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Bioforsk I Vol. 5 I Nr. 5 I 2010

EAPR 2010

14th triennial meeting of the Virology Section
of the European Association for Potato Research (EAPR)
Hamar, Norway 4th – 9th July 2010

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Book of abstracts

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The publisher of Bioforsk FOKUS is:
Bioforsk, Frederik A. Dahls vei 20, 1432 Ås
post@bioforsk.no

Editor in charge: Director of Research Nils Vagstad
Current issue: Bioforsk Plant Health and Plant Protection Division
Local editor: Director Ellen Merethe Magnus
Scientific editors: Carl Spetz and Dag-Ragnar Blystad

Bioforsk FOKUS
Vol 5 nr 5 2010
ISBN: 978-82-17-00664-0
ISSN 0809-8662

Cover photo: Tor Munthe
Repro & print: Kursiv Media AS

This issue can be ordered from:
Bioforsk Plantehelse, Høgskoleveien 7, 1432 Ås
plantehelse@bioforsk.no
Price: 300 NOK

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Preface

Dear Friends

It is my pleasure to welcome you to the 14th triennial meeting of the Virology Section of the European Association for Potato Research (EAPR). I am pleased to tell you that the organizing committee has worked very hard trying to make this meeting a very nice experience for all those attending, and we hope that this meeting is within your expectations. During this meeting, we have tried to cover various important aspects of plant virology ranging from the classical and applied to the more molecular. To this end, we have organized seven sessions which will cover the following topics: Resistance, virus transmission, emerging and quarantine diseases, diagnostics and detection methods, soil-borne viruses, plant-virus interactions and epidemiology and control.

On this occasion, we are honored to have prominent guest speakers from the plant virology community. Dr. Lute Bos will enlighten us with a talk on the history of potato research during the last 100 years, whereas Dr. Renate Koenig will present the complex evolutionary history of one of the most damaging potato infecting viruses, *Tobacco rattle virus*. In addition, Dr. Luis Salazar will tackle the effects of climate change in the patters of viral populations in potatoes. Mr. Åsmund Asdal (Norwegian Genetic Resource Center) will give a talk on how the Svalbard Global Seed Vault is being used to preserve the genetic diversity of potatoes, and as an introduction to our field excursion Dr. Borghild Glorvigen of the Norwegian Extension Service give an overview on the potato production in Hamar County. Last but not least, Dr. Anna Germundsson from the National Veterinary Institute of Norway will take us on a journey from being a plant virologist to becoming an animal virologist and what we can learn from each other.

If you pay some attention to the participant list you will notice that although this is a meeting of the European Association for Potato Research, we have many scientists from non-European countries. We have scientist representing North and South America, Africa, Asia and Oceania; which in fact covers the whole globe! Hopefully, this more diverse participation to the virology section meeting will continue in the future meetings, and establish stronger intercontinental bonds between scientists.

I would like to clarify one small detail regarding the table of content and the order of the abstracts. The abstracts are placed in alphabetical order according to the speaker, which in some cases is not the first author. We have tried to make it more visible by presenting the speaker's name in bold characters.

Finally, on behalf of the EAPR organizing committee: Dag-Ragnar Blystad, Erling Fløistad, Kari Munthe and myself, I welcome you to Hamar!

Carl Spetz
Chairman of the EAPR Virus Section

Table of Contents

Abstracts of presentations

Abstracts of Posters

Abstracts of presentations

Interception of botanical seed transmitted new potato viruses at the USDA-APHIS Plant Germplasm Quarantine Program

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While testing seedlings germinated from botanical potato seed accessions imported from South America, two viruses were detected, each in a different accession. These detections were possible through the use of molecular and biological testing procedures. The two viruses were named after the accessions JCM-23 and JCM-79. Seedlings from accession JCM-23 showed severe upper leaves deformation and necrosis, whereas accession JCM-79 seedlings showed necrosis and wilting in the lower leaves. Tests for both isolates included: mechanical inoculations onto 14 indicator plants, ELISA, and RT-PCR for most of the known isometric viruses affecting potatoes. Electron microscopy analysis suggested the presence of isometrical particles for both isolates. Isolate JCM-23 was not mechanically transmitted in several attempts, yet it infected healthy potato plants and tomatoes via grafting. Tubers harvested from infected plants did not show any symptoms. However, plants grown from such tubers showed strong necrosis, leaf deformation and rugosity ('frog' skin) as secondary symptoms. Tomatoes showed leaf deformations only in leaves closer to the scion. By contrast, isolate JCM-79 was easily mechanically transmitted to several different species of indicator plants. In *Nicotiana clevelandii*, isolate 79 showed local necrotic rings three days after inoculation with infectious sap. In *Nicotiana tabacum* cv. Samsum, isolate JCM-79 showed local necrotic rings and very distinctive systemic necrotic lines seven days after inoculation. Potatoes were infected with JCM-79 by grafting *N. clevelandii*. Semi-purification of both isolates yielded small isometric particles for both isolates which were observed using electron microscopy. Double stranded RNA (dsRNA) analysis uncovered two viral species in isolate JCM-23; a top 4 kb and a bottom 1.3 kb fragment. Just recently JCM-79 was identified as a putative new strain of *Cherry leaf roll virus*. Cloning and sequencing for JCM-23 is in progress. Both viruses are potentially dangerous seed-transmissible pathogens infecting potatoes which were intercepted by the USDA-APHIS-PPQ Plant Germplasm Quarantine Program, thus preventing the introduction of putative unknown foreign potato pathogens into the USA.

Studies on molecular and biological aspects influencing aphid transmission and control of *Potato virus Y*

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Potyviruses are a group of non-persistently transmitted viruses which require the virus encoded protein helper component-proteinase (HC-Pro) in order to be transmitted. The molecular mechanism of non-persistent transmission of plant viruses by aphid vectors is not fully understood. A better understanding of the potyvirus transmission mechanism requires more knowledge about the three components involved in the transmission process (virus/host/vector). In this work, we have identified aphid cuticle proteins (CUPs) that may be potential virus receptor proteins and the impact of the host plant on the vectoring ability of the aphid was investigated. Three CUPs that interacted *in vitro* with PVY HC-Pro were identified by screening a *Myzus persicae* cDNA library (Ramsey *et al*. 2007). Identified CUP protein genes were cloned and the recombinant proteins were purified and the interaction was confirmed to occur with HC-Pro of another potyvirus. The choice of host plant can influence virus transmission by aphids because virus concentration may vary and aphid vectors have different feeding preferences. We investigated the influence of different plants (potato, tobacco, oilseed rape and *Physalis floridana*) as virus sources and for aphid colony propagation. Our results suggest that the host plant used for maintaining aphids influenced their capacity for virus acquisition and there was also an influence of recipient plant host on virus transmission. The results will be discussed in the context of understanding the transmission process and methods of controlling non-persistent viruses. In addition, the data highlight the importance of the choice of plant species used in assessment of aphid vectoring ability.

Why is a Solanum weed affecting the aphid transmission of PVY in Idaho potato fields?

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Potato virus Y (Potyvirus: *Potyviridae*) (PVY), the most economically important virus affecting seed and commercial potato (*Solanum tuberosum* L.), production in the United States, is vectored by several potato-colonizing and non-colonizing aphid species in a non-persistent manner. The green peach aphid, *Myzus persicae* (Sulzer), and the potato aphid, *Macrosiphum euphorbiae* (Thomas), are the most efficient potato-colonizing aphid vectors of PVY. The bird cherry-oat aphid, *Rhopalosiphum padi* L., a cereal aphid that migrates in large numbers through potato fields during the middle of the growing season, is a potato non-colonizing aphid capable of transmitting PVY. Chemical insecticide applications against aphid vectors are one of the most commonly practiced PVY-control strategies. However, even the most intensive aphid control regime may not prevent spread of potato viruses unless measures are also taken to keep virus-source plants at a minimum. Hairy nightshade, *Solanum sarrachoides*, a prevalent annual solanaceous weed in the Pacific Northwest (PNW) of the United States, is an alternate host for PVY and a preferred host for *M. persicae* and *M. euphorbiae*.

Laboratory transmission experiments of PVY^{O} and PVY^{NTN}, two PVY strains present in the PNW, by *M. persicae*, *M. euphorbiae* and *R. padi* from hairy nightshade to potato plants indicated that the percentage transmission of PVY^{NTN} by *M. persicae* and *M. euphorbiae* was twice as high (46 and 34%, respectively) from hairy nightshade to potato than from potato to potato (20 and 14%). Although no significantly different, percentage transmission of PVY^{O} by *M. persicae* and *M. euphorbiae* was also higher (20 and 20) from hairy nightshade to potato than from potato to potato (14 and 16%). No effect of the inoculum source was observed in the transmission of either PVY strain by *R. padi*.

Additionally, three years of field experiments showed that transmission of PVY by the three previously mentioned aphid vectors was higher in plots that had a PVY-infected hairy nightshade plant as source of virus inoculum than in plots that had a PVY-infected potato plant.

These results show that even low aphid numbers combined with this abundant and ever-present virus inoculum source could result in seed-certification rejection with severe economic losses to seed growers, as well as cause a negative impact on commercial production. Moreover, nightshade plants can sometimes survive the winter in protected places in Idaho. Thus hairy nightshade plays an important PVY epidemiology role in potato cropping systems and should be considered in a comprehensive PVY management plan.

Norwegian efforts to conserve genetic diversity; from local potato varieties to the Svalbard Global Seed Vault

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Being a small country, highly dependent on germplasm and genetic resources from abroad and on equitable interactions between countries, Norway has the last 30 years put strong emphasis on the establishment of sustainable systems for conserving and access to plant genetic resources for food and agriculture.

As in most developed countries, our agriculture has evolved from the use of a large number of local landraces with significant genetic diversity to production based on a limited number of varieties from advanced plant breeding. Through this, many landraces in a number of crops were lost. The Nordic Gene Bank (now NordGen) was established in 1979, and remaining landraces and old varieties in cereals, forage, potatoes, vegetables and root crops from the Nordic countries were conserved in the gene bank.

Norwegian efforts for the establishment of international agreements in this field started with the UN World Commission on Environment and Development (1984-1987), which was chaired by our prime minister Gro Harlem Brundtland. From the CBD (Convention on Biological Diversity) coming into force in 1992 Norway has advocated international systems for exchange of genetic resources.

In line with this, the Nordic governments have stated that all plant genetic resources (PGR) should stay in the public domain, freely available for all uses. This is less restrictive than the practice outlined in the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA), which limits access to germplasm through the Multilateral System (MLS) of the treaty to a list of approximately 60 crops (generas) and to uses related to food and agriculture only.

However, Norway strongly supports the implementation and use of ITPGRFA as a tool for access and sharing of benefits arising from the use of PGR. To facilitate the implementation of the MLS, Norway has from 2009 contributed economically to the Access and Benefit Sharing Fund of the treaty equal to 0.1 percent of the annual turnover of domestic seed trade. A well functioning fund is an essential part of the treaty, and Norway has encouraged other countries to make similar contributions.

When the ITPGRFA was adopted in 2001 the idea of having a global security storage for seeds in the Norwegian archipelago Svalbard was relaunched. The Norwegian government accepted the challenge, and the Svalbard Global Seed Vault was opened in 2008. All gene banks are invited free of charge to put copy samples of their gene bank accessions in the vault. In 2010 the number of accessions in the vault mounts to more than 500.000.

The Norwegian Genetic Resource Centre was established in 2006, managing national programs for conservation and use of genetic resources in plants, domestic animals and forest trees respectively.

The PGR program is cooperating closely with NordGen, especially regarding conservation of germplasm in field collections and *in situ*, and also in distribution of varieties and landraces to a broad range of users. In addition to use in commercial breeding, the demand for conserved PGR material is increasing among niche farmers and hobby growers. Both NordGen and the national program distribute seeds and propagating material free of charge to all kind of users.

Potatoes are an example of a crop in which large diversity regarding conserved material is met by significant interest from increasing groups within growing, cultural history and cookery. About 200 old potato varieties have been taken care of, partly through gene bank and governmental programs and partly by hobby growers and collectors. In order to meet demands for potato varieties among different users, a national potato gene bank is being established through cooperation between Bioforsk and the Norwegian Genetic Resource Centre.

Molecular characterization of emergent PVY strains with high potential of replication

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To date, primary PVY infections are not considered as an important source for virus propagation. Recently, we observed in Switzerland an increase in potato lots rejected for seed certification due to a monitored level of virosis higher than 1% in post-harvest controls. Even more, with some lots, an exceptionally high level of virosis, up to 25% after harvesting, was observed. We hypothesized that emergent particular strains may have acquired a higher potential of replication leading to an increased capacity of virus transmission. We compared the replication of different PVY isolates from the same PVY sub-group. These samples were collected from field cultures, or from lots rejected for certification. After mechanical inoculation on *Solanum tuberosum* cv. Nicola or *N. tabacum* cv. Xanthii, samples of leaves were collected at various time points and levels of virus tested by quantitative real-time RT-PCR. In parallel, the accumulation of PVY capsid protein in leaves was visualized by Western Blot. With some particular strains, we were able to detect up to 20x more viral RNA at 4 days and one week post inoculation than with other strains. This difference was not due to a difference in infectious virus concentration in inoculums as tested by titration on *N. tabacum* cv. Xanthii. The genetic variability of the RNA-dependant RNA polymerase and the P1 genes, both implied in viral replication, is currently under investigation.

Pepino mosaic virus infection in potato and other solanaceous host and test plants

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One of the goals of the EU project PEPEIRA (funded under FP6 2007-10, involving 20 partners from 17 European countries) was to carry out a biological characterization of *Pepino mosaic virus* (PepMV). The objective of the study we report here was twofold: 1) to determine the most important biological characteristics of different PepMV isolates and strains present in the different EU member states, and 2) to determine the possible risks of PepMV strains and variants on tomato and other solanaceous crops.

The development of symptoms and infection rates in local cultivars of important solanaceous cultivated plants and indicator plants were assayed at different European geographic localities for three isolates of PepMV belonging to the European tomato (1066), the Chile2 (PCH06/104) and the US1 genotype. Presence of virus was tested by DAS ELISA and back inoculations to *Nicotiana benthamiana* and *N. occidentalis* 37B test plants.

In our study, the three selected genotypes of PepMV were inoculated to 16 potato (*Solanum tuberosum*) cultivars. In general, potato developed no symptoms when inoculated with the three genotypes, and leaves and roots were most often ELISA negative.

Bioforsk included the local Norwegian PepMV isolate TomA2001-1 in this study. This isolate gave systemic infection including in the tubers in five out of five plants of the cultivar 'Beate', but did not infect 'Bintje' or 'Juno'. All plants of 'Beate' showed mosaic symptoms.

We can conclude that potato, in general, is not readily infected by the most common isolates of PepMV occurring in Europe. The results from 'Beate' inoculated with the isolate TomA2001-1 showed that a systemic infection and transmission through the tubers can occur when specific isolates are combined with a sensitive cultivar.

