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INTRODUCTION

Collecting, conservation and utilization of plant genetic resources and their global distribution are essential components of international crop improvement programmes.

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant pests¹ along with the host plant. In particular, pathogens that are often symptomless, such as viruses, pose a special risk. In order to manage this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of concern.

The ever-increasing volume of germplasm exchanged internationally for research purposes, coupled with recent advances in biotechnology, has created a pressing need for crop-specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IPGRI to launch a collaborative programme for the safe and expeditious movement of germplasm, reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO, as the depository of the International Plant Protection Convention of 1951, has a long-standing mandate to assist its member governments to strengthen their plant quarantine services, while IPGRI's mandate – *inter alia*– is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

The purpose of the joint FAO/IPGRI programme is to generate a series of crop-specific technical guidelines that provide relevant information on disease-indexing and other procedures that will help to ensure phytosanitary safety when germplasm is moved internationally. The recommendations in these guidelines are intended for small, specialized consignments used in technical crop improvement programmes, e.g. for research, conservation and basic plant breeding programmes. When collecting germplasm, local plant quarantine procedures, for example pest risk assessment, should be considered.

These technical guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the elaboration of the guidelines in their private capacities and do not represent the organizations for whom they work. The guidelines are intended to be the best possible advice for institutions involved in germplasm exchange for research, conservation and basic plant breeding. FAO, IPGRI and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. They reflect the consensus of the contributing crop specialists, based on the best scientific knowledge available at the time. The experts who have contributed to this document are listed after this introduction.

¹ The word 'pest' is used in this document as it is defined in the International Plant Protection Convention. It encompasses all harmful biotic agents ranging from viroids to weeds.

The guidelines are written in a short, concise style, in order to keep the volume of the document to a minimum and to facilitate updating. Suggestions for further reading are given at the end, along with the references cited in the text (mostly for geographical distribution, media and other specific information). The information given on a particular pest is not exhaustive but concentrates on aspects that are most relevant to the safe movement of germplasm. Only pests which may be transmitted when germplasm is moved in the recommended form are described in these guidelines.

The present guidelines were initiated at an FAO-sponsored meeting held in Edinburgh, United Kingdom, 11-14 March 1996. The meeting was hosted by the Scottish Agricultural Science Agency.

Orthography for virus taxon names

In this document we have attempted to conform with the latest orthographical conventions for virus taxon names, but some of these names may require revision once the 7th ICTV (International Committee on the Taxonomy of Viruses) Report is published in mid-1999. The latest convention requires formal taxon names to be written in italics and have an upper case first letter. This had been required for family and genus names (e.g. family Comoviridae genus *Nepovirus*) and has now been extended to the species name (e.g. potato leafroll virus is now *Potato leafroll virus*).

Names of viruses classified provisionally by the ICTV, those not yet classified and synonyms are not to be in italics (Mayo 1998; Mayo and Horzineck 1998).

In this document “tentative” means that the virus has been classified provisionally by the ICTV. “Possible” means that the virus has not yet been considered by the ICTV for classification and the genus and family names stated are the views of the scientists working with the virus only.

Guideline update

In order to be useful, the guidelines need to be updated when necessary. We ask our readers to kindly bring to our attention any developments that possibly require a review of the guidelines, such as new records, new detection methods or new control methods.

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GENERAL RECOMMENDATIONS

1. These guidelines apply to cultivated *Solanum* spp. (e.g. *Solanum tuberosum*) stolon- and tuber-forming wild species, and closely related species that do not develop stolons or tubers (Hawkes 1990) and are intended for use by research workers and specialized institutions engaged in plant breeding, germplasm conservation and evaluation.
2. International movement of germplasm must comply with the regulatory requirements of the countries involved. Prospecting activities for conservation must be based on prior consultation with the relevant plant protection organizations (PPOs) and a knowledge of organisms in the relevant countries.
3. Information exchange between exporter and importer must be sufficiently transparent for a full evaluation of the pest risk. Wherever possible, material should be selected from the least risk source, e.g. an institution which maintains pathogen-tested germplasm. Indexing procedures and precautions taken to prevent infection and contamination of the germplasm after testing should be documented, e.g. in a 'Germplasm Health Statement' (Appendix I and II).
4. Growing of untested germplasm outside effective containment and/or isolation is not recommended. Collecting and conservation activities must be preceded by establishing the nearest point where the material can be placed in quarantine containment.
5. It is recommended to transfer pathogen-tested pollen, true potato seed (TPS³) and in vitro plants (including microtubers moved aseptically) only. In this way dissemination of fungi, arthropods and nematodes is reduced, and tests for these organisms, other than visual inspection, are not described in these guidelines.
6. Movement of pathogen-tested stem-cuttings or tubers is not recommended because of the risk of the material becoming infected after testing.
7. Pathogen-tested material should be derived from tested parents and maintained under conditions designed to prevent infection and contamination. Absence of pathogens should be established by applying the indexing procedures described in these guidelines and should be verified as appropriate.

3 True potato seed (TPS) in this document means sexually produced potato seed..

8. Risks can be reduced further by post-entry quarantine containment (e.g. insect-proof houses, out-of-season cultivation, growing in an isolated area with no links to potato material) and quarantine testing. Post-entry quarantine containment/testing is a requirement in many countries. Some countries, however, will accept the testing done by specified countries as being equivalent to their own and accept material without the need for post-entry quarantine containment/testing. Untested or infected material must be managed so as not to be a risk to tested material.
9. The volume of consignments should not be larger than is necessary to preserve the genetic usefulness of the germplasm. In the case of vegetative material and TPS, quantities recommended are 1-10 plants and 20-200 seeds respectively. Increasing quantities may lead to resource problems in containment and testing.
10. Some PPOs stipulate that each unit (in vitro plantlet, seed/seedling and pollen-derived plant) of germplasm is tested individually for quarantine pests, whereas other authorities allow, particularly for TPS, test results derived from bulked samples representative of the consigned germplasm. Probability tables for assessing the risk associated with different TPS sample sizes can be found in Appendix III.
11. Normally in vitro material contaminated with saprophytic organisms should be destroyed. However, where the material is rare or valuable, such contamination may be dealt with by the treatments described in these guidelines. Antibiotics and fungicides must not be added to the medium for shipment.

Movement of infected material or material of unknown health status

Some PPOs make provisions for the movement and propagation of such material for research and conservation purposes. Effective quarantine containment must be maintained at all times and at the earliest opportunity the plant health risk must be minimized by testing and, if necessary, by therapeutic treatment.

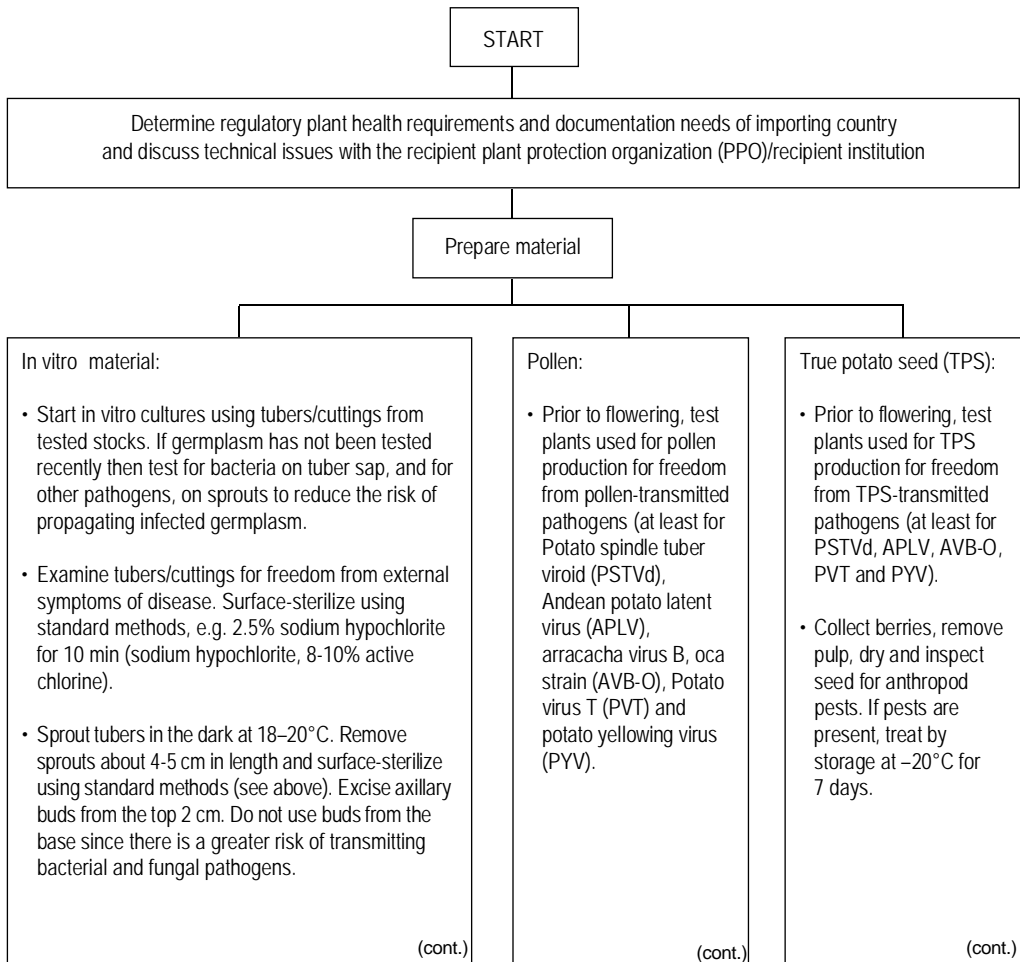
Therapy

Exceptionally, valuable infected material may be treated as recommended in these guidelines. Rigorous attention to quarantine containment must be applied during therapy. Material must only be released from containment after pathogen-freedom is confirmed using the testing procedures described. Recipients of the material should be informed of the pathogens eliminated.

TECHNICAL RECOMMENDATIONS

These are described in flow diagrams for the exporting and importing countries. Additional information on shipping in vitro material is described by Seabrook and Coleman (1988).

Exporting country



In vitro material:

Plant buds into sterile culture medium (Murashige and Skoog medium without growth regulators with 30 g/L sucrose and 8 g agar, or 10 g agar when there is international transfer).

- Apply stringent aseptic techniques and procedures including autoclaving instruments between lines (flame sterilization using methanol may not always be effective) and cutting over a sterile disposable surface.
- Chronologically record actions in handling germplasm so that material can be checked easily for cross-infection, should infected material be detected later.
- Test in vitro plants for pathogens before multiplication and plant material that tested negative in sterilized compost under containment. Test again for pathogens prior to flowering and observe for disease symptoms.
- If pathogens are detected which cannot be eradicated, the germplasm must be destroyed. If the germplasm is scarce or unique, maintain it separately under containment so as not to present a risk to other germplasm. Check for cross-infection as appropriate.
- Saprophytic bacterial or fungal contamination of scarce or unique material in vitro may be treated with antibiotics or fungicides. These and charcoal must not be added to the medium for shipment.

Pollen:

- Collect pollen and inspect for arthropod pests. If pests are present, treat by storage at -20°C for 7 days.

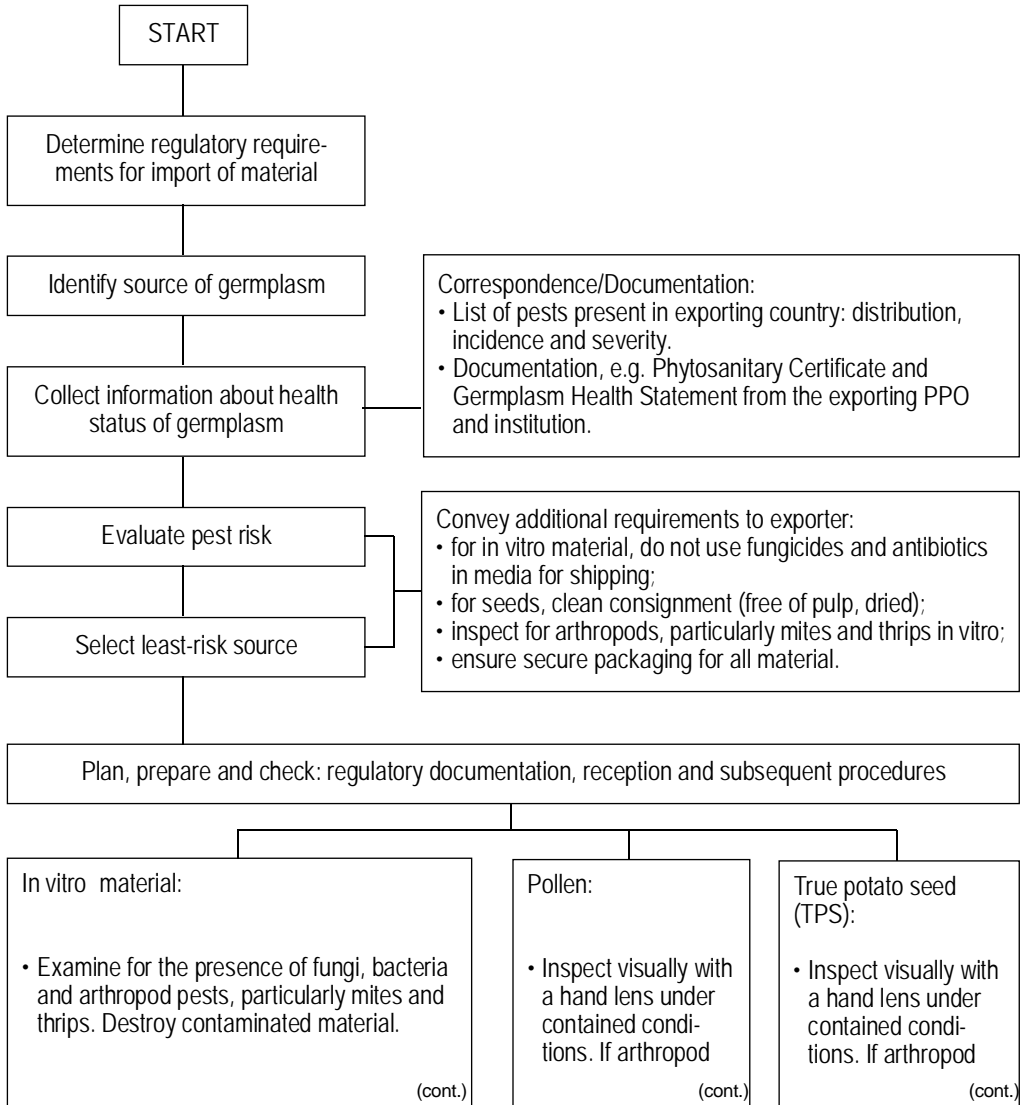
True potato seed (TPS):

- Surface-sterilize using standard methods (e.g. those described in "in vitro material") to kill external seedborne pathogens.
- If required by the exporting or importing plant protection organization, test a sample of the seeds before export for freedom from TPS-transmitted pathogens (at least for PSTVd, APLV, AVB-O, PVT and PVV).

Ensure a priori compliance with regulatory plant health requirements of the importing country including arranging for inspection and the issuing of a Phytosanitary Certificate to accompany the material.

Prepare additional documents such as a Germplasm Health Statement. Include any other relevant information. Send advance copies to recipient and include another with shipment.

Importing country



In vitro material:

- Apply stringent aseptic techniques and procedures, including autoclaving instruments between lines (flame sterilization using methanol/ethanol may not always be effective) and cutting over a sterile disposable surface.
- Chronologically record actions in handling germplasm so that material can be checked easily for cross-infection should infected material be detected later.
- Before multiplication, test in vitro plants for pathogens as specified by the PPO. Plant material that tested negative in sterilized compost in containment; observe for disease symptoms and test for pathogens again. If results are negative, release in vitro plants. If tubers are to be released, precautions must be taken to avoid cross-contamination between plants, should infection be found. If material is positive, assess risk of cross-contamination of other material and retest or destroy this as necessary.
- If in vitro facilities are not available, plant directly in sterilized compost in containment and observe and test as above.

Pollen:

pests are found, treat by storage at -20°C for 7 days.

- Grow pollinated plants and progeny seedlings derived from imported pollen sources in containment and test for pathogens as specified by the PPO (at least for PSTVd, APLV, AVB-O, PVT and PYV).

True potato seed (TPS):

pests are found, treat by storage at -20°C for 7 days.

- Establish seeds in vitro or germinate in sterilized potting compost and grow out under containment.
- Test seedlings for pathogens as specified by the PPO (at least for PSTVd, APLV, AVB-O, PVT and PYV).

If pathogens are detected which can not be eradicated, the material must be destroyed. If the material is scarce or unique it should be maintained separately under containment so as not to present a risk to other material held.

DETECTION

General information on tests for viruses, phytoplasmas and bacteria is presented below, with more specific information under each pathogen description.

Viroid

See description for Potato spindle tuber viroid.

Viruses

The primary tests to detect viruses are bioassay on indicator plants and serology using ELISA (enzyme linked immunosorbent assay) with polyclonal or monoclonal antibodies (Hill 1984; Torrance 1992; Salazar 1996). Several viruses (including unknowns) may be detected on a single indicator plant whereas ELISA is generally virus-specific. Other tests are based on nucleic acid detection (Jones 1992; Salazar and Querci 1992; Salazar 1996) or involve electron microscopy (Roberts 1986).

Each potato plant must be tested separately unless procedures for bulking have been evaluated. For bioassay and ELISA, plants should be sampled from at least two positions on every stem including a young, fully expanded leaflet at the top of each stem and an older leaflet from a midway position. Direct testing of tuber sap, sprouts or eye-plugs is unreliable and should be used as a preliminary test only. Leaflets from the same plant may be bulked. Ideally at least two different tests, such as bioassay and ELISA, should be conducted.

Indicator plants for viruses are given in Appendix IV and disease symptoms in recommended indicators are described under each pathogen description. Some promising new indicators – *Nicotiana glauca*, *N. miersii* and *N. occidentalis* (van Dijk et al 1987; van Dijk and Cuperus 1989) – are also described, but in general these have not been evaluated against a wide range of isolates. Other indicators also have been reported (Horváth 1985). Care should be exercised in selection of indicators since differences in susceptibility to virus infection between accessions of the same species may occur (van Dijk and Cuperus 1989). Inoculum may be prepared by grinding leaf tissue in tap water but if this is of poor quality, distilled or deionized water should be used. Some laboratories may prefer to use a phosphate inoculation buffer, e.g. 0.02M phosphate buffer, pH 7.4 + 2% w/v polyvinylpyrrolidone (PVP, MW 10000).

Inoculation buffer: 0.02M phosphate buffer + 2% polyvinylpyrrolidone (PVP)

solution 1: $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 89.0 g/L water

solution 2: $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 69.0 g/L water

Prepare a 0.5M stock solution of phosphate buffer by mixing 500 ml of solution 1 and 200 ml of solution 2. Adjust to pH 7.4 by adding one or the other solution.

To prepare the inoculation buffer, add 20 ml of the stock solution and 10.0 g of PVP (MW approx. 10 000) to 0.5 L water.

For inoculation, grind about 0.5 g of plant material in 5 ml inoculation buffer (ratio 1:10).

For several viruses, special buffers should be used (see virus descriptions).

Test plants are inoculated by rubbing plant sap on leaves lightly dusted with carborundum (400-600 mesh). Celite can also be used. Infection and symptom development may be affected by the condition of the test plant, cultural and environmental conditions. Indicator plants are most susceptible when young and actively growing; shading them (e.g. by covering with paper) prior to and after inoculation may enhance susceptibility and symptom development. Although increased test sensitivity has been achieved using air guns to propel the carborundum and sap (Laidlaw 1986, 1987), these are not recommended for quarantine viruses because of the increased risk of contaminating surfaces distant from the site of inoculation. Where viruses cannot be mechanically transmitted, grafting should be done (Hill 1984). Vector transmission is not recommended.

Double antibody sandwich (DAS) and triple antibody sandwich (TAS) ELISA procedures are commonly used for virus detection (Torrance 1992). Sensitivity and specificity of ELISA depends on the antibodies used. For many potato viruses these are available commercially (Appendix V) and ELISA protocols (Hill 1984; Copeland 1998) are often provided. In addition to virus-specific antibodies, antibodies have been produced which react with most aphid-transmitted potyviruses (Jordan and Hammond 1991). For most viruses, the sap extract is prepared in a standard sample buffer (PBS-Tween + 2% PVP).

Molecular hybridization techniques, using radioactive-labelled complementary DNA or RNA probes are available for many potato viruses, but their use has been generally limited to specialist laboratories (Barker and Torrance 1997). Non-radioactive probes based on digoxigenin are now available and their sensitivity is similar to that of radioactive probes (Pallás et al 1998; Webster and Barker 1998).

Confirmation of the presence of virus particles may be done by using electron microscopy and immunosorbent electron microscopy (ISEM) (Roberts 1986).

Isolation and purification of dsRNA and analysis by means of gel electrophoresis have been used to detect infections of plants with ssRNA viruses and may be useful to detect unknown viruses or where other detection methods are unavailable (Jones 1992).

To overcome the problem of sample deterioration, especially important where the testing laboratory is distant from the site of sampling, methods have been developed to allow trapping of virus particles/nucleic acid on membranes, which may then be despatched to the testing laboratory (e.g. dot/squash blots, NASH-nucleic acid spot hybridization) (Baulcombe and Fernandez-Northcote 1988; Gibson 1988; Lin et al 1990; Mitchell et al 1990; Barker et al 1992; Samson et al 1993).

For reverse transcription polymerase chain reaction (RT-PCR) protocols (Innis et al 1990), specific primers have been developed for a number of viruses infecting potato (Appendix VI). However, not all have been tested on isolates from potato yet. The use of PCR and degenerate primers is enabling the development of genera (or group) detection methods (Appendix VI). Although RT-PCR is a sensitive alternative to serological tests, more simplified and robust procedures are needed (Seal and Coates 1998), before it will find general use in routine application, where sensitive and more user-friendly methods are already available.

Phytoplasmas

Phytoplasmas are generally present in phloem sieve tubes only, may be unevenly distributed and present in low concentration, making detection difficult. To increase the chances of detection using the methods described below, where relevant, sap should be extracted from leaf midribs and petioles only.

Phytoplasmas may be maintained and propagated using dodder (*Cuscuta* spp.) or graft transmission to, e.g. *Capsicum annuum*, Madagascar periwinkle (*Catharanthus roseus*), *Datura stramonium*, tomato (*Lycopersicon esculentum*) or eggplant (*Solanum melongena*), and then by periodic grafting to young seedlings of these species.

Attribution of a disease to a phytoplasma can be based on symptom expression, association with arthropod vectors (particularly leafhoppers), electron microscopy of ultra-thin sections of phloem tissue for pleomorphic structures with a trilaminar unit membrane and sometimes disease remission following treatment with tetracyclines (Acikgoz 1993; Clark 1992). Electron microscopy alone is unreliable in poorly colonized tissue. Use of light microscopy and DNA-binding fluorochrome dyes, e.g. 4', 6-diamidino-2-phenylindole (DAPI) and Hoechst 33258 is more successful (Hiruki and da Rocha 1986; Dale 1988) and more suitable to mass screening than electron microscopy. These methods, however, cannot identify or differentiate between different phytoplasmas.

