen *et al.*, 2002). Whereas the weed *C. album* was only infected by manual sap-inoculation of leaves, the weed *S. nigrum* became infected both in leaves via mechanical sap inoculation and in roots by Sss in the hydroponic system. Because *S. nigrum* is a host for Sss (Nitzan *et al.*, 2009; Shah *et al.*, 2010), it is possible that this weed could be a source for maintaining PMTV in a field when potato is not grown.

1.2.5 Genome

PMTV is type member of the genus *Pomovirus* in the family *Virgaviridae* (Adams *et al.*, 2009). The genus *Pomovirus* also contains three other species: *Beet soil-borne virus* (BSBV), *Broad bean necrosis virus* (BBNV) and *Beet virus Q* (BVQ) (Adams *et al.*, 2009). Viruses that belong to the genus *Pomovirus* are morphologically similar to other rod-shaped viruses, i.e., tobnaviruses (*Tobacco rattle virus*, TRV), furoviruses (*Soil-borne wheat mosaic virus*, SBWMV) and benyviruses (*Beet necrotic yellow vein virus*, BNYVV). Pomoviruses and the other rod-shaped viruses differ mainly in their respective genome organization. Whereas pomoviruses represent a group of viruses with a tripartite genome, both tobnaviruses and furoviruses have a bipartite genome. The viral genome of benyviruses is organized into four or five components.

The particles of PMTV (18-20 nm in diameter and 100-150 or 250-300 nm in length) are tubular rod-shaped with a tripartite, positive-sense, single-stranded RNA genome (Scott *et al.*, 1994). The names of the three RNA segments are RNA-Rep (formerly RNA 1), RNA-CP (encoding the capsid protein CP, formerly RNA 2 or RNA 3, depending on the virus isolate) and RNA-TGB (containing the triple gene block TGB, formerly RNA 2 or RNA 3, depending on the virus isolate) (Adams *et al.*, 2012). All three RNA segments have identical tRNA-like structures at the 3' end (Savenkov *et al.*, 1999) (Fig. 4).

RNA Rep (ca. 6 kb) constitutes approximately half of the genome and is involved in virus replication. RNA-Rep encodes a replication protein (146 kDa) with methyltransferase and helicase domains as well as a polymerase readthrough protein (206 kDa), which includes the RNA-dependent RNA polymerase motif (Savenkov *et al.*, 1999).

RNA-CP (2.4-3.1 kb) encodes the coat protein (CP, 20 kDa) and the minor capsid protein (CP-RT, 89kDa), which is produced by suppression of the CP amber stop codon (Kashiwazaki *et al.*, 1995). The CP has encapsidation functions whereas the RT domain is involved in transmission of PMTV by the vector (Reavy *et al.*, 1998; Sandgren *et al.*, 2001). It has been shown that PMTV can move long-distance within the plant without the presence of the CP (Torrance *et al.*, 2009). Furthermore, the CP and the RT-CP are not necessary for replication, accumulation, cell-to-cell movement or long-distance movement of RNA-Rep and RNA-TGB in plants (McGeachy & Barker, 2000; Savenkov *et al.*, 2003). CP-RT has been shown to be located at one end of the virus particle (Cowan *et al.*, 1997) and is involved in attaching to a membrane of the Sss vector to support the movement of PMTV between the cytoplasm of the plant host and that of the vector (Adams *et al.*, 2001). Spontaneous deletions in the RT-domain of RNA-CP are known to occur. Such deletions have been found within the central part of the ORF including deletions in regions that are required for vector transmission (Reavy *et al.*, 1998; Sandgren *et al.*, 2001).

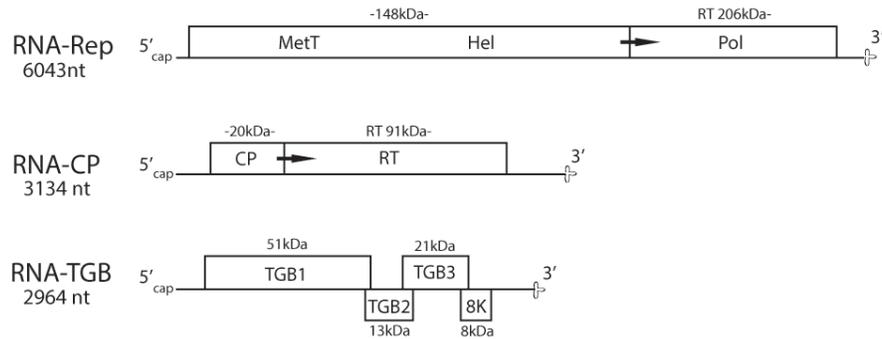


Figure 4. Genomic organization of *Potato mop-top virus* (PMTV).