Sweet pepper (*Capsicum annuum*) developed in general no symptoms when inoculated with the three genotypes, and leaves and roots were most often ELISA negative.

Eggplant (*S. melongena*), tomato (*S. lycopersicum*) and tobacco (*N. tabacum*) nearly always showed symptoms and were ELISA positive in both leaves and roots.

N. occidentalis 37B, *N. tabacum* 'Xanthi', *N. rustica, N. glutinosa, N. benthamiana* and *Chenopodium quinoa* were tested as indicator plants. In general bigger differences in symptom development between the three PepMV genotypes in the same plant cultivar could be observed than differences between cultivars infected with the same genotype.

Only few cases were recorded of possible influence of the geographic locality on the specific trials.

Phytoplasma diseases of potato

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During the earliest potato virus conferences at Wageningen/Lisse and Braunschweig between 1951 and 1960 some diseases of potato have already been reported that are now known to be caused by phytoplasmas. Phytoplasma infections are widespread but mostly occur incidentally and may do so in many plant species. They cause disease in potato every once in a while and may still create concern in the crop. The diseases were mostly mistaken for virus diseases because of the absence of any visible and cultivatable microorganism, of relationships of the pathogens with their plant hosts and vectors, and of their way of transmission. Part of the symptoms they induced, such as yellowing and degeneration, were also suggestive of virus infections. Many phytoplasma diseases have long been confusingly named yellows-type (virus) diseases. The naming of the diseases is erratic since their symptom expression is multifarious and often misunderstood.

In fact, in diseased plants there are two mostly concurrent syndromes, viz, (a) the non-specific degeneration that closely resembles the complex of symptoms resulting from phloem degeneration caused by true viruses as of potato leafroll, and (b) the highly characteristic morphological growth deviations that apparently result from hormonal disturbance of infected plants. The first set of symptoms usually include severe stunting, progressive plant decline, and even premature death. Then, the second syndrome frequently has no chance of showing up. The morphological aberrations characteristic of this syndrome comprise severe branching combined with negative geotropy (witches' broom growth), stunting, and, in flowering plants, a series of floral abnormalities such as phyllody, virescence, sterility, disturbed seed dormancy, and proliferation of axillary buds contributing to the witches' broom type of growth. These floral abnormalities result from a reversal of sexual plant development to intensified vegetative growth.

Usually, host ranges of the phytoplasmas are wide to extremely wide. Since 1967 electron microscopy of ultrathin sections of diseased tissues have revealed the association of many of the then still enigmatic diseases with very minute pleuropneumonia-like microorganisms (PLO or MLO, later called phytoplasmas) in the phoem of their vascular bundles. Detection and identification of such agents now mostly is by ELISA, PCR, and molecular methods but remains difficult. Their results often do not match with the symptoms the phytoplasmas cause and with their natural or artificial host ranges.

From potato degeneration to potato virology;
100 years of research on potato viruses and virus diseases

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During the dark age of plant pathology, when infectious diseases were at best ascribed to microorganisms - to be seen by light microscopy and be cultivated on agar - several diseases could not be associated with such pathogens. Potato crops then increasingly suffered from *degeneration* of plants and crops. It was considered to be a mere physiological phenomenon due to the unnaturalness of asexual plant propagation. No idea existed yet as to the existence and possible involvement of invisible and uncultivatable viruses in causing disease, and it was going to take until 1935 before the first virus could be isolated from organisms and be stored and studied in the laboratory.

Light microscopy between 1910 and 1913 by Dr. H.M. Quanjer at Wageningen, the Netherlands, revealed that leaf-curl or leaf-roll directly resulted from deterioration and necrosis of the phloem, and soon thereafter he found the disease to be graft-transmissible, qualifying the disease as a 'virus disease'. In 1920 Quanjer's graduate student J. Oortwijn Botjes discovered aphid transmission of leaf-roll virus, and Quanjer and his staff tentatively distinguished and identified an increasing number of 'viruses', and learned better to recognize diseased plants. This boosted the developing seed potato industry in the Netherlands, and the inspection and certification of seed potatoes was efficiently handled by the government-supervised Seed Potato Inspection and Certification Service (NAK, established in 1932). Since 1940 large-scale antiserum production was initiated by the Laboratory of Flower Bulb Research at Lisse, greatly drawing on research performed by Professor E. van Slogteren and co-workers' on the viruses of bulb crops and their detection by serology.

Quanjer's early pioneering investigations soon drew wide attention abroad as in the USA, Canada, Ireland, and Great Britain. Research on potato viruses in support of the seed potato production at Wageningen as well as at Cambridge and Rothamsted in Great Britain, and at Braunschweig in Germany also triggered and stimulated the study of viruses of other crops and contributed enormously to the development of plant virology at large as a new discipline.

The international scope of virus problems and a growing need of international consultation and cooperation in the rapidly expanding new field of potato virology led professors van Slogteren and T.H. Thung to initiate *International Conferences on Potato Virus Diseases* of which three were held at Wageningen and Lisse from 1951 till 1957, and a fourth at Braunschweig, Germany, in 1960. The latter was held concurrently with the First Congress of the European Potato Association (EPA, later EAPR), since then followed by other regular EAPR meetings. Meanwhile, the interests of potato virologists widened to plant virology at large, why a follow-up conference at Wageningen in 1965 broadly dealt with *Viruses of Plants*. From 1970 onwards, *General Virology Conferences* are now convened biennially by the Virology Division of International Union of Microbiological Societies (IUMS) as Virology Sections of the huge International Congresses of Microbiology. Next to these virology-based conferences there are meetings of some other crop-based working groups that convene under the umbrella of the International Society of Plant Pathology (ISPP) and the International Society of Horticultural Sciences (ISHS). Among plant virologists there is increasing ambiguity as to where they belong, but awareness grows that viruses are more than mere molecular agents. They originate(d), mutate and recombine in plants and crops, and as pathologists plant virologists should know what viruses are doing in the constantly changing ecological context of plants and crops.

For developing countries, valuable research on potato viruses is performed at the *Centro Internacional de la Papa* (CIP) in Lima, Peru in South America, with substations in climatically different zones. It maintains a large germ plasm collection of tuber-bearing potatoes for breeding purposes, and organizes workshops, courses and meetings for national scientists in the countries of its outreach.

Towards a sprout/seed-potato technology in developing countries:

An affordable export-import "seed" renewal alternative lessening the risks of virus and soil-borne pathogens spread

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The potato seed production in Brazil reaches no more than 4 successive field generations before >15% of the economically most important potato virus (PVY) takes place. Therefore, imported seed potato stocks are still necessary but considerably expensive. In order to meet small farmers seed-potato needs, attempts toward lowering costs of basic seed-potato stocks have been made via virological "on-farm" approaches, such as the innovative Sprout/Seed-Potato technology – (S/S-P) (Souza-Dias, 2006, Cultivar 39(VII):6-9). Under the (S/S-P), tons of sprouts, that are routinely removed from cold room stored tuber/seed-potato stocks (mainly imported, virus-free and basic lots), are directly planted inside aphid-proof screen-houses. Thus, large amount of, equally healthy, minituber/seed-potato lots (virus-free), are produced at lower cost than tissue culture (*in vitro*) systems. Therefore, millions of robust, vigorous sprouts (> 3cm high, weighting 0.4-2,0 g), which used to be discarded (useless by-product), are now an invaluable "seed". Productivity of >1 cm sized mini-tuber/sprout ranges from: 1.8 (cv. Atlantic) to 3.6 (cv. Bintje), which is comparable to others laboratory node/leaf-stem/bud cuttings systems. Mini-tubers produced from sprouts have been sold by US$ 0.12-0.15/unit, while from other tissue culture systems: US$ 0.19-0.25/unit. The Brazilian Federal seed-potato board has been updating the certification norms and, for the first time, will contemplate the S/S-P technology as official. After 7-yrs experimental import of sprout/seed-potato, we were able to demonstrate the viability of sprouts for long distance transport: export-import movement, via air-express services (Souza-Dias *et al*. 2008, 17th Trien. Conf. EAPR-Brasov, RO, p.184-187). Advantages of the sprout/seed-potato technology over these other laboratory (*in vitro*) systems, for large scale virus-free minituber/seed-potato production are: reduced freight cost, reduced somaclonal variation (mutations) and reduced risk of soil and tuber flesh borne pathogen-pest movement. In addition, the sprout/seed-potato technology do not need complex laboratory facilities, high light/energy consumption, and skilled labor. Based on these advantages, over the past 2 years, a Brazil-China and Brazil-Mozambique co-operative seed-potato production program has been shaping up, aiming to transfer and evaluate the sprout/seed-potato system. Furthermore we are expecting a Germany-Brazil-Benin/tri-country sprout/seed-potato co-operative program to be set soon. Despite the evidences favoring the sprout/seed-potato as a feasible, low-cost, large-scale mini-tuber/seed-potato production technology, it is still disregarded, on a worldwide basis, mostly in favor of *in vitro* (tissue culture) plantlet systems as propagating material (seed-potato). However, it is still viewed as "extremely useful in countries or locations which lack *in vitro* facilities". Therefore, our goal is to spread the sprout/seed-potato technology as a more affordable low-cost minituber/seed-potato stock alternative to countries in need of virus and pathogen free seed stocks for commercial production. Funded by: CNPq- 314018/2009-3, 578746/2008-5; Fundag – 13/002-3

A novel strain of *Potato virus Y*, PVY^{NTN-NW} predominating in potato fields in Syria and the simultaneous differentiation of PVY strains by multiplex PCR assay

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Potato virus Y (PVY) is the main potato virus in Syria which causes significant loses for both ware and seed potato production. PVY isolates from potatoes and weeds collected in Syria during 2002-2009 were characterized using biological, serological and molecular methods. According to their characteristics, Syrian PVY isolates grouped in 7 groups including those of the PVY^{NTN} and PVY^{N}W strains. Unlike PVY population in other regions, all PVY isolates tested had recombinant genomes. The majority of Syrian PVY isolates showed novel characteristics, hence they could not be included in any of PVY strains described previously. Of these novel isolates, an isolate group referred to as PVY^{SYR} was the most frequent in both potato and weed samples. A high rate of mixed infections with various PVY strains was detected also with isolates of PVY^{SYR} as the most common. Therefore intensive characterization was carried out for PVY^{SYR} to elucidate their origin, assess the significance and achieve final classification of this new isolate group. All PVY^{SYR} isolates tested induced tobacco veinal necrosis but reacted to a PVY^{O} monoclonal antibody, which are typical characteristics of the PVY^{N}W strain. In potato, however, all PVY^{SYR} isolates tested induced potato tuber necrotic ringspot disease (PTNRD) which is the characteristic phenotype of the PVY^{NTN} strain. The sequence analysis suggested that PVY^{SYR} had most likely emerged by genomic recombination events at the NIb/CP region between PVY^{NTN} and PVY^{N}W parents in Syria. Recombination analysis revealed that PVY^{SYR} isolates had two heterogeneous genotypes with different recombinant patterns, SYR-I and SYR-II, which did not affect their phenotypes and serotypes, given the second genotype as the most frequent. Owing to the shared properties of PVY^{SYR} with PVY^{NTN} and PVY^{N}W, PVY^{SYR} isolates represent a new recombinant strain within the PVY^{N} group that would be designated as PVY^{NTN-NW}. The high prevalence in both potatoes and weeds as well as the ability to induce PTNRD indicate to the significance of PVY^{NTN-NW} isolates and increase the necessity of their control. The specific and reliable detection method is an essential step to control PVY^{NTN-NW} and minimize its spread. Therefore, a multiplex polymerase chain reaction (PCR), that relies on a combination of previously published and newly designed primers was developed for the detection and identification of PVY^{NTN-NW} in single or mixed infections with the main PVY strains, PVY^{O}, PVY^{N}, PVY^{NTN} and PVY^{N}W. This PCR assay was also designed to detect the recombination points in the P1 region enabling the differentiation of the variable genotypes of the recombinant strains PVY^{NTN-NW}, PVY^{NTN} and PVY^{N}W. The reliability of this PCR assay was confirmed using a significant number of well characterized PVY isolates collected from Syria and Japan including those of PVY^{NTN-NW}, PVY^{O}, NA-PVY^{N}, PVY^{N}W and PVY^{NTN}. Application of "amplification based" methods will be growing in the future due to many non-questionable advantages offered by them to the detection of plant viruses. The multiplex PCR assay is a valuable tool for plant quarantine inspectors responsible for seed potatoes and interested in the identification of PVY strains particularly PVY^{NTN-NW}.

Characterization of a new virus transmitted through true seed of wild potato, *Solanum acaule*

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True seed of wild potato, *Solanum acaule* Bitt., was imported into the United States from Peru and entered into the potato germplasm program in Wisconsin, USA. After germination, one plant developed symptoms including chlorosis and necrotic lesions suggesting the presence of a virus. Leaves from that plant were transferred to the Potato Quarantine Program at USDA-APHIS in Beltsville, MD USA, for testing. A virus, designated JCM-79, was mechanically transmitted to alternate hosts including *Nicotiana clevelandii, N. debneyii,* and *Chenopodium amaranticolor*. A permit to handle foreign viruses was obtained by personnel at USDA-ARS, Prosser, WA USA, and the virus was maintained in a quarantine facility by mechanical inoculation to *N. clevelandii*, *N. tabacum* cv. Samsun NN, and *C. quinoa*. Symptoms were especially severe in *C. quinoa* and included necrotic lesions and apical tip necrosis. In Samsun NN tobacco, JCM-79 produced distinct chlorotic-necrotic rings on inoculated leaves and oak-leaf patterns and mosaic on upper leaves. The virus was partially purified from these three hosts by PEG precipitation, differential centrifugation, and sucrose density gradient centrifugation. Electron microscopic observations revealed the presence of isometric virus-like particles of approximately 25 nm in diameter. Purified virus-like particles were digested with sodium dodecyl sulphate and Proteinase K and nucleic acids were precipitated with ethanol. Agarose gel electrophoresis revealed two RNA molecules of approximately 8,000 and 6,500 nucleotides. These characteristics suggested JCM-79 might be a nepovirus or be a nepo-like virus. RT-PCR for *Cherry rasp leaf virus*, which was reported in potato in 2004, was negative. Oligo dT primed cDNA synthesis yielded a clone of approximately 1,600 bp that was sequenced (GU321989). The sequence most closely (~80%) matched that of *Cherry leaf roll virus* (CLRV), genus *Nepovirus*. Amplification with CLRV-specific RT-PCR primers yielded a product of 417 bp only from JCM-79 and CLRV-infected plants. This sequence (GU321988) was greater than 90% identical to the 3' untranslated region of isolates of CLRV from birch, walnut, and cherry. Additionally, ELISA tests with CLRV-cherry reagents (Bioreba, Inc.) were positive on JCM-79 infected materials. These data show that JCM-79 is a new variant of CLRV and could pose a threat to potato breeding programs since *S. acaule* has been used in crosses due its frost and pathogen resistance characteristics. The virus has been transmitted to true potato, *Solanum tuberosum*, by mechanical inoculation or grafting. Infected potato plants (cv. Alpha and Shepody) were symptomless but the virus could be detected by back-inoculation to *N. clevelandii* or Samsun NN tobacco. Characterization of JCM-79 is continuing and new information will be discussed.