Polyclonal and monoclonal antibodies have been produced against many phytoplasmas (Lin and Chen 1986; Shu et al 1990; Lee et al 1993a), including potato witches' broom (da Rocha et al 1986). Antibodies have been used in ELISA (Fos et al 1992), immunogold labelling and electron microscopy, immunofluorescence microscopy (Cousin et al 1989; Clark 1992) and tissue blotting on nitrocellulose membranes (Lin et al 1990). Antibodies to tomato stolbur (thought to be caused by the same phytoplasma that causes potato stolbur) have been produced and should detect potato stolbur but this has not been tested.

Probes also have been developed, but not specifically for potato phytoplasmas, although, a probe produced against clover proliferation phytoplasma will detect potato witches' broom phytoplasma (Lee et al 1991; Hiruki and Deng 1992).

PCR methods are the most sensitive and reliable. Extract nucleic acid using a phytoplasma enrichment procedure (e.g. Lee et al 1991; Ahrens and Seemüller 1992) and detect using universal phytoplasma primers. Until the reliability of universal primers detecting potato phytoplasmas is determined, it is advisable to use at least two different primer pairs to test a sample [e.g. R16F2/R16R2 (Lee et al 1993b), fU5/rU3 (Lorenz et al 1995) and P1/Tint (Smart et al 1996)].

More specific detection methods involve using phytoplasma-specific primers or differentiation on the basis of phylogenetic RFLP analysis of PCR-amplified 16S rDNA sequences (Lee et al 1993b; Schneider et al 1993). Provided that six or more frequently cutting restriction enzymes are used in the RFLP analysis, specific identification of the phytoplasma may be obtained. For new phytoplasmas, or phytoplasmas from a poorly studied region or crop, then it is preferable to use 12 or more enzymes to achieve identification (R. Davis, 1997, pers. comm.). Enzymes found valuable for these analyses include AluI, BamHI, BfaI, DraI, HaeIII, HhaI, HinfI, HpaI, HpaII, KpnI, MseI, RsaI, Sau3AI, TaqI and ThaI. Of the potato phytoplasmas, only potato witches' broom and stolbur have been differentiated using the PCR-RFLP method.

The search for phytoplasma-specific primers has led to evaluation of primers based on sequences of the 16S-23S rRNA spacer region (Smart et al 1996). This region appears to offer more variation than that of the 16S gene in which rRNA sequences of related phytoplasmas are very similar, in some cases. Similarly, finer distinctions may be made among some related phytoplasmas through analysis of sequences amplified in PCR from the ribosomal protein gene operon (Gundersen et al 1996). Primers previously designed for specific amplification of DNA from stolbur phytoplasma were recently found to prime amplification of DNA from other phytoplasmas (Davis et al 1997; Jomantiene et al. 1998); therefore, it may be advisable to supplement use of phytoplasma-specific primers with RFLP analysis of amplified DNA sequences.

Bacteria

Technologies for detection of quiescent or latent bacterial infection in potato germplasm include enrichment and growth in nutrient media, serology, and molecular methods that detect specific nucleotide sequences (De Boer et al 1996). General and/or selective media are commonly used for culture and presumptive recognition of most bacterial pathogens of potato (Schaad 1988). Enrichment and growth media formulations can be selected for specific multiplication of pathogenic species or for non-selective multiplication of plant-associated microorganisms generally (Perombelon and Hyman 1986). If proper aseptic technique is used, any microbial growth from potato tissue in enrichment medium is evidence of microbial contamination. Identification of any bacterium purified from the medium can be achieved by comparing its biochemical/physiological profile, carbon-utilization pattern, cellular fatty acid composition and/or genetic fingerprint with those of selected known cultures.

Bioassays in appropriate indicator plants are commonly used for simple detection of some bacterial potato pathogens, e.g. *Clavibacter michiganensis* ssp. *sepedonicus* in eggplant (*Solanum melongena*) seedlings and *Ralstonia solanacearum* in tomato (*Lycopersicon esculentum*) seedlings. Inoculation of infected plant extracts or presumptive isolates into

stem incisions between the cotyledons of seedlings (third fully expanded leaf stage) usually results in the development of typical wilt symptoms within 28 days. Further testing of stem tissue above the point of inoculation is recommended to ensure absence of latent pathogen populations in non-wilted indicator plants.

Polyclonal and monoclonal antibodies have been prepared to a wide range of plant pathogenic bacteria including potato pathogens, and these can be used in ELISA or immunofluorescence microscopy to detect the presence of specific pathogens. Antibodies directed to soluble antigens such as extracellular polysaccharides or lipopolysaccharides are best for ELISA whereas those directed to structural cell envelope antigens work best for immunofluorescence. An advantage of immunofluorescence is that it allows observation of bacterial density and cell morphology, whereas ELISA is better adapted for screening large numbers of samples (De Boer et al 1994). Sensitivity of both serological techniques is limited to the range of 10^3 to 10^4 cells/ml of plant extract.

Of the nucleic acid based methods, those involving DNA amplification by a polymerase chain reaction (PCR) are most sensitive for detection of specific bacterial species or subspecies (Slack et al 1996). Primers to either genomic or plasmid encoded sequences of the major bacterial potato pathogens have been published (e.g. Seal et al 1993). When PCR is used for screening germplasm, however, the possibility of false negative test results due to the presence of inhibitory plant compounds must be adequately addressed by using appropriate controls. Confirmation of amplified PCR products by molecular hybridization or analysis of restriction fragment length polymorphisms is recommended.

Specific cultural, serological and molecular methods have a role in identifying the presence of important potato pathogens. These methods, however, may restrict detection to specific strains or subgroups of a bacterial species. There may be a need to use growth media, antibodies and primers with lower specificity when screening germplasm to detect a wide range of pathogens.

THERAPY

Viroid

Cold treatment of *in vitro* plantlets (6-8°C for 3-4 months) has proved effective, but is laborious (Lizarraga et al. 1980).

1. Establish plantlets on Murashige and Skoog (MS) medium at 22-25°C, 16 h light + 8 h dark (light intensity 75 micromol m⁻² s⁻¹ is ideal) for 2-3 weeks and then reduce temperature to 6-8°C and incubate for 3-4 months.
2. Then remove meristems with no more than 1 leaf primordium onto MS medium and incubate at 22-25°C, 16 h light.
3. Once plantlets have established, test the top part of each plant for Potato spindle tuber viroid (PSTVd).
4. If negative, allow the base part to regrow and test again. If negative, allow it to regrow (after taking subcultures for retention and storage) and grow out under containment (in a glasshouse or growth room) and test several weeks later for freedom from PSTVd.
5. Finally the plants must be subjected to testing over a complete vegetative cycle.

Viruses

Combined thermo/chemotherapy (using the antiviral synthetic riboside, ribavirin) on *in vitro* plantlets is the most efficient method for virus elimination. Omission of either heat or ribavirin during therapy will for most viruses significantly reduce the percentage of plantlets testing virus-free.

If 10 plantlets are used, >50% should be expected to test virus-free after the first 4 weeks of heat treatment although the level of success will depend on the virus/isolate/*Solanum* spp./cultivar (Griffiths et al 1990; Slack and Tufford 1995).

1. Establish plantlets in *in vitro* MS medium at 22-25°C, 16 h light + 8 h dark (light intensity 75 micromol m⁻² s⁻¹ is ideal) for 4-6 weeks.
2. Subculture nodal cuttings onto MS medium amended with 20 mg/L ribavirin and grow for about 2-3 weeks until established.

3. Initiate heat treatment of a 4 h alternating 35°C light and 31°C dark cycle for 4 weeks (reduced light of 25 micromol m⁻² s⁻¹ has been most effective). For Potato leafroll virus (PLRV) and possibly other viruses, alternating temperatures of 40°C and 25°C may be more effective in elimination of virus, although plant mortality may increase. At these temperatures up to 100% elimination of PLRV may be obtained without using ribavirin (C. Jeffries, 1996, unpublished).
4. Examine plants weekly. If there is excessive mortality, shorten the treatment period.
5. After the treatment period, excise the topmost node from each plant onto MS medium and after establishment (as described above at 1) test the top part of the plant by ELISA. (Maintain the mother plants, which are on ribavirin, at 22-25°C, 16 h light + 8 h dark (light intensity 75 micromol m⁻² s⁻¹).
6. If the plant tested at (5) is negative, allow it to regrow and test the top part of the plant again.
7. If negative (after taking subcultures for retention and storage), grow the plant under containment (in a glasshouse or growth room) and subject to testing over a complete vegetative cycle.
8. If virus is detected, either:
 - (a) continue combined thermo/chemotherapy by subculturing apical nodes from mother plants (described above at 5) onto fresh MS+ribavirin medium or
 - (b) excise meristem-tips (about 0.25 mm long) onto MS medium, a process which may substantially increase the time required to obtain virus-free plantlets.

Note: ribavirin (trade name: Virazole) may be difficult to obtain commercially in some countries and the manufacturer (ICN Pharmaceuticals, 3300 Hyland Avenue, Costa Mesa, CA92626, USA) may need to be contacted directly (Tel: +1 714 545 0100; <http://www.icnpham.com>).

Phytoplasmas

In vitro methods using chemotherapy have not been reported for elimination of potato phytoplasmas. However, a procedure published for pear (*Pyrus communis*) (Davies and Clark 1994) may be expected to achieve elimination in potato.

1. Grow in vitro plants at 22-25°C, 16 h light + 8 h dark on MS medium supplemented with oxytetracycline >50 mg/ml and repeat subculturing using apical nodes onto fresh supplemented medium at 4-6 week intervals until elimination is achieved.

Alternatively:

2. Immerse tubers in hot water (50°C) for 10-15 min for potato witches' broom (Khurana et al 1988) and potato phyllody (Khurana et al.1979; 1988) followed by apical meristem culture; or

use combined thermo/chemotherapy (36°C 24 h light, or alternating 36°C, 16 h light / 30°C 8 h dark for 6-8 weeks and 500 ppm doxycycline applied by spraying tubers 2 or 3 times weekly) followed by apical meristem culture; or

allow nodal stem cuttings to stand in oxytetracycline for 24 h, then root and subject to chemotherapy followed by apical meristem culture.

3. Finally the plants must be subjected to testing over a complete vegetative cycle.

Bacteria

In vitro plantlets which are contaminated or infected with pathogenic bacteria should be discarded. Plantlets contaminated with saprophytic bacteria (and fungi) should normally be discarded, but valuable material may be rescued by appropriate therapy (Cassells 1988; Leifert et al 1991).

Although saprophytic bacterial (and fungal) contamination can occur in tissue cultures as a result of introduction from the environment through inadequate technique, some bacteria (e.g. *Bacillus*, *Corynebacterium* and *Pseudomonas* spp.) are endophytic. These are largely unaffected by surface-sterilization at the time of culture initiation from tuber shoot tissue (Cassells 1992), may remain latent for long periods in vitro and only become visible when conditions are suitable for multiplication. Detection of endophytic contaminants can be difficult and may involve testing and evaluating a wide range of media. Culturing the original tissue culture plant in Nutrient Broth or Richardson's (1957) medium has been used with some success, only propagating from those subcultures where the original tested negative. (See ring rot description for recipe of Richardson's medium.)

Incorporating combinations of antibiotics such as Sigma's antibiotic/antimycotic solution (catalogue no. A9909 containing 10 000 units penicillin, 10 mg/ml streptomycin, and 25 mg/ml amphotericin) at 2.5 ml/L into in vitro growth media inhibits growth of many microorganisms, but may not result in elimination, and is also mildly phytotoxic (Gilbert et al 1991). Therefore combinations of other antibiotics (and fungicides) may be needed. Ideally the microorganisms should always be isolated and tested against a range of antibiotics (and fungicides) before general use.

Therapy often fails to eliminate contamination entirely and must be combined with micropropagation from meristems. Antibiotics/fungicides must not be incorporated into the medium used for moving/exporting in vitro plantlets or tubers to avoid disseminating suppressed, and thus undetected, microorganisms.

Note: flame-sterilization with ethanol may be ineffective in preventing cross-contamination of some bacteria and ethanol may be a source of bacterial contamination (Schreiber et al 1996).

PATHOGEN DESCRIPTIONS

Viroid

Potato spindle tuber viroid (PSTVd)

Infectious unencapsidated, small, circular, single-stranded RNA, with considerable secondary structure, capable of autonomous replication when inoculated into a host. Commonly 359 nucleotides (Gross et al 1978) and rarely 358 and 360 (Herold et al 1992; Lakshman and Tavantziz 1993) in potato. 356 reported in a wild *Solanum* spp. (Behjatnia et al 1996) and tomato (*Lycopersicon esculentum*) (Puchta et al 1990) and 356 and 359 in pepino (*S. muricatum*) (Puchta et al 1990; Shamloul et al 1997). Isolates show small percentage differences in homology but a range of symptoms in potato. PSTVd is the only viroid known to infect potato naturally.

Significance

Yield losses vary with isolate, cultivar and growing conditions, from slight to serious (up to 64%) (Pfannenstiel and Slack 1980). True potato seed (TPS) and breeding programmes have proved vulnerable to infection. Continued interceptions suggest that some breeding stocks or genetic collections are not yet viroid-free.

Symptoms

Although symptoms may often be present these may be difficult to recognize, particularly before flowering time. Symptoms are strain/cultivar/environment-dependent and may vary from severe symptoms (reduction in plant size, changes in plant growth habit characterized by uprightness with dark green and rugose leaves) (Fig. 1) to mild and symptomless infection. Tubers may be reduced in size and may be misshapen with spindling and conspicuous eyes (Pfannenstiel and Slack 1980) (Figs. 2 and 3).



Fig. 1. Dwarfed plant with upright growth and rugose leaves, *S. tuberosum* cv. Norgold Russet. (S.A. Slack)

Hosts

- Natural: narrow. Primarily potato (and stolon- and tuber-forming *Solanum* spp. mainly in genebanks) but has been found in avocado (*Persea americana*) (Querci et al 1995), pepino (*S. muricatum*) and tomato (*Lycopersicon esculentum*) (Puchta et al 1990; Verhoeven and Roenhorst 1995).
- Experimental: wide. 94 species in 31 families.

Geographical distribution

China, parts of Eastern Europe including the former USSR, India and North America (Smith et al 1997). Systematic indexing over the last 10–20 years has apparently eliminated or significantly reduced PSTVd from commercial seed and ware production schemes in North America and parts of Eastern Europe. Recently isolated from a wild *Solanum* spp. growing in the Northern Territory of Australia (Behjatnia et al 1996).

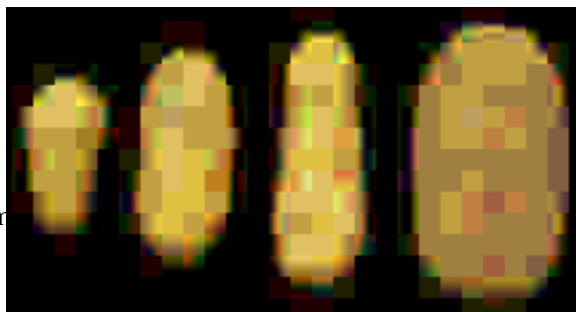


Fig. 2 (top). Spindle tubers with deep eyes, *S. tuberosum* cv. Russet Burbank (far right: healthy). (S.A. Slack)

Fig. 3 (bottom). Effect of increasing generation of infection on severity of tuber symptoms. Healthy tubers (top row), current season infection (2nd row), 3rd generation infected tubers (3rd row), *S. tuberosum* cv. Norgold Russet. (S.A. Slack)

Transmission

TPS (0-100% of seed may be infected) via infected pollen or ovules (Grasmick and Slack 1986; Singh et al 1992) and contact, but mainly by machinery in the field. Experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected with Potato leafroll virus has been reported (Salazar et al 1995; Querci et al 1996; Syller and Marczewski 1996; Querci et al 1997).

Detection

Material for testing should be incubated/grown at temperatures greater than 18°C, in the light for at least 2 weeks. Detect in sap or nucleic acid samples using ³²P- or digoxigenin-labelled DNA (Harris and James 1987; Welnicki and Hiruki 1992) or RNA probes (Salazar and Querci 1992; Podleckis et al 1993). RNA probes have proved to be more sensitive than DNA probes and the use of a RNase wash to clean filters reduces background (L. Salazar, 1996; C. James, 1996, pers. comms.). Return-PAGE (polyacrylamide gel electrophoresis) with silver staining (Huttinga et al 1987; Schroeder and Weidemann 1989) also provides very sensitive detection. Bioassay of samples on sensitive tomato cultivars, e.g. Sheyenne or Rutgers, may be done (Fig. 4). Symptoms are best at 25-28°C and high light intensity (Grasmick and Slack 1985), but mild strains may be missed and therefore inoculated plants should be tested by one of the methods described above. RT-PCR methods also have been published (Nolasco et al 1993; Levy et al 1994; Shamloul et al 1997; Weidemann and Buchta 1998).



Fig. 4. Severe strain infecting *Lycopersicon esculentum*. Rutgers. Dwarfing, rugosity, vein necrosis and rosette (right: healthy). (S.A. Slack)

VIRUSES

Only described are those viruses which have been found infecting potato and *Solanum* stolon- or tuber-forming species naturally. Therefore viruses such as Henbane mosaic virus (Horvath et al 1988), Tobacco etch virus (Valkonen et al 1996) and Tomato infectious chlorosis virus (Duffus et al 1996), which have been reported infecting potatoes only experimentally, are not included. Potato stunt disease (Cockerham and McGhee 1953) is not included since it has not been characterized and has not been reported again since its original discovery, nor is Belladonna mottle virus which has been found once in an unknown Andean cultivar (L. Salazar, 1996, pers. comm.) and the isolate is no longer available. An uncharacterized virus, isolated from *Solanum hannemannii* (Horváth et al 1993), which is thought to be related to Belladonna mottle virus (Salazar 1996), and southern potato latent virus (Brunt 1995) are not included, since little information is available.

Alfalfa mosaic virus (AMV) (genus *Alfamovirus*, family *Bromoviridae*)

Bacilliform. Four to five particle lengths (19, 29, 38, 49 and 58 nm) with the largest three particles required for infection. Diameter 18 nm.

Significance

Generally of little economic importance in potato. May cause problems locally if vectors move into potatoes from a reservoir host, particularly if a tuber necrosis-causing strain is involved.

Symptoms

Calico symptoms of bright yellow blotching or mottling of leaflets (Fig. 5). Leaflet necrosis is common with the balance of chlorosis to necrosis affected by the virus strain group. Some strains cause tuber necrosis which starts at the stolon attachment beneath the epidermis and eventually spreads throughout the tuber and is usually visible at harvest. Tubers may be misshapen, cracked and fewer in number. Tuber symptoms resemble those caused by Potato mop-top virus and/or Tobacco rattle virus infection.

Hosts

- Natural: wide. 47 plant species in 12 families. Potato is not a primary host and usually becomes infected when aphids move from a reservoir host like alfalfa (*Medicago sativa*) or clover (*Trifolium* spp.).
- Experimental: very wide. Over 300 species in 47 families.

Geographical distribution

Worldwide, but uncommon in potatoes.

Fig. 5. Calico symptoms in *S. tuberosum* (L.F. Salazar)



Transmission

Transmitted by 16 species of aphids including *Myzus persicae* in a non-persistent manner. Tends to be self-eliminating in tuber-to-tuber generations. Transmitted by pollen to botanical seed in alfalfa (up to 50% infected) and in experiments reported to be pollen-transmitted causing 0.9% infection in TPS of *Solanum tuberosum* (Valkonen et al 1992).

Detection

By ELISA and sap inoculation to *Chenopodium amaranticolor*, *C. quinoa* (chlorotic local lesions, and systemic chlorotic and necrotic flecks) which distinguish AMV from Cucumber mosaic virus and *Phaseolus vulgaris* (most cultivars (most strains necrotic or chlorotic local lesions, some strains systemic mottle, vein necrosis and leaf distortion)).

Andean potato latentvirus (APLV)(genus *Tymovirus*)

Isometric particles 28-30 nm in diameter. Three major serological strain groups are recognized: Hu, CCC and Col-Caj (Koenig et al 1979). The virus is a strain of Eggplant mosaic virus.

Significance

Little damage reported.

Symptoms

Usually latent in primary infections but occasionally causes mild mosaic and/or chlorotic netting of minor veins. Secondary infection often causes mild mosaic (Fig. 6). Sometimes chlorotic netting of the minor veins (Fig. 7) and slight rugosity of the leaves occur.

Hosts

- Natural: narrow. Potato and ulluco (*Ullucus tuberosus*) (Truskinov and Kosterova, 1995; Lizarraga et al 1996).
- Experimental: narrow. Mainly in Amaranthaceae, Chenopodiaceae, Cucurbitaceae and Solanaceae.



Fig. 6 (left). Mosaic, *S. tuberosum* cv. Mi Peru. (C.E. Fribourg)

Fig. 7 (right). Chlorotic netting of minor veins, *S. chacoense* (C.E. Fribourg)

Geographical distribution

Andean region of South America (Bolivia, Colombia, Ecuador and Peru) (Fribourg et al 1977; Koenig et al 1979).

Transmission

Mechanically (machinery) including plant-to-plant contact and flea beetle (*Epitrix* spp.). There are conflicting reports of whether APLV can be transmitted through TPS experimentally (Jones and Fribourg 1977; Jones 1982), but recently it was found infecting *Solanum acaule* TPS in a genebank (J. Roenhorst and J. Verhoeven, 1997, pers. comm.). Although pollen may be infected with APLV at very low levels, pollen to ovule transmission failed (Jones 1982).

Detection

By ELISA, but because of serological variability use a mixture of polyclonal antibodies raised to isolates from each of the strain groups and positive controls from each strain group (Schroeder and Weidemann 1990). Also detected by sap inoculation to *Nicotiana bigelovii* (best symptoms of local lesions and systemic mosaic in winter in northern Europe) and *N. clevelandii*, *N. debneyi* (systemic severe mosaic after 3-4 weeks, with sometimes vein netting in *N. clevelandii*). Better indicators have been suggested recently (Roenhorst and Verhoeven 1996): *N. benthamiana* (most isolates local chlorosis, chlorotic or necrotic lesions, systemic rugosity and/or mottle), *N. hesperis* 67A (usually local necrotic lesions and rings, systemic vein chlorosis and sometimes necrosis, leaf chlorosis, necrotic spots and leaf distortion; some isolates stunting and apical necrosis) and *N. occidentalis* P1 (local chlorotic or necrotic lesions, systemic vein clearing or vein chlorosis and leaf chlorosis, occasionally with necrotic lesions). Nucleic acid probes have been produced (CIP 1990).

***Andean potato mottle virus* (APMoV)(genus *Comovirus*, family *Comoviridae*)**

Isometric particles 28 nm in diameter. Serologically related strains B, C and H have been described (Salazar and Harrison 1978; Avila et al 1984). Non-potato infecting strains may exist (Valverde et al 1995).

Significance

Unknown, but may be significant.

Symptoms

Chlorotic blotches or severe mottle and rugosity (Fig. 8). Dwarfing of plants (Fig. 9) and delayed sprouting in sensitive cultivars.

Hosts

- Natural: narrow. Potato.
- Experimental: narrow. Restricted to the Solanaceae.



Fig. 8. Severe mottle, *S. tuberosum*(L.F. Salazar)

Geographical distribution

Andean region of South America [Chile (Contreras et al 1981), Ecuador (Smith et al 1997), Peru (Fribourg et al 1977)] and Brazil (Avila et al 1984). A non-potato, tabasco pepper infecting strain has been found in Honduras and Nicaragua (Valverde et al 1995).

Transmission

Mainly by plant-to-plant contact but cucumber beetle (*Diabrotica* spp.) also seems to play an important role (L. Salazar, 1996, pers. comm.).

