National Plant Diagnostic Network

Standard Operating Procedure for Plant Diagnostic Laboratories

Potato Wart
Synchytrium endobioticum

DRAFT 1.0

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Background:

Potato wart is caused by *Synchytrium endobioticum* (Schilberszky) Percival. *Synchytrium endobioticum* is thought to be indigenous to Peru, where the potato originated. In the early 1900's *S. endobioticum* was spread throughout Western Europe on breeding material from the Andean region of South American. The wart fungus was discovered in Newfoundland in 1909, where it has been under quarantine since 1912.

In the early 1900's the pathogen was present in defined locations within Maryland, Pennsylvania and West Virginia. It was presumably introduced on seed potatoes imported from Europe. Infestations in Pennsylvania and West Virginia were eradicated in the 1950s and 60s, respectively. Infestations in Maryland were thought to be eradicated but were found in one home garden in Allegany county during a survey in 1974. For five years bio-assays were conducted with susceptible potato cultivars and soil collected from the site where spores had previously been found. No symptoms were caused on the susceptible potatoes and the pathogen was considered to be eradicated from Maryland in 1994 (Putnam and Sindermann, 1994).

The pathogen is still found in soils in Newfoundland, particularly in home gardens. As a result strict quarantines specific to that area of Canada still exist. Much research on soil sampling and detection, the impacts of irrigation and temperature on disease incidence, and disease management has been done in Newfoundland. The Canadian Food Inspection Agency continues to sample from individual farms where potato wart has been confirmed. The disease is also present on isolated farms on Prince Edward Island where strict regulatory restrictions and surveying have been implemented (Canadian Food Inspection Agency).

Elsewhere the disease is found in Asia (Bhutan, India, Nepal), Africa (Algeria, South Africa, Tunisia), Europe (all countries except Portugal), South America (Bolivia, Chile, Falkland Islands, Peru) and New Zealand (EPPO, 2004).

Biology:

Synchytrium endobioticum in an obligate parasite in the order Chytridiales. This pathogen does not produce hyphae; structures include zoospores, sporangia and a resting structure. The pear shaped zoospores (2 – 4 μm) have a tail-like flagellum which is approximately seven times the length of the spore. Zoospores encyst and can subsequently germinate, producing an infection peg used to infect host epidermal cells. After infection host cells surrounding the infected cell enlarge, enclosing the pathogen. The fungus enters a short-lived, fast reproducing stage called the "summer sporangium". The summer sporangium produces many zoospores that are released and infect surrounding cells. This cycle is repeated throughout the growing season while conditions are favorable for infection. The host tissue can be extensively invaded. The swelling of cells surrounding the infected cells produces galls, or growths with a cauliflower-like-appearance.

Zoospores released from the summer sporangium can conjugate, producing a sexual entity that re-infects deeper cells, giving rise to a resting spore. The host cell wall remains closely attached, providing an additional layer on the already thick walled resting spore (EPPO, 2004). Resting spores can persist in soil for greater than 40 years and are found at depths of up to 50 cm.

When resting spores germinate the contents flow into a self contained sac. The resulting sporangium is expelled from the sac. The wall of the sporangium splits and releases 200 to 300 motile zoospores. Zoospores can survive for one to two hours in soil water before they encyst. Zoospores that encyst on susceptible host tissue form a penetration peg through which the contents flow into the host cell, beginning the cycle again.

Potato wart is spread by infected seed tubers, or by movement of infested soil. The thick walls surrounding the resting sporangia resist digestion by animals, therefore animals that have ingested infested plant parts may play a minor role in disease spread (EPPO, 2004). Infected tissues rot in the soil during the winter, releasing the resting spores. The wall of the resting spores is extremely chemo-resistant to common soil agents (CABI, 2003).

The relationship between weather and disease development is well-documented (Hampson and Coombes, 1997). Water is required for germination of both types of sporangia. A cold temperature requirement of approximately 160 days at or below 5°C limits the range of this pathogen. Laboratory studies found that infection does not occur at temperatures above 22°C, optimum temperatures for disease development are 16 –17°C (Nappfast).

There are several pathotypes (races) of *S. endobioticum*. These are differentiated by their virulence on potato cultivars. Pathotypes vary in their worldwide distribution. Pathotypes can be identified by the Spieckermann method, a bio-assay which takes eight weeks.

Symptoms:

Synchytrium endobioticum causes lumpy gall-like growths. Galls form on stem tissue, including the stem base, stolon bud, and tuber eyes. Less commonly galls form on foliage and flower tissues. Galls range in size (1 to 8 cm) and can vary in color. Young below ground galls are white and fleshy, as they age and begin to decay they turn a darker color. Above ground galls are green to brown, turning a darker color and decaying as they age. In severe cases tubers may be completely replaced by galls. Plants are not killed but sprouts can be damaged, limiting emergence from seed tubers.