A study of *Potato mop-top virus* in field and pot trials in Norway

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Potato mop-top virus (PMTV) causes spraing (unsightly brown arcs and rings in tubers of susceptible cultivars) and yellow chevrons or shortened internodes (mopping) in the leaves and stems of plants grown from infected tubers. Economic losses are due to poor tuber flesh quality leading to whole crop rejection. PMTV is prohibited in seed potatoes exported to some countries and detection of spraing symptoms in tubers will lead to whole seed consignments being rejected by national authorities. At present, economic losses occur primarily through the rejection of ware potatoes by processors and packers. In future however, it is likely that the disease will also impact on exporters of seed potatoes as an increasing number of seed importing countries are designating PMTV as a quarantine organism.

The virus is transmitted in nature by a soil-borne plasmodiophorid (*Spongospora subterranea*) that itself causes the disease powdery scab on tubers. The disease is prevalent in cool and damp conditions.

Some potato cultivars are particularly sensitive and PMTV-infected plants produce tubers with severe spraing symptoms. The cultivar Saturna is widely used in the Scandinavian potato processing industry and is a particularly sensitive cultivar. In Saturna and two other cultivars used for crisp production in Sweden, incidences of 25% spraing have been commonly reported and in Denmark incidences of 30-50% of Saturna tubers affected by spraing have been reported. Confirming other reports, some varieties appear to be systemically infected without exhibiting extreme symptoms. Preliminary results of field and pot trials examining infection rates within 3 sites over a two year period in Norway will be reported and the implications discussed.

Survey of PVY strains/variants in seed-potato multiplied in Czech Republic in last two years

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In the last decade there is possible to observe more severe infection pressure of main potato viruses in a number of European countries. Since 2000 in CR were four years (2002, 2006, 2007 and 2008) with higher infestation of seed potato with virus diseases, resulting in downgrading and rejection of more than 20% of seed potato acreage. Unusual were especially three contiguous years (2006-2008) with permanent and severe infection pressure. Because during seed potato certification in our country samples of all grades must obligatory undergo large-scale post-harvest greenhouse-ELISA tests, the results clearly demonstrated the infestation with particular viruses. From the main, most important potato viruses there was a striking prevalence of PVY over PLRV. Proportion of PVA, PVM and PVX was in certified seed significantly lower.

Our work was consequently aimed at the differentiation of virus strains or variants of PVY which was made as a part of research project. We knew from our previous studies conducted in seventies that there was the prevalence of isolates of PVY-O strain group and later gradual increase of PVY-N isolates, including PVY-NTN and PVY-N-Wi variants were noticed. Higher infection pressure of PVY-N variants was in CR confirmed again in 2006 at the beginning of high infectious period. From post-harvest certification tests 20 cultivars with higher PVY incidence were selected and using strain specific antibodies 65% out of 450 PVY infected plants were determined as PVY-N serotype and 34% as PVY-O/C serotype. Subsequent bioassay on tobacco plants revealed that while all the isolates of N serotype were displaying necroses and were ordered into PVY-N pathotype, only 14% of isolates of PVY-O/C displayed mosaic symptoms (being PVY-O pathotype), and all the rest ones evoked necrotic changes characteristic for PVY-N-Wi variants.

More extensive study in seed samples from official certification was accomplished in 2008. The PVY positive extracts in ELISA tubes were re-tested by PVY polyclonal antibodies (PRI Wageningen) and further by PVY-N and PVY-O/C serotype specific (Bioreba, Adgen) antibodies. A part of isolates were inoculated on tobacco and combination of results both serology and tobacco bioassays were used for strains/variants classification. Sensitive potato cultivars for PTNRD (Browning *et al*. 2004) and multiplex RT-PCR assay (Lorenzen *et al*. 2006) were also examined at limited number of isolates.

A total of 2.854 (53.6%) of the 5.326 PVY isolates collected in 2008 surveys of 1.174 seed-potato lots of 153 cvs. were characterised by serology as PVY-N serotype and 2.349 isolates as PVY-O serotype. On tobacco bioassays were tested 1.806 isolates of PVY-O serotype and from 1.614 infected plants 91.7% were displaying vein necrosis, being PVY-N-Wi variants. Only 8.3% of isolates were producing mosaic symptoms, characteristic to PVY-O strain group (pathotype). (Furthermore 70 PVY selected isolates of N serotype were inoculated on PTNRD sensitive potato cultivars (Hermes, Nadine, Nicola, Kobra) and all of them but one was able to produce necroses on tubers in greenhouse conditions, indicating to be PVY-NTN.)

Very similar monitoring was made also in the year 2009, with less severe infection pressure. A total of 746 (52.4%) of the 1.423 PVY isolates collected in 2009 surveys of of 126 cvs. were characterised by serology as PVY-N serotype and 656 isolates as PVY-O serotype. On tobacco bioassays were tested 395 isolates of PVY-O serotype and from 373 infected plants 93.6% were displaying vein necrosis being PVY-N-Wi variants. Only 6.4% of isolates were producing mosaic symptoms, characteristic to PVY-O strain group. Tobacco plants were also inoculated with 317 isolates of PVY-N serotype and it was found that all of infected plants (304) but two displayed vein necroses. Using multiplex RT-PCR assay (Lorenzen *et al*. 2006) unfortunately at limited number of isolates only, the amplicons characteristic of the PVY-O strain (267 and 689 nt), PVY-N-Wi (181 and 689 nt) and PVY-N-NTN (181 and 452 nt) respectively, were found.

The results unambiguously proved significant disappearing of original PVY-O isolates and enormous increase of PVY-N strain group variants.

The influence of storage temperatures on development of necrotic symptoms caused by PVY^{NTN} on tubers of potato cv. Igor

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PVY^{NTN} was first reported in central European countries and spread across the Europe by the end of the 20th century, causing large crop losses in several countries, including Slovenia. It causes necrotic symptoms on potato tubers of sensitive potato cultivars which make them unmarketable. Slovenian cultivar Igor was proven to be among the most susceptible and sensitive ones and severe necrotic symptoms are visible on most of the infected tubers.

Several diagnostic methods for PVY strain differentiation were developed so far, but none was proven to reliably differentiate tuber necrotic and necrotic strains. Therefore a reliable and sensitive biological assay for these strains is necessary, and the conditions responsible for reliable induction of tuber symptoms have yet to be defined. Only high temperature during growth and storage was pointed out as one of the factors which might influence their development so far.

The aim of our work was to study the influence of different temperatures on development of tuber necrosis caused by PVY^{NTN} during long term storage. Uninfected and infected tubers of cultivar Igor of two physiological ages were harvested – and stored under 12 different storage regimes. Three groups were stored at 4°C, 12°C and 25°C during whole experiment. Others were stored at 4°C and transferred to 25°C 1, 2, 3, 4, 6, 8, 12, 21 and 29 weeks after harvesting. Necrotic symptoms caused by PVY^{NTN} were evaluated on each tuber of all the treatments on a weekly basis. The evaluation was finished 35 weeks after harvesting. Each tuber was visually inspected for tuber necroses and marked.

Almost no necroses developed on tubers during storage at 4°C. After transfer to 25°C tuber necroses mostly developed within first 4 weeks. After transfer to high temperature practically no new necroses developed on tubers of two samples stored at 4°C for 21 and 29 weeks before the transfer to 25°C. In sample, stored at 12°C for the whole experiment, tuber necroses developed on less than 44% of tubers while at 25°C necroses developed on most of the tubers. The results confirm the effect of high temperature during storage on development of necrotic symptoms on the surface of tubers infected with PVY^{NTN} and show that it is possible to prevent formation of necroses caused by PVY^{NTN} on sensitive cultivars using proper storage management at low temperatures.

Stepwise development of an efficient method to control *Potato Virus Y* spread in seed potato fields

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Potato Virus Y (PVY) is a Potyvirus transmitted by aphids in potato fields. For susceptible cultivars, the association of mineral oil and aphicide is generally used to control PVY spread in seed potato fields. Mulching and border crops are also reported as alternative control methods. Four years of field trials have been carried out in Switzerland to compare common and alternative methods to control PVY spread. Four aphicides have been tested with foliage application: Lambda-Cyhalothrine, Triazamate, Pymetrozine and Imidacloprid. One commercial mineral oil and one commercial plant oils have been evaluated for virus control. Acibenzolar-S-methyl has been tested as elicitor. To reduce PVY transmission by aphid behaviour disruption, mulching, oat intercropping and film covering were tested eventually associated with mineral oil. Each control method has been tested at least one year. The results indicate that aphicides are effective to control the aphid populations in the field but inefficient to control PVY spread. Acibenzolar-S-methyl has no effect. Mineral oil has been shown to be efficient to control PVY spread but has no effect on aphid populations. Plant oils were less efficient than mineral oil in virus spread limitation. Mulching, oat intercropping and film cover reduce aphid populations on potato plants and PVY transmission. We concluded that the association of mineral oil with mulch or oat strengthens the control of aphid populations and PVY transmission on seed potato plants.

Initial findings on the Relative Vector Efficiency factors for the aphid transmission of *Potato virus A*

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In England and Scotland, an aphid monitoring scheme provides a risk assessment of virus spread for seed potato growers, and is mainly focused on *Potato virus Y* (PVY). The scheme is made up of a network of yellow water traps (YWT's) situated within seed potato crops in the main growing areas of Scotland and England and provides data on the number of aphids captured by species and the ability of each to transmit PVY. Growers maintain the traps and send the contents in for identification. The results are returned to the grower on the same day as sample arrival at the laboratory and are also incorporated into a website that has a map based interface for easy interrogation of results. The growers utilise this service and incorporate the data into their decision making processes on haulm destruction timing, insecticide use and product choice and as a marketing tool.

In recent years the incidence of *Potato virus A* (PVA) has been increasing and needs to be considered alongside PVY in the risk assessment system. There is little data available on the ability of aphids species to transmit PVA and in order to fill the gap transmission experiments on the most common aphid species captured in the traps have been undertaken. This uses a method recently reported by Verbeek *et al*. (2009) which enables the direct comparison of results for PVY and PVA transmission. The results and their incorporation into the risk assessment system will be discussed.

Animal, human and plant virologists – different backgrounds but the same aims

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Viruses in humans, animals, fish and plants cause big economic losses every year. While mammalians and fish can be vaccinated against important viruses, plants are dependent on resistant cultivars and good crop rotation. Independent on what strategy is being used to control the virus infection it is important to have knowledge and understanding about the virus, virus-host interaction, how the virus is transmitted and the epidemiology of the virus. Although there are many differences between a virus infecting an animal and a virus infecting a plant, many questions and techniques remain the same. I will talk about a journey from being a plant virologist to be an animal virologist. What can we learn from each other? How and in what area would we gain much more by working together? I will give some examples of collaboration between plant and animal/human virologists where plants have been used to gain novel information about the human virus. Furthermore, I will give an example of techniques frequently used today to identify “new” viruses in mammals and fish can be used to identify unknown viruses in plants.

Potato production in Hedmark county in Norway

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In Norway about 3.4% of the land is cultivated, while 1/3 of the country is covered by forest. The rest of the country is mountains and open moors, and some smaller areas where the 4.9 million people live. The cultivated land represents 1.03 million ha. The Norwegian potato production is produced on 1.4% of the total agricultural area. Grasslands represent 64% of the area, while cereals and oil seed is cultivated on 31% of the agricultural land (Statistics Norway, 2010). 3.7% of the total plant production is organic farming. Women stand for 25% of the work. About 2.3% of the working population works in agriculture, which constitutes 0.5% of the gross domestic product (Statistics Norway).

Traditionally, potatoes were produced on almost all farms in Norway. In 1959 potatoes were produced on about 90% of the farms, while in 2007 potatoes were only produced on about 7% of the farms. The potato production in Norway is concentrated in a few areas. The most important areas for volume production are around the Oslo fjord, in Jæren (southwest), in Nord-Trøndelag and the on both sides of the great lake Mjøsa. Hedmark, on the east side of Mjøsa, is the largest area for potato production with 37% of the total production. In 2008 Hedmark had 5302 ha of potato land. Potato consumption in Norway is (2008) close to 75 kg potatoes per person.

Hedmark County is situated in the southeastern part of Norway, bordering Sweden. The nature varies from beautiful mountains in the north, to green forests and wilderness in the east, and productive farmland in the middle area east of Mjøsa and in the south. Norway's largest river Glomma flows through Hedmark County, giving water to much of the farm land.

The potato production is situated in four different areas in Hedmark: Nord-Østerdal, Sør-Østerdal, Hedemarken and Solør-Odal. The potato production in Sør- and Nord-Østerdal is mainly of the variety Mandel. The potatoes are grown at altitudes from 200-500. The variety Mandel grown in the highest altitudes are sold as "Fjellmandel".

The areas of Hedemarken and Solør-Odal produce potatoes for the industry and table markets. The soil at Hedemarken is very rich, and has a high content of organic matter; while the soil of the Solør-Odal-area consists of loam and sandy loam, is free of stones and is easy to cultivate.

The most grown variety for chips is Saturna, but other varieties like Liva, Lady Claire, Bruse and Tivoli and others are also used. The main varieties used for the french fries industry are Innovator, Santana, Ramos, Oleva, Peik and Asterix. The major table potatoes varieties are Asterix, Beate, Folva and Mandel, but varieties like Laila, Pimpernel and Kerrs Pink still play a role in the shopping baskets. There is very little of early table potatoes grown in the area.

The seed potatoes in Norway are produced on totally 854 ha. The seed potato production is concentrated in just a few places in the country. Almost 72% of the seed potatoes are produced in the Hedmark County.

Identification of European PVY isolates by multiplex PCR with new set of primers

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Potato virus Y (PVY), is an important pathogen of potatoes, despite of decades of research and breeding for resistance. In our experiments, performed during year 2008 and 2009, 40% of field samples collected in Poland were infected with PVY. Many strains and strain variants of PVY were described around the world. A lot of recombinant isolates as well as typical PVY^{N}, PVY^{O}, PVY^{NA} and PVY^{C} were characterized but their naming and grouping differ among publications. Two most important recombinant strains are PVY^{N-Wi} and PVY^{NTN}. PVY^{N-Wi} isolates have at least one recombination junction in HC-Pro region and PVY^{NTN} at least 3 in HC-Pro, VPg and usually in CP. The isolates characterised as variants of this two strains could occur by independent recombination events but in similar mentioned above points and typically between similar parental strains, e.g. as it was described in Hu *et al*. 2009 (Hu X., Karasev A.V., Brown C.J., Lorenzen J.H. Sequence characteristics of potato virus Y recombinants. J. Gen. Virol. (2009), 90, 3033–3041).