Detection

By ELISA; although serologically related strains exist these are unlikely to cause problems in detection with antibodies raised to any strain detecting all strains (Schroeder and Weidemann 1990). Also detected by sap inoculation to *Nicotiana glauca*, *N. glauca*, *N. glauca* and *N. debneyi* (systemic mosaic).



Fig. 9. Severe mottle, rugosity and dwarfing of *S. tuberosum* plants (healthy plants at top). (L.F. Salazar)

Arracacha virus B - oca strain (AVB-O) (tentative: genus *Nepovirus*, family *Comoviridae*)

Isometric particles 26 nm in diameter. An oca- and potato-infecting strain of Arracacha virus B which is only distantly related serologically to the type strain AVB-T.

Significance
Unknown.

Symptoms
Symptomless infection in potato.

Hosts

- Natural: narrow. Potato and oca (*Oxalis tuberosa*)
- Experimental: wide. Eight dicotyledonous families.

Geographical distribution
Bolivia (Atkey and Brunt 1982) and Peru (Jones and Kentan 1981) in oca (*O. tuberosa*) and Peru in potatoes (Jones 1981). Recently found infecting 10% of native cultivars in a clonally propagated genebank at International Potato Centre, Peru (L. Salazar, 1997, pers. comm.).

Transmission
By TPS (2-12% infected) grown from infected plants or infected pollen infecting healthy ovules (Jones 1982). Transmission by TPS (0.2%) recently confirmed (L. Salazar 1997, pers. comm.). Infected pollen does not appear to infect the plant pollinated (Jones 1982). Not easily transmitted to potato by sap inoculation (Schroeder and Weidemann 1990). Vector unknown.

Detection
By ELISA and sap inoculation to *Chenopodium amaranticolor* (local necrotic lesions and systemic mild mosaic followed by recovery) but preferably *C. murale* (systemic chlorotic mottle followed by necrosis of the tip and upper leaves).

Beet curly top virus (BCTV) (genus *Curtovirus*, family *Geminiviridae*)

Isometric particles both single (18–20 nm) and geminate (32–35 x 18–20 nm).

Significance

Occurs rarely in potatoes and is usually of little economic importance. However, a high incidence of infection can occur in localized areas, causing severe disease problems.

Symptoms

Primary foliar symptoms are usually restricted to the terminal parts of one or more stems and include pinched, retarded growth of leaves with leaflets cupped, misshapen and chlorotic. Infected tubers are small and symptomless. Sprouting often fails or is slow with extremely dwarfed plants produced - “green-dwarf disease” (Fig. 10). Symptoms include a rosette appearance, with stems stiff and erect and with leaflets bunched and cupped. Secondary foliage symptoms may be confused with phytoplasmas, especially potato purple toproll (aster yellows).



Fig. 10. “Green dwarf disease”, *S. tuberosum* cv. Baronesa. (R.A.C. Jones)

Hosts

- Natural: wide. Including, bean (*Phaseolus* spp.), beet (*Beta vulgaris*), various cucurbits, pepper (*Capsicum* spp.), potato, spinach (*Spinacia oleracea*) and tomato (*Lycopersicon esculentum*). Weed species can be virus reservoirs.
- Experimental: wide. More than 300 species in 44 families.

Geographical distribution

Arid and semi-arid regions of the Middle East, the Eastern Mediterranean basin, North, Central and South America, but usually the virus only causes localized problems in potato in southeastern South America and western North America (Jones et al 1982).

Transmission

Transmitted in a persistent manner by leafhoppers, including *Circulifer tenellus* in North America and *C. opacipennis* in the Mediterranean basin. The virus is phloem limited.

Detection

By ELISA and by graft (or dodder) transmission to *Beta vulgaris* (systemic vein clearing with leaves curling inward and upward) and most cultivars of *Cucumis sativus* (seedlings are killed, older plants are stunted, malformed with leaves rolling upward). Cannot normally be mechanically transmitted. Nucleic acid probes have been produced (Stenger 1995; Creamer et al 1996).

***Cucumber mosaic virus (CMV)* (genus *Cucumovirus*, family *Bromoviridae*)**

Isometric particles 30 nm in diameter. Isolates are divided into two subgroups, I and II, on the basis of serology, RNA nucleotide and coat protein peptide sequences (Palukaitis et al 1992; Wahyuni et al 1992). Valkonen et al (1995) suggests that there are at least two strain groups based on hypersensitive reaction in potato.

Significance

Little economic importance in potato.

Symptoms

Chlorosis, mottling and blistering of foliage (Fig. 11).

Hosts

- Natural: wide. Infects many crop plants including banana (*Musa* spp.), cucumber (*Cucumis sativus*), pepper (*Capsicum* spp.), squash (*Cucurbita* spp.), tomato (*Lycopersicon esculentum*) and many leguminous and ornamental species. Adjacent crops and weed species are often reservoir hosts.
- Experimental: very wide. Over 1000 species in at least 85 families (Horváth 1979, 1980).

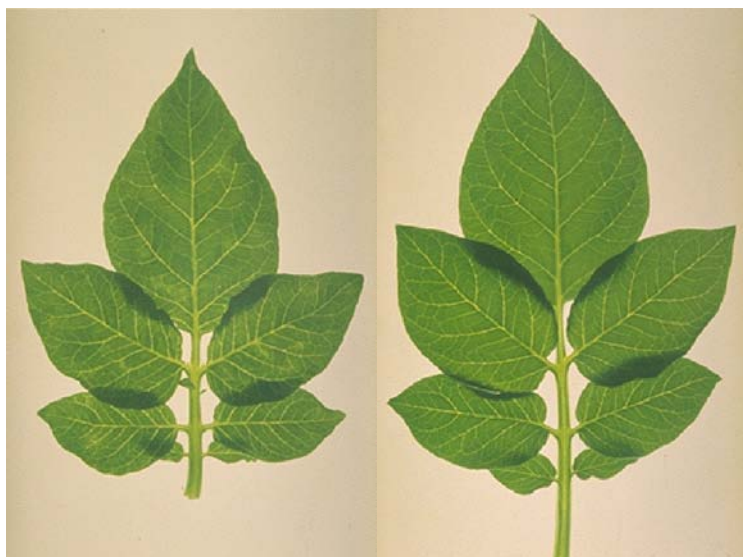


Fig. 11. Left: mild chlorosis and mottle, cv. Jemseg; Right: healthy, *S. tuberosum* cv. Jemseg. (J.P.T. Valkonen)

Geographical distribution

Worldwide in many crops but only occasionally reported in potato crops from, e.g. Europe (MacArthur 1958), Egypt (Abdel-Aziz 1995), India (Sangar and Agrawal 1986) and Saudi Arabia (Al-Shahwan et al 1997).

Transmission

Over 75 aphid species are reported as vectors of which the most important is *Myzus persicae*. Transmitted in a non-persistent manner. Transmission through botanical seed has been reported for a number of plant species (Sharma and Chohan 1974; Yang et al. 1997) but not for potato.

Detection

By ELISA, but owing to serological variability use a mixture of polyclonal antibodies raised to isolates from each subgroup. Chlorotic and necrotic local lesion hosts are respectively *Chenopodium amaranticolor* or *C. quinoa* and *Vigna unguiculata* (cowpea) and systemic hosts are *Nicotiana glutinosa* and *N. occidentalis* P1 (chlorosis). Some strains may infect *V. unguiculata* systemically and *N. occidentalis* P1 often shows local symptoms of chlorotic to bronze mottling. Nucleic acid probes have been produced (Hu et al 1995).

Potato aucuba mosaic virus (PAMV) (genus Potexvirus)

Flexuous filamentous particles 580 x 11 nm.

Significance

Not commonly found in potato and economically not important.

Symptoms

These are variable but leaf symptoms include occasional bright yellow spots (Fig. 12) to more extensive flecking, blotching and mottling (Fig. 13), necrotic spots between the veins, top necrosis and stunting. Tuber symptoms of external and internal necrosis and also net necrosis occur during storage at high temperature (20-24°C), but only in some cultivars. Surface symptoms may resemble those caused by Potato mop-top virus (PMTV).



Fig. 12. Mild symptoms of occasional yellow spots, *S. tuberosum* cv. Ninetyfold. (SASA)



Fig. 13. Bright yellow spots, flecks and blotches, *S. tuberosum* cv. Ulster Premier. (SASA)

Hosts

- Natural: narrow. Potato and probably crimson and subterranean clovers (*Trifolium incarnatum* and *T. subterraneum*) (Kollmer and Larson 1960).
- Experimental: wide. Nine families (de Bokx 1975).

Geographical distribution

Worldwide, but uncommon in potato.

Transmission

Aphids (*Myzus persicae*) in a non-persistent manner from plants which are co-infected with Potato virus A or Potato virus Y. Also mechanically (e.g. machinery) including by plant-to-plant contact (E. Fernandez-Northcote, 1996, pers. comm.).

Detection

By ELISA and sap inoculation to *Capsicum annuum* cvs. Long Red (Kassanis and Govier 1972), Plovdivsky Lyut (Kratchanova 1976) or Early Wonder (necrotic local lesions and systemic vein clearing and necrosis) and *Nicotiana glutinosa* (systemic mottling and vein banding). For differentiation between PAMV and PMTV, *C. annuum* can be used as PMTV does not infect it systemically. Nucleic acid probes have been produced (LeClerc et al 1992).

Potato black ringspotvirus (PBRSV) (genus *Nepovirus*, family *Comoviridae*)

Isometric particles 26 nm in diameter. Tobacco ringspot virus-calico strain (TRSV-Ca) is a strain of PBRSV (Salazar and Harrison 1978b).

Significance

Low importance in potato.

Symptoms

Leaf symptoms vary with cultivar and virus strain. Systemic necrotic spots or ringspots with PBRSV (Fig. 14) and bright yellow blotches (calico) with TRSV-Ca (Fig. 15).

Hosts

- Natural: narrow. Arracacha (*Arracacia xanthorrhiza* oca (*Oxalis tuberosa*) and potato.
- Experimental: wide. Infects species in eight dicotyledonous families (Salazar and Harrison 1978a).

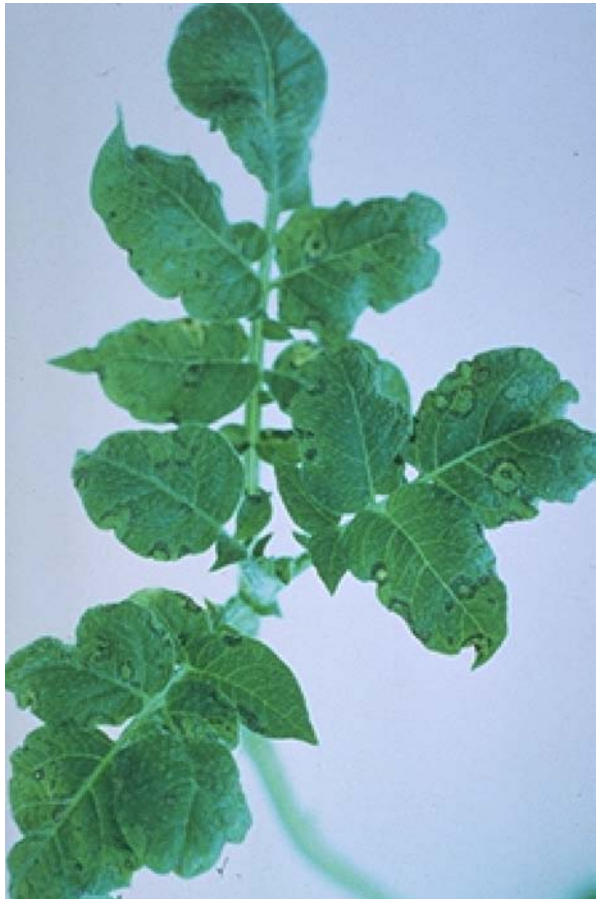


Fig. 14. Systemic necrotic ring spots, *S. tuberosum* (L.F. Salazar)

Fig. 15. Calico symptoms in *S. tuberosum* caused by the TRSV-Ca strain. (L.F. Salazar)



Geographical distribution
Peru (Fribourg 1977; Salazar and Harrison 1977).

Transmission

Unknown (nematode vector suspected). TRSV-Ca transmitted through 2-9% TPS grown from infected plants. Although infected pollen was found, the virus did not infect seed or the plant following pollination of healthy ovules (Jones 1982).

Detection

By ELISA and sap inoculation to *Chenopodium amaranticolor*, *Quinoa* (necrotic local lesions and systemic apical necrosis; plants may be killed), *Nicotiana tabacum* v. White Burley (locally a few small necrotic spots or no symptoms and systemic necrotic ringspots and line patterns; young plants may be killed), *N. benthamiana* or *N. occidentalis* P1 (local and systemic necrotic lesions).

Potato deforming mosaic virus (Brazil)(PDMV)(possible: genus *Begomovirus*, family *Geminiviridae*)

Geminate particles 30 x 18 nm. First described in Brazil by Daniels and Castro (1985) and then by Costa et al (1988) who mistakenly named it Potato yellow mosaic virus a name already assigned to another virus which is molecularly distinct from PDMV (Vega et al 1992). The name potato deforming mosaic also has been assigned to a disease of unknown etiology in Argentina (Delhey et al 1981) and it may be that the Argentinian and Brazilian diseases are caused by the same virus because of trade in potatoes between the two countries. Recently a newly described virus, Tomato yellow vein streak (ToYVSV) (Faria et al 1997) has been shown to cause PDMV symptoms when vector-inoculated to potato (Souza-Diaz et al 1996), but further work needs to be done to establish the relationship between PDMV and ToYVSV (S. Slack, 1997, pers. comm.).

Significance

Up to 35% yield reduction in the cultivar Baronesa.

Symptoms

Leaf deformation and yellow mosaic (Figs. 16 and 17).

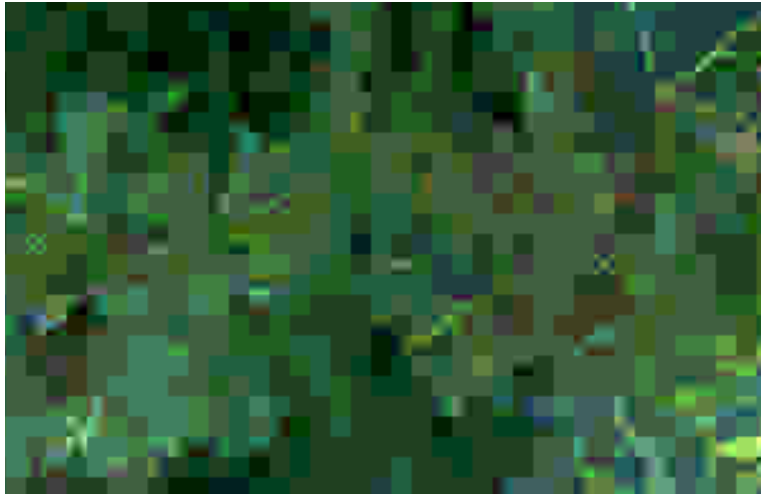


Fig. 16 (top). Primary infection. Severe distortion of apical leaves (bottom: healthy), *S. tuberosum* cv. Bintje. (J.A.C. de Souza-Dias)

Fig. 17 (bottom). Secondary infection in *S. tuberosum* cv. Achat. Severe distortion and yellow mosaic. (J.A.C. de Souza-Dias)



Fig. 18. Symptoms in
S. sisymbriifolium
 natural host
 (left: healthy).
 (J.A.C. de Souza-Dias)



Hosts

- Natural: narrow. Potato, *Solanum chacoense* and *S. sisymbriifolium* (Fig. 18) in rio Grande do Sul state, Brazil.
- Experimental: narrow. *Datura stramonium* and tomato (*Lycopersicon esculentum*)

Geographical distribution

Southern Brazil (Daniels and Castro 1985; Costa et al 1988).

Transmission

Bemisia tabaci (tobacco whitefly).

Detection

By ELISA and graft transmission to *Datura stramonium* and *Lycopersicon esculentum*. Rutgers (yellow mosaic and leaf distortion). Cannot be transmitted by mechanical inoculation.

Potato latent virus (PotLV) (possible: genus *Carlavirus*)

Filamentous rod with modal lengths of 525 and 625 nm. Found originally in *Solanum tuberosum* cv. Red La Soda (Bratney et al 1996).

Significance
Not known.

Symptoms
Symptomless in those cultivars tested.

Hosts

- Natural: only potato known so far.
- Experimental: wider host range than for Potato virus M or Potato virus S since *Nicotiana bigelovii*, *N. rustica*, *N. tabacum* and *Physalis pubescens* (= *P. floridana* Rydb.) are infected systemically.

Geographical distribution
North America but only limited testing has been done to determine distribution (Bratney et al 1996).

Transmission
Myzus persicae (J. McDonald, 1996, pers. comm.). Whether it can be contact transmitted in nature is not known.

Detection
By ELISA. Use of indicator plants is unreliable. *Nicotiana bigelovii* and *Chenopodium murale* may give transient symptoms of respectively faint systemic mottle and local chlorotic spots and *N. occidentalis* P1 mild chlorotic mottling.

Potato leafroll virus (PLRV) (genus *Polerovirus*, family *Luteoviridae*)

Isometric particles 24 nm in diameter, almost exclusively confined to phloem cells. Beet western yellows virus (BWYV) also has been reported to occur in leafroll-affected plants but there is increasing evidence that BWYV does not infect potato (Ellis and Stace-Smith 1995). Tomato yellow top virus (TYTV) which has been reported to infect potato and to produce more severe symptoms than PLRV on tomato (Hassan and Thomas 1984), is a virus closely related serologically to PLRV (Thomas 1984). In the 7th Report of the International Committee on the Taxonomy of Viruses (in preparation) TYTV is listed as a synonym/strain of PLRV. Solanum yellows virus (SYV) (Brunt 1989) is also listed as a synonym/strain of PLRV (M. Mayo, 1998, pers. comm.). A more precise definition of the taxonomic status of TYTV and SYV will require further study.

Significance

PLRV causes severe yield loss (up to 90%) and, in some cultivars a quality reduction due to internal damage to tubers (net necrosis).

Symptoms

Primary infection can cause yellowing of apical leaves which may become rolled and assume an erect habit (Fig. 19). Symptoms may be absent, particularly if infection occurs late in the season. Secondary symptoms are stunting and upward rolling of leaflets, especially those on lower leaves (Figs. 19 and 20) which are leathery and may break easily when crushed. Upper leaves may be pale in colour. In some cvs. anthocyanin production may lead to reddening or purpling of leaves. Cultivars may develop net necrosis in the tuber flesh (Douglas and Pavek 1972) (Fig. 21). In some species, particularly *Solanum tuberosum* subsp. *andigena*



Fig. 19 (top). Secondary infection: stunting and rolling of lower leaves (left). Primary infection: yellowing of apical leaves (centre). Healthy (right), *S. tuberosum* cv. Piraquara. (J.A.C. de Souza-Dias and S.A. Slack)



Fig. 20 (bottom). Secondary infection with characteristic leaf rolling in base leaves, *S. tuberosum* (SASA)

stunting and yellowing without leaf rolling is observed, a disease known as ‘enanismo amarillo’ in the Andes (Fig. 22).

Hosts

- Natural: narrow. *Solanum* spp. (including uncultivated species), tomato (*Lycopersicon esculentum*) (Jones et al. 1991) and ulluco (*Ullucus tuberosus*) (Lizarraga et al. 1996). *Datura stramonium* and *Capsella bursa-pastoris* may act as a virus reservoir.
- Experimental: narrow. About 20 species largely in the Solanaceae and a few non-solanaceous hosts including *C. bursa-pastoris*, *Gomphrena globosa* and *Montia perfoliata*.

Geographical distribution

Worldwide.

Transmission

Several aphid species transmit in a persistent circulative manner. *Myzus persicae* is the most efficient and important vector. *Aulacorthum solani* and *Macrosiphum euphorbiae* transmit PLRV but not as efficiently as *M. persicae*.

Detection

By ELISA; simultaneous incubation of sap and conjugate in the microtitre plate (“cocktail ELISA”) may give a useful increase in sensitivity (vanden Heuvel and Peters 1989). However, detection in some potato genotypes may be difficult because of “restricted virus multiplication” (Barker and Harrison 1985) (see also description for Saq’O). Also detected by graft transmission to *D. stramonium* (systemic interveinal yellowing), *Physalis pubescens*. (= *P. floridana* Rydb.) (systemic interveinal chlorosis, older leaves slightly rolled, plant stunted) and susceptible *S. tuberosum* lines (e.g. DTO-33, Maris Piper, Russet Burbank – see Symptoms). The virus is not transmitted by mechanical inoculation. Nucleic acid probes have been produced (Robinson and Romero 1991; Smith et al. 1993; Loebenstein et al. 1997).

Fig. 21 (top). Net necrosis, *S. tuberosum* (S.A. Slack)



Fig. 22 (bottom). Stunting and yellowing in *S. tuberosum* subsp. *andigena* cv. Sani imilla (enanismo amarillo disease). (L.F. Salazar)



Geographical distribution

Mainly found in cooler climates of the Andean region of South America, e.g. Peru (Salazar and Jones 1975), Canada, China, Japan (Imoto et al 1981) and Northern Europe, e.g. UK (Calvert and Harrison 1966). Although reported to occur in the Netherlands (Van Hoof and Rozendaal 1969), has not been recorded since (G. van den Bovenkamp, 1998, pers. comm.).

Transmission

By motile zoospores of the plasmodiophorid fungus *Spongospora subterranea* sp. subterranea which causes powdery scab of potato tubers. Virus-carrying *S. subterranea* sp. subterranea produces long-lived resting spores (cystosori) that persist in soil and can retain infectious virus for many years. The virus is transmitted through a variable proportion of tubers, often less than half.

Detection

ELISA can be used to detect the virus in indicator plants but may not be reliable for potato in which virus is usually in low concentration or erratically distributed. Also detected by sap inoculation to *Chenopodium amaranticolor* or *C. quinoa* (concentric brown local lesions and in addition necrosis bordering veins in *C. quinoa* and *Nicotiana benthamiana* or *N. debneyi* (systemic chlorotic mottling or necrotic line "oak leaf" patterns with some isolates). Indicator plants can be used in bait tests to detect soilborne virus (Arif et al 1994). Nucleic acid probes have been produced (Mills 1987).

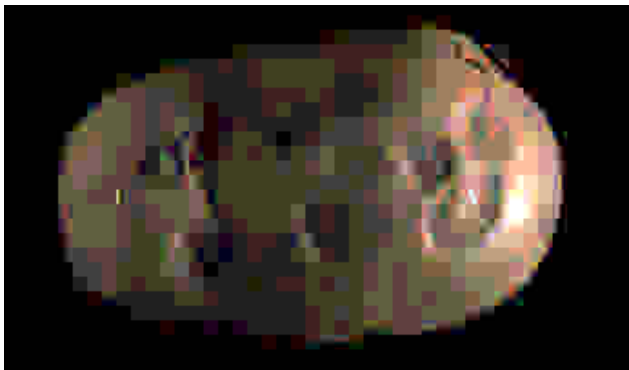


Fig. 25 (top). Lesions with central powdery scab pustule, *S. tuberosum* cv. Estima. (SASA)



Fig. 26. (right) Yellow blotches and incomplete chevrons, *S. tuberosum* cv. Maris Piper. (SASA)

Potato rough dwarf virus (PRDV) (possible: genus *Carlavirus*)

PRDV seems identical serologically to potato virus P (PVP) described from Brazil since PVP antiserum detects PRDV (M. Colavita, 1998, pers. comm.). However, reactions in indicator plants appear to differ: PRDV does not infect *Lycopersicon esculentum* and PVP does not infect *Chenopodium* spp. PRDV is serologically distinct from Potato virus S ordinary strain (PVS^O) (Butzonitch et al 1995) and potato latent virus (PotLV) (J. Martin, 1997, pers. comm.). PRDV is similar to Potato virus S Andean strain (PVS^A) in causing systemic infection in *Chenopodium* spp., although its host range is wider and it is not detected using PVS^A specific primers (Martin and Kerlan 1998). Work is underway to determine the relationship between PRDV and PVP and the other carlaviruses affecting potato, Potato virus M (PotLV) and PVS (Martin and Kerlan 1998; C. Jeffries, 1998, pers. comm.).

