Canada/US-Management Plan for Potato Viruses that Cause Tuber Necrosis

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1. Introduction and Goals

The Canadian Food Inspection Agency (CFIA) and the U. S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) announced, on December 23, 2002, that a joint potato virus management plan will be developed to maintain high quality seed potato production.

This plan proposes immediate measures to manage the risks associated with several pests that occur in both countries, including Potato mop top virus (PMTV), Potato virus Y (PVY) complex and Tobacco rattle virus (TRV), through seed potato certification measures. Through surveys and research, data will be collected and evaluated to better understand the biology of these viruses and their vectors, thus enabling the establishment of further control measures.

CFIA and APHIS are working cooperatively to protect each country's agricultural and environmental resources and agree to take actions that are least disruptive to trade. CFIA and APHIS also agree to work collaboratively with respective seed potato certification agencies and potato organizations to harmonize and make transparent, seed certification procedures to control viruses causing internal tuber necrosis. Successful management of viruses that cause internal tuber defects and their respective vectors will contribute to maintaining high seed potato phytosanitary quality and minimize impacts on commercial potato production.

This plan replaces the previous PVY^{N} management plan dated March 2, 1994 and amended October 5, 2001. The objective of this plan remains to facilitate trade of potatoes within and between Canada and the USA, while minimizing the impact on the tobacco industry caused by the tobacco-necrotic isolates of PVY infecting the potato crop. Additionally this plan has the objective to manage the incidence of PMTV and TRV and minimize the impact of these viruses on US and Canadian potato industries.

2. Definitions and Descriptions

Crop:
A variety and class of seed potatoes, growing in an aseptic environment, a protected environment or in one or more fields of a farm unit.*

Farm Unit:
a. A single tract of land, operated for the production and marketing of seed potatoes under the control of one grower, or
b. A number of separate tracts of land, operated as a single unit, with the use of common equipment, facilities or storage, for the production and marketing of seed potatoes under the control of one grower.

Field:

Identifiable area of land on which seed potatoes of a particular variety and class are planted or have been produced.

Flush-Through Production System:
System of seed potato production in which material is allowed to remain in the system for a limited number of generations.

Inspection:
Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations.* This includes visual examination as part of a federally recognized quality assurance program.

Lot:
A population of certified seed potatoes of one variety and class that is identified by one certificate number.

Nuclear material:
Plant material produced in an aseptic or protected environment.

Positive Lot, Crop or Field:
A lot, crop or field, where the presence of a pathogen has been confirmed, based on recognized tests.

PVY Complex:
Potato virus Y (PVY) is the type-species of the *Potyvirus* genus. It is a positive strand monopartite RNA virus that infects primarily solanaceous plants including potato, tomato, tobacco, and pepper. PVY occurs as a complex of virus strains that can be distinguished by reactions on tobacco and potato cultivars. Recognized strains covered by this plan include, but are not limited to:

Strain Group	Symptoms	Other Distinguishing Characteristics
PVY^{O}	Mosaic, leaf and stem-necrosis, leaf drop or veinal necrosis in potato	Mosaic in tobacco, mild to severe mosaic in potato; hyper sensitive response in potato with Ny and Nc genes
PVY^{N}	Mosaic or mild mosaic in potato, veinal necrosis in tobacco	Serologically distinguishable from $PVY^{O/C}$; coat protein sequence phylogenetically distinct from $PVY^{O/C}$
PVY^{NTN} note: may include several distinct isolates/groups	Mosaic or veinal necrosis in tobacco, necrotic arcs and ring spots in tubers of some potato cultivars	Serologically distinguishable from $PVY^{O/C}$; coat protein sequence phylogenetically distinct from $PVY^{O/C}$
$PVY^{N:O}$	Symptoms may vary.	Some strains are not serologically or phylogenetically distinct from $PVY^{O/C}$

Quality Assurance System: A planned and regularly monitored framework of controls, based on documented procedures, which is applied to critical operational activities in the production of potatoes or the performance of a service (e.g. diagnostics) or activity (e.g. conducting a test) to ensure operational efficiency and reliable compliance with specified quality standards. (NAPPO RSPM 3, 2002)

Shipping Point Inspection:
Inspection of seed potatoes shortly before or at time of shipment to determine the product complies with seed potato grade standards recognized by the US and Canada seed potato regulatory agencies.

Test:
Official examination, other than visual, to determine if pests are present or to identify pests*.

Tuber Necrosis:
Necrotic dark brown arcs, rings and/or flecks in the tuber tissue often associated with necrotic rings on the tuber surface. Tuber necrosis is also referred to as spraing depending on the virus.