We propose a multiplex PCR assay that recognizes a strain of virus despite difference among variants. The new set of primers was tested on previously characterized population of European PVY isolates. With this method we were able to identify natural and artificial mixtures of strains.

Table 2: Fragments produced during multiplex reaction.

Size	Primers	Typical for strains:
362	ynaf1236 + yna1598r	PVY^{NA}
495	ynf2064 + yn2559r	PVY^{N}
627	yof5764 + yn6391r	PVY^{NTN}
797	yof5764 + yo6561r	PVY^{O}, PVY^{N-Wi}
1100	ynf2064 + yo3165r	PVY^{NTN}, PVY^{N-Wi}
1556	yof1609 + yo3165r	PVY^{O}
1929	ynaf1236 + yo3165r	additional product for FrKV15

The PCR reaction was carried out using Maxima Hot Start *Taq* DNA Polymerase (Fermentas) in 20µl of reaction that contained 0,5 unit of polymerase (0,1 µl), 2µl of 10x buffer [200 mM Tris-HCl (pH 8.3), 200 mM KCl, 50 mM $(NH_4)_2SO$], 1,5 µl of 25mM $MgCl_2$, 0,5 µl of dNTPs (10mM each) and 0,25 µl of each 10 µM primer and virus cDNA. PCR program consisted of denaturing at 95°C for 5min; 30 cycles of 95°C for 30sec., 60°C for 30sec. and 72°C for 2,5 min.; ended with final extension at 72°C for 7 min. The low cost of the reaction is provided by use of 1% agarose gels, standard 1kb marker (GeneRuler 1kb DNA ladder, Fermentas) and standard hot start polymerase. It is worth mentioning that proposed assay recognises all considered PVY^{NTN} isolates including Gr99, N-Nysa, Wi 156 and Wi 156var. The assay can not distinguish mixed infection of PVY^{O} and PVY^{NTN} from mix of PVY^{N-Wi}, PVY^{O} and PVY^{NTN}.

Table 1: Primers designed for multiplex PCR.

Primer	Sequence of primers	Specific for strains:
ynaf1236	GCC AGT GAT TTG CTC AAA GTA TTA CA	PVY^{NA}
yna1598r	GGC GGA TAG CTT ATT CCT GAA G	PVY^{NA}
yof1609	GGA ATC TGT ATT TGT CGT GCG A	PVY^{O}
ynf2064	TTC TGT TAC ATT AAC ATT TTC CTC GC	PVY^{N}, PVY^{NTN}, PVY^{N-Wi}
yn2559r	TTC ATC CAG TAG CAA TTG CTT CA	PVY^{N}
yo3165r	TTA TTA AAT CGC TCG CTC AAT CCT	PVY^{O}, PVY^{NTN}, PVY^{N-Wi}
yof5764	CGC CAT GCT CGT GAC AAA	PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, PVY^{NA}
yn6391r	CCC ATA CAT TTC AGA CGT TCC A	PVY^{N}, PVY^{NTN}, PVY^{NA}
yo6561r	ATC TTT CGG CAT TTT GAT GAG G	PVY^{O}, PVY^{N-Wi}

Some features of PVY resistance transferred from *Solanum tarnii* to *S. tuberosum*

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New strains of *Potato virus Y* (PVY), a serious threat to the worldwide production of potato, are frequently being reported. The strains PVY^{NTN} and $PVY^{N}W$ in particular, cause large necrotic ring like blemishes on the tubers in some years. Due to the abundance of viruliferous aphids it is proving difficult for growers to produce virus free seed potatoes without the use of pesticides.

There are dominantly-inherited resistance genes to PVY in cultivated and wild potato species, which determine whether plants show a hypersensitive response (*Ny*) or extreme resistance (*Ry*) to virus infection. Several single dominant genes (Ry_{adg}, $Ry_{chc,}$ R_{hou} and Ry_{sto}) determine the resistance to PVY. Mainly genes for extreme resistance from *S. andigena* and *S. stoloniferum* are used in potato breeding programs. Of the more than 200 potato cultivars currently grown in Germany only 18 are extremely resistant to PVY. PCR markers based on Ry_{adg} and Ry_{sto} can be used to select those cultivars that are resistant to PVY. Although the resistance due to these genes has proved to be stable new resources should be identified as there could be a break down in the resistance. Other reasons are (1) the similarity of the genetic background of all potato cultivars, which has resulted from continuously crossing of similar potato cultivars, and (2) the high cost of plant protection. Therefore, new sources of resistance have been introduced into cultivated potato from the Mexican wild species *S. tarnii*, *S. cardiophyllum* and *S. pinnatisectum*, which belong to the Section *Petota* of the genus *Solanum* L. Somatic hybrids generated by protoplast electrofusion have been back crossed with the commercial cultivars Delikat, Sonate and Baltica. Their progeny were mechanically inoculated in a greenhouse and in the field with different strains of PVY and subsequently tested for infection with virus using ELISA. Segregation analysis of the second back-cross (BC) generation indicates that these genes for resistance are dominant. It is thought that genes for extreme resistance to PVY from different wild potato species share similar sequences. PCR markers were used to determine whether the new resistance differs from that originating from *S. andigena* and *S. stoloniferum*.

The Ry_{sto}-specific SSR-marker STM0003 revealed that the R-alleles (130 bp, 170 bp fragments) are only present in *S. stoloniferum* and *S. cardiophyllum* but not in *S. tarnii* and the resistant somatic hybrids. In contrast, marker Rysc3 specific for Ry_{and}, revealed another R-allele (320 bp fragment) in *S. tarnii* and the resistant somatic hybrids, and in some of the resistant and susceptible BC1 clones. In resistant BC2 progenies no marker signal was recorded. The amplification products from *S. tarnii*, its resistant somatic hybrid, resistant BC1 progenies, *S. andigena* and *S. stoloniferum* were cloned and sequenced. The sequences of the amplified fragments are identical to that of the Ry-1 gene from *S. andigena*.

These results indicate that the available markers are not specific to the resistance gene from *S. tarnii*. Probably, the PVY resistance gene from *S. tarnii* is a novel gene. Currently segregating populations are being used to develop a specific molecular marker to further characterize this new source of extreme resistance to PVY.

In rare cases a weak positive ELISA-reaction was observed for some clones of PVY after mechanical inoculation. To determine whether this was an unspecific reaction a qPCR method was developed to detect PVY more accurately. Scions of different accessions with extreme resistance to PVY, including recently generated ones, were grafted onto infected potato rootstocks in order to achieve an infection pressure as high as possible. None of the scions gave positive results for a PVY infection. It is concluded that an examination whether a resistance is extreme or not requires absolute quantification methods, which primarily are represented by qPCR. However, for the detection of RNA-viruses with qPCR samples of plant material necessarily have to be prepared for RNA extraction and established methods are time-consuming and costly. Therefore a low-cost, high-throughput and fast sample preparation method was adapted without the need for any toxic compounds - the immuno-capture (IC) technique. Thus, the advantages of both ELISA with its high-throughput and qPCR with its accuracy can be combined.

Fitness analysis using artificial mutated PVY isolates

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Potato virus Y (PVY) is an important plant pathogen which causes major damage to several agronomically important crop species. This ssRNA virus is transmitted by aphids in a non persistent manner and infects many species of the family *Solanaceae*. Potato strain isolates of PVY have been classified into two main groups, according to i) their ability to induce vein necrosis symptoms (PVY^N), or not (PVY^O), on *Nicotiana tabacum* cv. Xanthi and ii) their biological properties on some *Solanum tuberosum* ssp. *tuberosum* varieties. Following the first description of PVY, non necrotic isolates remained dominant. However, during the last twenty years, epidemiological studies have highlighted an increase of the proportion of PVY^N isolates in natural populations. Understanding the processes that have led to the recent prevalence of necrotic genotypes in PVY populations is an important challenge for research programs studying this virus. A reverse genetic approach has highlighted the essential role of two amino acids in the viral genome for PVY induced necrosis. Moreover, recent published data has shown that the majority of PVY isolates present in fields possess genomes resulting from recombination between PVY^N- and PVY^O-like sequences. Therefore, the acquisition of the necrotic ability and/or the emergence of recombinant genomes could be associated with a higher fitness of both PVY^N and recombinant isolates. To test the influence of these nucleotides and the impact of recombination on the fitness of PVY isolates, single and mixed inoculations were performed using non-necrotic PVY^O-139, necrotic PVY^N-605 and point mutated versions of PVY^N-605 (PVY^{KRED}, PVY^{KR} and PVY^{ED}) as well as artificial recombinant (PVY^N-605/PVY^O-139) viral genomes.Tobacco plants susceptible or not to viral induced necrosis were inoculated. Specific quantification tools have been used in order to measure, for each isolate/plant combination, the competitive success in host plants. The results obtained demonstrate the influence of both the capacity for necrosis and the role of other sequence(s) of the complete PVY genome on viral fitness.

Validation of ELISA and bioassay used in potato quarantine testing to meet the requirements of ISO 17025

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The UK Potato Quarantine Unit (UKPQU), SASA is responsible for carrying out testing on potato material from 3rd countries as specified in Commission Directive 2008/61/EC. The testing done exceeds this regulation and is based on EPPO standard P3/21 "Phytosanitary procedures. Post-entry quarantine for potato". For viruses *in vitro* plants are tested once and glasshouse plants twice using ELISA for APLV, APMV, AVB-O, PBRSV, PotLV, PLRV, PMTV, PVA, PVM, PVP (PRDV), PVS, PVT, PVV, PVX, PVY, PYV, TBRV and TSWV. Glasshouse grown potato plants are tested once using the indicator plants *Chenopodium amaranticolor* (Ca), *C. murale* (Cm), *C. quinoa* (Cq), *Nicotiana benthamiana* (Nben), *N. bigelovii* (Nb), *N. clevelandii* (Nc), *N. debneyi* (Nd), *N occidentalis*-P1 (No) and *N. tabacum* cv.White Burley (Nt). Since March 2007 potato quarantine testing at the UKPQU has been accredited to ISO 9001, but now the UKPQU has applied for accreditation of ELISA and bioassay to ISO 17025; Method validation is a critical part of this accreditation. Guidance on method validation is available in EPPO standard PM7/98. In order to determine test sensitivity for ELISA, virus infected sap was tested after dilution in healthy potato sap from 0 - 400 times followed by a 10^{-1} dilution in extraction buffer. For bioassay infected sap was diluted in healthy potato sap from 1:5 - 1:50, then diluted 1:25 or 1:50 in water (to represent the amounts of water normally added prior to inoculation) and then inoculated to 2 plants of each of 3 indicator plant species selected from the range of plants normally used. Using ELISA all viruses other than TSWV (detected at 1:50 sap dilution) were detected (threshold 2 times the healthy control) at the maximum sap dilution of 1:400. For some viruses the maximum level of sensitivity was obtained after 40 min (APLV) and 75-120 min (APMV, PotLV, PVP) but for the remainder (AVB-O, PBRSV, PotLV, PRDV, PVA, PVM, PVS, PVT, PVV, PVX, PVY, PYV, TBRV and TSWV) overnight incubation was required. For bioassay (Table 1) all viruses except PYV, were detected at a 1:5 dilution of infected sap:healthy sap followed by a dilution in water of 1:50 indicating that the procedures used within the UKPQU are fit for purpose. At the maximum dilution tested at least one indicator plant species allowed reliable detection (on both plants) of all the viruses (except PVA, PVM, PYV, TBRV). The universal indicator *N. occidentalis*-P1 (Verhoeven & Roenhorst, 2003) allowed the detection of all viruses except AVB-O and PYV although for some viruses (PVA, PVT, PVV) it was not as susceptible as other plants used.

[1]Virus followed by propagation host in parenthesis; [2]indicator plant followed by maximum sap dilution/ maximum water dilution at which symptoms were observed in parenthesis; St=*Solanum tuberosum*; Pf= *Physalis floridana* (for other host plants see text); *1 out of 2 indicator plants of a species showing symptoms, if no asterisk both plants showed symptoms; (-) no indicator plant showed symptoms

Verhoeven JTJ, Roenhorst JW (2003) Detection of a broad range of potato viruses in a single assay by mechanical inoculation of herbaceous test plants. EPPO Bulletin 33;305-311.

Table 1. Detection of potato viruses using bioassay

Virus [1]	Bioassay[2]	Virus [1]	Bioassay[2]
APLV (Nb)	Nb(50/50),Nc(50/50),No(50/50)	PVS (St)	Nd(-),Cq(50/50),No(50/50)
APMV (Nc)	Nb(50/50),Nc(50/50),No(50/50)	PVT (Ca)	Ca(50/50),Cq(50/50),No(50/20*)
AVB-O (Cm)	Cm(5/50),Cq(50/50),No(-)	PVV (St)	Nd(50/50),Nt(50/50),No(50/20)
PBRSV (Nt)	Cq(50/50),No(50/50), Nt(50/20* 25/20)	PVX (St)	Nben(50/50),Nt(50/50),No(50/50)
PotLV (St)	Nb(-),Nd(50/20*),No(50/50)	PVY (St)	Nben(50/50),Nt(50/50),No(50/50)
PRDV (St)	Ca(-),Cq(50/50),No(50/50)	PYV (Pf)	Nben(-),Nt(-),No(-)
PVA (St)	Nben(25/20*,5/50),Nc(-), Nd(5/20*),No(5/50*), Nt(50/20*,5/50)	TBRV (St)	Cq(5/50),Nt(5/50),No(25/50)
PVM (St)	Nc(-),Nd (-),No (50/50*, 25/50)	TSWV (Nt)	Nben(50/50),Nt(50/50*,50/20),No(50/50)
PVP (St)	Ca(50/50),Cq(50/50),No(50/50)		

Molecular studies on *Tobacco rattle virus* (TRV) infections in various ornamental plants and potatoes suggest complex evolutionary histories of tobraviral RNA 2 recombinants

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Tobraviral genomes consist of an RNA 1 which contains the genes necessary for replication, movement and silencing suppression and an RNA 2 which contains the coat protein gene (ORF 2a) and up to three additional RNA 2-specific genes of which at least ORF 2b is needed for nematode transmission. In addition to their 5' RNA 2-specific part tobraviral RNAs 2 contain a 3' terminal part which shows a high percentage of sequence identity to a tobral viral RNA 1. Both the RNA 2-specific and the RNA 1-related RNA 2 portions may vary considerably in size and composition. On the basis of their nucleotide compositions the coat protein genes on TRV RNAs 2 from potatoes can be divided into three only distantly related groups. One group is represented by the Dutch isolates PLB and PSG which are related to the isolate PPK20 from nematodes in Scotland, the second one consists of German isolates, e.g. TRV Ros, which are related to isolates from nematodes in England and from *Hosta* and the third one is formed by the US isolates ORY and MI. Rather different groupings, however, are recognized when the RNA 1-related parts in the RNAs 2 of these isolates are compared. This suggests that recombinations must have taken place. Such recombinations were followed in more detail in a tobravirus infection in *Alstroemeria*, a vegetatively propagated ornamental plant. It contained one TRV RNA 1 which was associated with seven different RNAs 2. The 5' RNA 2-specific part of all these RNAs 2 showed almost 100% sequence identity with that of RNA 2 of the TRV isolate TCM from tulip, but in some of them it was shorter than in the TCM isolate whereas in others it was longer suggesting that deletions had taken place. In those RNAs 2 in which more deletions had occurred the 3' end resembled that of the cognate *Alstroemeria* TRV RNA 1, but in the others it resembled that of Pea early browning virus (PEBV) RNA 1 which was not detected in the infected plants. Tobraviral RNA 2 recombinants which contain TRV- and PEBV-derived sequence elements in various lengths have previously been described by others. It seems that by the exchange of RNA 2-specific genome elements, tobraviruses may acquire properties which are favourable for their infectivity for specific hosts or their transmission by different nematode species or races. In *Alstroemeria* and tulip the exchange of originally PEBV-related genome elements by TRV-derived ones may open the wide host range of TRV RNAs 2 also to those derived from PEBV which has a much narrower host range.