Slightly flexuous filamentous particles, 638 nm x diameter not reported.

Significance

Of little importance locally. Incidence of 4% and 0.4% in Spunta and Kennebec, the two most important cultivars grown in Argentina. The cultivar Sierra Volcán, which is only grown in the breeding collection at Balcarce Experimental Station, may be 60% infected. All nuclear stock in vitro material and basic seed potatoes are routinely tested. PRDV has not been found infecting certified material.

Symptoms

The cultivars Achat, Jaërla, Kennebec, Primicia and Sierra Volcán show dwarfing of the plant and a thickening of old leaves which become rough (Fig. 27). Some cultivars (Americana, Araucana, Atlantic, Granola, Huinkul and Spunta) show no symptoms. Ballenera and Bintje show moderate symptoms.

Hosts

- Natural: narrow. Potato.
- Experimental: narrow. Equivalent to PVS^O but with additional hosts and hosts with different symptoms. Hosts which may be infected systemically by PRDV and not PVS^O: *Nicotiana benthamiana*, *N. bigelovii*, *N. edwardsonii* and *Petunia hybrid* (all no symptoms), *Impatiens balsamifera* (occasional chlorotic and necrotic patches) and *N. megalosiphonifera* (leaf rugosity and vein clearing). Under some conditions, however, PVS^O may also infect *N. benthamiana* and *N. megalosiphonifera*. (Butzonitch, 1998, pers. comm.). Hosts with different symptoms: *Chenopodium amaranticolor* (PRDV - systemic infection, no symptoms; PVS^O - local chlorotic lesions), *C. quinoa* (PRDV - systemic chlorotic lesions; PVS^O - local chlorotic lesions), *N. occidentalis* (PRDV - systemic mottle and rugosity; PVS^O - systemic vein clearing) and clone 'A6' (*S. demissum* x *S. tuberosum* cv. Aquila) (PRDV - local conspicuous chlorotic lesions on detached leaves; PVS^O - no symptoms). PRDV does not infect *Capsicum annuum*, *Lycopersicon esculentum* and *N. tabacum* even after grafting.

Geographical distribution

Argentina, limited to Balcarce region of Buenos Aires province (Butzonitch et al 1995). Also found in *Solanum tuberosum* cv. Red Pontiac received from Uruguay (Calderoni 1978; M. Colavita, 1998, pers. comm.).

Transmission

In the laboratory, mechanically through sap inoculation using carborundum and water, and inefficiently by aphids (*Myzus persicae*). Modes of transmission in the field have not been tested.

Detection

By ELISA (limited quantity of antibody available from I. Butzonitch or M. Colavita and also in preparation by J. Martin). Sap inoculation of most indicator plants is unreliable with the possible exception of clone 'A6' (*S. demissum* x *S. tuberosum* cv. Aquila) (local conspicuous chlorotic lesions on detached leaves), and *Chenopodium quinquefolium* (inoculate well-grown plants, systemic chlorotic spots after 40 days). PCR is reliable using carlavirus specific primers such as those described by Badge et al (1996) (J. Martin, 1997, pers. comm.), but the homologous antibody must then be used to specifically identify PRDV.



Fig. 27. Severe dwarfing of the plant and thickening and roughening of old leaves, *S. tuberosum* cv. Kennebec. (right: healthy plants). (I. Butzonitch and M. Colavita)

Potato virus A (PVA) (genus *Potyvirus*, family *Potyviridae*)

Flexuous filamentous particles 730 x 11 nm. Occurs in distinct strain groups based on hypersensitive response in potato (Valkonen et al 1995).

Significance

PVA occurs less frequently than Potato virus Y (PVY). Yield reduction can be up to 40%. Severe disease in combination with the Potato virus X and/or PVY.

Symptoms

Mild mosaic, roughness of surface and wavy leaf margin or no symptoms, depending on cultivar.

Hosts

- Natural: narrow. Potato.
- Experimental: narrow. Mainly species belonging to the Solanaceae.

Geographical distribution

Worldwide, but not found in the Andean region of South America (Salazar 1990).

Transmission

By aphids (many species) in a non-persistent manner.

Detection

By ELISA and sap inoculation to *Nicotiana tabacum* cv. Samsun or White Burley (systemic vein-clearing, and respectively, diffuse mottling and dark green vein-banding) and *Solanum demissum* 'A' or clone 'A6' (*S. demissum* x *S. tuberosum* cv. Aquila) which are good local lesion hosts.

Potato virus M (PVM)(genus *Carlavirus*)

Slightly curved filamentous particles 650 x 12 nm. Serologically distinct strains seem to exist since a strain of PVM (PVM-ID) has been described recently which is poorly detected by some PVM antibodies. Antibodies produced to PVM-ID are PVM-ID specific (Cavileer et al 1998).

Significance

Yield reduction in potatoes is usually low, at worst 15-45%. Economically important in Eastern Europe and Russia where some cultivars may be 100% infected.

Symptoms

Often symptomless. Causes mottle, mosaic, crinkling and rolling of leaves (paracrinkle, Fig. 28), and stunting of shoots. Symptoms mainly occur in plants infected at very young stage. Severity is influenced by virus isolate and potato cultivar (Fig. 29).

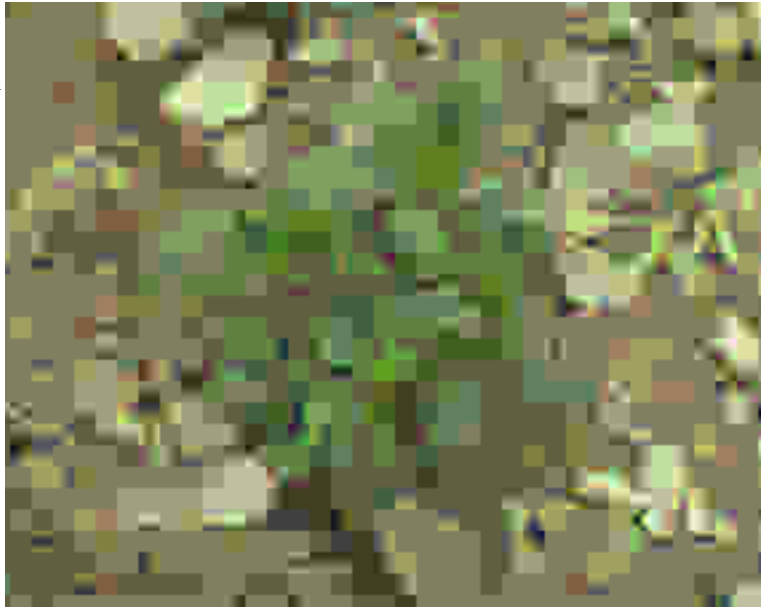
Hosts

- Natural: narrow. Mainly Solanaceae, of which potato is the most important.
- Experimental: narrow. Including species of Chenopodiaceae, Leguminosae and Solanaceae.



Fig. 28. Mild mosaic, crinkling and leaf rolling (paracrinkle), *S. tuberosum* (L.F. Salazar)

Fig. 29. Severe strain.
Severe mosaic, leaf
necrosis and dwarfing of
plant, *S. tuberosum*
(L.F. Salazar)



Geographical distribution

Worldwide but not found in the Andean region of South America (Salazar 1990).

Transmission

For most isolates, natural spread is by aphids in a non-persistent manner. Some isolates, however, may be transmitted mechanically (e.g. machinery) including plant-to-plant contact.

Detection

By ELISA and sap inoculation to *Datura metel* (chlorotic or necrotic local lesions, followed by systemic rugosity and chlorotic mottle), *Nicotiana debneyi* (necrotic ring-like local lesions) or *Phaseolus vulgaris* cv. Red Kidney (local lesions). To separate PVM and Potato virus S (PVS) in mixed infections see the PVS description.

Potato virus P (PVP) (possible: genus *Carlavirus*)

Serologically distinct from Potato virus M (PVM) and Potato virus S (PVS), PVP seems identical serologically to potato rough dwarf virus (PRDV) described from Argentina since PVP antiserum detects PRDV (M. Colavita, 1998, pers. comm.). However, reactions in indicator plants appear to differ: PRDV does not infect *Lycopersicon esculentum* and PVP does not infect *Chenopodium* spp. Work is underway to determine the relationship between PRDV and PVP and the other carlaviruses affecting potato: potato latent virus, PVM and PVS (Martin and Kerlan 1998; C. Jeffries, 1998, pers. comm.).

Slightly flexuous filamentous particles, length 640 nm x diameter not reported.

Significance

Important locally in Brazil. Cultivars Baronesa and Macaca infected at levels of 84%, with an average of 20% (Daniels et al 1993; Daniels and Carvalho 1994).

Symptoms

Symptomless on potato and other hosts tested so far.

Hosts

- Natural: narrow. Potato.
- Experimental: narrow but wider than for PVS since *Datura metel*, *D. stramonium*, *L. esculentum* and *Nicotiana glutinosa* could be symptomlessly infected. *Chenopodium* spp. could not be infected (L. Salazar, 1998, pers. comm.).

Geographical distribution

Brazil (Rio Grande do Sul state) (Daniels et al 1993; Daniels and Carvalho 1994).

Transmission

Experimentally transmitted by aphids (*Myzus persicae*)

Detection

By ELISA (limited quantity of polyclonal antibody available from J. Daniels). Infected indicator plants do not produce symptoms.

Potato virus S (PVS)(genus *Carlavirus*)

Slightly flexuous filamentous particles 660 x 12 nm. Two strain groups have been recognized, designated PVS^O (ordinary) and PVS^A (Andean), based on non-systemic and systemic infection in *Chenopodium* spp.

PVS^A = pepino latent virus (Dolby and Jones 1988).

Significance

The most frequently found virus in potato. Yield reduction is usually low, at worst 10–20%, but might be slightly worse in combination with Potato virus X(PVX).

Symptoms

PVS^O is symptomless on the majority of cultivars, with occasional mild leaf symptoms of rugosity, vein deepening and leaf bronzing (Fig. 30); PVS^A has been reported to cause more severe symptoms (Rose 1983; Dolby and Jones 1987).

Hosts

- Natural: narrow. Pepino (*Solanum muricatum*) (Dolby and Jones 1988; Verhoeven and Roenhorst 1995) and potato.
- Experimental: narrow. Including species of *Chenopodiaceae* and *Solanaceae*.

Geographical distribution

PVS^O worldwide. PVS^A reported from the Andean region of South America, (Hinostroza-Orihuela 1973), Germany (Dolby and Jones 1987), Netherlands (Rose 1983), New Zealand (Fletcher 1996) and USA (Slack 1983).



Fig. 30. Bronzing and necrotic spots on upper leaf surfaces of older leaves, *S. tuberosum* cv. Duvira. (J.A.C. de Souza-Dias)

Transmission

Commonly spread mechanically (e.g. machinery) including plant-to-plant contact. Some isolates are spread in a non-persistent manner by aphids, particularly *Myzus persicae* and *Aphis nasturtii*. PVSA has been reported to be more efficiently transmitted by aphids than the ordinary strain (Slack 1983).

Detection

By ELISA. Recently monoclonal antibodies specific to PVSA have been developed (Čerovska and Filigárová 1995) which seem reliable (J. Morris, 1998, pers. comm.). Also by sap inoculation to *Chenopodium amaranticolor*, *C. murale* or *C. quinoa* with *C. murale* possibly detecting a greater range of isolates (de Bokx 1970). Both PVS^O and PVSA induce chlorotic local lesions and additionally PVSA systemic chlorosis and necrosis. Although *Nicotiana debneyi* has been reported as an indicator of PVS, symptom expression (systemic mosaic) may be absent and it may not be infected by some isolates. Symptoms in *N. occidentalis* P1 include local mild chlorotic or necrotic lesions and systemic mild curling of leaf margins, sometimes in combination with small necrotic lesions and mild leaf chlorosis. Nucleic acid probes to PVS have been produced (Foster and Mills 1990).

To separate Potato virus M (PVM) and PVS in mixed infections the following species may be used: *Lycopersicon esculentum* which is a systemic host of PVM but not in general of PVS [Note: PVS may infect some tomato cultivars, e.g. Linda and Nevski, but Red Cherry seems immune (Horváth 1972)]; *L. humboldtii*, *L. pimpinellifolium*, *L. pyriforme*, *L. racemiflorum*, *L. racemigerum* (Horváth 1971, 1972), *N. hesperis* (Beemster and de Bokx 1987) and *Solanum tuberosum*. Saco which are susceptible to PVM but not PVS.

Potato virus T (PVT) (genus *Trichovirus*)

Filamentous usually flexuous particles 640 x 12 nm, showing characteristic substructure consisting of criss-cross or rope-type patterns (Fig. 31).

Significance
Unknown.

Symptoms
Symptomless in potato. Yellow (calico) symptoms reported by Jones et al (1982) but has not been observed since (L. Salazar, 1997, pers. comm.).

Hosts

- Natural: narrow. Mashua (*Tropaeolum tuberosum*), moca (*Oxalis tuberosa*), potato and ulluco (*Ullucus tuberosus*) (C. Lizarraga, 1997, pers. comm.).
- Experimental: narrow. Infects species in the Amaranthaceae, Chenopodiaceae, Leguminosae and Solanaceae.

Geographical distribution

Bolivia (Abad 1979) and Peru (Salazar and Harrison 1977, 1978).



Fig. 31. Elongated particles with characteristic substructure, *S. tuberosum* (L.F. Salazar)

Transmission

Mechanically (e.g. machinery) including plant-to-plant contact and by TPS with 0-59% seed infection reported experimentally (Jones 1982). Also seed-transmitted in *Chenopodium quinoa*, *Datura stramonium* and *Nicandra physalodes*. Pollen-transmitted to ovules but infected pollen does not appear to infect the plant pollinated (Jones 1982). Has been detected in a TPS Potato Germplasm Collection at the International Potato Centre, Peru (CIP 1991).

Detection

By ELISA; simultaneous incubation of sap and conjugate in the microtitre plate ("cocktail ELISA", see the description for Potato leafroll virus) may give a useful increase in sensitivity (R. Burns, 1996, pers. comm.). Good polyclonal antisera are difficult to prepare and therefore monoclonal antibodies have been produced (Vernon-Shirley et al. 1993). Also detected by sap inoculation to, e.g. *Chenopodium amaranticolor* or *C. quinoa* (sometimes local chlorotic spots and respectively systemic leaf necrosis and mosaic followed by top necrosis in high or low light intensity in both indicator plants), or *Phaseolus vulgaris* cv. The Prince (necrotic ringspots in leaves shaded heavily after inoculation, and systemic necrosis followed by recovery). Nucleic acid probes have been produced (CIP 1992).

Potato virus U (PVU) (genus *Nepovirus*, family *Comoviridae*)

Isometric particles about 28 nm in diameter, serologically unrelated to 17 other nepoviruses.

Significance
Unknown.

Symptoms

Though the virus was isolated from a plant with bright yellow leaf markings, the symptom could not be reproduced after top-grafting scions from four potato cultivars onto infected *Nicotiana tabacum* cv. Xanthi plants (Jones et al 1983).

Hosts

- Natural: unknown.
- Experimental: wide. Infects 44 species in seven families.

Geographical distribution

Isolated only once from a potato plant (unknown cultivar) in the Comas valley, Junin, Peru at 3600 m above sea level (Jones et al 1983).

Transmission

Experimentally transmitted by an unknown *Longidorus* spp. and mechanically to potato with difficulty.

Detection

Antibodies to PVU are unavailable and detection by ELISA has not been evaluated. Detected by sap inoculation to *Chenopodium amaranticolor* C. quinoa (chlorotic and necrotic local lesions and systemic mottle and leaf deformation) and *N. tabacum* cvs. Samsun or White Burley (systemic chlorotic ringspot and line patterns followed by recovery).

Potato virus V (PVV) (genus *Potyvirus*, family *Potyviridae*)

Flexuous filamentous particles 700-720 x 12-13 nm. Isolates have been called Potato virus Y-C G1 in the Netherlands (Rozendaal et al 1971), Potato virus YC AB in Ireland (Calvert et al 1980), and UF in Peru (Fribourg and Nakashima 1984). PVV is listed as a virus species in the 7th Report of the International Committee on the Taxonomy of Viruses (in preparation) (M. Mayo, 1998, pers. comm.). However, it may be a potato-infecting strain of Peru tomato mosaic virus

Significance

In Europe few cultivars are naturally infected, and the majority of these are symptomless or have only mild symptoms. In Bolivia damage is severe in some native cultivars.

Symptoms

Virtually symptomless in the majority of cultivars. A few develop mosaic and necrotic spotting of lower leaves, others develop severe systemic necrosis and leaf dropping.

Hosts

- Natural: narrow. Potato and tomato (*Lycopersicon esculentum*)
- Experimental: narrow. Mainly species belonging to the Solanaceae.

Geographical distribution

Bolivian highlands (Alvarez and Fernandez-Northcote 1996), Peru (Fribourg and Nakashima 1984) and Northern Europe, e.g. France, Germany, the Netherlands (Rozendaal et al 1971) and the United Kingdom (Calvert et al 1980; Jones and Fuller 1984).

Transmission

Several aphid species, e.g. *Macrosiphum euphorbiae* and *Myzus persicae* in a non-persistent manner.

Detection

Although the virus can be detected by ELISA, commercially available PVV polyclonal antibodies produced against European isolates may not detect all isolates of PVV (Alvarez and Fernandez-Northcote 1996). Also detected by sap inoculation to *L. esculentum* (systemic vein clearing), *Nicotiana debneyi* (chlorotic local lesions and systemic vein clearing, mosaic and chlorotic spots and rings) and *Solanum demissum* (systemic necrosis).

Potato virus X (PVX) (genus *Potexvirus*)

Filamentous particles 515 x 13 nm. PVX has been separated into:

serotypes PVX^O (common), and PVX^A (Andean) which includes strains PVX_{cp} and PVX_{HB} (same as pathotype HB, see below) (Fernandez-Northcote 1990);

pathotypes 1 and HB. Pathotype 1 contains the four strain groups of Cockerham (see below) and HB contains strains, e.g. strain HB from Bolivia (Moreira et al 1980) which break Rx extreme resistance (immunity) genes (Fernandez-Northcote 1990) and

strain-groups 1, 2, 3 and 4 which differ in virulence to potato genotypes with hypersensitive resistance genes Nx and Nb (Cockerham 1955, 1970).

Recently a strain MS has been described from Argentina which breaks Rx resistance in *Solanum acaule* PI 175395 and the cultivar Serrana INTA (Tozzini et al 1994). However, Serrana INTA has been reported to contain only hypersensitive resistance genes (Fernandez-Northcote 1990) and therefore further work is needed to confirm the Rx resistance-breaking properties of this strain.

Significance

Yield reduction usually 15–20%. Mixed infections with other viruses, particularly Potato virus A and Potato virus Y may result in extreme losses.

Symptoms

Mild mosaics and mottles (Figs. 32 and 33), but some strains cause severe or rugose mosaic. Tuber necrosis occurs in some cultivars.

Hosts

- Natural: narrow. *Nicotiana glauca* (Sangar et al 1980), potato and tomato (*Lycopersicon esculentum*). Co-infections with Tomato mosaic virus causes double streak in tomatoes.
- Experimental: wide. More than 240 species in 16 families.

Geographical distribution

Serotype PVX^O worldwide, common occurrence. Serotype PVX^A restricted: cp strain to the south-central Andes of Peru and the HB strain mainly to the Bolivian plateau around Lake Titicaca (Moreira et al 1980; Fernandez-Northcote and Lizarraga 1991).

Transmission

Readily transmitted mechanically (e.g. machinery) including plant-to-plant contact. Transmissions by zoospores of the fungus *Synchytrium endobioticum* (Nienhaus and Stille 1965) and by biting insects (grasshoppers) (Munro 1981) has been reported.

Detection

The two serotypes PVX^O (includes PVX-MS) and PVX^A, can be detected and differentiated by ELISA using monoclonal antibodies (Torrance et al 1986) and nucleic acid probes (Querci et al 1992, 1993a, 1993b). Some commonly available polyclonal antibodies to PVX^O may not detect some isolates of PVX^A particularly when the virus is at low concentrations (Fernandez-Northcote and Lizarraga 1991). Polyclonal antibodies have been produced at International Potato Centre, Peru which will reliably detect both serotypes. Also detected by sap inoculation to several *Nicotiana* spp., e.g. *benthamiana*, *glutinosa* and *tabacum* cvs. Samsun and White Burley (necrotic spots/ringspots on inoculated leaves and systemic necrotic spots, mosaic or veinal chlorosis). *N. occidentalis* P1 gives local and systemic necrotic lesions. Some isolates of PVX^A may only give a mild mosaic in *N. glutinosa*. *Gomphrena globosa* a useful local lesion host, but the HB strain may only give local, symptomless infection.



Fig. 32 (left). Mild mosaic, *S. tuberosum* (S.A. Slack)

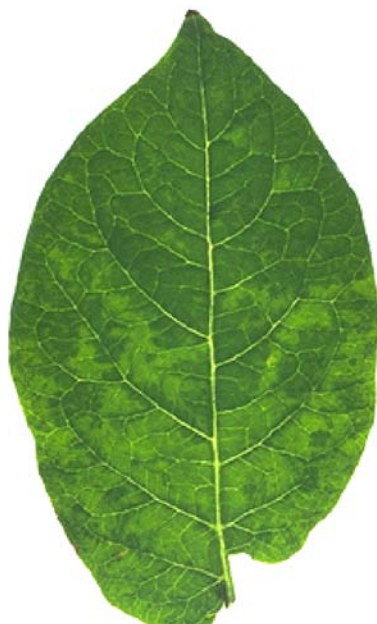


Fig. 33 (right). Mottle, *S. tuberosum* v. Wilja. (SASA)

Potato virus Y (PVY) (genus *Potyvirus*, family *Potyviridae*)

Flexuous, long filamentous particles 740 x 11 nm. PVY isolates have been grouped into different strain groups on the basis of local and systemic symptoms on *Nicotiana* spp., *Physalis pubescens* (= *P. floridana* Rydb.) and potato: C, the stipple streak strain; O, the common strain; N, the tobacco vein necrosis strain (including NTN, the so-called tuber necrotic strain of N). A further strain group, Z, also has been suggested (Jones 1990). The understanding of NTN is complex and still not resolved. Groupings based on multiple sequence comparisons of the coat protein region mirror the main strains, with PVY^O, PVY^N and PVY^{NTN} groups being clearly identified together with a fourth group which contains PVY^C isolates. All isolates in the PVY^{NTN} group have been identified as recombinants between PVY^O and PVY^N. However, PVY^N isolates which do not have the recombinant coat protein also cause tuber necrosis (Boonham et al. 1998). It has been suggested that the tuber-necrotising property may be located at the 3' end, in the last 6000 nucleotides of the PVY genome (Glais et al. 1998).