* FAO Glossary of Phytosanitary Terms, 2002

3. Virus Management Methods

3.1 Potato Virus Y (PVY) Complex

3.1.1 Nuclear Material

In-vitro material

- All plants in a tissue culture bank must be tested and found free of PVY.
- Any material introduced into tissue culture must be tested and found free of PVY.
- Any tissue culture material testing positive for PVY cannot be used to produce certified seed potatoes, unless the virus is eliminated.

Protected Environment (greenhouse / screenhouse/growth chamber)

Certification rules concerning production in protected environment must include the following

- Seed potato production in a protected environment will be tested at least annually for PVY at a target rate of 1% of the plants, with a minimum of 5 plants per crop/lot. It is recommended that testing takes place every crop cycle.
- Any crop/lot grown in a protected environment that tests positive for PVY must be destroyed, unless it is found to be PVY^{O} alone, in which case it can be downgraded.
- If aphid vectors are detected in the protected environment, when the crop is growing, testing must be conducted to determine freedom from PVY.

3.1.2 Field Grown Seed

- Inspection for PVY complex symptoms will be included in seed certification agencies' inspection procedures.
- A sample of suspect plants observed during field inspection will be tested to determine if the symptoms are caused by PVY. ~~:~~ If PVY presence is confirmed the tolerances for mosaic must be enforced.

3.1.3 Field Generation 2

All Field Generation 2 (FG2) material will be sampled and tested for the presence of PVY and PVY^{N}. A total of 400 leaves will be collected and tested from up to 10 seed lots/crops per farm unit. The collection of the 400 leaves will be proportional and random across the crops or lots to be tested.

- Tolerances of the state and federal certification programs apply.
- If a farm unit has less than 10 FG2 crops/lots the sample size for the farm will still be 400 leaves.

- If the farm unit has more than 10 FG2 crops/lots an additional sample of 400 leaves will be submitted for testing from every group of 10 lots or less.
- If crops/lots are found positive for PVY^N, all FG2 crops/lots on that farm, and lots/crops that are progeny of the same seed lot as the positive crops/lots, must be sampled and tested at a rate of 400 leaves per lot.
- Regulatory action will only be taken on FG2 crops/lots. PVY^N positive crops/lots of FG2 can only be used for the production of commercial crops, not for recertification.

The testing of FG2 crops/lots may be done either during the growing season or post harvest. If done post-harvest, leaves from grown-out plants or sprouts from tubers must be used.

- Leaf testing will be done by ELISA using an appropriate protocol. An ELISA positive for PVY^N will be considered a suspect positive. Confirmatory tests must be conducted with PCR and/or bioassays.

3.2 Potato Mop Top Virus (PMTV)

3.2.1 Nuclear Material

- All plants in a tissue culture bank must be tested and found free of PMTV.
- Any material introduced into tissue culture must be tested and found free of PMTV.
- Any tissue culture material testing positive for PMTV cannot be used to produce certified seed potatoes, unless the virus is eliminated.

3.2.2 Field Grown Seed

- Inspection for PMTV related symptoms will be included in seed certification agencies' inspection procedures. Training material on PMTV symptoms in plants will be provided to all field inspectors.
- Plants symptomatic for PMTV must be eliminated before final inspection.
- PMTV will also be regulated through the inspection of seed shipments.

3.3 Tobacco Rattle Virus (TRV)

3.3.1 Field Grown Seed

- Inspection for TRV related symptoms will be included in seed certification agencies' inspection procedures. Training material on TRV symptoms in plants will be provided to all field inspectors.
- Plants symptomatic for TRV must be eliminated before final inspection.

- TRV will also be regulated through the inspection of seed shipments.

3.4 Alfalfa Mosaic Virus (AMV)

- Tubers exhibiting necrosis caused by AMV will be scored as an internal defect and be allowed for recertification providing the tolerance of 2.0% for internal necrosis is not exceeded.

3.5 Shipping Point Inspection

3.5.1 Seed for Commercial Production

- All seed shipments moving from a production State or Canada for commercial production must be inspected by federally recognized inspectors or a federally authorized cooperator from a facility under a quality assurance system and must meet the tolerances for internal necrosis of Table 1 below. If a lot exceeds this tolerance, the grower will have the option to re-grade the lot or remove it from the seed certification system.

3.5.2 Seed for Recertification

- All seed shipments moving from a production State or Canada for recertification must be inspected by a federal, state or provincial inspector for tuber necrosis. If a lot exceeds the tolerance of 0.5% necrosis caused by PVY complex, PMTV or TRV (see Table 1 below), it is not eligible for recertification but may be shipped for commercial purposes.