Diagnosis and discovery of viruses using siRNA deep sequencing

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Vegetative propagated crops such as potatoes are prone to build up of virus infections. To ensure that material is pathogen free expensive and lengthy indexing protocols need to be applied, delaying the international exchange of germplasm and breeding material, which is a major activity of the International Potato Center (CIP). Plants defend themselves against viruses by RNA silencing which involves the generation and use of small interfering RNA (siRNA): short RNA sequences of 20-25 nt derived from the viral genomic or sub-genomic RNA. Recently we developed a new technique based on deep sequencing and assembly of plant derived small RNAs, to rapidly identify viral infections in plants. The technique could not only identify known viral pathogens, occurring at extremely low titers, but also novel viruses, without the necessity of any prior knowledge. We are currently assessing the applicability of the technique to be used in routine indexing of plants using representative samples infected with various known as well as unknown viruses. Results from individual and bulk sequencing of infected plant samples will be presented including the identification of novel viral potato pathogens.

Nature and epidemiology of potato viruses PVA and PVY in Scotland – Do they have the same aphid vectors?

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Potato virus Y (PVY) is a non-persistently transmitted potyvirus that is a major cause of crop loss in potatoes in many parts of the world, responsible for yield depression of up to 80% and total crop loss when in combination with other potato viruses. In Scotland, virus testing in support of the Seed Potato Classification Scheme (SPCS) over the period 1998-2009 has shown PVY to be responsible for, on average, 39% of all potato virus symptoms. Most natural spread of PVY can be attributed to aphids, with the Peach–potato aphid *Myzus persicae* recognised as the most efficient vector.

Like PVY, *Potato virus A* (PVA) is a non-persistently transmitted potyvirus, but because it has traditionally been associated with mild symptoms, it has received very little attention in epidemiological studies. Most aphid species colonising potatoes are recognised as vectors, but the potential of non-colonising species to vector PVA is largely unknown although there has been at least one report of a non-colonising aphid, *Brachycaudus helichrysi,* vectoring PVA. In many parts of the world where potatoes are grown, PVA is not one of the more prevalent viruses. However, in the UK this virus has increased in incidence, particularly over the last 20 years. In Scotland, virus testing in support of the SPCS over the period 1998-2009 has shown PVA to be responsible for 20% of virus symptoms. Compared with PVY, PVA is prevalent in far fewer varieties, e.g. Desiree, Hermes and Estima, but as these are now some of the most popular varieties currently grown in the UK, the impact of PVA has become of increasing economic significance. Field trials were carried out to assess the relative timing of transmission of PVY and PVA and to relate this to aphid activity. The variation observed between the different years indicates that the timing of transmission of both viruses is not constant. In all seasons analysed a measure of aphid vector pressure from aphid suction trap provided a good explanation of the changes in the timings of the extent of PVY transmission. The epidemiology of PVA is less clear cut with the information from only two of the years (2008 and 2009) suggesting a good relationship between the aphid vector pressure and PVA transmission. As the patterns of changing incidence of PVY and PVA within the Scottish Seed Potato Classification Scheme have differed in recent years, it seems likely that there are some significant differences in the epidemiology of these two potyviruses and that these differences may lie in the species of aphids that play a significant role in their transmission. Ongoing work will help to elucidate whether these differences lie in the species of aphids responsible for their transmission, or elsewhere. Recent results on the analysis of the PVY population structure will be presented.

Transmission of the economically highly important citrus and grapevine pathogens: stolbur phytoplasma and *Candidatus* Liberibacter spp. to potatoes; towards the need of a virus-like epidemo-pathological approach

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Over the last 5 years "Zebra Chip" (ZC) a potato disease caused by *Candidatus* Liberibacter spp. has become a major phytosanitary concern for the potato industry in Central and North America. Diseased tubers show mosaic pattern of light-dark cream color turning dark at frying. ZC is transmitted by the potato psylid (*Bactericera cockerelli Sulc.*, syn. *Paratrioza cockerelli*)

In Brazil, the potato ZC disease has not been reported yet. However, *Ca.* Liberibacter americanus (CLam) is present in Brazil and also *Ca.* Liberibacter asiaticus (CLas), causing one of the most damaging diseases of the citrus orchards: Huanglongbin (HLB). The strong association of CLam and CLas led us to investigate to the hypothesis whether or not CLam and or CLas could infect potato plants and causing the ZC. Attempts to inoculate CLam and CLas in potato plants have been done via top grafting with apical stem of infected vinca plants (*Catharantus roseus*) and via cuscuta (*Cuscuta campestris*) from CLas infected citrus plants, inside a quarantine greenhouse.

Results from molecular *Ca.* Liberibacter sp. analyses performed 40-50 days after inoculation, showed CLam and CLse in vinca tissues (scion) and cuscuta (haustorien) but not in inoculated potato plants. These results are not considered conclusive yet. Potato inoculations of CLam and CLas at sprouts of seed-tubers and younger/emerging plants including transmission via insect vector are underway. Positive or negative results from these proactive approach, toward an eventual interaction of citrus HLB/potato ZC is expected to be of value (alert) for the official phytosanitary inspection service as well as preventive control strategies in Brazil.

Potato stolbur caused by a phytoplasma of the stolbur (16Sr-XII-A) group is a serious disease in South-east Europe, Russia and the Mediterranean area. Since 2006, it has been detected in Germany. The disease influences potato yield and especially tuber quality by discoloration of crisps and potato chips during the frying process. The stolbur phytoplasma is strongly associated with the host plant bindweed (*Convolvulus arvensis*) and transmitted by *Hyalesthes obsoletus* Sig. to potato. This planthopper also transmits the causal stolbur phytoplasma of Bois Noir in grapevine. During the last 20 years, infestations of *H. obsoletus* have steadily increased, and Bois Noir has become one of the economically most important diseases of grapevine in Europe. It is hypothesized due to changing climate conditions that the vector recently spread to areas with previously unfavorable climate. Therefore, the risk for occurrence of stolbur beyond former expansion regions in potato fields is surmised. It becomes critical to determine whether the *H. obsoletus* transmitted stolbur phytoplasma, causing Bois Noir on grapevine is capable of infecting potatoes. Therefore, a transmission experiment was carried out. Healthy potato tubers of certain cultivars were planted in a Bois Noir diseased vineyard of the 'Cabernet Dorsa' cultivar with a high density of *C. arvensis*. At occurrence of disease symptoms, leaves and stems of the infected potatoes, bindweed plants and foliar samples of the grapevines surrounding the potato planting were collected and the phytoplasma analysed in PCR. Grapevine and bindweed were highly infected by stolbur phytoplasma and also the potato cultivar samples between 50 and 100%.

If climate conditions further develop to the benefit of *H. obsoletus,* vigilance on monitoring its incidence and the potential risk for transmission of phytoplasmas is recommendable not only in vineyards but also in potato fields.

Potato spindle tuber viroid transmission by thrips and honey bees

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During the last few years infection of several species of ornamental plants with *Potato spindle tuber viroid* (PSTVd) has been recorded. Concern of risk of transmission of viroids from ornamentals to the important food crops tomato and potato has been raised. It is well known that viroids are transmitted mechanically by sap. There are a few records of direct transmission by bumblebees and by aphids through encapsidation of the viroid RNA in the *Potato leaf roll virus*. Until now no investigations of the possible role of thrips and honey bees as vectors for PSTVd have been published.

Experiments with thrips (*Thrips tabaci and Frankliniella occidentalis*)

PSTVd isolate S1 from *Solanum jasminoides* and B1 from *Brugmansia* sp were used as inoculum. The experiments with the two thrips species were carried out separately. Thrips were reared on non-infected plants; starved for 2 h and transferred to a Petri dish with PSTVd infected plant tissue for 24 h; transferred to a non-infected receptor plant for 2-3 days and eliminated afterwards. Receptor plants were incubated 5-8 weeks in a glasshouse before testing for PSTVd by conventional or real-time PCR. The experiments comprised transmission from PSTVd infected *S. jasminoides* and *Brugmansia* sp. to non-infected *S. jasminoides* and tomato. No transmission of PSTVd from infected to non-infected plants was shown.

Experiments with honey bees (*Apis mellifera*)

PSTVd isolate S1 was used as inoculum. The plant species included in the transmission trials comprised *Nicotiana glutinosa*, *Brugmansia* sp. and tomato.

All plants were at the flourishing stage. Mini glasshouses with 6 infected and 6 non-infected receptor plants were used. Two houses with infected and non-infected plants and as control a house with 12 non-infected plants were included. A mini honey bee hive with 1500 workers with brood, but without a queen was placed in each mini glasshouse. The honey bees were fed with sugar solution. The bees were kept in the glasshouse for two weeks and then removed. Four to five weeks later the receptor plants were tested for PSTVd by real-time PCR.

The following experiments were carried out where honey bees were allowed to forage on PSTVd infected and non-infected flourishing plants: Infected and non infected *N. glutionosa;* infected *N. glutinosa* and non-infected tomato; infected *Brugmansia* sp. and non infected *Brugmansia* sp.
No transmission of PSTVd was shown under the experimental conditions used.

Main conclusions

The results show that the two thrips species do not vector PSTVd. The risk of honey bee transmission of PSTVd from viroid infected ornamentals to tomato crops is probably negligible, because the tomato flowers are unattractive to honey bees. Furthermore, the risk of other non-floral mechanical damages combined with possible viroid transmission caused by honey bees is considered very low, because of the low weight and power of a single honey bee.

Acknowledgements

The activities were granted through the EU ERA-network EUPHRESCO and financed by the Ministry of Food, Agriculture & Fisheries, Danish Food Industry Agency and the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management.

New knowledge on *Potato mop-top virus* in Denmark

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Introduction
The authors participated in 2005-08 in a transnational project Enhanced control of potato mop-top virus in the Nordic and Baltic Sea region. The results in the presentation are from the Danish project activities.

Distribution of PMTV in Denmark
Soil samples were collected 2006-07 from seed potato growers. The soil was sampled below graders giving average samples each covering whole fields. The samples were tested using *Nicotiana benthamiana* as bait plant and testing the roots with DAS-ELISA. PMTV was detected in most parts of Denmark, where professional potato production takes place.

Estimation of the propagation rate of PMTV under field conditions
From the mapping of PMTV, the incidence on the island Lolland showed to be of special interest, because contract production of seed potatoes was first initiated in 2000. However, the farmers must have started earlier, so a period of potato production can be estimated to around 12 years. PMTV was recorded at 3 out of 7 locations. The occurrence of visual spraing was still very low. The farmers ran a 3 years crop rotation (potato every 4th year). With the assumption that PMTV has been introduced to Lolland with seed tubers, it can be concluded that it takes about 3 potato crops to propagate PMTV to a detectable level, but where visual spraing is not yet a real problem.

The importance of weed as natural hosts of PMTV
Two common weed species, *Solanum nigrum* (black nightshade) and *Chenopodium album* (pigweed or white goosefoot), known to be experimental hosts for PMTV, were collected from PMTV infected fields at 5 locations and mainly at the edge of the fields. Of the 222 collected species of *S. nigrum*, 89 (40%) were ELISA-tested positive for PMTV in the roots, while no virus was detected in roots of *C. album*. This shows that *S. nigrum* is a serious natural propagation host of PMTV, especially in potato free years during crop rotation.

Dispersal of PMTV to new fields
PMTV is dispersed to new fields with seed tubers, but it has not been elucidated whether the transmission is owed to the virus infection of the tubers themselves or to the adhering soil containing viruliferous sporeballs of *S. subterranean*.

Experiments were carried out, where tubers were harvested from PMTV infested fields. The tubers were ELISA-tested in the stolon end for PMTV. The adhering soil was removed and baited for PMTV using *N. debneyi* with subsequent ELISA-test of the roots. The tubers were washed and surface disinfected and planted in pots in PMTV free growth medium with and without addition of non-viruliferous sporeballs of *S. subterranean*. The plants were grown to maturity where after the plants and the tubers were removed and the soil was baited for PMTV using *N. debneyi*.

The results showed that PMTV was dispersed with the adhering soil removed from both virus infected and non infected tubers. PMTV was also dispersed with the washed and disinfected tubers. Addition of non viruliferous sporeballs of *S. subterranean* to the growth medium increased significantly the number of positive bait plants obtained.

In conclusion, PMTV is dispersed both with the adhering soil containing viruliferous sporeballs of *S. subterranea* and with virus infected tubers, and the concentration of sporeballs in the soil influences the infection rate. These results underline the role of contaminated soil in dispersal of PMTV. Therefore, the only means to avoid dispersal of PMV with seed tubers is to grow seed potatoes in fields free of PMTV.

Acknowledgements
The activities were financed by Nordic Joint Committee for Agricultural Research.

Leafhopper and aphids associated with potato in Alaska, USA: Species composition, seasonal abundance, and potential virus vectors

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Due to its geographical isolation and climatic constraints, Alaska is considered relatively free of diseases and insect pests; therefore growers in the state are exploring the potential of producing seed potato for export. However, the biology of agricultural insect pests in the circumpolar region is lacking or poorly understood. Research conducted from 2004 to 2006 in the main potato (*Solanum tuberosum* L.) production areas of Alaska resulted in the identification of 41 leafhopper species associated with agricultural settings. Twenty species were identified in association with potato. Two species, *Davisonia snowi* (Dorst) and *Macrosteles fascifrons* (Stål), made up approximately 60% of the total number of individuals collected, representing 34 and 26%, respectively. Both species, *M. fascifrons* and *D. snowi* generally arrived in fields by late May to early June and numbers peaked by late June to July. In all years *M. fascifrons* populations peaked earlier than *D. snowi*. Three of the species collected [*Balclutha punctata* (Fabricius), *M. fascifrons,* and *Scaphytopius acutus* (Say)] are known vectors of phytoplasmas of potatoes and other agricultural crops or have the potential to cause mechanical damage to potatoes. This report represents the first extensive study of Cicadellids from potatoes in Alaska. Preliminary data on potential aphid vectors associated with potato include known vector species such as *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer), *and Rhopalosiphum padi* (L.).