Significance

Yield losses reach 10-80%. Disease is more severe in combination with other potato viruses, particularly Potato virus X

Symptoms

Symptoms include mild and severe mosaic (Fig. 34), rugosity, crinkling, dropping of leaves (leaf drop streak, Fig. 35), severe systemic necrosis and dwarfing. N isolates usually cause only slight leaf symptoms (Figs. 36 and 37). NTN isolates cause severe superficial tuber necrosis (potato tuber necrotic ring spot disease, Figs. 38, 39 and 40) and may also cause necrotic foliar symptoms. Some N isolates may also cause tuber necrosis.

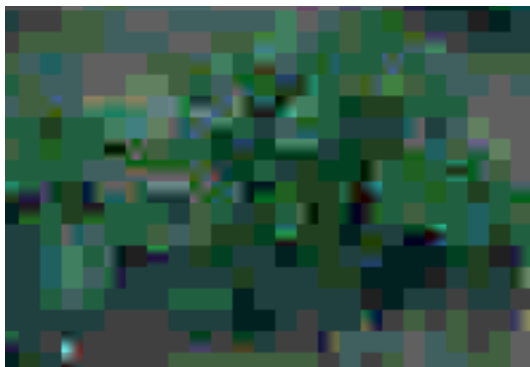


Fig. 34 (top). Severe mosaic, rugosity and dwarfing of plant, *S. tuberosum* (SASA)

Fig. 35 (bottom). Leaf drop streak, *S. tuberosum* v. Kennebec. (SASA)

Hosts

- Natural: wide. Up to nine families, including important crops such as pepper (*Capsicum* spp.), potato, tobacco (*Nicotiana* spp.), tomato (*Lycopersicon esculentum*) and tree tomato (*Cyphomandra betacea*) and some ornamental plants (e.g. *Dahlia* and *Petunia* spp.) and some weeds (e.g. *Physalis* spp., *Solanum dulcamara* and *S. nigrum*).
- Experimental: wide. More than 400 species in 30 families.

Geographical distribution

PVY C strain group in Australia, Ecuador (Fernandez-Northcote 1990), Europe, India, North America (Ellis et al 1997), South Africa and New Zealand; N strain group in Africa, Europe (Todd 1961), South America (Argentina, Chile, Colombia and Peru: Fernandez-Northcote 1990), New Zealand (Fletcher 1989) and with localized outbreaks in North America (Singh 1992; Singh et al 1993) which have been subject to eradication programmes; NTN in Europe, Israel and Lebanon (EPPO 1997), and probably elsewhere; O strain group worldwide.

Transmission

Many aphid species transmit in a non-persistent manner. Although aphid transmission is the most important means of spread in the field, Banttari et al. (1993) indicate that all PVY strains may be spread mechanically, including plant-to-plant contact. In Chile, some N isolates are said to be spread by plant-to-plant contact in tobacco and tomato (L. Salazar, 1998, pers. comm.).

Detection

By ELISA with polyclonal antibodies raised against isolates from either strain group detecting all strain groups. Monoclonal antibodies (MAbs) are available which will detect most strains (Fernandez-Northcote and Gugerli 1987) but reliable differentiation of strains has proved more difficult. MAbs specific to or detecting most isolates of PVY^C, PVY^N



Fig. 36 (top). Mild mosaic and leaf crinkling, *S. tuberosum* (PPS)

Fig. 37 (bottom). Mild mosaic, *S. tuberosum* cv. Craigs Royal (right: healthy). (SASA)

and PVY^O with little cross-reaction to other strains have been reported recently (Ellis et al. 1996; McDonald and Singh 1996a). Nucleic acid probes can be used for detection and differentiation (Baulcombe and Fernandez-Northcote 1988) of the different strains. Serological differences have been reported between PVY^{NTN} strains and other members of the PVY^N group using a monoclonal antibody but a greater range of isolates need to be examined to determine the reliability of this method (Cervovská 1998). Methods to detect PVY^{NTN} have been published, using RT-PCR (Weidemann and Maiss 1996) or PCR followed by restriction analysis (Glais et al 1996), and detection of all the main strains (O, N and NTN) by RT-PCR (Boonham et al. 1998). However, the reliability of these molecular methods still needs to be determined since some isolates may or may not cause tuber necrotic symptoms and are negative or positive in one or the other of these tests (McDonald and Singh 1996b; I. Browning, 1997, pers. comm.; Boonham et al. 1998).

Also detected by sap inoculation to *Solanum demissum* or clone 'A6' (*S. demissum* *S. tuberosum* cv. Aquila) which are local lesion hosts. PVY-81 found in South Africa does not cause necrosis in 'A6' (Thompson et al 1987). *S. demissum* PI 230579 is a better local lesion host than 'A6' (Webb and Wilson 1978). All strains may be detected by *N. benthamiana* (systemic vein clearing, mottle and rugosity) and *N. occidentalis* P1 (local mild chlorotic lesions sometimes; systemic vein clearing, mottle, chlorosis and stunting). *N. tabacum* cv. White Burley is the most useful to differentiate the N strain group (systemic vein banding and severe veinal necrosis) from the other strain groups (vein banding and mottle).



Fig. 38 (top). Raised surface lesions, *S. tuberosum* (J. Horváth)

Fig. 39 (middle). Raised surface lesions, *S. tuberosum* (CSL)

Fig. 40 (bottom). Cross-section showing characteristic minimal penetration of the tuber flesh, *S. tuberosum* (CSL)

Potato yellow dwarf virus (PYDV) (genus *Nucleorhabdovirus*, family *Rhabdoviridae*)

Bacilliform particles 380 x 75 nm, closely associated with the cell nucleus.

Significance

No economic importance. Virus tends to self-eliminate in tuber-to-tuber generations.

Symptoms

Secondary foliar symptoms include dwarfing, brittleness and chlorosis (Fig. 41). Leaflet margins roll upward while the longitudinal axis curves downward. Pith necrosis of stems is common. It starts apically and may extend to the entire stem. Tubers are small, misshapen, may not sprout and are few in number with generalized necrosis (Fig. 42).

Hosts

- Natural: narrow. Potato and the common weed ox-eye daisy (*Chrysanthemum leucanthemum* var. *pinnatifidum*) which is a virus reservoir.
- Experimental: wide. Species in at least seven dicotyledonous families.

Geographical distribution

Canada and northern United States, although no reports in potatoes for 50 years (S. Slack, 1997, pers. comm.) and Saudi Arabia (Al-Shahwan et al 1997). A rhabdovirus similar to PYDV has been reported from South Russia and the Ukraine (Kozar and Kurbala 1978; Vlasov and Larina 1982).



Fig. 41. Severe dwarfing and chlorosis, *S. tuberosum* v. Chippewa. (S.A. Slack)

Fig. 42. Generalized tuber necrosis in *S. tuberosum* (right: heat necrosis). (S.A. Slack)



Transmission

Leafhoppers transmit in a persistent, propagative manner. Two strains reported: one transmitted by *Aceratagallia sanguinolenta* and the other by *Agallia constricta* with *Agallia quadripunctata* able to transmit both.

Detection

By ELISA and sap inoculation to *Nicotiana clevelandii* or *N. glutinosa* (local lesions followed by systemic mosaic and yellowing) and *N. rustica* (bright yellow local lesions followed by systemic mosaic).

Potato yellow mosaic virus (PYMV) (genus *Begomovirus*, family *Geminiviridae*)

Bigeminate (bisegmented) particles 18–20 nm in diameter. Biological properties are very similar to Tomato yellow mosaic virus (ToYMV) described by Uzcátegui and Lastra (1978), but characterization of ToYMV at the molecular level and comparisons with PYMV still need to be done. ToYMV has never been reported infecting potatoes naturally. Recently, a number of virus isolates were obtained in Venezuela from tomato showing symptoms generally associated with geminivirus infection: golden or yellow mosaic, mottling and crumpling. Most of these isolates showed close sequence homology to PYMV leading the authors to conclude that they were tomato-infecting isolates or strains of PYMV (Guzman et al 1997). In addition, sequence analysis has shown that isolates from tomato from a number of Caribbean islands (Guadeloupe, Martinique and Puerto Rico) are closely related to PYMV (Polston et al 1998). Whether the tomato-infecting isolates infect potato is not known.

PYMV is molecularly distinct from Tomato golden mosaic virus (Roberts et al 1988) which does not appear to infect potato.

Significance
Unknown.

Symptoms
Bright yellow mosaic, leaf distortion (Fig. 43) and dwarfing.



Fig. 43. Severe leaflet distortion and yellowing, *S. tuberosum* cv. Desiree. (R.H.A. Coutts)

Hosts

- Natural: narrow. Potato and Solanum spp. and tomato (*Lycopersicon esculentum*)
- Experimental: narrow. Mainly solanaceous hosts.

Geographical distribution

Venezuela in potato (Roberts et al 1986). Guadeloupe, Martinique, Puerto Rico, Trinidad, Tobago and Venezuela in tomato (Polston and Anderson 1997).

Transmission

Bemisia tabaci (tobacco whitefly).

Detection

By ELISA and sap inoculation to *Nicotiana glauca* cv. Xanthi-nc or *Petunia hybrida* most cultivars (chlorotic spots in inoculated leaves, most clearly seen in *P. hybrida* and systemic golden-yellow mottling in newly developing leaves and gross deformation and dwarfing of plants).

Potato yellow vein virus (PYVV)(possible: genus *Crinivirus*, family *Closteroviridae*)

Closterovirus-like filamentous particles observed in purified preparations but modal length not determined because of particle degradation. The sequence homology of PYVV heat shock protein is close to those of Lettuce infectious yellows virus, sweet potato pseudo-yellows virus and sweet potato sunken vein virus (Salazar et al 1998).

Significance

More than 50% yield reduction (Saldarriaga et al 1988).

Symptoms

Initially only leaf veins are bright yellow (sometimes this can also be seen in in vitro plants, Fig. 44); later the entire leaf lamina becomes yellow (Fig. 45). Under appropriate environmental conditions the whole plant may become bright yellow. Infected plants do not always produce symptoms.

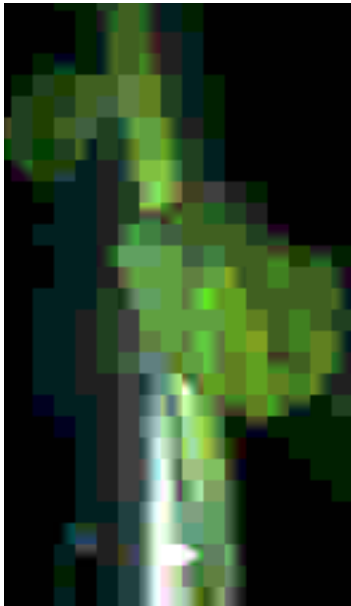


Fig. 44 (left). Bright yellow veins, in vitro plant, *S. tuberosum* cv. Saco. (SASA)

Fig. 45 (right). Bright yellow veins, with whole plant becoming increasingly yellow, *S. tuberosum* (L.F. Salazar)

Hosts

- Natural: narrow. Potato. Wild *Lycopersicon* spp., *Polygonum mepalense* and *Solanum nigrum* are symptomless hosts.
- Experimental: Unknown.

Geographical distribution

Colombia (Fig. 46), Ecuador (Silberschmidt 1954) and Peru (Cajamarca) (Salazar et al 1998).

Transmission

Trialeurodes vaporariorum (glasshouse whitefly) (Saldarriaga et al 1988).

Detection

By graft transmission to *S. tuberosum* clone A6. Symptoms in potato are particularly characteristic but infected plants do not always produce symptoms (see Symptoms above). Cannot be transmitted by mechanical inoculation. Until recently double-stranded RNA analysis (Valverde et al 1986) and RT-PCR using degenerate closterovirus primers (Citrus tristeza virus and beet-pseudo yellows virus) were the only diagnostic methods available. Now a nucleic acid probe for use in nucleic acid spot hybridization has been developed and work is in progress to develop virus-specific antibodies (Salazar et al 1998).



Fig. 46. Whole potato crop (*S. tuberosum*) infected with PYVV, Colombia. (N.W. Simmonds)

Potato yellowing virus (PYV) (possible: genus *Alfamovirus*, family *Bromoviridae*)

Bacilliform with 4-5 particle sizes, between 21-60 nm x 18 nm. Serologically unrelated to Alfalfa mosaic virus (Fuentes 1991).

Significance
Unknown.

Symptoms

Main symptoms include mosaic, yellowing (Fig. 47), and premature senescence of the plant. Symptomless infections may occur. Germination of TPS infected with PYV may be impaired (Valkonen et al 1992b).

Hosts

- Natural: narrow. Potato.
- Experimental: wide. 44 of 51 species in seven plant families (Fuentes and Jayasinghe 1993).



Fig. 47. Yellowing and premature senescence, *S. tuberosum* (L.F. Salazar)

Geographical distribution

Widespread in Peru (Fuentes and Jayasinghe 1993) and Chile (Valkonen et al 1992a). Found in potatoes from Bolivia (L. Salazar, 1998, pers. comm.).

Transmission

Transmitted semi-persistently by *Myzus persicae*. Also transmitted through the ovule of infected plants to infect seed (20% of TPS infected), but pollen from infected plants failed to set berries in healthy plants of *S. brevidens* (Valkonen et al 1992b). Transmission by TPS (17%) confirmed recently (L. Salazar, 1997, pers. comm.).

Detection

By ELISA and sap inoculation to *Capsicum annuum*, e.g. cvs. Gold Spike, Golden Calwonder, Jalapeno (systemic leaf distortion, yellow mosaic and vein clearing), *Nicotiana tabacum* cv. Samsun (systemic line patterns, mosaic and chlorotic spots) or *Physalis pubescens*. (= *P. floridana* Rydb.) (systemic yellowing and leaf distortion). Sap inoculation using water is unreliable; use 0.01M 2-mercaptoethanol at pH 6.5 or graft transmit to *P. pubescens*.

Solanum apical leaf curling virus (SALCV) (tentative: genus *Begomovirus*, family *Geminiviridae*)

Tri-segmented particles about 50 x 18 nm.

Significance

Only of limited significance in localized areas; seems best adapted to tropical regions.

Symptoms

Leaf rolling and distortion of apical leaves (Fig. 48). Tubers may fail to sprout or may produce a combination of vigorous and hairy sprouts. Symptoms can be confused with those of phytoplasmas or primary infection with Potato leafroll virus.

Hosts

- Natural: narrow. Black nightshade (*Solanum nigrum*), cape gooseberry (*Physalis peruviana*) potato, *Nicandra physalodes* and *S. basendopogon*
- Experimental: narrow. Only Solanaceae.

Geographical distribution

Mid-elevation jungle of Peru (Hooker and Salazar 1983).

Transmission

Transmitted experimentally only by grafting. Vector is unknown.

Detection

By ELISA and by graft transmission to *Datura stramonium* or *D. tatula* (pronounced yellowing of the small veins in newly formed leaves 7-10 days after grafting, and later vein yellowing with tip leaves curled downward and dwarfing of the whole plant; severe chlorosis in older infections). Cannot be transmitted by mechanical inoculation.



Fig. 48. Leaf rolling and distortion of apical leaves, *S. tuberosum* cv. Revolución. (L.F. Salazar)

Sowbane mosaic virus (SoMV) (genus *Sobemovirus*)

Isometric particles 26-28 nm in diameter. Virus found only in the potato cv. Puebla sent to International Potato Centre, Peru from Mexico for virus elimination (L. Salazar, 1997, pers. comm.).

Significance
Rare in potatoes.

Symptoms
Very mild mosaic.

Hosts

- Natural: narrow. Mostly species of the Chenopodiaceae. Potato.
- Experimental: narrow. Mainly Chenopodiaceae but also some species in the Amaranthaceae, Cucurbitaceae, Leguminosae and Solanaceae.

Geographical distribution
Worldwide in Chenopodiaceae. Rare in potato. Found once in a line from Mexico (L. Salazar, 1997, pers. comm.).

Transmission
Probably transmitted mainly mechanically and infrequently by insects, e.g. *Circulifer tenellus* and *Liriomyza langei*. Transmitted by seed, up to 70% in *Chenopodium* spp. (Bennett and Costa 1961; Dias and Waterworth 1967) and pollen (Francki and Miles 1985). TPS and pollen transmission have not been evaluated for potato.

Detection
By ELISA and sap inoculation to *Chenopodium amaranticolor*, *C. murale* and *C. quinoa* (chlorotic local lesions, systemic yellow flecking, star shaped patterns and leaf deformation).

Tobacco mosaic virus (TMV) (genus *Tobamovirus*)

Rod-shaped particles 300 x 18 nm.

Significance

Not a problem in potatoes.

Symptoms

Various. Yellow 'V-shaped' symptoms in leaves similar to those obtained with Potato mop-top virus (Fig. 49). Interveneal yellowing, rigid leaves, mild mottling and severe stunting also reported (Hansen 1960; Phatak and Verma 1967).

Hosts

- Natural: wide. Including several crops and both herbaceous and woody plants.
- Experimental: wide. More than nine families.

Geographical distribution

Worldwide distribution in crops other than potato. In potato occasionally found in China, India (Phatak and Verma 1967; Khurana and Singh 1983), in native cultivars in the Peruvian Andes (L. Salazar, 1997, pers. comm.) and in Saudi Arabia (Al-Shahwan et al. 1997). Reported infecting *Solanum commersonii* in Argentina (Hansen 1960).

Transmission

Highly infectious. Spreads very easily mechanically (e.g. on machinery during cultural operations) including plant-to-plant contact. Although not proved for potato, transmitted by botanical seed (but not through the embryo) of some plant species (Mink 1993).

Detection

By ELISA and sap inoculation to *Nicotiana glutinosa* (local necrotic lesions are produced at temperatures below 25°C; at higher temperatures systemic symptoms may be produced as well) or *N. tabacum* cvs. Samsun or White Burley (systemic mosaic). Use *N. tabacum* cvs. Samsun or White Burley to distinguish TMV (no local symptoms) from Tomato mosaic virus (local necrotic lesions). Nucleic acid probes have been produced (Palukaitis and Symons 1980; Bar-Joseph et al. 1986).



Fig. 49. Yellow V-shapes, *S. tuberosum* (L.F. Salazar)

Tobacco necrosis virus (TNV)(genus *Necrovirus*, family *Tombusviridae*)

Isometric particles 26 nm. Various strains have been reported (Adam et al 1990) representing two serotypes, A and D (Babos and Kassanis 1963).

Significance

Not significant in potato, infected tubers produce healthy plants.

Symptoms

Only produces tuber symptoms. The skin shows superficial light to dark brown circular lesions with radial or reticular cracks and some blistering may occur (Figs. 50 and 51). During storage, lesions turn darker, enlarge and become sunken.

Hosts

- Natural: wide for serotype-D. More than nine families, including potato and many other crop species, of which some become systemically infected. Narrow for serotype-A, mainly *Phaseolus vulgaris* but no information available on whether this serotype infects potato.
- Experimental: very wide. More than 30 families. Infects many species of which most only produce local lesions.

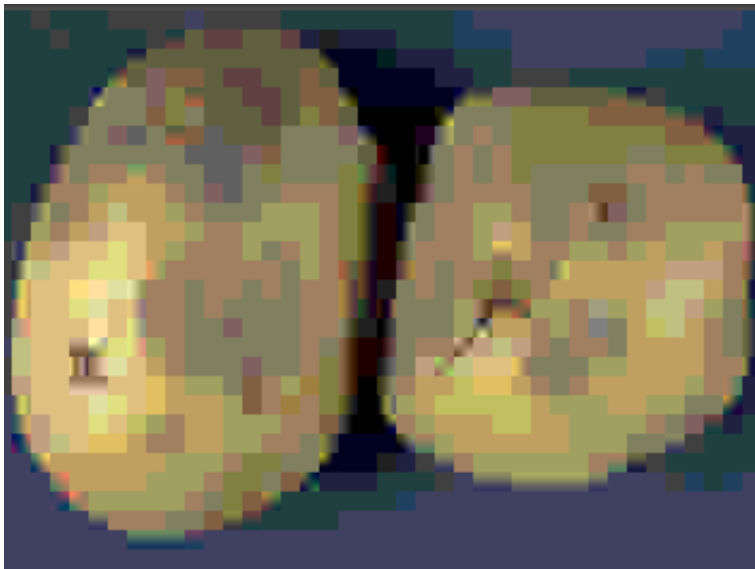


Fig. 50. Slightly sunken necrotic lesions with cracking, *S. tuberosum* (PPS)

Fig. 51. Surface lesion with characteristic limited penetration of tuber flesh, *S. tuberosum* cv. Stroma. (SASA)



Geographical distribution

Worldwide distribution in other crops particularly in irrigated fields and unsterilized soil in glasshouses. In potato only reported from Europe and North America (Jones et al. 1982).

Transmission

Transmission is by zoospores of the root-infecting fungus *Olpidium brassicae* (causal agent of 'club root' disease of brassicas), dependent on suitable combinations of virus strain, fungus race and host species. No survival in resting spores.

Detection

Because of the uncertainty about whether serotype-A strains infect potato, antibodies from each serotype should be used in ELISA. Sap inoculation to, e.g. *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana clevelandii*, *N. glauca*, *N. tabacum* cvs. Samsun, White Burley or *Phaseolus vulgaris* cvs. e.g. "Dubbele Witte" or "Dubbele Witte zonder Draad" (necrotic local lesions) is considered to be more reliable than ELISA.

Tobacco etch virus (TRV) (genus *Tobravirus*)

Rigid, tubular particles of two predominant lengths, 190 nm and 50-115 nm x 22 nm. Normal particle-producing isolates (M-type) have two species of genomic RNA (RNA-1 and RNA-2) and are readily transmitted to test plants by inoculation with sap and by nematodes. Other isolates (NM-type) have only one RNA species (RNA-1), do not produce particles, are transmitted with difficulty by inoculation with sap and are not transmitted by nematodes.

Significance

There may be appreciable loss of saleable yield because of spraing damage to tubers.

Symptoms

Three categories are observed depending on the reaction of potato cvs. growing in virus-containing soil. (1) A proportion of tubers develop spraing (Figs. 52 and 53) and virus (predominantly NM type cultures) can be isolated. Plants grown from these tubers exhibit distortion, stunting and mottling (Fig. 54), typically confined to one or a few shoots and known as 'stem mottle' (Fig. 55). (2) A few brown flecks develop in tubers and virus (M type cultures) can be isolated. Transient yellow chevrons (Fig. 56) are sometimes detected on a few leaflets of some stems grown from the tubers and virus is detected in leaves on all stems of these cultivars

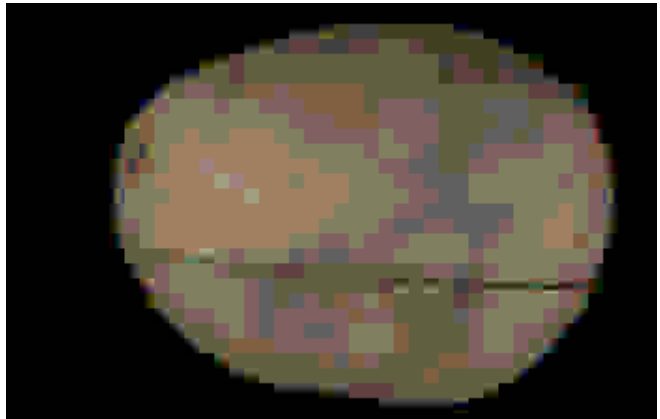


Fig. 52 (top). Necrotic rings, *S. tuberosum*(SASA)

Fig. 53 (bottom). Internal tuber symptoms of "spraing", *S. tuberosum*(SASA)

which appear to be tolerant of infection. (3) Some cultivars are resistant to infection and no symptoms or virus can be detected in foliage and tubers.

