Detection of *Potato Mop-Top Virus* in potato tissues and soil

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Introduction

Potato mop-top virus (PMTV) is soil-borne and has tubular rod-shaped particles. It is found in potato growing areas in Europe, Canada, South America and Asia that have a cool wet climate. It was recently identified in the USA (2002) and an extensive survey revealed that it was widespread in potato producing states.

The symptoms induced by PMTV vary greatly with potato cultivar. Typical tuber symptoms of brown lines, arcs or rings (spraing) in tuber flesh and raised external lines appear in the year of infection when the virus is transmitted to potato by the soil-borne 'fungus' *Spongospora subterranea* f. sp. *subterranea,* the causal agent of powdery scab disease. Plants grown from infected tubers show yellow markings and chevrons in the leaves some cultivars display shortening of internodes (mop-top). Substantial yield losses (ca. 20 percent) have been reported in sensitive cultivars due to decreased tuber production and quality. Symptoms of PMTV can be confused with those induced by *Tobacco rattle virus* (a nematode transmitted virus) and reliable diagnosis is needed since control measures differ.

Virus-carrying Spongospora resting spores can remain viable in soil for many years and potatoes were infected with PMTV when planted in a field 18 years after potatoes were last grown. There is no effective, environmentally safe chemical control of *S. subterranea* and the best method to prevent spread of PMTV is to plant virus-free tubers on PMTV-free land.

Internal and external lines induced by PMTV in potato tubers

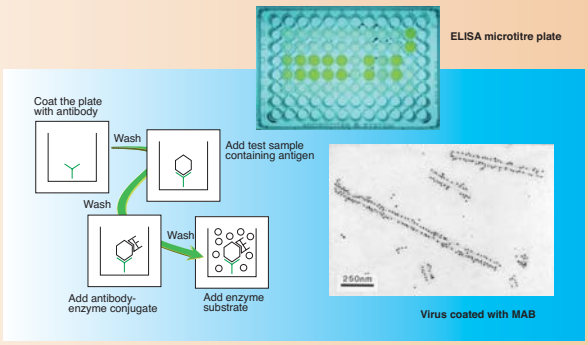
Symptoms of TRV in potato tubers cv. Duke of York

Yellow markings of PMTV on potato cv. Desiree grown from infected tubers.

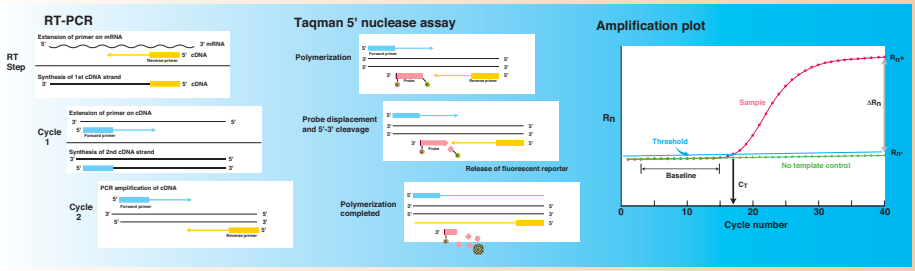
Modern methods to detect and identify PMTV in potato tissues

Several sensitive methods to detect and identify PMTV have been devised at SCRI.

The ELISA method is based on antibodies that are specific for PMTV coat protein. The advantage of this method is that sample preparation procedures are simple and fast enabling many thousands of tests to be done in a short time. ELISA is a robust and sensitive technique and relatively inexpensive. The ELISA devised by SCRI was used by APHIS (USDA Animal and Plant Health Inspection Service) in their 2002 survey of USA seed stocks.

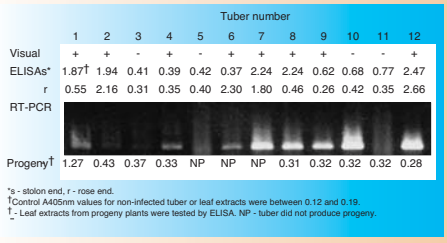


A nucleic acid based test called reverse transcription-polymerase chain reaction (RT-PCR) can also be done. This method utilises pairs of short oligonucleotide primers specific for regions of PMTV nucleic acid sequence to amplify the region of interest. The reaction product is analysed in an agarose gel. An alternative assay incorporating fluorescent probes in a PCR based assay can give a graphical output (amplification plot) as the assay progresses (known as a 'real time' PCR or Taqman assay). These nucleic acid based methods are extremely sensitive but sample preparation is more cumbersome limiting the number of samples that can be tested in a short time.



Comparison of ELISA and RT-PCR

We have compared ELISA and RT-PCR in tests on potato tubers and leaves. Both tests readily detected PMTV. However, we found that the distribution of PMTV in potato leaf and tuber samples was erratic with samples assayed positive and negative from the same tuber. We have also found that there are large numbers of symptomless tubers in infected stocks, therefore tests based solely on presence or absence of symptoms will underestimate infection. We found that storage of tubers at an elevated temperature (20˚C) for 4 weeks prior to testing increased the number of samples scored positive. Such pre-treatment may improve the accuracy of post harvest tuber tests.



Comparison of the numbers of tubers with virus symptoms with the numbers that were assessed positive in ELISA

Potato Cultivar	Symptoms present	ELISA Positive
Arran Pilot	35	54
Pentland Crown	3	36
Saturna	3	29
Brodick	6	10
Pentland Marble	4	5

Detection of PMTV in soil samples

It is possible to test soil samples to detect virus-carrying *S. subterranea* by transplanting seedlings of *Nicotiana debneyi* into pots containing air-dried soil sample mixed with potting compost. The 'indicator plants' show symptoms of necrotic rings and lines in the leaves in 4-6 weeks and the duration could be decreased by testing the roots of the bait plants by RT-PCR after approx. 2-3 weeks. If reliable methods for germination of *S subterranea* resting spores were found, the germinated zoospores could be tested directly. Using ELISA we have detected PMTV within zoospores of virus-carrying *S subterranea*. In future, it should be possible to devise tests to extract *S. subterranea* resting spores from soil, extract nucleic acids and test by RT-PCR or Taqman.