Localization of *Potato virus Y*^{NTN} RNA and viral particles in potato plants

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There were numerous methods developed for the detection of *Potato virus Y*^{NTN} (PVY^{NTN}), the causal agent of potato tuber necrotic ringspot disease (PTNRD), in plants. Diagnostic methods are mainly based on ELISA, RT-PCR, bioassays and RT real-time PCR. On the other hand, there is very limited data about the distribution of PVY within the plants. Different approaches can be used in manner to localise viral proteins in plant tissues or within the cells, such as electron microscopy and immuno-tissue printing, however *in situ* hybridisation is the only method which enables the localisation of target RNA within tissue. Therefore we introduced a complex approach to localize PVY^{NTN} RNA and PVY^{NTN} viral particles in the same potato plants. Recently developed RT real-time PCR PVY detection system (Kogovšek *et al*. 2008) enabled us to identify the tissues of systemically infected sensitive potato plants of cultivar Igor, containing the highest amounts of PVY^{NTN} RNA, what we compared with relative concentrations of viral particles in the same tissues by negative staining transmission electron microscopy (TEM). Besides, ultrathin sections of resin embedded potato tissues were investigated by TEM for the subcellular localization of PVY proteins. For better insight of viral RNA accumulation, the in-situ hybridization method for detection of PVY^{NTN} RNA in potato tissues was developed. There was a very good correlation between the results obtained by all four methods used.

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

Do pospiviroid infections in solanaceous ornamentals pose a risk for potato?

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Recently many latent infections by *Potato spindle tuber viroid* (PSTVd) and other pospiviroids have been detected in vegetatively propagated ornamentals. In a survey in the Netherlands in 2006 PSTVd infections were found in over 40 and 70% of the professionally grown lots of *Brugmansia* spp. and *Solanum jasminoides*, respectively. Phylogenetic studies showed that PSTVd genotypes form four separate groups relating to the vegetatievely propagated crops *Brugmansia spp.*, *Physalis peruviana* (Cape gooseberry), *S. jasminoides* and *S. tuberosum* (potato). Genotypes from seed-propagated *S. lycopersicum* (tomato) did not form a separate cluster but grouped in the clusters of *P. peruviana*, *S. jasminoides* and *S. tuberosum*. These results indicated that latently infected plants of these crops have been sources of infection for tomato. Studies on mechanical transmission and stability of the genome of PSTVd during repeated passages in potato and tomato indeed supported this hypothesis. In addition, it appeared that tomato became more easily infected than potato and that mechanical transmission was hardly successful at a temperature of 15°C. The latter explains the failure of mechanical transmission in potato in former field experiments. Therefore, PSTVd infections in ornamentals only pose a limited risk for outdoor potato crops, especially in Northern Europe. The risks posed by other pospiviroids are expected similarly low, which is supported by the absence of outbreaks in potato. As a consequence seed potatoes still can be seen as the main pathway for introduction and spread of pospiviroids in potato crops. Therefore, production of pospiviroid-free seed potatoes remains the best practice to control these diseases.

Observed and predicted variations of potato virus patterns due to climate change

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Throughout the times the potato has been affected by several diseases among which those caused by viruses are the most important. At the beginning of the XX century major virus diseases in the potato crops in North America were leaf curl (caused by PLRV), mosaics induced by the severe necrotic strains of PVX, and yellowing and necrotic symptoms induced by viruses such as PVG (or PVF) and AMV (the so-called calico virus in North America). In Europe the situation reported was somewhat different. In addition to PLRV viruses such as PVX, PVY, PVS and PVA were common. These viruses caused important losses in potato. The *Potato spindle tuber viroid* (PSTVd) was also the cause of important yield decrease in North America and some countries in Europe as well. Later in the XX century and more likely due to the production and export of seed from Europe virus diseases spread to other countries in other continents. It is worth mentioning at this time the case of PVY-NTN and similar strains that induce ringspots in the tubers of several cultivars. These strains are common in the Andean potatoes though the ringspot disease that it causes in several countries is not commonly observed in the Andes due to its particular weather conditions and the fact that most varieties grown up there belong mainly to *Solanum tuberosum* ssp. *andigena* which appears to be tolerant to this virus disease. It is very tempting to suggest that these strains spread from the Andes to Europe together with the potato in the XVI century where they remained “hidden” until they found opportunities to establish and spread.

Changes in climate due to global warming and the increased cultivation of the potato in several regions of the world, mainly in tropical or semi-tropical countries, have led to changes in virus patterns. This was more likely due to changes in vector type and species in the crop. For example, the widespread of whiteflies (*Bemisia* sp. and *Trialeurodes* sp) in potato. These insects were only limited to small regions in some countries such as the case of *Trialeurodes vaporariorum* in some provinces in Colombia and Ecuador and *Bemisia tabaci* in Central America has helped the spread of viruses such as *Potato yellow vein virus* (PYVV) and *Tomato yellow leaf curl virus* (TYLCV), respectively. In this region the common tuber symptoms (spraing -like) observed in North America and Europe caused by *Potato mop-top virus* (PMTV) do not develop even in susceptible *S. tuberosum* ssp. *tuberosum* cultivars.

Spread of known virus vectors such as thrips and psyllids in potatoes are changing the virus patterns in potato crops in many countries. *Tomato spotted wilt virus* (TSWV) and possibly other tospoviruses which are transmitted by thrip species (*Thrips* sp and F*rankliniella* sp mainly) are finding ways to spread in some countries in South America helped by the increase in cultivation of other susceptible crop species such as peppers, artichokes and tomatoes. This change of crop species in some regions will certainly lead to further changes in virus patterns in potatoes in a climate changing environment.

Rapid development of virus resistant cultivars using genotypes carrying Rx and Ry genes in triplex condition

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Using different breeding strategies (introgression, crosses, backcrosses, etc.) new varieties that combine high yielding potential, resistance to pests and diseases and good quality are the aims of breeding programs. However, the breeding process is long, costly and the new developed varieties are not easily accepted by the farmers, especially in the Andes. Varieties with resistance to viruses are essential to maximize yield in the Andes. Potato farmers in the Andes are somewhat reluctant to use the new varieties and prefer to keep on producing older cultivars. Therefore, we propose to use a classical breeding strategy in order to develop a variety, which morphologically looks like one variety already known by producers but that will combine the additional positive characters of immunity to PVY and PVX available in *Solanum tuberosum* genotypes in multiplex condition. The strategy consists in crossing a known highly adopted variety (parent/variety A) with a PVY and PVX immune genotype carrying the Ry and Rx genes in triplex condition (parent B) and select in the segregating progeny all the clones that will combine the morphological and agronomical characteristics of variety A and the resistance of parent B. By knowing exactly how the end-product should look like we are hoping to accelerate the breeding process mainly by reducing the time needed for evaluation of resistance in every step of the selection and decrease its cost by doing a more drastic selection and carrying less material from one generation to the other. The adoption of the variety-like cultivar (variety C) will also be accelerated because of its resemblance to a traditional variety already known by the market chains actors: farmers, wholesalers, consumers and processors. The time required for the development, identification and diffusion of like-variety cultivar could be reduced from the 10 or more years needed for a traditional variety down to even 4-6 years under the Peruvian conditions. In this presentation we describe how the variety “Musuq Tomasa” was developed using this strategy. First evaluation results of this new potato like-variety are also reported.

An enhanced detection method for *Tobacco rattle virus*

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The potato (*Solanum tuberosum*) is the 4th most important commercial crop grown worldwide with an annual production of approximately 300 million tons. In Norway, potato is the second most important crop being grown in 18 000 ha and cultivated all over the country ranging from the southern county of Aust-Agder to the northernmost county of Finnmark. Besides being used for consumption (i.e. table potato, crisps) potatoes are also important for the production of starch and alcohol. The annual potato production in Norway during the last four years has been approximately 290 000 tons/year with an estimated value of 493 million kr/year.

Potato production in Norway has been hampered during the last 15 years with the occurrence of spraing symptoms (brown rings and arcs) in the flesh of potato tubers. These symptoms render the tubers unacceptable for the production of crisps, french fries and for the fresh market, resulting in economic losses for the growers. Spraing in potato tubers are caused mainly by two potato-infecting viruses, *Potato mop top virus* (PMTV) and *Tobacco rattle virus* (TRV). Currently, there is no reliable method to detect TRV in Norway. TRV is transmitted by nematodes of the genera *Trichodorus* and *Paratrichodorus* and can also be transmitted by seed potatoes. Symptomless infection of potato tubers occurs. Consequently, TRV can spread to nematode-infested fields by the use of symptomless infected seed potato tubers. A PCR-based detection method was developed in collaboration with the University of Hamburg. This method was based on TRV specific primers and also internal control primers amplifying the NAD gene. Successful PCR amplification of a region of TRV was achieved from leaf tissues samples infected with TRV. In addition, a successful RNA extraction method from root tissue was developed in our lab. Root samples from various fields used for the production of seed potato have been screened for TRV. A more sensitive nested PCR assay has been implemented to enhance even more the sensitivity. This method consists of a nested PCR assay with internal TRV-specific primers. Moreover, a multiplex PCR-based detection method utilizing potato tuber has been developed in collaboration with Graminor and the University of Hamburg. This method has the objective of screening for TRV and PMTV (both important soil-borne viruses) in seed potatoes.

The *Ny-1* allele dosage and hypersensitive resistance to PVY (*Potato virus Y*) in potato

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Potato virus Y (PVY) is one of the most important viruses in potato. Breeding of resistant cultivars is one of the most effective strategies to achieve protection against PVY. There are two main types of resistance to PVY: extreme resistance (ER) and hypersensitive resistance (HR). The gene *Ny-1* confers HR for the common (PVY^O) and necrotic (PVY^N) strains (Szajko *et al*. 2008). *Ny-1* mapped on potato chromosome IX in cultivar Rywal. Expression of HR was temperature-dependent. The virus was effectively localized at 20°C. At 28°C, plants were systemically infected but no symptoms were observed. Throughout 5 years of field experiments plants of cv. Rywal produced PVY-free tubers. Therefore, we postulate that *Ny-1* can be useful for potato breeding as an alternative donor of PVY resistance.

A tetraploid potato displays tetrasomic inheritance and plants can be simplex (Aaaa), duplex (AAaa), triplex (AAAa) or quadruplex (AAAA) with respect to a given gene. The objective of this study was to investigate the effect of *Ny-1* allele dosage on defense responses in potato leaves after infection with PVY^N. *Ny-1* duplex clones were selected from a cross between Rywal and PVY-resistant F1 hybrid, using real time PCR. The results of experiments for symptom expressions and presence of PVY RNA in inoculated potato leaves will be presented.

Szajko K, Chrzanowska M, Witek K, Strzelczyk- Żyta D, Zagórska H, Gebhardt C, Hennig J, Marczewski W (2008) The novel gene *Ny-1* on potato chromosome IX confers hypersensitive resistance to *Potato virus Y* and is an alternative to *Ry* genes in potato breeding for PVY resistance. Theor Appl Genet, 116: 297-303.

Potato mop-top virus, a soil-borne virus affecting potato production in northern Europe

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Potato mop-top virus (PMTV; genus *Pomovirus*; family *Virgaviridae*) is distributed in the potato growing areas in the Americas, Japan and northwestern Europe. In the Nordic countries, the internal necrotic arcs in tubers (spraing symptoms) caused by PMTV constitute a severe quality problem as the affected tubers are unsuitable for the French fry and chip (crisp) industries and are rejected by supermarkets and the food industry. However, occurrence of PMTV in other countries of the Baltic Sea region was unknown and was studied by a research consortium consisting of 21 research institutions and a few companies in 2005-2008 (Santala *et al*. Ann. Appl. Biol., in press). Harmonized sampling and virus detection procedures were introduced by the Nordic laboratories. Bioassays and serological and molecular methods were employed to detect PMTV in potato tubers and soil samples. Intensive and systematic surveys in Poland detected a single PMTV-infected tuber in 2008. Other tubers expressing spraing symptoms were infected with *Tobacco rattle virus*, which also occurred in Russia according to the bioassays. In the Baltic countries and northwestern Russia, no PMTV was detected, within an exception of minitubers in a screenhouse in Latvia in 2005. Surveys in the Nordic countries showed that only the main seed potato production area in northern Sweden and the High Grade seed potato production zone in Finland were negative for PMTV. However, these data should not be interpreted as if all fields in other areas were contaminated with PMTV. The project increase awareness of a high percentage of PMTV-infected but symptomless tubers in many cultivars and that breeding for resistance and inspection of seed potatoes should not rely on indexing symptoms only. Movement of viruliferous sporangia of the soil-borne vector (*Spongospora subterranea* f.sp. *subterranea*) on seed tubers was realised as a risk for further dispersal of PMTV in the region.

Determination of aphid transmission efficiencies for N, NTN and Wilga strains of *Potato virus Y*

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Potato virus Y (PVY, genus *Potyvirus*, family *Potyviridae*) causes high economic losses worldwide, especially in the production of seed potatoes (*Solanum tuberosum)*. PVY control systems rely on measuring virus pressure and vector pressure in the field. Calculation of the vector pressure is based on the relative efficiency factors (REFs) of aphid species, which express the transmission efficiency of aphid species in relation to the efficiency of *Myzus persicae*, the most efficient vector of PVY.
In the Netherlands, in the 1980s, aphid's REFs were determined using aphids caught alive in the field. Thus, experiments were conducted using limited numbers of aphids and only during the potato growing season. We have now developed a system which allows us to test virus transmission whole year round, using aphid clones reared in insect chambers.

Using the new system, we determined the aphids' relative transmission efficiency factors (REFs) for six isolates of the PVY strains PVY^{N}, PVY^{NTN} and PVY^{N-Wi}. Biotype Mp2 of *M. persicae* showed comparable average transmission efficiencies for all isolates, and was used as an internal control to determine the REFs of 18 other aphid species. The newly determined REFs for PVY^{N} were comparable to previously reported values. New REFs for the PVY^{NTN} strains were overall comparable to the REFs for PVY^{N}, except for *Aphis frangulae* and *Schizaphis graminum*. For PVY^{N-Wi} six aphid species showed higher REFs (*Acyrthosiphon pisum, A. fabae, Aphis nasturtii, Aphis* spp., *P. humuli* and *R. padi*). Only *A. frangulae* shows a lower REF for PVY^{N-Wi}. In addition three aphid species (*Aulacorthum solani*, *Myzus ascalonicus* and *S. graminum*) for which no REF was determined earlier were found to be capable to transmit PVY and their REFs were determined.