Hosts

- Natural: wide. Infects many crop plants including potato, e.g. gladiolus (*Gladiolus* spp.), hyacinth (*Hyacinthus* spp.), lettuce (*Lactuca sativa*), narcissus (*Narcissus* spp.), spinach (*Spinacia oleracea*), sugar beet (*Beta vulgaris*), tobacco (*Nicotiana* spp.), tulip (*Tulipa* spp.) and many weed species which act as virus reservoirs.
- Experimental: very wide. Over 400 species in more than 50 monocotyledonous and dicotyledonous families although in many instances the infection does not become systemic and remains in the roots.

Geographical distribution

Europe (Böning 1931), China (Harrison and Robinson 1984), Japan (Tomaru and Nakata 1967), New Zealand (Jones and Young 1978), the former USSR (Harrison and Robinson 1984), and North (Oswald and Bowman 1958; Walkinshaw and Larsen 1958; Cornelissen et al 1986), Central and South America (Harrison and Robinson 1984).



Fig. 54 (top). Slight yellow mottle, *S. tuberosum* cv. Achat. (J.A.C. de Souza-Dias)

Fig. 55 (bottom). Yellow mottle, *S. tuberosum* cv. Wilja (stem mottle). (H. Barker)



Transmission

Several species of trichodorid nematodes (*Paratrichodorus* spp. and *Trichodorus* spp.) are vectors and retain virus for many months but not through moults. Reported to be transmitted through TPS at very low levels (Horváth et al 1996).

Detection

Serological detection is unreliable because of considerable diversity in the particle proteins of different isolates. Therefore sap inoculation to *Chenopodium amaranticolor* (necrotic local lesions), *C. quinoa* (spreading irregular necrotic lesions) or *Phaseolus vulgaris* (The Prince (local pinpoint lesions) is recommended. Lesions on *C. quinoa* may not be as diagnostic as those on *C. amaranticolor* and *P. vulgaris*. Other test plants are not recommended because symptoms are variable and may be absent depending on virus isolate. NM isolates, which do not produce nucleoprotein particles, are poorly transmitted by mechanical inoculation unless it is done using extracted RNA (Harrison and Robinson 1981). Grind leaves in 1:1 mixture of buffer (10 mM Tris, 1 mM EDTA, pH8) to water-saturated phenol. Take aqueous layer and remove phenol by washing with chloroform followed by precipitation with ethanol at -18°C. Resuspend pellet in buffer with bentonite (protects against RNase degradation) and inoculate. Labelled cDNA probes based on RNA-1 (Robinson 1989), and RT-PCR (Robinson 1992; Crosslin and Thomas 1995; Weidemann 1995) are also reliable.



Fig. 56. Yellow chevrons, *S. tuberosum* v. Up to Date. (SASA)

Tobacco streak virus (TSV)(genus *Illavirus*, family *Bromoviridae*)

Quasi-isometric particles 22-35 nm in diameter.

Significance

Little economic significance in potato.

Symptoms

Primary symptoms are necrotic concentric rings on leaves or necrotic lesions on stems. Top necrosis on some stems is also common. Secondary symptoms include severe leaf deformation and vein thickening.

Hosts

- Natural: wide. Many species in over 30 monocotyledonous and dicotyledonous families.
- Experimental: wide. Many species in over 30 monocotyledonous and dicotyledonous families.

Geographical distribution

Probably distributed worldwide but only reported in potato from Brazil (Costa et al 1964) and Peru (Salazar et al 1981).

Transmission

Thrips (*Frankliniella occidentalis* and *Thrips tabaci*) are reported as vectors in some crops but in potato the vector is unknown. Transmission through the tubers is rare. Transmitted by seed and pollen of some plant species (Mink 1993), but not proven for potato.

Detection

By ELISA and sap inoculation to *Nicotiana tabacum*v. White Burley (local necrotic spots or rings and systemic necrosis and line patterns; plants may recover from the necrotic symptoms) or *Vigna unguiculata*subsp. *cylindrica*(catjang) (local reddish necrotic or chlorotic lesions followed by systemic necrosis or mottle).

***Tomato black ring virus (TBRV)* (genus *Nepovirus*, family *Comoviridae*)**

Isometric particles, 28 nm in diameter with angular outlines. Some isolates have satellite RNAs. TBRV from two antigenically distinct serogroups infect potato, the first contains potato bouquet and pseudo-aucuba strains and the second, the beet ringspot strain.

Significance

Little economic significance in potato, since infection is often sporadic and may be self-eliminating in tubers. Individual plants with severe stunting may show 80% yield loss. Even those with no apparent symptoms may show a 30% yield loss.

Symptoms

Primarily infected plants are usually symptomless but one or more stems may show black necrotic rings or spots (Fig. 57). Secondarily infected plants may be symptomless or stems may be severely dwarfed with leaf curling, cupping and ringspots (Fig. 58). Although symptoms may be dependent on cultivar, some isolates of the beet ringspot strain seem to cause severe dwarfing whereas others induce few or no symptoms (C. Jeffries, 1996, pers. comm.). The pseudo-aucuba strain causes bright yellow leaf markings. Infected tubers may be severely cracked and distorted.

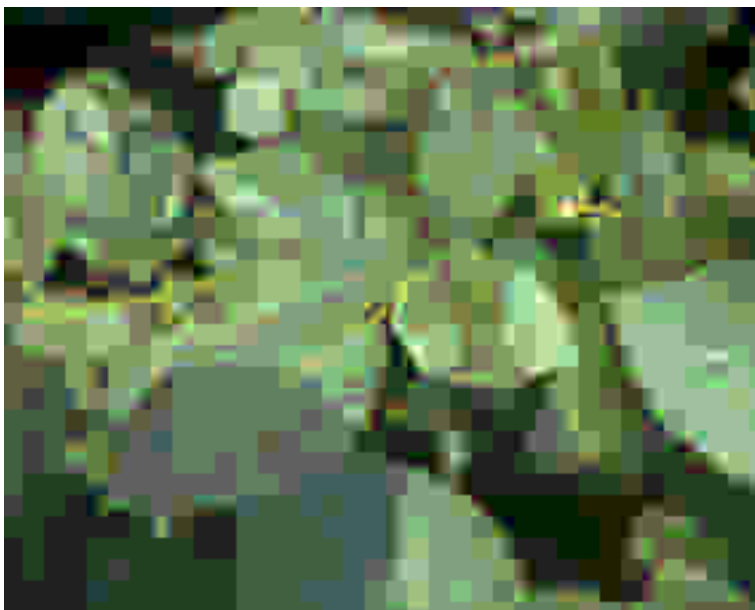


Fig. 57. Ringspots, *S. tuberosum* cv. Croft. (C. J. Jeffries)

Fig. 58. Leaf rolling and rosetting of upper leaves, and dwarfing of plant, *S. tuberosum* v. Croft. (C.J. Jeffries)



Hosts

- Natural: wide. Including important crop plants such as *Vitis vinifera* tree fruit and small fruit species, vegetables, ornamentals and many weed species. Potato is probably a poor host.
- Experimental: very wide. At least 76 species in 29 dicotyledonous families.

Geographical distribution

Various crop plants infected, mainly in Europe but also in Asia, Africa, North and South America (Smith et al 1997). Occasionally reported infecting potatoes in Europe, e.g. Germany (Köhler 1950), UK (Harrison 1957, 1958) and Poland (Kudamatsu et al 1981).

Transmission

By nematodes (*Longidorus* spp.). Most important means of survival of TBRV is in weed seeds. It is transmitted to a high degree through botanical seed of many plant species (Murant and Lister 1967) including TPS (C. Jeffries, 1996, pers. comm.).

Detection

By ELISA, using a mixture of antibodies to the main two serogroups because there is considerable antigenic variation. Also by sap inoculation to *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana rustica*, *N. tabacum* v. White Burley (chlorotic or necrotic local lesions and systemic necrosis or chlorotic mottle, although leaves produced later in *N. rustica* and *N. tabacum* may be symptomless) or *N. occidentalis* P1 (local necrotic lesions and bronzed rings, systemic mild chlorosis with small necrotic lesions and growth reduction). Nucleic acid probes have been produced (Bretout et al 1989).

Tomato mosaic virus (ToMV) (genus *Tobamovirus*)

Rod-shaped particles 300 x 18 nm.

Significance

Not a problem in potato.

Symptoms

Mosaic/mottle in *Solanum bulbocastanum* (Fig. 59).

Hosts

- Natural: fairly wide. Pepper (*Capsicum* spp.), tomato (*Lycopersicon esculentum*) and *S. bulbocastanum* and other Solanaceous crops including potato
- Experimental: wide. More than nine families susceptible.

Geographical distribution

Worldwide but only occasionally reported from potato and *Solanum* spp. Found infecting cv. Astilla in Hungary (Juretic et al 1977; Horváth et al. 1978) and *S. bulbocastanum* growing in a glasshouse in the Netherlands (J. Verhoeven, 1997, pers. comm.).

Transmission

Highly infectious. Spreads very easily mechanically (e.g. on machinery during cultural operations) including plant-to-plant contact. Although not proven for potato, transmitted by botanical seed (but not through the embryo) of some plant species.

Detection

By ELISA and sap inoculation to *Nicotiana glutinosa*, *Nabacum* cvs. Samsun or White Burley (local necrotic lesions are produced at temperatures below 25°C; at higher temperatures systemic symptoms may be produced as well). To distinguish ToMV from Tobacco mosaic virus (TMV) see the description for TMV. Nucleic acid probes have been produced (Palukaitis and Symons 1980).



Fig. 59. Mottle, *S. bulbocastanum* (PPS)

Tomato spotted wiltvirus (TSWV)(genus *Tospovirus*, family *Bunyaviridae*)

Enveloped particles, roughly spherical, ranging from 70-110 nm in diameter, and with knob-like surface projections formed by glycoproteins. Three RNA segments forming the genome are encapsidated by the nucleocapsid protein into three pseudospherical nucleocapsids.

Significance

Important in localized areas where the vector (and virus) occurs primarily in other crops [e.g. tomato and sweet pepper (*Capsicum annuum*)] or in natural vegetation but then moves to potato. In the northwestern/central plains of India (Khurana et al. 1997) 90% of plants may be infected in some cultivars in some years. In South Africa, production of potatoes in some areas (Vryburg in the North West Province and Barclay West in the Northern Cape Province) was seriously affected in the 1997/98 growing season after viruliferous thrips spread from nearby crops of paprika (*Capsicum annuum*) (G. Thompson and M. Cloete, 1998, pers. comm.).

Symptoms

Primary infection results in top and/or stem necrosis with the formation of concentric rings or spots on leaves and stems (Fig. 60). Shoots which are not killed may have a stunted rosetted appearance and chlorotic ringspots on leaves. Only one or a few stems on a plant may be infected and therefore only some progeny tubers may be infected. Tubers may be few, sometimes small and malformed with necrotic damage (Fig. 61). They may



Fig. 60. Necrotic spots and stem necrosis, *S. tuberosum* cv. Itarare. (J.A.C. de Souza-Dias)

have reduced sprouting or fail to sprout. Some stems grown from infected tubers may be healthy, or stems may have latent infection or produce symptoms which are similar to those of primary infection.

Host range

More than 900 species in over 90 monocotyledonous and dicotyledonous families have been shown to be susceptible to TSWV either by natural infection or experimentally. Twenty percent of them belong to the Solanaceae of which 47 susceptible *Solanum* species have been recorded (Peters 1998).

- Natural: very wide. Including crop species such as pea (*Pisum sativum*), groundnut (*Arachis hypogaea*), soyabean (*Glycine max*), sweet pepper (*Capsicum annuum*), tobacco (*Nicotiana* spp.), tomato (*Lycopersicon esculentum*) and many ornamental species.
- Experimental: very wide. Almost all species of the Asteraceae and Solanaceae tested are susceptible.

Geographical distribution

Wide distribution (Smith et al. 1997). Reported on potatoes in the field mainly from Argentina (Granval de Millan et al. 1998), Australia (Magee 1936; Norris and Bald 1943; Moran et al. 1994), Brazil (Costa and Kiehl 1938), central India (Khurana et al. 1997) and South Africa (G. Thompson and M. Cloete, 1998, pers. comm.). In Europe, has been found infecting potatoes in Portugal in 1993 and 1997 after spread of thrips and virus from tomato (D. Louro and C. Sera, 1998, pers. comm.) and in the Netherlands has caused damage to potatoes grown adjacent to infected greenhouse-grown tomato crops (Verhoeven and Roenhorst 1994).



Fig. 61. Internal tuber necrosis, *S. tuberosum* (PPS)

Transmission

At least eight species of thrips – *Frankliniella bispinosa*, *F. fusca*, *F. intonsa*, *F. occidentalis*, *F. schultzei*, *Thrips palmi*, *T. setosus* and *T. tabaci* – act as vectors in a persistent and propagative manner, but only some of these (e.g. *F. occidentalis*, *F. schultzei*, *T. palmi* and *T. tabaci*) are probably important in transmission to potato. *Thrips palmis* is a pest on potatoes in Brazil.

Detection

By ELISA but care must be taken over false positives. Antisera to the nucleocapsid (N) protein is highly specific (Resende et al. 1991; De Avila et al. 1993), antisera to the complete virus or other proteins are less specific (Adam et al. 1996). Also detect by sap inoculation to *Nicotiana benthamiana*, *N. glutinosa*, *N. occidentalis*, *N. tabacum* cvs. Samsun and White Burley (large slightly sunken necrotic local lesions and systemic necrosis) which are useful as systemic hosts. *Petunia hybrida* can give specific local lesions within 3 days of inoculation, but is less susceptible than *N. benthamiana*. Dark red or blue/purple flowering *petunia* varieties must be used. Incubation for a few days in the dark enhances susceptibility. Use *Datura stramonium* plants when co-infection with Potato virus Y is suspected and *N. glutinosa* when Tobacco mosaic virus is suspected to occur. However, inoculation of extract from infected plants is not always successful as TSWV is highly unstable in vitro. The use of freshly prepared inoculation buffers containing sodium sulphite or polyvinylpyrrolidone (PVP), e.g. 0.01M potassium phosphate buffer, pH 7.0-7.4 + 0.01M Na₂SO₃ or 0.02M phosphate buffer, pH 7.4 + 2% PVP (MW 10 000), is recommended (Best 1968; Mumford et al. 1996a; Verhoeven et al. 1996). The recipe for the phosphate buffer + PVP is shown on page 21. Chilling the mortar and pestle, and buffer, washing the leaves some minutes after inoculation, and incubation of the plants 24 h in the dark before inoculation may be beneficial.

Nucleic acid probes have been produced (Huguenot et al. 1990). RT-PCR is reliable (Adam et al. 1996; Mumford et al. 1996b).

Other tospoviruses have been found infecting potato and *Solanum* spp.: Groundnut ringspot virus infecting potato in Argentina (Granval de Milan et al. 1998), Impatiens necrotic spot virus infecting *Solanum bulbocastanum*, *S. fendleri*, *S. mochiquense*, *S. oplocanense* (causes local necrotic lesions) and *S. muricatum* (very mild mosaic) in glasshouses in the Netherlands (Verhoeven and Roenhorst 1995); Tomato chlorotic spot virus in Argentina and Brazil (Nagata et al. 1995; Granval de Milan et al. 1998) and a tospovirus serologically related to Groundnut bud necrosis virus infecting potato in India (*S. Paul Khurana* and *R. Naidu*, 1996, pers. comm.). Since these tospoviruses do not react with TSWV antibodies, care should be taken during diagnosis. The most reliable indicators for all tospoviruses are *N. benthamiana* and *N. occidentalis* P1 (local and systemic necrotic lesions and necrosis) (*J. Verhoeven* and *J. Roenhorst*, 1997, pers. comm.).

Wild potato mosaic virus (WPMV) (genus *Potyvirus*, family *Potyviridae*)

Flexuous filamentous particles 685-800 nm long.

Significance

Not a problem in potato. Affects some solanaceous plant species.

Symptoms

Severe leaf mosaic and deformation in *Solanum chancayense* and chlorotic mosaic, rugosity and enations in *S. muricatum*.

Hosts

- Natural: narrow. Pepino (*S. muricatum*), tomato (*Lycopersicon esculentum*) and *S. chancayense*.
- Experimental: narrow. Includes *Nicotiana* spp. and *Solanum* spp. but *S. tuberosum* cultivars could not be infected.

Geographical distribution

Coastal area of Peru near Lima (Lachay hills) (Jones and Fribourg 1979). Also reported infecting field crops of tomato in Germany; a result of cross-infection from infected pepino (*S. muricatum*) which had been imported from South America (Adam et al 1995).

Transmission

Experimentally, non-persistent transmission by *Myzus persicae*.

Detection

By ELISA using specific or potyvirus-group antibodies. Also by sap inoculation to *Nicotiana bigelovii*, *N. glauca* and *N. occidentalis* P1 (systemic mosaic).

UNCHARACTERIZED VIRUS AND VIRUS-LIKE DISEASES

Potato deforming mosaic disease (Argentina)

Unknown etiology, but see comments for potato deforming mosaic virus (Brazil).

Significance

Yield reduction between 20-30%.

Symptoms

Leaf deformation and severe mosaic (Fig. 62).

Hosts

- Natural: narrow. Potato.
- Experimental: narrow. Potato.

Geographical distribution

Argentina but symptoms have not been observed for many years and the disease is said to be no longer present (J. Daniels, 1997, pers. comm.).

Transmission

Whitefly ?

Detection

No test available, by symptoms in potato only.



Fig. 62. Mosaic and leaf deformation, *S. tuberosum* cv. Serrana (left: healthy). (L.F. Salazar)

Saq'O disease

An unknown phytoplasma and a native strain of Potato leafroll virus (PLRV) are associated with the disease, but it is not yet known whether they are the causal agent(s).

Significance

Up to 40% yield reduction and degradation of tuber quality including flavour changes.

Symptoms

In cv. Runa leaves are chlorotic, reduced in size, rolled and show purple coloration underneath leaf tips (Fig. 63). Leaves of cv. Waycha are slightly chlorotic and broadened and there is a proliferation of aerial stolons. In both cultivars there is a proliferation of stems, stolons and roots (Figs. 64 and 65). Small tubers have superficial eyes and are discoloured internally and externally.



Fig. 63. Leaf rolling, chlorosis and purple colouration, *S. tuberosum* cv. Runa. (L.F. Salazar)

Hosts

- Natural: a disease so far only studied in the native cvs. Runa and Waycha but may occur in others.
- Experimental: unknown.

Geographical distribution

Bolivia (PROINPA 1993).

Transmission

Disease symptoms can be reproduced by graft transmission and through tubers.

Detection

Two months after graft transmission to *Datura stramonium* the PLRV isolate produces symptoms and can be detected by ELISA. Neither putative agent is easily detected directly from infected potato plants by ELISA or PCR (E. Fernandez-Northcote, 1996, pers. comm.).



Fig. 64 (top).
Proliferation of stems
and stolons,
S. tuberosum cv. Runa.
(E. Fernandez- Northcote)

Fig. 65 (bottom).
Proliferation of stems and
stolons, with many small
tubers formed,
S. tuberosum cv. Waycha
(right: healthy).
(E. Fernandez-Northcote)



PHYTOPLASMAS

Phytoplasmas, previously known as mycoplasma-like organisms (MLOs), are cell wall-less prokaryotes in the class Mollicutes. None has been isolated and cultured in a cell-free medium *in vitro*. Provisionally one can distinguish six phytoplasmas on potato based on symptom expression in hosts and their vectors: potato marginal flavescence (PMF), potato phyllody (PP), potato purple toproll (PTR), potato purple-top wilt (PTW), potato stolbur (PS) and potato witches' broom (PWB). Recently, however, some phytoplasmas including PS and PWB and the closely related clover proliferation phytoplasma have been differentiated from other phytoplasmas on the basis of phylogenetic RFLP analysis of PCR-amplified 16S rDNA sequences (Lee et al 1993; Schneider et al 1993) and sequence analysis of 16S rDNA (Seemüller et al 1994; Davis et al 1997). Respectively these have been placed in the Stolbur and Clover Proliferation Phytoplasma Groups. For the other potato phytoplasmas, in the absence of serological or molecular data, it is difficult to decide how to group or separate them but PMF may possibly belong to the Stolbur Group, PP the Clover Proliferation Group and PTR and PTW the "aster yellows" group (typically yellow or purple foliage, with leafhopper vectors *Macrostelus* and *Hyaalsthes* spp. and usually not tuber-transmissible). In North America, "aster yellows" has been called potato purple top wilt, yellow top, bunch top, purple dwarf, apical leafroll, haywire, latebreaking virus, blue stem and moron, but it may be that these are discrete diseases caused by different phytoplasmas.

Potato marginal flavescence (PMF)

Cause

The classification/identity of the phytoplasma associated with PMF has not been determined but, despite certain differences, it seems to be closer in symptoms to stolbur than purple-top wilt.

Significance

Minor.

Symptoms

PMF symptoms are the most severe of the phytoplasmas affecting potatoes in India. In warm dry conditions (20-28°C, RH 40-60%), symptoms appear soon after emergence: mild chlorosis of the leaflet margins of the upper leaves which remain very small (Fig. 66). The chlorosis intensifies progressively, leaf blades become thick, rough and puckered. Growth is stunted (Fig. 67) because of short internodes with small leaves having narrow leaflets partly overlapping each other. Infected plants produce few, small tubers which often remain "blind" or emerge very late.

Hosts

- Natural: narrow. Potato
- Experimental: wide. Many solanaceous crops and periwinkle (*Catharanthus roseus*) but not white clover (*Trifolium repens*)

Geographical distribution

India (restricted to certain seed potato production farms in the Shimla Hills) (Nagaich et al 1974; Khurana et al 1988).

Transmission

Seriana equata (leafhopper).

Detection

No specific serological or molecular methods have been developed. Therefore use traditional methods (Khurana et al 1988). Although not tested, PMF should be detectable and differentiable by PCR using phytoplasma-universal primers, followed by RFLP analysis.



Fig. 66 (top). Chlorosis of the leaflet margins and rough lamina, *S. tuberosum* cv. Kufri Jyoti (experimentally infected). (S.M. Paul Khurana)



Fig. 67 (bottom). Late emergence, extreme stunting and symptoms from emergence (left). Delayed expression of severe symptoms with small leaflets (right), *S. tuberosum* cv. Kufri Jyoti. (S.M. Paul Khurana)

Potato phyllody (PP)

Cause

The classification/identity of the phytoplasma associated with PP has not been determined but it may be related to potato witches' broom phytoplasma.

Significance

Minor.

Symptoms

Characteristic symptoms are enlarged phylloid flowers, "green snakehood" like carpels, extreme hairiness, flattening of stems with prominent wings and development of chlorotic, small compound leaves having small leaflets but enlarged folioles⁴ (Fig. 68). Phylloid flowers often proliferate into vegetative shoots. After a prolonged period of infection, the potato plants develop several naked androecia as small yellow rosettes on the stem, and also axillary aerial tubers.

Hosts

- Natural: narrow. Potato.
- Experimental: narrow. Eggplant (*Solanum melongena*), periwinkle (*Catharanthus roseus*), tobacco (*Nicotiana sp.*) and tomato (*Lycopersicon esculentum*)

Geographical distribution

India (southern plateau area) (Khurana et al 1988).

Transmission

Vector not known.

Detection

No specific serological or molecular methods have been developed; therefore use traditional methods (Khurana et al 1988). Although not tested, PP should be detectable and differentiable by PCR using phytoplasma-universal primers, followed by RFLP analysis.