Table 1: Eligibility of Seed potatoes related to the Percentage of Internal Tuber Necrosis, apparently caused by PVY complex, PMTV or TRV

Percentage Internal Tuber Necrosis Scored*	**Seed intended for Recertification**	**Seed intended for Commercial Production**
Less than or equal to 0.5%	Eligible	Eligible
Between 0.5 and 2.0%	Eligible if negative lab test for $PVY^{N/NTN}$, PMTV, TRV	Eligible
More than 2.0%	Not eligible	Not eligible

Notes:
* Internal tuber necrosis will be calculated as percent incidence {(number of tubers with internal necrosis/number of tubers sampled) x100}.

4. Scoping Survey for PVY Complex

A multi year scoping survey, starting with the 2004 crop will be conducted, coordinated by APHIS and CFIA, in each state and province, where seed potatoes are certified. The seed lots of the oldest classes in production will be surveyed to determine the incidence and makeup of any PVY complex at that point in the seed potato production system. The data will be kept confidential and be reviewed annually on a country-wide basis. The data will be used confidentially to help determine if the FG2 management protocol is effectively managing virus levels of PVY complex. No regulatory action will be taken on lots found to be positive in this survey. PVY certification requirements will not be changed without proper notification. See Appendix 1 for details of the survey and testing protocols.

5. Research Needs

Both parties acknowledge the need for and value of multiple virus testing protocols**.** Such protocols should be encouraged as a research goal and new protocols incorporated into the plan when available.

PVY complex

- Research on the susceptibility of US and Canadian potato varieties to $PVY^{N/NTN.}$
- Research on recombinants between PVY^{O} and PVY^{N} to develop appropriate tests for these and any other PVY^{N} isolates.
- Research on the population dynamics and prevalence of recombinant strains to determine if changes in the population of strains are occurring.
- Research on environmental factors that influence symptom expression to develop a better understanding of PVY strain symptoms.

PMTV

- Research on susceptibility to PMTV to determine if the pathogen causes an economic impact on US and Canadian potato varieties. If any varieties are shown to be extremely sensitive to PMTV, a specific management plan for those varieties should be developed.
- Research on the control of powdery scab since control of the vector will lead to control of PMTV.
- Research on improved testing methodologies for PMTV to improve the detection of the pathogen.

TRV

- Research on improved testing methodologies for TRV to improve the detection of the pathogen.
- Research on the control of the TRV vector since control of the vector will lead to control of TRV.
- Research on the efficiency of the nematode vector to acquire the virus from infected potato plants to determine a better understanding of the dynamics of the vector/virus interaction.

6. Training needs

- Inspectors involved in shipping point and field inspection of seed potatoes will be trained annually by state, federal, provincial and/or university officials on symptoms of $PVY^{N/NTN}$, PMTV and TRV in tubers and plants to assist them in their inspection efforts.
- APHIS and CFIA will develop a manual to differentiate spraing symptoms from other causes of internal necrosis.
- An appendix of pest management practices for controlling viruses addressed in this plan and their vectors will be added upon completion.

7. Implementation

- It is essential that APHIS and CFIA participate in the implementation of this plan, and oversee the application of all of its elements. Seed potatoes produced and certified by a federal or state certification agency must comply fully with the content of the management plan.
- It is understood that some elements of the management plan will require amendments to regulations, rules, policies and inspection manuals. Therefore, every certification agency, including CFIA and APHIS, must make the necessary amendments to achieve full implementation of the plan within 12 months from the date of signature.

8. Revision

- This plan will be reviewed after three years by APHIS and CFIA or as needed at the request of either party. Results of all reviews and any recommended modifications to this plan will be shared with federal, state and provincial regulatory officials, the potato industry and the tobacco industry for comment.

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9. Endorsement

The Canadian Food Inspection Agency and the United States Department of Agriculture Animal and Plant Health Inspection Service agree to full implementation of this management plan with an ultimate goal of using existing seed certification programs to control viruses that cause tuber necrosis.