Global transcriptomic approach to study the role of the salicylic acid in response of potato to PVY infection

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The application of microarray technology has been demonstrated to be a powerful tool for analysing gene expression profiling. The great advantage of this approach is that numerous genes can be monitored simultaneously, while the traditional methods of expression analysis allow only a few genes to be examined at the same time.

Hypersensitive response (HR) is an efficient defence strategy in plants that restricts pathogen growth. The HR can be activated during host as well as non-host interactions. Recently, we have identified in potato (*Solanum tuberosum* L.) cv. Rywal a novel gene *Ny-1*, which is responsible for an arrest of PVY particles at the infection sites. This is the first known gene from potato that confers HR to both common (PVY^0) and necrotic (PVY^N) strains of PVY (Szajko *et al*. 2008 *Theor. Appl. Genet*. 116, 297-303). The HR is correlated with an increase in biosynthesis of salicylic acid (SA) which is one of the most important signal molecules in plant response to viral infection. However, the exact function of SA in the HR still remains unclear.

To analyse gene expression during HR, we have used Agilent custom arrays containing over 40 000 unique potato genes developed by the POCI (Potato Oligo Chip Initiative) Consortium. We have monitored global gene expression in PVY-inoculated and mock-treated plants at various time points after treatment. We have also investigated the role of the SA in the plant-viral interactions by comparing the response of the wild-type potato (cv. Rywal) and transgenic lines impaired in SA biosynthesis (potato cv. Rywal/*nahG*).

We have found that in the absence of SA, activation of the large part of genes involved in plant defence is strongly delayed and weakened. Additionally, we have observed that in Rywal/*nahG* plants after viral infection the energy balance is shifted from energy assimilation towards mobilization of stored sources, i.e. from anabolism to catabolism. More data from aforementioned experiments will be also presented.

This research was supported by the Polish Node of the Potato Genome Sequencing Consortium contract no. 47/PGS/2006/01. Additionally, it was supported by FEBS Short–Therm Fellowship to K. Witek.

Development of a multiplex RT-PCR procedure for indexing common potato viruses in seed potatoes in Canada

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Five potato viruses, *Potato virus S* (PVS), *Potato virus X* (PVX), *Potato virus A* (PVA), *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) are common across Canada and their incidence is normally low in commercial potatoes. Generally, PVX, PVA and PVS do not cause significant damage to potato. However, PVY (*Potyvirus*) and PLRV (*Polerovirus*) are two major potato pathogens and can be highly destructive depending on the combination of virus isolates, potato cultivars and vectors. All these viruses have a single-stranded, plus sense RNA genome. They are mainly transmitted through infected tubers and may also be transmitted by aphid vectors (except PVX) or mechanically (except PLRV) during the growing season. PLRV is introduced by aphids in a persistent non-propagative manner into the vascular tissues of plants and, except in plants co-infected with certain other virus, *e.g.* PVY, remains largely restricted to the phloem tissues. However, PVA, PVY and PVS are transmitted by aphids in a non-persistent manner and not restricted in the phloem tissues of plants.

The exportation of Canadian seed potatoes to several countries requires that the seed tubers be tested for these viruses and the overall virus incidence of seed potatoes must be below a certain level determined by the import countries. ELISA is the standard procedure in place for detecting these viruses and this method is simple, rapid and quantitative. ELISA for detecting PLRV and PVY, however, is not sensitive enough to detect low virus titres that may be present in dormant tubers. Therefore, ELISA is only performed on extracts of sprouted tubers or progeny plants. The entire testing process is time consuming and labour intensive and the slow process is sometimes the major factor that prevents the sale of seed potatoes to certain foreign markets.

A mxRT-PCR procedure was developed in this study for the simultaneous detection of these viruses in dormant potato tubers. Potato materials (leaves, dormant tubers) infected with one or more viruses were used for extracting total RNA. Composite samples containing tissues of leaves or tubers infected with different viruses were also prepared to have various virus combinations. Several potato cultivars were evaluated. Tri-Reagent method was used for extracting total RNA as described previously with the addition of an extra step to grind tissues in extracting buffer.

Primers were designed to target all strain types of these viruses and the PCR amplicons were between 79 bp and 486 bp. Both the one-step and two-step RT-PCR were evaluated and the latter was adopted in the mxRT-PCR protocol. Oligo $d(T)_{12-18}$ and a mixture of gene specific reverse primers were both evaluated. Oligo $d(T)_{12-18}$ proved to be adequate for synthesising the first strand cDNAs from RNAs of all these viruses including PLRV whose genomic RNA does not have significant stretches of polyadenylate. The cDNA templates were then mixed with 23 µl of PCR mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.2% blotto, 0.6 mM of each dNTP, 0.1 – 0.4 µM (depending on virus) of each virus specific primer, 3.5 mM of $MgCl_2$ and 5 units of Taq DNA polymerase. The PCR parameters and the following gel electrophoresis were essentially the same as previously described except that the annealing temperature was 60°C which was optimum for all primers used.

Reliable detection of these viruses in the total RNA extracts of either potato leaves or dormant tubers was achieved by using this mxRT-PCR procedure. As little as 1 fg viral RNA in dormant potato tubers was readily detected without the need to break tuber dormancy. Virus was easily detected in composite samples of 200 (PVY and PLRV) to 400 (for PVX, PVS and PVA) dormant tubers without compromising the sensitivity of the test. The test results showed that the mxRT-PCR was highly specific and produced no false amplification of non-targeted viruses and *Potato spindle tuber viroid*. The mxRT-PCR protocol developed can be recommended for use in seed potato certification for directly screening dormant potato tubers on a large scale for meeting the requirements of export markets.

Changes in PVY population in Poland

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Historical data: The first PVY^{N} isolate was found in 1956 in Poland by Berbeć on tobacco. The PVY^{O} isolate named LW was identified by Chrzanowska in 1970 on potato cv. Lipiński Wczesny. Till 1982 PVY^{O} strain had composed up to 80-90% of the PVY population. A shift of PVY strain spectrum had happened in Poland since 1983-1986. In the period from 1987 to 1992 PVY^{N} strain had become predominant reaching up to 87% of the population. The $PVY^{N}W$ isolate named Wi was identified on potato cv. Wilga in 1984 by Chrzanowska, and since then the $PVY^{N}W$ strain had dominated in the PVY population till 2007. Occurrence of $PVY^{N}W$ in the population decreased to 32% in 2008. The PVY^{NTN} isolate named 12/94 was found by Chrzanowska in 1994 on tobacco bait plant grown in the potato field at Młochów. $PVY^{N/NTN}$ strain gradually increased from 10 up to 20% of the PVY population from 1994 to 2003. From 2004 to 2008 a significant increase of $PVY^{N/NTN}$ was noted, and it reached 66% of the population. The PVY^{O} was rarely detected since 1996-1997 (5%) and dropped to 2% in 2008. The isolate Wi ($PVY^{N}W$) and 12/94 (PVY^{NTN}) were sequenced and described as the recombinant types between PVY^{O} and PVY^{N} strain.

In the present study, a total of 281 PVY isolates, collected from 1995 till 2009 were tested. Based on ELISA with PVY monoclonal cocktail and PVY^{N}-specific antibodies (Bioreba) and, in addition, on symptoms on *Nicotiana tabacum* cv. Samsun, 155 isolates were classified as $PVY^{N}W$ and 126 isolates as $PVY^{N/NTN}$ strains. Among the 281 studied isolates, 112 isolates were characterized by a triplex RT-PCR method developed by Rigotti and Gugerli (2007). The tested isolates were mainly classified as the $PVY^{N}W$ Wi-P and recombinant PVY^{NTN} strain types. Neither PVY^{N} nor non-recombinant PVY^{NTN} strain was found among tested isolates. The isolate 12/94 and two similar ones were not detected by the triplex RT-PCR method (no band) and were designated as recombinant PVY^{NTN} 12/94 type strain in our work. Besides *N. tabacum*, *Chenopodium amaranticolor* was used as an additional test plant with necrotic reaction caused by PVY^{O} and PVY^{C} (local lesions, LL) and lack of symptom by PVY^{N}. In 100 $PVY^{N}W$ isolates (64.5%) out of 155 tested, consistency between the molecular and biological data was found. However, serotype of 10 isolates (6.5%) changed from PVY^{O} to PVY^{N} and 23 isolates (14.8%) exhibited vein clearing symptoms (VCl) on tobacco instead of vein necroses (VN). For the $PVY^{N/NTN}$ strain, majority of the isolates, 76 (60.3%) out of 126 tested, induced severe LL on *C. amaranticolor*, a characteristic reaction of PVY^{O} strain. Whereas, 23 isolates (18.3%) of $PVY^{N/NTN}$ induced only a few LL and 13 ones (10.3%) were without symptom. Moreover, we provide biological evidence that two amino acid changes (K-400 to R-400, E-419 to D-419) of the viral genetic determinants for vein necrosis in *N. tabacum* in the HC-Pro cistron indeed correlate with the loss of vein necrosis phenotype of a previously sequenced recombinant PVY^{NTN} isolate namely Gr99. Unpredictable variations in serological and biological features of PVY tested isolates were also observed for 22 (14.2%) $PVY^{N}W$ and 13 (10.3%) PVY^{NTN} isolates. We conclude that the detection methods used in this study are reliable and currently the PVY^{NTN} strain is the dominant form of PVY population in Poland. The PVY^{NTN} isolates can be further divided based on their ability to induce LL on *C. amaranticolor*.

Abstracts of Posters

Quantitative approach of aphid-mediated transmission of PVY isolates

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Potato virus Y (PVY) is an important plant pathogen which causes major loss in several important crop species. This (+)ssRNA virus is transmitted by aphids in a non persistent manner and infects many species of the family *Solanaceae*. Potato strain isolates of PVY have been classified into different groups and subgroups, according to i) their ability to induce vein necrosis symptoms (PVY^N) or not (PVY^O) on *Nicotiana tabacum* and ii) their biological properties on some *Solanum tuberosum* ssp. *tuberosum* varieties. During the last twenty years, epidemiological studies have highlighted an increase of the proportion of necrotic isolates in natural populations. Understanding the processes that have led to the prevalence of these isolates in PVY populations is an important challenge for research programs studying this virus. Among the different factors involved in the emergence and increased prevalence of a viral entity, aphid-mediated transmission is important as it can be considered as one of the key steps in the spread of the pathogen in the environment. The molecular bases of plant/virus/aphid interactions involved in plant-to-plant transmission have been well described in previous studies. However, not much information is available on the quantitative parameters related to host changes induced by the virus. Using specific quantitative tools (real-time PCR) and reference isolates (PVY^N-605 and PVY^O-139), we designed an experimental procedure to analyze the impact of both qualitative and quantitative parameters of viral sources (infected *N. tabacum* leaves) on the efficiency of aphid-mediated transmission of PVY isolates. The results of these studies indicate that the viral concentration required for an efficient plant-to-plant transmission is affected by both the isolate and the location of the infected leaf used as viral source.

Detection of potato viruses using antibodies against recombinant viral proteins

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Cloning and expressing of plant viral genes coding for structural and non-structural proteins has become an important strategy for obtaining large amounts of antigens with uniform concentration and stable properties.

We report here the strategy for production of polyclonal antibodies against recombinant proteins of viruses which infect potatoes, namely antibodies against coat proteins (CP) of *Potato virus A* (PVA), *Potato virus Y* (PVY), *Potato mop-top virus* (PMTV), *Potato virus X* (PVX) and *Potato leafroll virus* (PLRV), and also against non-structural proteins of PMTV and PLRV. At present we are working on antibodies for safety detection of *Potato virus M* (PVM).

The obtained sera and antibodies were tested for the detection of mentioned pathogens in laboratory hosts (tobacco species) and natural host *Solanum tuberosum*. The obtained antisera have been successfully used for plant virus detection by Western blot analysis and indirect PTA ELISA, but they have failed in DAS ELISA. Our antibodies did not recognize native epitopes, but only epitopes which were affected by some denaturation steps.

Nevertheless, the obtained polyclonal antibodies could be a very good tool not only for virus detection but also for the study of their life cycle.

This research was supported by the grant No. MSMT 1M06030 of the Ministry of Education, Youth and Sports of the Czech Republic.

Chimeric *Potato virus X* as a tool for peptide display and experimental vaccine development

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Expression of heterologous proteins in plants represents an attractive strategy for vaccines production combining cost-effectiveness and safety. We investigated the possibility to transiently express *human papillomavirus* type 16 (HPV-16) epitopes-based vaccine in plants using potato virus X-based vector and to induce an immune response against HPV.

The optimized expression of recombinant *Potato virus X* (PVX) coat proteins (XCP) carrying different epitopes from HPV-16 was developed. Epitopes derived from the L2 minor capsid protein and E7 oncoprotein were joined as N-terminal or C- terminal fusions with XCP of a *Potato virus X* based vector and these recombinant proteins were initially expressed in *E. coli* to prove their ability to form virus-like particles (VLPs). Then, the transient expression in plants using *Agrobacterium tumefaciens* mediated inoculation was performed. To increase the level of the produced proteins the transgenic *Nicotiana benthamiana* plants expressing the *Potato virus A* HC-Pro gene were tested. Immunogenicity of these recombinant viruses was tested after immunization of mice. Recombinant viruses were injected subcutaneously or administered by a tattooing device. In animal sera the antibodies against the XCP and the L2 epitope were found after both methods of vaccine delivery.

This research was supported by the grant No. 521/09/1525 of the Czech Science Foundation.

Finding of *Eggplant mottled dwarf virus* in potato in Slovenia

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Eggplant mottled dwarf virus (EMDV) was first reported in 1969 from Italy. Affected eggplant showed severe stunting, pronounced mottling and crinkling of the leaves and generalized unfruitfulness. The virus was characterized and the name EMDV was proposed. The virus occurs in northern Africa, southern Europe and the Middle East. It was found in different hosts including eggplant, cucumber, tomato, tobacco, pepper, muskmelon, potato, honeysuckle and *Hibiscus rosa-sinensis*. *Anaceratagallia laevis* and *A. ribauti* were identified as vectors of EMDV in cucumber in France and *Agallia vorobjevi* as a vector in Iran.

Previous year crops of potato from different parts of the country were planted in 2004 on location in central Slovenia to compare the frequency of virus infections in planted material. Severe dwarfing, leaf curling and reduced leaf size were observed on some plants of potato cvs. Bistra, Pšata, Desiree and Discovery and on advanced clone KIS 94-1/5-14 from the Agricultural Institute's breeding program. Tubers were very small and few in number, in cross sections of tubers brown spots were observed. The symptoms were observed only on potatoes originating from Rakičan in north-eastern Slovenia. A range of test plants were inoculated with the sap of two symptomatic plants of cvs. Pšata and Desiree. Local and systemic symptoms were observed on *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. rustica*, *N. tabacum*, but only local lesions developed on *Datura stramonium*. No symptoms were observed on *Chenopodium quinoa*. Rhabdovirus-like particles were observed in sap of symptomatic *N. benthamiana*, *N. clevelandii* and *N. tabacum* using electron microscopy. Antisera against EMDV and *Potato yellow dwarf virus* (PYDV) were kindly supplied by Dr. S. Winter, DSMZ, Braunschweig, Germany. DAS-ELISA with above mentioned antisera confirmed the infection of potato and symptomatic *N. rustica* with EMDV. Although EMDV infects different important crops and causes severe symptoms in potato its low incidence in crops makes it a less important pathogen.