RNA-TGB (ca. 3 kb) encodes four proteins. Three overlapping genes form the triple gene block (TGB1 51 kDa, TGB2 13 kDa and TGB3 21 kDa) and encode proteins, which are required for virus movement (Torrance *et al.*, 2009; Cowan *et al.*, 2012; Samuilova *et al.*, 2013). TGB1 is an RNA-binding protein that interacts with the viral RNA to form a viral ribonucleoprotein (vRNP) complex. TGB2 and TGB3 are integral membrane proteins to promote the intra-cellular transport of vRNP to the plasmodesmata (Torrance *et al.*, 2011). Furthermore, a recently published study showed an association of TGB2 and vRNA with chloroplasts (Cowan *et al.*, 2012). These results indicate that the

viral replication might be associated with the chloroplast membranes and that the TGB2 has an important role in targeting the virus to the chloroplasts (Cowan *et al.*, 2012). The fourth protein 8K (cysteine-rich protein, 8kDa) is the smallest protein and it appears to enhance the virulence of the virus and may act as a weak RNA silencing suppressor (Lukhovitskaya *et al.*, 2005; Lukhovitskaya *et al.*, 2013)

1.2.6 Diversity

Characterised PMTV isolates from Europe, North America and Asia share a high level of sequence identity (Cerovska *et al.*, 2003; Lambert *et al.*, 2003; Nielsen & Nicolaisen, 2003; Xu *et al.*, 2004; Cerovska *et al.*, 2007; Latvala-Kilby *et al.*, 2009; Budziszewska *et al.*, 2010; David *et al.*, 2010; Crosslin, 2011; Hu *et al.*, 2013; Whitworth & Crosslin, 2013). Two distinguishable variants of RNA-CP and RNA-TGB sequences have been found that occur in different combinations and as mixed infections (Nielsen & Nicolaisen, 2003; Latvala-Kilby *et al.*, 2009). Sequence analyses of PMTV isolates from Colombia and Peru have revealed a close relationship to PMTV isolates from other parts of the world (Mayo *et al.*, 1996; Gil *et al.*, 2011; Osorio-Giraldo *et al.*, 2013). Furthermore, virus isolates related to PMTV have been detected in potato in Colombia suggesting a new tentative pomovirus infecting potatoes (Gil *et al.*, 2011; Osorio-Giraldo *et al.*, 2013).

1.3 Other viruses causing necrotic symptoms in potatoes

Necrotic diseases in tubers are not always associated with PMTV infection. Several other viruses can be the causal agents for internal and external tuber necrosis, such as *Tobacco rattle virus* (TRV, genus *Tobravirus*, family *Virgaviridae*), *Potato virus Y*, notably PVY^{NTN} (genus *Potyvirus*, family *Potyviridae*) and *Tobacco necrosis virus* (TNV, genus *Necrovirus*, family *Tombusviridae*).

TRV can cause symptoms in infected potato tubers that are similar and often indistinguishable from those caused by PMTV. TRV is a soil-borne virus, which is transmitted by nematodes of several species within the genera *Trichodorus* and *Paratrichodorus* (Walkingshaw *et al.*, 1961; Robinson & Harrison, 1989) and it is widely distributed in potato-production areas throughout Europe and North America (Weingartner & Shumaker, 1990; Mojtahedi *et al.*, 2001). An early survey of TRV and its vectors showed a wide distribution of both TRV and three species of *Trichidorus* nematodes in southern Sweden (Persson, 1968). Later surveys found an extensive spread of trichoroid nematodes in the Nordic region also as far north as the Polar Arctic

circle (Eriksson & Insunza, 1986). Since the 1970s, no more investigations have been carried out to determine the presence of TRV in the Nordic potato-growing areas although the incidence of spraing disease in tubers has increased in Sweden.