4 Folioles are subleaflets spaced on the rachis between successive pairs of leaflets.

Fig. 68. Chlorotic, hairy leaves and enlarged phylloid sepals and "green, snakehood" like modified carpels, *S. tuberosum* cv. Kufri Kuber (experimentally infected). (S.M. Paul Khurana)



Potato purple toproll (PTR)

Cause

The classification/identity of the phytoplasma associated with PTR has not been determined.

Significance

Minor because of restricted distribution, but in some years 50% infection can be found in certain cultivars, e.g. Kufri Jyoti.

Symptoms

Symptoms often appear late (40-50 days) after planting in the cool, wet season (15–20°C, RH 50-90%). Rolling of the base of leaflets of young top leaves, usually with light to intense pink or purple pigmentation. May be preceded by reduced leaflet size and marginal chlorosis. Infected plants become stiff, have shortened internodes and develop many axillary shoots, often with aerial tubers. Mother tubers remain firm until harvest. Small progeny tubers are produced on short stolons. On storage, hairy sprouts develop from a large number of infected tubers.

Hosts

- Natural: narrow. Potato and white clover (*Trifolium repens*)
- Experimental: wide. Mainly solanaceous crops. Also periwinkle (*Catharanthus roseus*)
It does not infect “aster-yellows” susceptible aster.

Geographical distribution

Northwestern hills of India (Khurana et al 1988).

Transmission

Transmitted in nature by *Alebroides dravidanum* and experimentally also by *Orosius albicinctus*

Detection

No specific serological or molecular methods have been developed. Therefore use traditional methods (Khurana et al 1988). Although not tested, PTR should be detectable and differentiable by PCR using phytoplasma-universal primers, followed by RFLP analysis.

Potato purple-top wilt (PTW)

Cause

The classification/identity of the phytoplasma associated with potato PTW has not been determined.

Significance

Minor importance except for occasional outbreaks in midwestern USA following the harvest of spring grains and maturing of native grasses and broadleaf weeds.

Symptoms

Stunting, bunched apical growth, rolling of upper leaflets associated with yellowing or development of reddish-purple coloration of leaves. Formation of aerial tubers, proliferation of axillary buds, stunting, vascular discoloration, wilting and premature death of infected plants have been observed. Stored potatoes may not germinate or may germinate with the formation of numerous hair-like sprouts (stems). Plants infected in early growth often develop aerial tubers or swollen stems.

Hosts

- Natural: very wide. Several hundred plant species including vegetable, ornamental, field crops and weeds belonging to over 50 families.
- Experimental: very wide, including asters and periwinkle (*Catharanthus roseus*)

Geographical distribution

North America (Wright et al 1981; Smith et al 1997), Australia (Harding and Teakle 1985) and possibly elsewhere.

Transmission

By leafhoppers *Macrostelus quadrilineatus* (*M. fascifrons*) in North America and by other *Macrostelus* spp. elsewhere.

Detection

No specific serological or molecular methods have been developed. Therefore use traditional methods (Khurana et al 1988). Although not tested, PTW should be detectable and differentiable by PCR using phytoplasma-universal primers, followed by RFLP analysis.

Potato stolbur (PS)

Thought to be caused by the same phytoplasma that in Europe causes tomato stolbur (Smith et al 1997), pepper stolbur (Schneider et al 1993) and grapevine yellows disease known in various countries as Vergilbungskrankheit (Germany), bois noir (France) or southern European (Mediterranean) grapevine yellows (Seemüller et al 1994; Davis et al. 1997).

Cause

Stolbur phytoplasma represents a distinct group in classification schemes based on phylogenetic RFLP and sequence analysis of rDNA. It has been suggested that stolbur phytoplasma be named as a distinct new “candidatus” species, “CandidatusPhytoplasma solani”.

Significance

May be severe in some regions with up to 86% of a crop affected. Outbreaks occur in cycles and are favoured by hot dry summers which stimulate vector migration.

Symptoms.

Plant stunting, and rolling and purple or yellow discoloration of the upper leaflets. Proliferation of axillary buds and formation of aerial tubers or swollen stems may also occur. Lower stems often develop cortical necrosis, sloughing of tissue and premature death. Flaccid (gummy) tubers may form. Tubers may give rise to normal or spindly sprouts (hair sprouting).

Hosts

- Natural: wide. Typically attacks Solanaceae (45 species). Principal economic hosts are eggplant (*Solanum melongena*), a pepper (*Capsicum annuum*), potato and tomato (*Lycopersicon esculentum*). May also infect species in other families, e.g. bindweed (*Convolvulus arvensis*) and grapevine (*Vitis vinifera*).
- Experimental: wide. As above including periwinkle (*Catharanthus roseus*)

Geographical distribution

Europe (Smith et al 1997). Has also been reported in Israel (Zimmerman-Gries 1970) and Turkey (Citir 1985).

Transmission

The principal vector of stolbur phytoplasma in Europe appears to be the leafhopper *Hyalesthes obsoletus*

Detection

Although not tested should be detected by immunofluorescence microscopy (Cousin et al. 1989) and ELISA (Fos et al 1992) using antibodies prepared to tomato stolbur, nucleic acid hybridization (Lee et al 1991) or PCR using phytoplasma-universal primers, followed by RFLP analysis (Schneider et al 1993) for specific identification. Although stolbur group-specific primers (fSTOL/rSTOL1) have been published (Maixner et al 1995) these have been found to amplify DNA from non-stolbur phytoplasmas (Jomantiene et al. 1998).

Potato witches' broom (PWB)

Cause

In classification schemes based on phylogenetic RFLP analysis of DNA, PWB phytoplasma (Canada) together with clover proliferation phytoplasma (Canada) and beet leafhopper virescence phytoplasma (associated with potato witches' broom in the USA) form a distinct phylogenetic group (Lee et al 1991) and putative *Phytoplasma* species. It is not known whether the Canadian PWB is the same species as those occurring in other geographical areas.

Significance

Generally of minor importance because potato is not the preferred host.

Symptoms

Symptoms are cultivar-dependent. Characteristic symptoms are stunted, multistemmed plants that have many axillary branches (Figs. 69 and 70). The leaves are rounded and may be chlorotic. An abnormally large number of small tubers is produced. Tubers have a shortened dormancy period and may produce hair sprouts (Fig. 71).



Fig. 69 (top). Stunted multi-stemmed plants, *S. tuberosum* cv. Kerr's Pink (right: healthy). (SASA)

Fig. 70 (bottom). Stunted multi-stemmed plant, *S. tuberosum* cv. Kerr's Pink. (SASA)

Hosts

- Natural: narrow. Including alfalfa (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*), potato and red and white clover (*Trifolium pratense* and *T. repens*).
- Experimental: narrow. As above including periwinkle (*Catharanthus roseus*).

Geographical distribution

Potato witches' broom disease has been reported from the Andes of South America (L. Salazar, 1997, pers. comm.), Asia, Europe and North America (Smith et al 1997).

Transmission

In Europe, the leafhopper *Sclerorachus dasiduis* is a vector of PWB. In North America, *S. dasiduis* and *S. balliare* are the vectors. In the Peruvian Andes a psyllid (*Russelliana solanicola*) is associated with PWB (L. Salazar, 1997, pers. comm.). It is thought that the vectors are unable to acquire PWB from potato. Tubers perpetuate PWB.

Detection

Non-specific tests such as electron and fluorescent microscopy have been described (Hiruki and da Rocha 1986). More specific methods are immunofluorescence microscopy (da Rocha et al 1986), nucleic acid hybridization [probes prepared against clover proliferation phytoplasma hybridize with PWB DNA (Deng and Hiruki 1990, 1991)] and PCR using phytoplasma-universal primers, followed by RFLP analysis for specific identification (Lee et al 1993) or reamplification of the PCR products with the specific Clover Proliferation Group primer 1A/1B in nested-PCR (Deng and Hiruki 1991; Khadhair et al 1997). Methods involving PCR are the most sensitive.



Fig. 71. Hair sprouts, *S. tuberosum* (SASA)

BACTERIA

For detection of bacteria it is recommended to use at least two different methods from those described.

Bacterial slow wilt

Cause

Erwinia chrysanthemum Burkholder et al.

Description

Gram-negative, pectolytic (some strains very weakly pectolytic), rod-shaped bacterium. Depending on biovar, optimum growth between 27 and 35°C, indole and phosphatase positive.

Significance

Causes considerable foliage disease in warm weather. Tuber soft rot in storage and in seed tubers can be high. Incidence has recently increased in some temperate European countries.



Fig. 72. Stem symptom (blackleg type), *S. tuberosum* (CIP)

Symptoms

Causes blackleg-like symptoms under warm conditions (Fig. 72), or stem pith decay (Fig. 73) with slow wilt, yellowing and desiccation of foliage (Lumb et al 1986). Also causes soft rot of tubers in field and in storage (Fig. 74).

Hosts

- Wide host range, biovars 1, 3, 7 and 9 mainly associated with potato disease. Some biovars show a narrow host range, others (like biovar 3) a very wide host range (Samson et al 1987).

Geographical distribution

Cosmopolitan. Biovar 3 predominates on potato in tropical regions. Biovars 1, 7 and 9 are associated with potato in Europe (Smith et al 1997).

Biology and transmission

Carried mostly on latently infected seed potato tubers. Found in surface water in some regions (Cother and Gilbert 1990).



Fig. 73 (top). Cut stem showing pith decay, *S. tuberosum*(CSL)

Fig. 74 (bottom). Soft rot of tubers, *S. tuberosum* (CIP)

Detection

Not all strains grow well on crystal violet pectate medium. "Growth factor medium" has been used for isolation (Allefs et al 1995). Serological detection is limited by cross-reactivity of antisera with lipopolysaccharide of saprophytic pseudomonads (van der Wolf et al 1993). PCR methods are available (Bakker et al 1995; van der Wolf et al 1995).

Growth factor medium (Allefs et al. 1995)

K ₂ HPO ₄	0.4 g
MgSO ₄ · 7H ₂ O	0.05 g
NaCl	0.1 g
NH ₂ PO ₄	0.5 g
Glucose	1.0 g
Yeast extract	3.0 g
Agar	15.0 g
Distilled water	1.0 L

Autoclave at 121°C for 15 min

Bacterial wilt or brown rot

Cause

Ralstonia solanacearum (Smith) Yabuuchi et al; syn. *Burkholderia solanacearum* (Smith) Yabuuchi et al; syn. *Pseudomonas solanacearum* (Smith) Smith.

Description

Gram-negative, rod-shaped bacterium. Species divided into five biovars and five races; biovar 2-A (race 3) is most commonly found on potato. Causes brown rot (also called bacterial wilt).

Significance

Very destructive on potato, highly contagious and persistent in the field.

Symptoms

The main symptom is wilting (Fig. 75) often accompanied by vascular browning. Cut stems ooze bacterial slime. In tubers, browning and necrosis of vascular tissue (brown rot) often occurs and bacterial ooze may be present in vascular tissue, and externally on eyes (Figs. 76a, 76b). The action of pectolytic enzymes may result in cavities developing around the vascular ring (Fig. 76c). Infection can remain latent in stems and tubers.



Fig. 75. Bacterial wilt, *S. tuberosum* (CIP)

Hosts

- Very wide host range with more than 40 families listed (Hayward 1991; Hayward and Hartman 1994). Biovars 1, 2-A, 2-T, 3 and 4 (races 1 and 3) cause disease on potatoes. Biovar 2-A (race 3) mostly specific to potato and some related Solanaceae (e.g. *Lycopersicon esculentum*, *Solanum nigrum*, *S. dulcamara* and to some extent on *S. melongena* and *Capsicum* spp.). Potato cultivars vary in susceptibility.

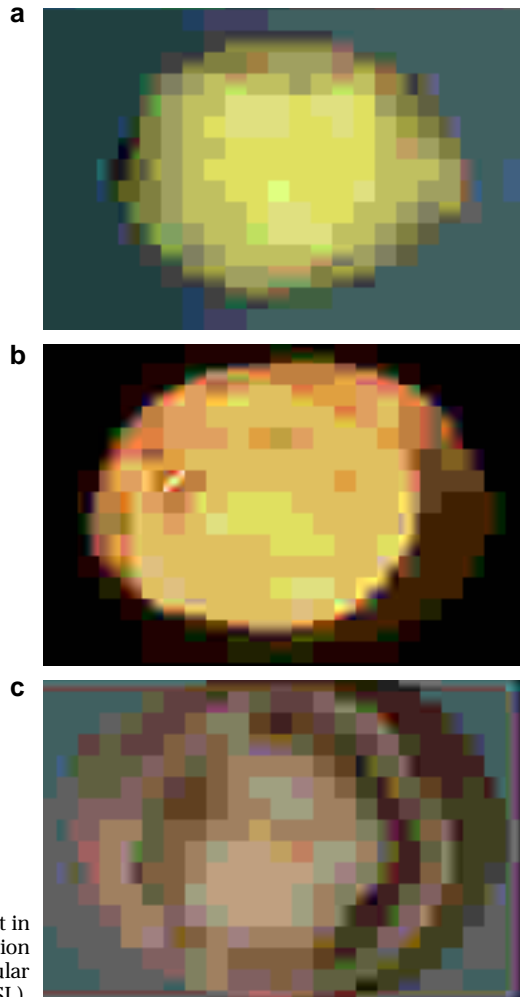


Fig. 76. Brown rot development in *S. tuberosum* tubers (various stages): (a) discolouration of vascular ring (CSL); (b) bacterial ooze from vascular ring (CIP); (c) cavities around vascular ring. (CSL).

Geographical distribution

Widespread in tropics and subtropics. Biovar 2-A (race 3) occurs in Europe as far north as 56° and in upland and subtropical regions of South America as far south as 38° (Smith et al. 1997; Hayward et al 1998). Biovar 1 (race 1) occurs on potato in the southern states of the USA.

Biology and transmission

Disseminated in vegetative propagating material. Present in vascular tissues, on the surface and in lenticels of latently infected seed potato tubers. Resistant germplasm (e.g. accessions of *Solanum phurejense*) develop vascular infections which remain localized in roots or lower stem. Possibility of spread on infected TPS requires further investigation.

Detection

Large variety of detection methods currently available. These include: culture on semi-selective media (Englebrecht 1994; Elphinstone et al 1996), ELISA (Robinson-Smith et al 1995), immunofluorescence (Janse 1988), bioassay in susceptible tomato seedlings (Janse 1988), and DNA amplification using specific polymerase chain reactions (Seal et al 1993). Maintenance of tubers and in vitro plantlets at high temperature (25-30°C) induces symptom development in latently infected material.

Modified semi-selective SMSA medium for *R. solanacearum* (Englebrecht 1994) as adapted by Elphinstone et al. (1996).

Bactopeptone (Difco)	10.0 g
Bactagar (Difco)	15.0 g
Casamino acids (Difco)	1.0 g
Glycerol	5.0 ml
Distilled water	1.0 L

After autoclaving at 121°C for 15 min and cooling to 40°C add filter-sterilized solutions of the following ingredients to reach the final concentrations given:

2,3,5-triphenyl tetrazolium chloride	50.0 mg per L
Polymixin B sulphate	100.0 mg (600 000 U) per L
Bacitracin†	25.0 mg (1250 U) per L
Chloramphenicol	5.0 mg per L
Crystal violet	5.0 mg per L
Penicillin	0.5 mg (825 U) per L

† It may be necessary to increase the concentration of bacitracin to 100-300 ppm to reduce contaminating saprophytic bacteria (this does not affect recovery of *R. solanacearum*)

Blackleg or softrot

Cause

Erwinia carotovora subsp. *atroseptica* (van Hall) Dye.

Description

Gram-negative, rod-shaped pectolytic bacterium. Does not grow above 35°C. Produces acid from α -methyl-glucoside and reducing substances from sucrose. Causes blackleg and soft rot. Predominant strains are serogroup I, but other serogroups occur. The related heterogeneous subspecies *carotovora* (Jones) Bergey is of lesser concern because it is ubiquitous and opportunistic. It causes soft rot disease in storage and seed piece decay; some strains cause blackleg-like symptoms.

Significance

Widespread occurrence; disease incidence generally low.

Symptoms

Black soft rot of the stem, invariably originating from the seed tuber. Young diseased plants are stunted and chlorotic, older plants wilt or desiccate (Fig. 77). Progeny tubers may develop soft rot (with dark margins), usually originating at the stolon (Figs. 78 and 79).

Hosts

- Almost exclusively restricted to potato. Related strains infect other plant species.



Fig. 77. Stem symptom, *S. tuberosum* (S.H. De Boer)

Geographical distribution

Cosmopolitan (Bradbury 1986; Smith et al. 1988).

Biology and transmission

Primarily transmitted by latently infected seed tubers. Other plant parts can be infected/contaminated with the bacterium. Infection of TPS is not reported.

Detection

Commonly detected using selective pectate media, e.g. crystal violet pectate medium (Cuppels and Kelman 1974; Perombelon and Burnett 1991) often with anaerobic enrichment techniques (Meneley and Stanghellini 1976). ELISA, immunofluorescence and immunofluorescent colony staining (Jones et al 1994) are available for serogroup I strains. Monoclonal antibodies with specificity to serogroup I have been produced (De Boer and McNaughton 1987; Gorris et al 1994). A PCR method has been developed (De Boer and Ward 1995).



Fig. 78 (top). Tuber surface symptom, *S. tuberosum* (K. Benlioglu)



Fig. 79 (bottom). Internal tuber symptom, *S. tuberosum* (S.H. De Boer)

Ring rot

Cause

Clavibacter michiganensis subsp. *sepedonicus* (Spieck. and Kotth.) Davis et al, syn. *Corynebacterium sepedonicum*

Description

Slow-growing gram positive, coryneform bacterium.

Significance

Very destructive on potato when cutting seed. Highly contagious.

Symptoms

In very severe cases, fairly typical vascular wilt in field similar to that caused by *R. solanacearum*. In other cases areas between leaf veins eventually become chlorotic and margins become necrotic (Fig. 80). This may be confused with senescence late in the season. A dwarf rosette type symptom has been described in some cultivars (shortened internodes and stunting of the plant). Tuber infection is through the stolon. If the tuber is cut across the heel end, narrow glassy/cream-yellow zones may be seen in the vascular tissue near the stolon end. As the infection progresses a narrow yellowish to light brown zone surrounding the vascular tissue may develop (Fig. 81a). Upon squeezing, a creamy bacterial exudate can be expelled from cut vascular tissues and tissue outside the vascular ring may easily separate from the inner tissues. Cheesy rotting starts at the stolon end of the vascular ring which does not usually discolour until secondary invaders begin to colonize. Cavities may develop around the vascular ring (Figs. 81b and 81c). Severely affected tubers show periderm cracking (Fig. 82). Latent infection occurs particularly in tolerant cultivars.



Fig. 80. Wilt, necrosis and marginal leaf rolling, *S. tuberosum* (S.H. De Boer)

Hosts

- Naturally occurring on potato. Artificially infects tomato and eggplant. Association with sugarbeet roots and seed has been reported (Bugbee et al 1987; Bugbee and Gudmestad 1988).

Geographical distribution

North America, eastern and northern Europe (e.g. Denmark, Finland, Germany, Russia, Sweden and Ukraine) and sporadic in northern Asia (Smith et al 1997).

Biology and transmission

Primarily transmitted through vascular infection (may be latent) of seed potato tubers. Persistent on equipment and in storage. Latent pathogen populations in symptomless in vitro plantlets of some potato cultivars can survive several generations.



Fig. 81 (top). Ring rot development in *S. tuberosuntubers* (various stages): (a) discolouration around vascular ring (CSL); (b) cavitation starting around vascular ring (S.H. De Boer); (c) cavities around vascular ring. (S.H. De Boer)

Fig. 82 (bottom). External cracking of tuber periderm, *S. tuberosum* (S.H. De Boer)

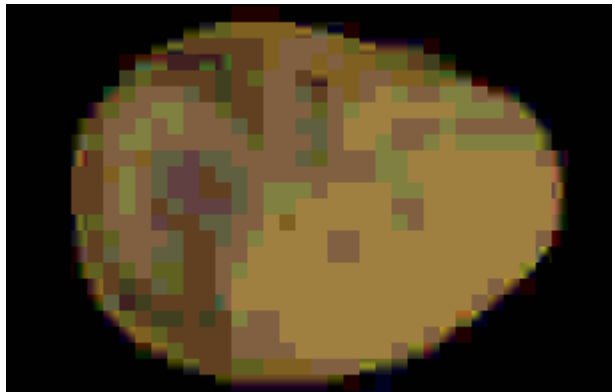
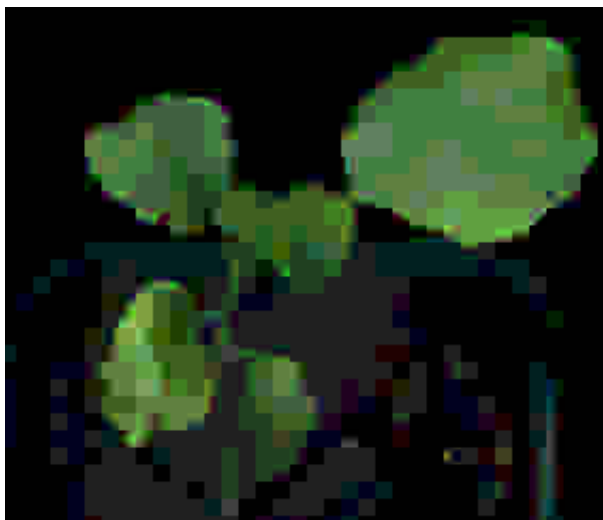


Fig. 83. Symptoms in eggplant: flaccid areas at leaf margins and wilting.
(S.H. De Boer)



Detection

Sensitive detection methods are required since pathogen density is often low in latent infections (Slack 1987). The European Union-approved method is based on immunofluorescence and bioassay in eggplant (*Solanum melongena*, g. cv. Black Beauty, Fig. 83) seedlings (Anon. 1990). Serological methods are commonly used to screen and index pathogen-free seed stocks from tissue culture programmes (Zink 1991). Monoclonal antibodies are available for immunofluorescence (De Boer and Wieczorek 1984) and ELISA (De Boer et al 1988). The monoclonal antibodies used in ELISA, however, may fail to detect non-fluid forms of the organism (Baer and Gudmestad 1993). PCR methods are under development (Schneider et al 1993; Rademaker and Janse 1994; Li and De Boer 1995; Slack et al 1996). Nutrient broth, such as Richardson's (1957) medium, can be used to detect bacterial contamination (including *Clavibacter michiganensis* ssp. *sepedonicus*) in in vitro plantlets.

Richardson's medium (1957)

Yeast extract	1.0 g
Casein hydrolysate	1.0 g
Dextrose	1.0 g
Calcium pantothenate	0.1 g
Distilled water	1.0 L

Autoclave at 121°C for 15 min

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Appendix III

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Appendix VI

Virus genera and virus-specific PCR detection methods

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APPENDIX I.