A library of monoclonal antibodies for an improved serological characterization of PVY isolates

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Potato virus Y (PVY), type-member of the genus *Potyvirus* (family *Potyviridae*) is known as a high variable viral species with different strains (e.g. PVY^{N}, PVY^{O} and PVY^{C}) and variants (e.g. PVY^{NTN} and $PVY^{N}W$). The PVY diversity has been mainly studied at both biological (aggressiveness and virulence) and molecular levels (mutations and recombination). However, only few data are available on serological variability within PVY species. Thus, in addition to the main Y^{N} and $Y^{O/C}$ serogroups, other original serotypes have been described for selected members of PVY strains. However, most of available serological diagnostic tools are based on the use of monoclonal antibodies allowing detection of isolates according to PVY^{N} or $PVY^{O/C}$ strains. These tools are not adapted for the accurate detection and description of PVY populations. Consequently, the main objectives of this project are to describe the immunological diversity of PVY isolates and to perform epitope characterization for selected ones. To reach these aims, a library of monoclonal antibodies is under construction. Using information from both data bank and our own sequencing programs, sequence alignments of the N-terminal region of the PVY coat protein were carried out. This approach revealed that PVY^{N} and PVY^{O} isolates clustered in 22 and 39 groups, respectively. According to this described molecular diversity, mixtures of PVY^{N} or of PVY^{O} isolates were prepared and independently used to immunize mice. First results from this wide serological PVY screening project will be presented and discussed.

Interactions between *Potato virus Y*, its principal vector *Myzus persicae* and host plants

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Potato virus Y (PVY) has long been known as the most important virus in potato and tobacco crops. Characteristic of PVY is its great potential for the evolution, which results in the high incidence of the virus variants showing novel biological, molecular and/or serological properties. Natural vectors of PVY are aphids, and *Myzus persicae* is its principal vector. The virus is transmitted by aphids in a non-persistent non-circulative manner. This means that PVY acquisition from an infected plant and inoculation to a healthy plant can be performed during short feeding probes made by aphids in the epidermal tissue to assess plant suitability as a host. The vector remains viruliferous for a short period of time because the virus is easily lost during subsequent probes. In the field, the extent of PVY spread can be facilitated or reduced by a large variety of biotic and abiotic factors. However, little is known about the interactions between potential aphid vectors of PVY and commonly occurring in recent years virus variants classified as PVY^{NTN} and PVY^{N-W}.

Our studies were undertaken to evaluate the efficiency of transmission by *M. persicae* of 12 geographically different isolates/variants of PVY. In the years 2008-2010 three distinct timed probe experiments were carried out under greenhouse conditions. The virus isolates comprised six variants classified into subgroups PVY^{NTN} and PVY^{N-W}, and six isolates represented traditional PVY^{O} and PVY^{N} strains. Some of the latter were used as reference isolates. The sources of PVY inoculums for aphids were infected plants of potato cv. Irga. The virus was acquired by apterous aphids during a 7-minute vs. 3-day acquisition access period (AAP). Three plant species were used as the assay plants: *Nicotiana tabacum* var. Samsun, *Physalis floridana* and *Solanum nigrum*. Twenty seedlings of each species per experimental unit and 7 aphids per seedling were applied. Significant differences between some isolates in the efficiency of transmission by *M. persicae* were found. In general, transmissibility of PVY^{NTN} and PVY^{N-W} isolates was markedly higher than that of the reference PVY^{N} and PVY^{O} isolates. Transmission efficiency of some PVY isolates taken up by aphids during a 3-day AAP, compared to a 7-minute AAP, appeared to be unexpectedly high. The results obtained imply that wingless *M. persicae* colonizing PVY-infected plants can transmit the virus to neighbouring healthy plants with a relatively high efficiency. In attempts to elucidate the reasons of different transmissibility of the isolates by *M. persicae*, quantification of the viral RNA in samples of aphid vectors is evaluated using real-time RT-PCR. The aim of our recent studies was also to evaluate transmissibility of PVY isolates by *M. persicae* from source plants simultaneously infected with two isolates, in pairs consisting of PVY^{NTN} isolate and PVY^{O} isolate. Two plant species: *N. tabacum* var. Samsun and *S. nigrum* were applied both as the sources of virus inoculums for aphids and assay plants. The aphids acquired PVY during a 7-minute AAP. In transmission tests, 20 seedlings of each of the assay plants per replication and one aphid per seedling were used. Both source plants and assay plants were examined for the presence of PVY isolates using monoclonal antibodies (MoAbs) against PVY^{N}, MoAbs against PVY^{O} and multiplex RT-PCR assay reported by Lorenzen *et al.* (2006). The results obtained proved that one individual of *M. persicae* is capable of simultaneous transmitting two isolates of PVY. A mechanism of the transmission remains to be elucidated.

This study is a part of work supported by the Ministry of Science and Higher Education, grant N31001432/1114.

Lorenzen, J. H., Piche, L. M., Gudmestad, N. C., Macham, T. & Thiel, P. 2006. A multiplex PCR assay to characterize *Potato virus Y* isolates and identify strain mixtures. Plant Disease, 90, 7, 935-940.

Endemic Isolates of *Potato spindle tuber viroid* in Russia and their evolution

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Potato spindle tuber viroid (PSTVd) is a highly structured circular RNA molecule that contains approx. 360 nt and, unlike most viral RNAs, lacks mRNA activity. The disease caused by PSTVd ('spindle tuber') was first described in the former USSR in the early 1930's, but the unique features of its causal agent were only discovered in 1971 (Diener & Raymer, 1971). A quarantine pathogen for most European countries, PSTVd remains widespread in Russia and other CIS countries.

Our laboratory has studied PSTVd since 1974, but only three years ago could we begin to compare the molecular properties of Russian isolates thanks to financial support provided by USDA/ARS. Over the last three years we have recovered many Russian PSTVd isolates originally collected in the late 1980s and early 1990s. Several of these isolates had been propagated annually in the field, while others were stored as RNA solutions at low temperature. Analysis of 70 PSTVd isolates from our collection has identified 30 different sequence variants. Seven of these isolates originated from CIS or other countries outside Russia. Of 23 Russian PSTVd isolates with different primary structures, two were identical to isolates described earlier from other countries; i.e., PSTVd.004 from Germany (GenBank M14814) and PSTVd.009 from the USA (GenBank M88677). Comparison of Russian isolates with the type strain (i.e., PSTVd-Intermediate, Genbank V01465) and 53 other naturally-occurring PSTVd isolates deposited in Genbank revealed four groups of variants.

The first group of variants that we term the 'Australian population' is represented by 8 closely related isolates, each containing approx. 20 changes compared to PSTVd-Intermediate strain. The second group of variants includes 33 isolates (11 from Russia) whose distinguishing features include the presence of an A/U substitution at position 120 and the deletion of a single adenine residue between positions 121-123. The third or "Russian population" contain 12 exclusively Russian isolates and differs from the third population by the presence of an A/C rather than A/U substitution at position 120. Members of the last group of 28 isolates do not exhibit any common differences from the type strain and thus can be considered as derivatives of this strain.

Group 4 contains a large number of isolates causing severe to lethal symptoms in 'Rutgers' and other sensitive tomato cultivars. Groups 2 and 3, in contrast, contain many isolates closely related to PSTVd-mild strain (GenBank M14814) and few (if any) severe isolates. Russian isolates in groups 2 and 3 exhibit similar patterns of sequence changes. A model illustrating the possible evolutionary relationships between these four groups of PSTVd variants will be presented.

A new approach for studying the genomic variability and recombination patterns of *Potato virus Y* and *Potato virus M*

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Recombination point mutations and small insertions or deletions are all major source of diversity especially in RNA viruses. The reason why these mutations happen so often is probably as a result of high error rates of the RNA synthesis during the replication of the RNA viruses. However, recombination frequency is normally affected by several factors, including host genes, the viral replication proteins and various features of the viral RNA templates involved.

Potato virus Y (PVY) is the type member of the genus *Potyvirus* (family *Potyviridae*) which forms the largest and most economically important of the plant virus groups recognized by the International Committee on Taxonomy of Viruses. The PVY genome is a monopartite, linear, single-stranded, positive-sense RNA of about 9.7 kb in length. PVY has a high degree of genetic variability (identity range 82%-98% from BLAST) and is also subject to recombination. Since the early 1980s, a number of PVY recombinants have been documented, including PVY^{NTN-}, PVY^{N-}-Wi and as a result, since 1984, the importance of PVY^{N} has been increasing again in Europe.

Potato Virus M (PVM), is a member of the genus *Carlavirus* and its genome is comprised of filamentous particles composed of multiple copies of the coat protein and a monopartite positive-sense ssRNA of approximately 8.5 kb in length. Although this virus has been described by Schultz and Folsom in 1923 little research has been done to understand its genomic variability. Recently, using the CP gene, it has been reported that all known isolates of the virus can be placed into two distinct groups.

Correct detection and elimination of viruses in potato stocks is vital to potato health and production across the world. Therefore, seed certification programs are important for the detection, quantification, and suppression of major viral diseases. The appearance of new virus isolates or strains will result in the need to update the spectrum of standard tests so they can be highly efficient. Although many PVY recombinants have been reported, mechanisms of selection of quite a limited number of recombination junctions are poorly understood and physical factors affecting recombination, especially in PVY, are still not very well known. In the case of PVM much less has been reported.

To examine the PVY and PVM complex at the sequence level and to assess the extent of inter-strain variation, we propose to perform deep-sequencing of full virus genomes using a high-throughput sequencing technique based on pyrosequencing (GS-FLX, Roche). Pyrosequencing has come to the fore as a much more high throughput methodology than traditional Sanger sequencing. Sequences from multiple isolates will be compared with the help of both phylogenetic and recombination analysis to assess any differences present. Once genomic differences are detected, biological differences (symptoms, transmission efficiency etc.) between the distinct sequence types identified will be evaluated. Factors affecting sequence variation both in natural production and in artificial systems will also be examined.

Validation of DAS-ELISA for detection of *Andean potato latent virus* detection in potato leaf material

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To monitor the presence of quarantine viruses and viroids in the total column of potato production, extensive testing occurs in the Netherlands. Next to broad range testing of (imported) gene bank material, new varieties and pre-basic seed is tested. In these stages the material is tested for the presence of viroids and three viruses, *Andean potato latent virus* (APLV), *Andean potato mottle virus* (APMoV) and *Potato black ringspot virus* (PBRSV).

In the process of applying for an ISO 17025 accreditation, we started with validation of the detection of the tymovirus APLV in potato using DAS-ELISA. This validation was based on the 'National directive for validation of detection and identification methods for plant pathogens and pests'. This direction is based on NEN7777 and EPPO PM7/98 and has been composed by the laboratories testing in plant health in the Netherlands.

In this particular validation it has been shown that this DAS-ELISA for APLV is very suitable for screening of potato leaf material. However, since in the determination of the analytical specificity also closely related tymoviruses reacted, for identification confirmation with a second method is necessary.

Validation of methods for detection of APMoV, PBRSV, pospiviroids and other viruses will follow in the near future.

Salicylic acid plays a critical role in *Potato virus Y* spreading in potato plants carrying *Ny-1* gene

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A prototypic plant pathogen interaction system used to study disease resistance is tobacco and *Tobacco mosaic virus* (TMV). In this pathosystem, the resistance response comprises two phenomena, known as the hypersensitive response (HR) and systemic acquired resistance (SAR). The mechanisms that are involved in HR and SAR are not completely understood.

We studied resistance response of *Solanum tuberosum* ssp. *tuberosum* cv. Rywal to PVY infection. We identified a novel locus *Ny-1,* that determines the HR response of *S. tuberosum* cv. Rywal plants to PVY. The *Ny-1* locus has been mapped (Szajko *et al*. 2008 Theor. Appl. Genet. 116: 297-303.) on potato chromosome IX, linked by 2cM to the locus *GP41*.

In contrast to most characterised potato cultivars, Rywal develops local HR reaction after PVY inoculation. These plants, are resistant to known: ^{N}, ^{0}, ^{NTN} PVY strains, when grown in growth chambers at 20°C. Such resistance response successfully limits virus replication and its spreading. Using RT-PCR approach we were able to detect presence of PVY particles only in the inoculated parts of plants. The HR and virus localisation correlates with an increase in salicylic acid (SA) biosynthesis and PR gene induction. Additionally, these reactions are abolished when plants are growing at constitutive ≤28°C temperature or in transgenic *nahG* plant with reduced level of SA.

This research was supported by the Polish Node of the Potato Genome Sequencing Consortium contract no. 47/PGS/2006/01.

The current PVY population affecting potatoes in Finland

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Potato virus Y (PVY) is one of the most common viruses in potato. It causes serious problems in the production and quality of potatoes. To investigate the PVY population in potatoes in Finland, we collected 21 PVY isolates from seed potatoes and seven additional isolates from potato fields in 2006 and 2007. Biological, serological and molecular methods were applied to characterize the isolates. Results showed that the isolates fell into two strain groups (PVY^{N} and PVY^{O}) according to the responses on potato cultivars Pentland Crown (Ny:nc), Pentland Ivory (Ny:Nc), King Edward (ny:Nc) and *Nicotiana tabacum* cv. Samsun. Interestingly, several isolates which caused necrosis on *N. tabacum* Samsun also induced necrotic blotches on locally and systemically infected leaves of Pentland Ivory and/or Pentland Crown which contains the *Ny* resistance gene specific to PVY^{O}, but the symptoms differed clearly from the necrotic local lesions and no systemic infection caused by PVY^{O}. Serological results were consistent with the biological assays with one exception: isolate 182-14 caused veinal necrosis on tobacco but was detected with a PVY^{O}-specific monoclonal antibody (Mab1129), which is similar to the PVY^{NW} strain group. The 5'- and 3'-proximal sequences of the PVY genome including 5'UTR, P1, HC-Pro, CP and 3'UTR were determined in the PVY isolates. In 14 out of 21 isolates from seed potatoes and five out of seven isolates from other potato crops were found to contain recombination events which have been previously reported in PVY^{NTN} strain group, within the CP encoding sequence. One isolate was tested on many potato cultivars and found to induce tuber necrosis symptoms in cultivars Nicola and Annabelle. Only the isolate 182-14 was found to contain a recombination event in the P1 encoding region. Phylogenetic analysis indicated that 182-14 has a PVY^{N}-like HC-Pro but PVY^{O}-like CP. In conclusion, PVY^{N} strain group was the predominant one in seed potatoes in Finland and hence likely to be also most common in ware and industrial potato crops; only four isolates belonged to PVY^{O} strain group.