PVY is the most common viral pathogen found in potato, and it infects plants of a wide range of species, primarily within the family Solanaceae (Danci *et al.*, 2009). PVY is distributed all over the world and causes losses in potato production in the form of reduced yield or quality (Singh *et al.*, 2008). The virus is transmitted non-persistently by aphids of more than 50 species (Radcliffe & Ragsdale, 2002). Three main distinct groups of strains of PVY have been described (Singh *et al.*, 2008): the common group PVY^O, the stipple streak group PVY^C and the tobacco vein necrosis group PVY^N. As a result of genomic recombination between viruses of the strain groups PVY^O and PVY^N, additional necrotic strains have emerged, including the recombinant PVY^{NTN}, PVY^{NTN-NW} and PVY^{NW} (Glais *et al.*, 2002; Chikh Ali *et al.*, 2007; Chikh Ali *et al.*, 2010). PVY^{NTN} is the causal agent of potato tuber necrotic ringspot disease (PTNRD). The disease was first described from Hungary in 1979 where 50 to 70% of the tubers of two cultivars were affected by PTNRD (Beczner *et al.*, 1984). Since the 1970s, different variants of PVY^{NTN} have appeared and spread across Europe and occur now also in North America and Japan (Singh *et al.*, 2008; Karasev & Gray, 2013), but the presence of PVY^{NTN} in Sweden has still to be confirmed.

TNV is another soil-borne virus and the causal agent of several crop diseases worldwide. It is vectored by zoospores of the fungal obligate parasite *Olpidium brassica* (Teakle, 1962). When infecting potato, TNV may cause necrotic symptoms on the tubers, which has been described as ABC disease. The name ABC is associated with three types of tuber symptoms from dark brown patches and radial or reticular cracks on the skin to light brown lesions with often parallel cracks (Noordam, 1957). TNV occurs very rarely in potato crops. In Sweden, necrotic symptoms related to the ABC disease have been recorded in the potato variety Mandel in 1968 (Nilsson, 1986), but the presence of TNV was then not tested.

1.4 Control of PMTV

No chemical treatments against viruses are available on the market that can be applied directly in the field. Plants that are infected systemically by viruses will stay infected all their life, which has consequences, especially for vegetatively propagated crops, e.g., potato. Therefore, preventive methods, adapted for each plant crop and for each virus species, are the only solution for controlling virus

diseases and limiting the economic damage. These methods include plant breeding for virus resistance, vector control management, crop sanitation by removing diseased plants and improved production of virus-free planting material.

Control of PMTV in infested fields is difficult because the vector Sss is a soil-borne parasite and the two possible solutions are: control of powdery scab and control of PMTV using resistant potato cultivars.

Not many methods are available for controlling Sss and powdery scab, and they have only limited effects against PMTV. Chemical treatments for seeds and soil as well as soil fumigation are common methods for controlling *S. subterranea*. However, most of them are not approved in Europe because of the high toxicity and unfavourable environmental effects, e.g. seed treatment with mercury-containing products and methyl bromide for soil fumigation. The use of fungicides, such as fluazinam, manozeb and zinc compounds for soil and seed treatments, is insufficient for controlling Sss. In Europe, the only solution to reducing the infection rate of PMTV in the field might be the use of resistant varieties to Sss. Unfortunately, this approach is not effective. The incidence of powdery scab and PMTV infection in tubers has shown no correlation (Sandgren *et al.*, 2002; Montero-Astúa *et al.*, 2008), which may be explained by infection of root and stolons by Sss and not only tubers. A potato cultivar might have a high tuber resistance to Sss, but a low root resistance (Merz, 2008). Only immunity to Sss in tubers, roots and stolons may exclude infection with PMTV. So far, no cultivars are known to be classified as immune to Sss (Merz *et al.*, 2012). To date, testing for resistance in potato germplasm has been done only for a few wild tuber-bearing potato species and stable genetic resistance to Sss in roots (Spongospora root galling) has been identified (Nitzan *et al.*, 2010). These results are promising for the use in future breeding programs and the development of commercial cultivars.

For PMTV, no potato cultivars are known to be resistant to PMTV infections. However, differences in the development of spraing symptoms have been observed in long-term trials with different potato varieties (Latvala-Kilby *et al.*, 2009). The cultivars Saturna, Nicola and Bintje are widely used in Scandinavia and they are sensitive to the development of spraing symptoms. On the other hand, Saturna is resistant to TRV infections and therefore often used as an indicator of PMTV infections in the field. Some cultivars can be considered to be tolerant to spraing disease with only few tubers developing symptoms. However, tolerant tubers contain a high titre of PMTV and are likely to increase the infection rate in the field. Therefore, the use of tolerant cultivars is not a sustainable solution for controlling spraing disease. Breeding for resistance could be considered as an important goal. A few breeding lines,