INFORMATION REQUIRED IN POTATO GERmplasm HEALTH STATEMENT

1. Title: "Potato Germplasm Health Statement "
2. Reference number for each statement issued.
3. The address of the institution issuing the statement.
4. The name and address of the consignee.
5. Information on the material to be shipped
 - 5.1 Name of the cultivar, line or species.
 - 5.2 Country of origin if different from the supplier.
 - 5.3 Type of material, e.g. in vitro plants, true potato seed or pollen.
 - 5.4 Pathogens against which the material has been tested and the results.
 - 5.5 Full description of the material tested and how it relates to the material shipped.
 - 5.6 The method used for testing, e.g. ELISA, bioassay (state indicator plants used).
 - 5.7 The number of times the material was tested by each method.
 - 5.8 Whether plants were observed over one vegetative cycle for disease symptoms and the results.
 - 5.9 For true potato seed the % level of sampling and testing if appropriate.
 - 5.10 The pathogen(s) eliminated, if the material has been derived from a pathogen elimination programme.
 - 5.11 Precautions taken to ensure that since testing the material has not become infected or contaminated.

6. Declaration:

"This statement provides information on the plant health status of the germplasm listed. It is not a substitute for the Phytosanitary Certificate issued by the Plant Protection Organization of the exporting country. The material listed has been tested by competent staff using procedures recommended in the FAO/IPGRI Technical Guidelines for the Safe Movement of Potato Germplasm. This statement is issued for guidance only and does not infer any legal responsibility".

Signature
Position

Date

APPENDIX II.

Plant Health Statement No.

Specimen Potato Germplasm Health Statement

This statement provides information on the plant health status of the germplasm listed. It is not a substitute for the Phytosanitary Certificate issued by the Plant Protection Organization of the exporting country.

Name and address

Institution issuing statement:	Consignee:
--------------------------------	------------

Name and origin of cultivar, line or species and type of material, e.g. in vitro plants, true potato seed (TPS) or pollen.

Name:	Country of Origin:	Type of material shipped:
-------	--------------------	---------------------------

Pathogens tested and test method

Full description of material tested	Pathogen	Test method 1		Test method 2		
		Method	No. of times tested	Method	No. of times tested	Result

List of indicator plants used if not recorded above

(cont.)

Potato plants were observed over one vegetative cycle for disease symptoms:	YES	NO
Potato plants were free from symptoms of disease: If NO, please explain:	YES	NO

% level of
sampling
of true seed:

If the material has been derived from a pathogen-elimination programme, indicate the pathogen(s) eliminated:

Precautions taken to ensure that since testing, the material has not become infected or contaminated:

The material listed has been tested by competent staff using procedures recommended in the FAO/IPGRI Technical Guidelines for the Safe Movement of Potato Germplasm. This statement is issued for guidance only and does not infer any legal responsibility.

Signature: _____ Date: _____ Position: _____

APPENDIX III.

PROBABILITY TABLES OF FAILING TO DETECT INFECTION IN SEED LOTS

When seeds are drawn (without replacement) from small seedlots, often the sample is a high percentage of the total seedlot. Consequently, the chance that each individually sampled seed is infected will be altered by whether previously sampled seeds from the same seedlot were positive or not. Therefore the chance of successively sampled seeds testing positive does not remain constant. In such circumstances, the chance of finding no infected seed in a sample of size M from a seedlot of size N containing $N \times X/100$ infected seeds is given by the hypergeometric distribution (Freund 1970).

Percentage chance of finding none infected from the sample is

$$\frac{(N - NX/100)! (N-M)!}{(N - NX/100 - M)! N!} * 100$$

where $N! = N \times (N-1) \times (N-2) \times \dots \times 2 \times 1$ and $0! = 1$

("!", called by mathematicians "factorial", is defined by: $N! = N \times (N-1) \times (N-2) \times \dots \times 2 \times 1$
When $N=0$ we have a special case. $0! = 1$)

Percentage chance of failing to detect any infection in a sample of M seeds out of a seedlot of 25 seeds (N) when the true infection of the seedlot ranges from 4-48%.

		Seedlot size (N) - 25						
		True infection (%)			True infection (%)			
No. tested (M)		4	24	48	No. tested (M)	4	24	48
2		92.00	57.00	26.00	14	44.00	0.26	—
4		84.00	30.64	5.65	16	36.00	0.05	—
6		76.00	15.32	0.97	18	28.00	0.00	—
8		68.00	6.99	0.12	20	20.00	—	—
10		60.00	2.83	0.01	22	12.00	—	—
12		52.00	0.97	0.00	24	4.00	—	—

Percentage chance of failing to detect any infection in a sample of M seeds out of seedlot sizes of 100 and 200 seeds (N) when the true infection of the seedlot ranges from 1-50%.

No. tested (M)	Seedlot size (N) - 100				Seedlot size (N) - 200			
	True infection (%)				True infection (%)			
	1	4	25	50	1	4	25	50
5	95.00	81.19	22.92	2.81	95.05	81.36	23.33	2.97
10	90.00	65.16	4.79	0.06	90.23	65.84	5.21	0.08
15	85.00	51.64	0.90	0.00	85.53	52.98	1.11	0.00
20	80.00	40.33	0.15	0.00	80.95	42.36	0.23	0.00
30	70.00	23.38	0.00	0.00	72.19	26.57	0.01	0.00
40	60.00	12.44	0.00	0.00	63.92	16.18	0.00	0.00
50	50.00	5.87	0.00	0.00	56.16	9.54	0.00	0.00
60	40.00	2.33	0.00	–	48.89	5.00	0.00	0.00
70	30.00	0.70	–	–	42.14	2.95	0.00	0.00
80	20.00	0.12	–	–	35.88	1.53	0.00	0.00
90	10.00	0.01	–	–	30.13	0.74	0.00	0.00
100	–	–	–	–	24.87	0.34	0.00	0.00
110	–	–	–	–	20.13	0.14	0.00	–
130	–	–	–	–	2.14	0.02	0.00	–
150	–	–	–	–	6.16	0.00	0.00	–
170	–	–	–	–	2.19	0.00	–	–
190	–	–	–	–	0.23	0.00	–	–

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Indicator plant	PLRV	PMTV	PRDV	PVA	PVM	PVP	PVS	PVT	PVU	PVV	PVX	PVY	PYDV
<i>Beta vulgaris</i>													
<i>Capsicum annuum</i>													
<i>Chenopodium amaranticolor</i>	L						LS†	L?S	LS		L		
<i>Chenopodium murale</i>				L			L	S			L		
<i>Chenopodium quinoa</i>	L	St†		S			LS†	L?S	LS		L		
<i>Cucumis sativus</i>								SR					
<i>Datura metel</i>				S	LS			S			S	S	
<i>Datura stramonium</i>	G							S		LS	S		
<i>Datura tatula</i>													
<i>Gomphrena globosa</i>					L?		L				L?		
<i>Lycopersicon esculentum</i>				S				S		S	S	S	
<i>Nicotiana benthamiana</i>		S		S				S		S	LS	S	
<i>Nicotiana bigelovii</i>				S	S					S	S	S	LS
<i>Nicotiana clevelandii</i>				S	L			S?		LS	S	S	
<i>Nicotiana debneyi</i>	S			S			S?			S	LS	S	LS
<i>Nicotiana glutinosa</i>				S						S	LS	S	LS
<i>Nicotiana hesperis-76A</i>													
<i>Nicotiana miersii</i>													
<i>Nicotiana occidentalis-P1</i>							LS				LS	L?S	
<i>Nicotiana rustica</i>													LS
<i>Nicotiana tabacum</i> "Samsun"				S					SR		LS	S	
<i>Nicotiana tabacum</i> "White Burley"				S					SR	S	LS	S	
<i>Nicotiana tabacum</i> "Xanthi-nc"													
<i>Petunia hybrida</i>													
<i>Phaseolus vulgaris</i>					L			LSR					
<i>Phaseolus pubescens</i> L.													
(= <i>P. floridana</i> Rydb.)	G			S							LS	LS	
Potato clone A6			L	L			L				L	L†	
<i>Solanum demissum</i> "A"				L						S			
<i>Solanum demissum</i> "Y"										LS		L	S
<i>Solanum tuberosum</i>	G												
<i>Vigna unguiculata</i>													
<i>Vigna unguiculata</i> subsp. <i>cylindrica</i>													

(cont.)

In developing this table only symptoms with diagnostic characteristics were considered. Susceptibility without symptoms or non-susceptibility of a host to virus infection were not considered.

- L Local symptoms
- S Systemic symptoms
- R Symptoms may be followed by recovery so that no symptoms are seen.
- ? Infection or symptoms variable, depending on, e.g. virus isolate
- G Virus not transmitted or difficult to transmit by mechanical inoculation. Use grafting or dodder as appropriate (refer to individual pathogen descriptions)
- L,S,G** Most recommended indicators. These are referred to in the relevant pathogen description.

† Symptoms best in winter in Northern Europe

‡ Only PVS Andean isolates systemic

§ Also reported as potato chlorotic stunt virus

¶ *S. demissum* PI 230579 has been reported to be a better local lesion host than “A6”

†† Inoculate well-grown plants, systemic spots after 40 days.

Pathogen	Adgen	Agdia	Bioreba	BM	CIP	DSMZ	IPO-DLOSAN-OFIMisc.	¹
<i>Potab virus S</i>	M	P	P	P	P	P	P	P
<i>Potab virus T</i>	M	P			p ^{3,9}	p ^{14,T2,16}		
<i>Potab virus U</i>								
<i>Potab virus V</i>	M,P	P	M,P		p ⁹	P		
<i>Potab virus X</i>	M ⁴ ,P	P	P	P	P	P	P	P
<i>Potab virus Y</i>	M ¹⁵	M,P	M ¹⁵ ,P	P	P	P	P	M,P
<i>Potab yellow dwarf virus</i>						P		
<i>Potab yellow mosaic virus</i>								
Potato yellow vein virus								
Potato yellowing virus					p ⁹			
Solanum apical leaf curling virus					p ⁹			
<i>Sowbane mosaic virus</i>					p ⁹			
<i>Tobacco mosaic virus</i>	P	P	P		p ⁹	P	P	P
<i>Tobacco necrosis virus</i>						P	P	
<i>Tobacco rattle virus</i>		P				P, T ¹⁶		
<i>Tobacco streak virus</i>	P	M, P			p ⁹	P		
<i>Tomab black ring virus</i>		P				P	P	
<i>Tomab mosaic virus</i>	P	P				P	P	P
<i>Tomab spotted wilt virus</i>	M,P	M ⁵ ,P	M,P		p ^{3,9}	M ⁵ ,P,T ¹⁶		P p ⁵
<i>Wild potab mosaic virus</i>						P		
GROUP TEST								
<i>Begomovirus</i>						T ¹⁶		
<i>Potyvirus</i>		M				M, T ¹⁶		
<i>Tospovirus</i>						M ⁵ ,T ¹⁶		
Uncharacterized virus and virus-like diseases								
1	Potato deforming mosaic (Argentina)							
2	SaqO							

Pathogen	Adgen	Agdia	Bioreba	BM	CIP	DSMZ	IPO-DLOSAN-OFIMisc.	¹
Phytoplasmas								
Potato marginal flavescence								
Potato phyllody								
Potato purple toproll								
Potato purple-top wilt								
Potato stolbur ¹⁷	P	P						M ⁹
Potato witches' broom								
General phytoplasma test			T					
Bacteria								
Bacterial slow wilt (<i>Erwinia chrysanthemi</i>)	P	M					p12	p10
Bacterial wilt or Brown rot (<i>Ralstonia solanacearum</i>)	P ^{6,12}	M ⁷			p ^{3,9}		p12	p10
Blackleg or soft rot (<i>Erwinia carotovora</i> subsp. <i>atroseptica</i>)	M ¹⁰	P,M					p12	P ¹⁰ , PCR
Ring rot (<i>Clavibacter</i> <i>michiganensis</i> subsp. <i>sepedonicus</i>)	P	P,M ⁸					p10	p10

All antibodies are for use in ELISA unless otherwise specified.

¹ I. Butzonitch for potato rough dwarf antisera; J. Daniels for potato virus P antisera and D. Peters for tomato spotted wilt virus and other tospovirus antisera (see list of contributors for contact details)

² Available soon

³ For use in nitro-cellulose membrane or dot blot ELISA only

⁴ Two monoclonal antibodies available: all strains or PVX^{HB} specific

⁵ Antibodies available to all four serogroups of tospoviruses

⁶ All biovars

⁷ Only for race I

⁸ Two monoclonal antibodies available, one for use in ELISA the other for immunofluorescence microscopy

⁹ Only limited quantities of antibodies available

¹⁰ For use in immunofluorescence microscopy

¹¹ May be advertised under the name Red La Soda Virus

¹² For use in ELISA and immunofluorescence microscopy

¹³Two antibodies available, one for each serotype

¹⁴Only for use in electron microscopy

¹⁵Including PVY^N specific

¹⁶PCR-based test

¹⁷Antibodies prepared to tomato stolbur (not tested whether they will detect potato stolbur)

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APPENDIX VI.

VIRUS GENERA AND VIRUS-SPECIFIC PCR DETECTION METHODS

Genus	Specificity †	Reference ‡
Alfamovirus	Group	–
	Alfalfa mosaic virus	Bariana et al. 1994
	Potato yellowing virus	–
Begomovirus	Group	Deng et al 1994; Rojas et al. 1993; Briddon and Markham 1995; Wyatt and Brown 1996
	Potato deforming mosaic virus (Brazil)	–
	Potato yellow mosaic virus	–
	Solanum apical leaf curling virus	–
Carlavirus	Group	Badge et al. 1996
	Potato latent virus	G. Foster, 1996, pers. comm.
	Potato rough dwarf virus	–
	Potato virus M	Badge et al. 1996
	Potato virus P Potato virus S	Badge et al. 1996
Comovirus	Group	Brioso et al 1996
	Andean potato mottle virus	–
Crinivirus	Group	Karasev et al 1994; Tian et al 1996
	Potato yellow vein virus	–
Cucumovirus	Group	–
	Cucumber mosaic virus	Rizos et al 1992; Nolasco et al 1993; Wylie et al 1993; Blas et al 1994; Hu et al 1995; Ilardi et al 1995; Singh et al 1995b
Curtovirus	Group	–
	Beet curly top virus	–

Genus	Specificity †	Reference ‡
Iarvirus	Group	–
	Tobacco streak virus	–
Nepovirus	Group	–
	Arracacha virus B-oca strain	–
	Potato black ringspot virus	–
	Potato virus U	–
	Tomato black ring virus	–
Polerovirus	Group	Robertson et al. 1991
	Potato leafroll virus	Hadidi et al 1993; Nolasco et al 1993; Spiegel and Martin 1993; Singh et al 1995a; Schoenet al 1996; Singhet al 1996
Pomovirus	Group	–
	Potato mop-top virus	Arif et al. 1994
Potexvirus	Group	Lim et al 1993
	Potato aucuba mosaic virus	–
	Potato virus X	–
Potyvirus	Group	Langeveld et al 1991; Colinet and Kummert 1993; Pappu et al 1993; Colinet et al 1994; Bateson and Dale 1995; Colinet et al 1995; Gibbs and Mackenzie 1997
	Potato virus A	–
	Potato virus V	–
	Potato virus Y	Barker et al. 1993; Singh and Singh 1996; Singh et al. 1996
	Wild potato mosaic virus	–
Necrovirus	Group	–
	Tobacco necrosis virus	–
Nucleorhabdovirus	Group	–
	Eggplant mottled dwarf virus	–
	Potato yellow dwarf virus	–

Genus	Specificity †	Reference ‡
Sobemovirus	Group	–
	Sowbane mosaic virus	–
Tobamovirus	Group	–
	Tobacco mosaic virus	Drygin et al 1992
	Tomato mosaic virus	Castello et al 1995
Tobravirus	Group	
	Tobacco rattle virus	Robinson 1992; Weidemann 1995; Crosslin et al. 1995; MacFarlane 1996
Tospovirus	Group	Dewey et al 1996; Mumford et al 1996
	Tomato spotted wilt virus	Nolasco et al 1993; Mumford et al 1994
Trichovirus	Group	Kummert et al 1995
	Potato virus T	–
Tymovirus	Group	–
	Andean potato latent virus	–

† Some group detection methods may detect other genera in the same family.

‡ Only those authors in bold have worked with potato virus isolates.

APPENDIX VI.

SUMMARY OF VIROID AND VIRUS PROPERTIES

Key to columns of virus properties:

- 1 Particle morphology (Bac = bacilliform; Iso = isometric; Seg = segmented (i.e. geminiviruses that occur as bi- or tri-geminate particles in addition to the single particles, bigeminate are usually the predominant and most stable form); Fil = filamentous; Rod = rod or tubular shaped; Quasi-iso = quasi-isometric; Env = enveloped particles.
- 2 Number of distinct particle sizes.
- 3 Particle diameter in nm.
- 4 Particle length for rods, filaments, segmented (Geminiviruses) and bacilliform particles in nm. N/A = not applicable.
- 5 Vector type.
- 6 Persistence of virus in vector.
- 7 Transmission through pollen, true potato seed (TPS), transmission from plant-to-plant (contact), self-elimination from tubers by poor transmission.
- 8 Geographical distribution (may not reflect distribution in potatoes)

NB Entries in *italics* (Columns 1-8) are based on provisional or controversial data or, if data have not been gathered, by comparison with other similar viruses.

Pathogen	Acronym	Genus	Properties							
			1	2	3	4	5	6	7	8
Viroid										
<i>Potato spindle tuber viroid</i>	PSTVd	None	Circular ssRNA only	4-5	18	19-58	Aphid-borne when plants co-infected with PLRV	Non-persistent	Contact. Pollen. TPS	Worldwide
Virus										
<i>Alfalfa mosaic virus</i>	AMV	<i>Alfavirus</i>	Bac	4-5	18	19-58	Aphid	Non-persistent	Self-eliminating. Pollen. TPS	Worldwide
Andean potato latent virus, a strain of <i>Eggplant mosaic virus</i>	APLV	<i>Tymovirus</i>	Iso	1	28-30	N/A	Beetle	Non-persistent	Contact. Pollen. TPS	Central and S. America
<i>Andean potato mottle virus</i>	APMoV	<i>Comovirus</i>	Iso	1	28	N/A	Beetle	Non-persistent	Contact	S. America
Arracacha virus B-oca strain	AVB-O	tentative <i>Nepovirus</i>	Iso	1	26	N/A	Unknown		Pollen. TPS	S. America
<i>Beet curly top virus</i>	BCTV	<i>Curtovirus</i>	Seg	2	18-20	32-35	Leafhopper	Persistent		American continent, Eastern Mediterranean, Middle East Worldwide
<i>Cucumber mosaic virus</i>	CMV	<i>Cucumovirus</i>	Iso	1	30	N/A	Aphid	Non-persistent		Mediterranean, Middle East Worldwide
<i>Eggplant mottled dwarf virus</i>	EMDV	<i>Nucleorhabdovirus</i>	Bac	1	76	190	Aphid			Mediterranean, Middle East Worldwide
<i>Potato aucuba mosaic virus</i>	PAMV	<i>Potexvirus</i>	Fil	1	11	580	Non-persistent transmission by aphids in plants co-infected with PVY or PVA. Contact			Mediterranean, Middle East Worldwide

(cont.)

Pathogen	Acronym	Genus	Properties							
			1	2	3	4	5	6	7	8
<i>Potato black ringspot virus</i>	PBRV	Nepovirus possible	Iso	1	26	N/A	Nematode	Persistent	Pollen, TPS	S. America S. America
Potato deforming mosaic virus (Brazil)	PDMV	Begomovirus	Seg	2	18	30	Unknown			
Potato latent virus	PoLV	possible Carlavirus	Fil	2	12	525 and 625	Aphid	Non- persistent	Contact	N. America
<i>Potato leafroll virus</i>	PLRV	Polerovirus	Iso	1	24	N/A	Aphid	Persistent		Worldwide
<i>Potato mop-top virus</i>	PMTV	Pomovirus	Rod	2	18-20	100-150 250-300	Fungus	Persistent	Self- eliminating	Cooler climates in N. and S. hemi- spheres
Potato rough dwarf virus	PRDV	possible Carlavirus	Fil	1	12	638	Aphid	Non- persistent	Contact	S. America
<i>Potato virus A</i>	PVA	Potyvirus	Fil	1	11	730	Aphid	Non- persistent		Worldwide, but not Andes
<i>Potato virus M</i>	PVM	Carlavirus	Fil	1	12	650	Aphid	Non- persistent		Worldwide, but not Andes
Potato virus P	PVP	possible Carlavirus	Fil	1	12	640	Aphid	Non- persistent	Contact	Worldwide, but not Andes
<i>Potato virus S</i>	PVS	Carlavirus	Fil	1	12	660	Aphid	Non- persistent	Contact	S. America
<i>Potato virus T</i>	PVT	Trichovirus	Fil	1	12	640	None		Contact	Worldwide
<i>Potato virus U</i>	PVU	Nepovirus	Iso	1	28	N/A	Nematode	Persistent	Contact, TPS	S. America
<i>Potato virus V</i>	PVV	Potyvirus	Fil	1	12-13	700-720	Aphid	Non- persistent		S. America Europe, S. America
<i>Potato virus X</i>	PVX	Potexvirus	Fil	1	13	515	None		Contact	Worldwide
<i>Potato virus Y</i>	PVY	Potyvirus	Fil	1	11	740	Aphid	Non- persistent		Worldwide
<i>Potato yellow dwarf virus</i>	PYDV	Nucleorhab- dovirus	Bac	1	75	380	Leafhopper	Persistent	Self- eliminating	N. America
<i>Potato yellow mosaic virus</i>	PYMV	Begomovirus	Seg	2	18-20	30	Whitlily	Persistent		S. America

(cont.)

Pathogen	Acronym	Genus	Properties								
			1	2	3	4	5	6	7	8	
Potato yellow vein virus	PYV	possible <i>Citrivirus</i>	Fil				Whitely	Persistent			S. America
Potato yellowing virus	PYV	possible <i>Alfavirus</i>	Bac	4-5	18	21-60	Aphid	Semi-persistent			Pollen, TPS S. America
Solanum apical leaf curling virus	SALCV	tentative <i>Begomovirus</i>	Seg	3	18	50	Unknown				S. America
<i>Sowbane mosaic virus</i>	SoMV	<i>Sobemovirus</i>	Iso	1	26-28		<i>Insect</i>				Contact Widespread Pollen, TPS
<i>Tobacco mosaic virus</i>	TMV	<i>Tobamovirus</i>	Rod	1	18	300	None				Contact. Worldwide Pollen, TPS
<i>Tobacco necrosis virus</i>	TNV	<i>Necrovirus</i>	Iso	1	26	N/A	Fungus	Non-persistent			Worldwide
<i>Tobacco rattle virus</i>	TRV	<i>Tobravirus</i>	Rod	2	22	50-115, 190	Nematode	Persistent			TPS Mainly N. hemisphere
<i>Tobacco streak virus</i>	TSV	<i>Ilarivirus</i>	Quasiiso	1	22-35	N/A	N/A	<i>Thrip</i>			Persistent Self-eliminating Worldwide
<i>Tomato black ring virus</i>	TBRV	<i>Nepovirus</i>	Iso	1	28	N/A	Nematode	Persistent			Widespread Pollen, TPS
<i>Tomato mosaic virus</i>	ToMV	<i>Tobamovirus</i>	Rod	1	18	300	None				Contact. Worldwide Pollen, TPS
<i>Tomato spotted wilt virus</i>	TSWV	<i>Tospovirus</i>	Env	1	70-110	N/A	Thrip	Persistent			Widespread S. America
<i>Wild potato mosaic virus</i>	WPMV	<i>Potyvirus</i>	Fil	1	11	685-800	Aphid	Non-persistent			
Uncharacterized virus and virus-like diseases											
Potato deforming mosaic (Argentina)		unknown					Unknown				Argentina
Saq'O		Polerovirus + phytoplasma					<i>Aphid</i> and <i>leafhopper</i>				S. America