CFIA	USDA APHIS
Date:	Date:

10. References

NAPPO PVY^N Testing Protocols
Superficial Necrosis, caused by Virus (PTNRD), UN/ECE paper, TRADE/WP.7GE.6/2004/12,

Appendices

- Scoping Survey for PVY Complex

Appendix 1

Scoping Survey for PVY Complex

1. **Purpose:** To conduct a scoping survey to assess the presence of different strains of PVY and their incidence in seed potatoes in Canada and the United States. The specific objectives of the survey are:
   a. to estimate the relative incidence of each strain or novel isolate of PVY (PVY^{O}, PVY^{N}, $PVY^{N:O}$, $PVY^{EU\text{-}NTN}$, $PVY^{NA\text{-}NTN}$, and others),
   b. to assess the correlation between strain type and symptom expression in potato

2. **Principles:** To ensure uniformity and comparability of survey results, the survey will be conducted in both Canada and the United States to conform to the following principles:
   a. every seed producing state/province will be sampled,
   b. the survey will include as many commercial potato cultivars as possible,
   c. every seed farm in all seed producing states/provinces will be sampled,
   d. older field generations of seed potatoes on each farm will be sampled,
   e. the sampling rate in each state/province will be between 100 to 400 tubers per lot.,
   f. the number of samples taken in Canada and the US will be proportional to the size of their respective seed potato industries, estimated to require about 80,000 tubers for Canada and 100,000 tubers for the US.
   g. the survey will utilize an ELISA test (see below) for initial detection of PVY in tuber sprouts,
   h. samples will be preserved to allow subsequent strain characterization,
   i. testing will be done on samples representing the 2004 crop and subsequent crops,
   j. samples will be collected randomly within the seed potato lots selected for testing,
   k. results of the survey will be reviewed by September 1, 2005 and subsequent years,
   l. confidentiality of individual grower results will be maintained.

3. **Procedure:** The survey will be conducted in three stages of testing.
   a. PVY-positive tubers or plants will be selected on the basis of an initial ELISA test using monoclonal 4C3. every seed producing state/province will be sampled,
   b. All 4C3-positive tubers/plants will be retested by ELISA using monoclonal 1F5 to determine whether the serotype is PVY^{O} or $PVY^{N,}$
   c. The P1 gene of PVY will be amplified by RT-PCR from all 4C3-positive tubers/plants and sequenced to determine subtype. Selected PVY isolates representing each of the recombinant and strain types will be observed or assayed for symptomology on potato foliage and tubers. The following criteria for the testing procedures have been adopted to ensure uniformity and comparability of results.

4. **Sample preparation** (although the survey can be conducted using either tuber

sprouts or leaves of grow-out plants, testing of tuber sprouts appears to be most feasible and economical).

a. any method (natural or chemically induced) of breaking tuber dormancy is acceptable,
b. sprouts must be taken from the bud end of the tuber,
c. one to three sprouts will be tested from each tuber for each test,
d. sprouts must be at least 0.5 cm but not more than 6.0 cm long,
e. sprouts from up to three tubers may be combined into a composite sample for the initial ELISA test,
f. a fresh sprout extract will be used for the second ELISA test,
g. either a third sprout or sap from the second sprout will be used for RT-PCR amplification of the PVY P1 gene,
h. sprout sap to be used for RT-PCR amplification must be kept on ice and used within 24 hr.

5. **ELISA**.
   a. the initial ELISA test to select PVY infected tubers will utilize monoclonal antibody 4C3,
   b. the second ELISA test to determine PVY serotype will utilize monoclonal antibody 1F5,
   c. the ELISA tests are to be a triple antibody sandwich (TAS) format,
   d. coating antibody for both ELISA tests will be a polyclonal antibody that reacts with known PVY strains,
   e. duplication of test wells is NOT required,
   f. each ELISA plate requires a minimum of one control well containing a PVY^{O} isolate, one control well containing a PVY^{N} isolate, and four negative control wells containing sap from PVY-free sprouts,
   g. all negative control wells must have absorbance values of <0.100 for the results of the ELISA plate to be considered valid (the ELISA plate should be blanked on a well or wells containing buffer only),
   h. the positive/negative threshold will be 0.100 unless the mean of the negative controls is >0.033 in which case the threshold will be 3X the mean of the negative controls,

6. **RT-PCR amplification and sequencing**
   a. sprout sap will be combined with buffer and spotted in quadruplicate on nitrocellulose membranes as specified in the dot blot procedure,
   b. RNA will be extracted from dot blots and cDNA generated by reverse transcription using Primer A (Nie and Singh, 2002),
   c. cDNA will be amplified by PCR using Primers S1 and A (Nie and Singh 2002) for 30-35 cycles,
   d. sequencing of amplicons will be contracted to a sequencing facility to obtain uniform and consistent results,

e. P1 gene sequences will be aligned and analyzed using standard bioinformatics protocols will be determined.

Reference: Nie, X. and Singh, R.P. 2002. A new approach for the simultaneous differentiation of biological and geographical strains of potato virus Y by uniplex and multiplex RT-PCR. J. Virol. Methods 104:41-54.