such as NY99, have shown a low incidence of PMTV-infected tubers and a low incidence of spraing development (Sandgren *et al.*, 2002). However, the genetic stability of resistance to PMTV remains to be studied. Screens for detection of resistance to PMTV have been carried out on cultivated and wild potato germplasm by Graminor AS (Norway) and Danish Potato Breeding Foundation (LKF Vandal), respectively. A cross between *Solanum vernei* and *S. tuberosum* indicated that resistance was inherited from *S. tuberosum* parents as found by LFK Vandel (I). A quantitative trait locus (QTL) for resistance was found to be located on chromosome IX in *S. tuberosum* which is a promising result as it renders unnecessary to backcrosses for several generations and these findings can be used for molecular marker-assisted selection (MAS) (I). However, additional experiments are needed to map the resistance locus and use the markers for MAS in further crosses.

2 Aims of the study

The incidence of spraing disease in potato crops has increased during the last decades in Sweden and in the other Nordic countries including Denmark, Norway and Finland, and the disease has turned into to a severe problem for potato production. One important goal for agriculture is to prevent further spread of pathogens into new fields and geographical areas and to find a solution to control diseases. Therefore, the main objective for this thesis is to increase the knowledge on the current geographical distribution, epidemiology and genetic diversity of PMTV in northern Europe.

The specific objectives were to:

- Determine the distribution of PMTV in Sweden as well as the incidence of PMTV and other necrosis-inducing viruses in symptomatic and non-symptomatic potato tubers of different varieties.
- Determine the distribution of PMTV in the Nordic countries and in other countries of the Baltic Sea region, which form a high risk zone for the spread of PMTV because they are economically connected and share the same ecological and climatic conditions.
- Characterise sequences of PMTV isolates to enhance the knowledge on viral diversity and evolution.
- Determine whether alternative weed hosts could be a source for maintaining PMTV in a field.

3 Results and discussion

3.1 Distribution of PMTV in the Baltic Sea region (paper I+II)

The geographical distribution of PMTV was determined throughout the Baltic Sea region. Analyses of potato tubers and soil collected from potato fields showed that PMTV is widely distributed in the potato-growing areas within the Nordic countries including Sweden, Norway, Finland and Denmark. Only the High Grade seed production zones in Sweden and Finland, which are concentrated to the northern parts of these countries, seem to be free of PMTV. In Sweden, 36 of 110 (35%) tested farms located in 19 of 21 counties were affected by PMTV and the virus was found both in soil as well as in tubers. This is further north than observed in a previous study (Sandgren, 1995).

These results indicate that PMTV has been introduced into new fields and has become prevalent in potato fields throughout Scandinavia. The fast dispersal of PMTV and the establishment into new geographical areas create a risk zone for increased problems caused by PMTV. It is likely, but not confirmed, that PMTV could be dispersed by wind into new fields in viruliferous resting spores of Sss, which has been shown for another soil-borne pathogen infecting potato, *Synchytrium endobioticum* (Hampson, 1996). Under controlled conditions, PMTV can be dispersed via soil in viruliferous resting spores of its vector *S. subterranea* adhering to seed tubers (Kirk, *unpublished data*). It has been shown that the infestation rate in a field had a stronger correlation with the PMTV content in adherent soil than with the amount of PMTV in seed tubers (Davey *et al.*, 2008; Davey *et al.*, 2013). These observations are very important for the understanding of PMTV epidemiology as they show the role of contaminated soil in virus dispersal. Thus, seed potatoes can be considered to be free of PMTV when grown in non-infested fields. PMTV is also transmitted from infected seed tubers to the daughter tubers but it spreads very slowly and irregularly. However, the transmission rate was greatly enhanced by the presence of the vector Sss (Kirk, *unpublished*

data). This knowledge underscores the role of the vector for the infection rate of PMTV in the field.

Compared to the Nordic countries, the occurrence of PMTV in Poland, the Baltic countries and northwestern Russia is very limited. PMTV was detected in only two samples, in minitubers produced in Latvia, and in one tuber grown in Poland. Neither in Russia, Estonia nor Lithuania, PMTV was found to be present in potato fields. The presence of the vector Sss is necessary for the establishment of PMTV in the field. Sss is not common or very rare in these countries (J. Kaspas, IHAR, *personal communication*). It is possible that soil conditions might be different from the Nordic countries and that they are not optimal for becoming severely infested with Sss and PMTV. On the other hand, the import of seed potatoes to Poland and the Baltic countries from countries affected by PMTV started recently and the time for PMTV and Sss to establish in new fields may not have been sufficient. New surveys in the following years would be necessary for monitoring the presence of PMTV and its vector in these regions.

3.2 Incidence of symptomless potato tubers infected with PMTV (paper I+II)

During the investigations a high occurrence of symptomless tubers was observed for several varieties infected with PMTV in Sweden, Denmark and Finland. The high frequency of latently infected tubers is well known and has been reported in previous studies from Scotland (Sokmen *et al.*, 1998), Denmark (Nielsen & Mølgaard, 1997) and Finland (Kurppa, 1989; Latvala-Kilby *et al.*, 2009). The absence of spraing symptoms in infected tubers demonstrates that only visual inspections are not sufficient for PMTV detection. Tubers that harbour latent infection are serious sources for introducing the virus into new fields when Sss is present. These results show the importance of certification schemes with analysed seed tubers to control the virus and to prevent the spread of this pathogen and its vector into new areas.

3.3 Incidence of PMTV and TRV in tubers with spraing (paper I+II)

In Sweden, the incidence of PMTV and TRV was analysed in 217 tubers (representing 28 varieties) with suspected infection and the results showed that the two viruses were equally common in symptom-expressing tubers. PMTV was detected in 102 tubers (11 varieties) with spraing, whereas TRV was found in 100 tubers (19 varieties) with spraing or advanced corky symptoms. The

high number of inspected potato varieties with spraing symptoms indicates that most of the varieties in the Swedish production are susceptible to both viruses and may express symptoms. However, for most of the tubers, it was not possible to distinguish if the symptoms were caused by PMTV or TRV, as previously observed also by Engsbro (1984) and Molgaard and Nielsen (1996). One unique finding in this study was the co-infection of TRV and PMTV in tubers of one potato variety, but no visual differences in symptom development in the tubers were observed compared to single infection with either virus. In nature, co-infections of plant viruses are frequent, and may increase the disease severity in the host plants as found for *Potato virus X* (PVX) and PVY (Pacheco *et al.*, 2012). However, possible synergistic effects in mixed infections with PMTV and TRV were not studied in the present survey and remain to be explored in future studies.

In Finland, all tubers with spraing symptoms were infected only with PMTV. In contrast, only TRV was found in spraing-expressing tubers collected from different areas in Poland. In the Leningrad province of Russia, tubers with necrotic symptoms were found, but analyses showed that they were not infected with PMTV. *Nicotiana* bait plants that were grown in soil samples collected from the same Russian fields where the symptomatic tubers had been found, developed systemic symptoms in the leaves similar to those caused by TRV (Robinson, 2003). However, the presence of TRV was not confirmed using a virus-specific test. In Lithuania and Estonia, all inspected tubers were without symptoms and tested negative for PMTV.

3.4 Presence of TRV and TNV in Sweden (paper I)

TRV was found to be present from southern and up to the central part of Sweden. This is further north than observed in an earlier study, where TRV was found mainly in the southern part of Sweden (Persson, 1968). However, it has been shown that the vectors of TRV, the trichodorid nematodes, are present also in the northern parts of Sweden (Eriksson & Insunza, 1986). Therefore, the virus has the potential to become prevalent in potato fields in these areas. More detailed investigations are necessary to determine the exact incidence of TRV and its vector nematodes in the northern part of the country.

Potato tubers of cv. Melody with necrotic symptoms related to the ABC disease were obtained during the survey of PMTV and TRV. Analysis confirmed the presence of TNV in symptomatic potato tubers for the first time in Sweden. TNV occurs in many parts of Europe, including Sweden, and it is the causal agent for many diseases in several crops. Still, the ABC disease in

potato is rarely seen in Europe and has been reported only once before in Sweden (Nilsson, 1986).

3.5 Detection of necrotic strains of PVY in potato (unpublished)

Three variants of PVY were detected in five potato samples from Öland and Uppsala. A plant grown in the greenhouse from a tuber (cv. unknown) with external necrotic symptoms developed mild crinkling and mild mosaic symptoms on the leaves (Fig. 5). The leaves tested positive for PVY in a serological test and molecular analyses by multiplex RT-PCR (Chikh Ali *et al.*, 2010) indicated infection by a virus of the strain PVY^{NTN} in the plant. In three potato plants (cv. King Edward), PVY was detected in both tubers and leaves using ELISA analysis. Among the tested samples two different PVY strains, PVY^{NTN} and PVY^N, were identified. The potato plants infected with PVY^{NTN} displayed severe leaf crinkle and strong mosaic symptoms on the leaves while infection with PVY^N was not associated with symptoms. Further, the presence of an additional strain, PVY^{NW}, was identified in the leaves of one potato plant (cv. unknown) with mild leaf crinkle and mild mosaic symptoms (Table 1).



Figure 5. Symptomatic potato leaves infected with *Potato virus Y^{NTN}* (PVY^{NTN}). Left: Mild leaf crinkle and mild mosaic, Right: Severe leaf crinkle and severe mosaic.

The presence of two necrotic PVY strains, PVY^{NTN} and PVY^{NW}, in Swedish potato was demonstrated for the first time. PVY, the most important virus infecting potato, occurs worldwide and can cause severe yield losses. The presence of numerous biological strains makes the situation more complex. In Europe and other parts of the world, necrotic strains of PVY are widespread and are becoming more prevalent in areas where seed potato is grown (Crosslin

et al., 2006; Rigotti *et al.*, 2011; Tian *et al.*, 2011). Some of them are able to induce tuber necrosis in potatoes. In contrast to the soil-borne viruses PMTV and TRV, PVY can quickly spread within a field during one season since it is vectored by aphids of several species. In Sweden, earlier studies have shown that the bird-cherry oat aphid (*Rhopalosiphum padi*) was the main vector for PVY together with aphids of many other species (Sigvald, 1989).

Table 1. *Presence of recombinant strains of Potato virus Y (PVY) in collected samples.*

Sample	PVY strain	Symptoms	Variety	Origin
Plant 1	PVY ^{NTN}	Severe leaf crinkle and mosaic	King Edward	Öland/Kalmar
Plant 2	PVY ^{NTN}	Severe leaf crinkle and mosaic	King Edward	Öland/Kalmar
Plant 3	PVY ^N	No symptoms	King Edward	Öland/Kalmar
Plant 4	PVY ^{NTN}	Mild to severe leaf crinkle and mild to severe mosaic, tuber necrosis	Not known	Öland/Kalmar
Plant 5	PVY ^{NW}	Mild leaf crinkle and mild mosaic	Not known	Uppsala

Due to the annual high incidence of vector aphids in Sweden and the predominance of the PVY^N strain group in Finnish seed and ware potatoes (Tian *et al.*, 2011) the necrotic strains of PVY may be frequent also in Sweden. However, more and detailed investigations have to be carried out to determine the prevalence of PVY^{NW} and PVY^{NTN} in Sweden.

3.6 Diversity and evolution of PMTV (paper III)

Molecular characterization and phylogenetic relationship analyses were carried out for 43 new PMTV sequences originating from potato tubers and field soil from Sweden, Denmark and the USA. The analyses were based on nearly complete RNA-CP (including CP gene and RT-domain) and 3'-proximal RNA-TGB (including 8K gene and 3'UTR) sequences and include also previously reported PMTV. Moreover, the large number of available PMTV sequences allowed the analysis of molecular signatures of selection pressure based on the coding regions.

The results of this study showed a limited diversity of PMTV based on RNA-CP and RNA-TGB and confirmed previous findings for PMTV isolates from America, Asia and Europe (Mayo *et al.*, 1996; Latvala-Kilby *et al.*, 2009; Budziszewska *et al.*, 2010; Osorio-Giraldo *et al.*, 2013; Whitworth & Crosslin, 2013). According to the phylogenetic analyses, no strict geographical grouping was obtained for isolates from Europe, Asia and North America. These results indicate that the virus may not spread directly through the vector *Sss* to new locations, but almost certainly is distributed through infected seed tubers.

However, sequence analysis revealed the presence of a heterogeneous PMTV population in the field. Two distinguishable types for both RNA-CP and RNA-TGB were found in all studied samples. Four RNA molecule combinations of RNA-CP and RNA-TGB were present, and the combination of RNA-CP-II and RNA-TGB-B was found to be most prevalent among the combinations. The existence of different combinations of RNA-CP and RNA-TGB types suggests the exchange of entire genome segments between variants, known as reassortment (Koenig *et al.*, 2011; Koenig *et al.*, 2012).

The limited diversity of PMTV RNA-CP, which is required for vector transmission, may be a result of the fact that PMTV has only a limited host range and the only known vector for PMTV is *Sss*. Only small variations in the genotype of PMTV isolates were found for the CP gene and the RT-domain and no significant evidence for positive selection pressure was observed in these coding regions. In contrast, the genes of RNA2 of TRV, which are associated with nematode-transmissibility, are highly variable and differ greatly in length and sequence (Ploeg *et al.*, 1993; MacFarlane *et al.*, 1996; Vassilakos *et al.*, 2001). TRV, another soil-borne potato-infecting virus, has a broad host range and is transmitted by several species of the nematode genera *Trichodorus* and *Paratrichodorus* (Ploeg *et al.*, 1992) suggesting specific adaptations of TRV to different vector and host species.

In contrast to the CP gene and the RT-domain, the sequence of the 8K gene was less conserved and contained substitutions on codon positions where they led to several amino acid exchanges mostly concentrated to the central part of the protein. This part is highly hydrophobic, which suggests that the 8K protein may act as an integral membrane protein (Lukhovitskaya *et al.*, 2005). However, no significant selection pressure was found for the 8K protein indicating only a low frequency of non-synonymous changes. It has been demonstrated that the 8K protein increases the symptom development in test plants when expressed from heterologous viruses (Lukhovitskaya *et al.*, 2005). Therefore, we studied if the expression of spraing in tubers may correlate with certain RNA-TGB types. According to the analysis, no correlation in spraing development was found with the type of RNA-TGB. Both types of RNA-TGB were detected in symptom-expressing and symptomless tubers. However, because the 8K protein acts as a virulence factor the amino acid differences may affect the pathogenicity of these isolates, which might be of interest for future studies.

3.7 Detection of PMTV in the weed *C. album* (paper IV)

Over a period of two years, weed samples were collected randomly in one PMTV-infested field. Potato had previously been grown in the field and it was then affected by PMTV infections causing spraing. The field was instead converted to cultivation of strawberries (*Fragaria × ananassa*). In total, 580 root samples of eleven weed species and 38 root samples of the cultivated strawberry plants were collected (Table 2).

Table 2. Collected plant species from virus infested field used for PMTV tests.

Scientific names of plant species	Common plant names	Number of collected plants
<i>Anthriscus sylvestris</i>	Cow parsley	10
<i>Artemisia vulgaris</i>	Common wormwood	10
<i>Cirsium arvense</i>	Creeping thistle	10
<i>Chenopodium album</i>	White goosefoot	480
<i>Epilobium hirsutum</i>	Hairy willowherb	10
<i>Fragaria × ananassa</i>	Strawberry	38
<i>Rumex acetosella</i>	Red sorrel	10
<i>Solidago virgaurea</i>	European goldenrod	10
<i>Taraxacum officinale</i>	Common dandelion	10
<i>Trifolium pratense</i>	Red clover	10
<i>Tripleurospermum perforatum</i>	Wild chamomile	10
<i>Urtica dioica</i>	Common nettle	10

Roots of *C. album* were found to be infected with PMTV (10 out of 480 plants), while no infection was detected in roots of the other weed species or in cultivated strawberries. In addition, we were able to detect PMTV in roots of *C. album* grown in PMTV-infested soil in the greenhouse under controlled conditions. This is to our knowledge the first time that a PMTV infection has been detected in *C. album*. These results are surprising because in previous studies roots of *C. album* did not become infected with PMTV through virus-infested soil (Jones & Harrison, 1969; Foxe, 1980) or through a hydroponic system with viruliferous zoospores of Sss (Andersen *et al.*, 2002).

The incidence of PMTV in *C. album* was found to be low, which suggests that this weed might not be involved in the fast spread of PMTV throughout Sweden. On the other hand, PMTV-infected roots of *C. album* might be an important virus source for transmission. *C. album* is a natural host for Sss (Jones & Harrison, 1969; Andersen *et al.*, 2002) and PMTV is known to be transmitted by zoospores of Sss (Arif *et al.*, 1995). Therefore, *C. album* might be an important factor for maintaining PMTV in a field even when potato is not cultivated.

3.8 Genetic diversity of PMTV isolates collected from *C. album* (paper IV)

This study presents the first sequence data of PMTV collected from the weed *C. album*. The nucleotide sequences of three PMTV isolates from *C. album* were determined for the 3'-proximal part of RNA-TGB, including the 8K gene and the 3'UTR. Furthermore, the nucleotide sequences of three clones of a PMTV isolate from *C. album* were analysed for a partial region of the RT-domain of RNA-CP.

The PMTV isolates from *C. album* shared a high nucleotide sequence identity both for RNA-TGB (99.6-100%) and RNA-CP (99.9-100%). Comparison with published sequences of PMTV isolates from potato tubers, soil and test plants collected from Europe, Asia, North America and South America also showed a high level of sequence identity. Phylogenetic trees based on RNA-TGB and RNA-CP revealed a close relationship between PMTV isolates from potato and *C. album*. This indicates that the same genotype of PMTV is infecting potato and the weed *C. album*.

A deletion was observed in three clones of the same PMTV isolate from *C. album* that corresponded approximately to deletions found in previous studies. Deletions in the RT-domain of PMTV are known to occur spontaneously both naturally as well as generated through mechanical transmission on test plants (Reavy *et al.*, 1998; Sandgren *et al.*, 2001). It has been shown that deletions in the RT-domain region may lead to the inability of Sss to acquire and transmit PMTV (Reavy *et al.*, 1998), which means a 'dead end' for PMTV spread. Further studies on the PMTV isolate from *C. album* would be of interest for testing the transmissibility by zoospores of Sss.

4 Conclusion

- PMTV is widely distributed throughout Scandinavia including Sweden, Norway, Denmark and Finland and it has become prevalent in the potato-production areas of these countries. Only the High Grade seed production zones in Sweden and Finland seem to be free of PMTV.
- Poland, the Baltic countries and northwestern Russia (Leningrad province) are still free of significant problems with PMTV.
- Analysis of spraing-expressing tubers in Sweden showed that it was not possible to determine visually if the symptoms were caused by TRV or PMTV and indicated that TRV has become prevalent in southern and central parts of Sweden.
- A high incidence of symptomless tubers infected with PMTV demonstrated that only visual inspections are insufficient for recognition of the virus.
- Analysis of PMTV sequences revealed the presence of a heterogeneous PMTV population in the field. Two distinguishable types were found for both RNA-CP and RNA-TGB, each type showing only limited genetic variability and with no significant positive selection pressure acting on the coding sequences.
- PMTV was detected for the first time in roots of the weed *C. album*. The low incidence of PMTV in this weed indicates that *C. album* might not be involved in the fast spread of PMTV throughout Sweden, but that it might be an important factor for maintaining PMTV in the field.
- Molecular analyses of RNA-CP and RNA-TBG sequences of PMTV showed that the same genotype is infecting both potato and *C. album*.

- A deletion was found in the RT-domain of a PMTV isolate from *C. album* suggesting that it has lost the ability to be acquired and transmitted by its vector Sss.
- TNV and two necrotic strains of PVY (PVY^{NTN}, PVY^{NW}) were to our knowledge for the first time detected in potato tubers in Sweden.

5 Future perspectives

- Spraing is a serious problem both for farmers and the potato-processing industry. The content and formation of brown rings and arcs in potato tubers are not fully understood and might be of interest of further studies. It is likely that after infection with PMTV changes in plant metabolism processes lead to the development of symptoms. On the other hand, infection with viruses can activate host defense mechanisms, which can result in the appearance of symptoms.
- Sss is the key for introducing and establishing PMTV in new fields. The survey did not focus on the presence of Sss in the countries of the Baltic Sea region and this pathogen has only been given limited attention in this region. More information is required for studying the prevalence of Sss in potato-growing areas to prevent further spread of PMTV into new areas.
- The main focus should be the breeding of potato cultivars resistant to PMTV. The identification of suitable sources of resistance via enhanced screening of cultivated potato germplasm is a promising approach as shown in previous studies. Unfortunately, most breeding companies show only limited interest because problems with spraing disease are restricted mainly to the Nordic countries. However, it has been shown that spraing and PMTV occur even in North America and in the expanding potato-production areas of Asia. It might be only a matter of time until PMTV has become prevalent in these regions.
- In a previous study, a relative good level of resistance to PMTV has been obtained in potato cultivars transformed with the CP gene of PMTV. It has been shown that the incidence of PMTV in CP-transgenic tubers was lower than in non-transgenic potato. To

identify host factors that are involved in the infection cycle of PMTV and used them as resistance factors in potato would be helpful in obtaining resistant potato plants.

- Infection of potato with TNV and PVY^{NTN}/PVY^{NW} was demonstrated for the first time in Sweden. Further studies are required for determining the importance of these pathogens for the Swedish potato production.

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