Galls on potato tuber.
Photo by M.C. Hampson

Internal view of gall.
Photo by M.C. Hampson

Potato wart abnormalities on inoculated stem.
Photo by M.C. Hampson

Aerial stem infection.
Photo by M.C. Hampson

Importance:

Due to the significant losses this disease can cause and the long-term persistence of the pathogen in soil it is an important quarantine pest throughout the world. Naturally, the disease spreads slowly, so regulatory restrictions have been used effectively to limit the spread of this disease.

Exclusion of this pathogen from non-infested areas is the most efficient method of disease control. Resting spores can persist in soil for long periods of time. Chemical soil treatments are not a viable option to eradicate the resting spores from infested soil. OEPP/EPPO suggests that infested fields should not be used for potato production for twenty years (OEPP/EPPO, 2004). Regulatory agencies must deschedule previously infested fields before they can again be used for potato production. Descheduling requires analysis of soil sample for resting spores as well as bioassays with susceptible potato cultivars.

If the pathogen were introduced into the United States predictive models suggest that potato wart survival is possible in the Northeast, Rocky Mountains, Northern Plains and the Lake States (Nappfast). *Synchytrium endobioticum* was put on the U.S. Select Agent and Toxin List in 2002. This subjects *S. endobioticum* to significant additional regulation in the U.S., with the goal of further protecting U.S. potato production.

References:

CABI, 2003. Crop Protection Compendium. CAB International.

EPPO, 2004. Plant Quarantine database. Data Sheets on Quarantine Pests, '*Synchytrium endobioticum*'. Paris, France: European and Mediterranean Plant Protection Organization.

Franc, G.D. 2001. Potato wart In: Compendium of Potato Diseases, 2nd edition. APS Press

Hampson, M.C., Wood, S., and I.A. MacLatchy, 1994. Wart. In: Diseases and Pests of Vegetable Crops in Canada, edited by R.J. Howard, J.A. Garland, and W.L. Seaman. The Canadian Phytopathological Society.

Nappfast Pest Assessment: *Synchytrium endobioticum,* (Potato Wart). http://www.nappfast.org/casestudies_files/potato_wart.pdf

Putnam, M.L. and A.B. Sindermann, 1994. Eradication of potato wart disease from Maryland. American Potato Journal 71:743-747.

OEPP/EPPO, 2004. Diagnostic protocols for regulated pests, Synchytrium endobioticum. Bulletin OEPP/EPPO Bulletin 34, 214-218.

Protocol:

Take the appropriate steps, listed below, when plant material suspected of *S. endobioticum* is submitted to your laboratory.

1-Submitter Shipping:

Sample submission may be directly from a grower questioning the cause of symptomatic plants or from regulatory personnel that have reasons for suspecting a possible infection.

1. Suspected plant material may be collected as leaves, stems, or pods. Leaf samples should be placed between moist paper towels or paper in order to keep them flat. Suspect plant material may be placed in a ziplock bag and stored in cool conditions. Material may also be stored in a paper bag and stored in ambient conditions to prevent molding. When refrigeration is available the paper bag should be stored in a ziplock bag. Be careful not to contaminate the outside of the collection bags.

2. Include with the sample on a piece of paper the collection information (date, exact field location and sample location within the field, county name, host plant, collector's name and phone number). Form PPQ 391 should be completed and submitted with the sample to the state or university diagnostic facility within the state of collection. Samples are not to be directly submitted to the USDA Beltsville laboratory.

2-NPDN Laboratory Receipt and Examination:

Upon arrival, contact submitting entity and acknowledge receipt of sample. The suspect plant material should be examined within a certified biological safety cabinet. Any tools, supplies, and miscellaneous materials used during the examination must be separated and placed in sealed plastic bags awaiting sterilization by a certified autoclave. The surface of all materials must be disinfected prior to the removal from the biological safety cabinet.

3-Sample Storage:

While examination and testing is being conducted, suspect plant material must be stored in access controlled cabinets and/or refrigerators.

Keeping the suspect plant material for extended periods of time is not recommended. Plant material should be destroyed using the methods described in section #7, Sample Destruction. Sample destruction is recommended within 2 weeks of submission to your facility is no confirmation has been reported.

4-Sample Screening:

Diagnostic screening of suspect samples will be carried out by triage laboratories (the State's or cooperating university diagnostic laboratory). This screening includes identification *of S. endobioticum* based on morphological characteristics to genus level. If these facilities cannot perform this screening, samples can be referred to the appropriate NPDN Regional Center (contact information below). If a positive result is obtained through diagnostic screening, confirmatory testing by an approved laboratory to species level may be required.

Great Plains Region:
Joy Pierzynski/Judy O'Mara
Kansas State University
Department of Plant Pathology
4024 Throckmorton Hall
Manhattan, KS 66506
(785) 532-1383

North Central Region:
Jan Byrne
Michigan State University
Diagnostic Services
114 Center for Integrated Plant Systems
East Lansing, MI 48824
(517) 355-3504

Southern Region:
Richard Cullen
Plant Disease Clinic
UF Bldg 78 Mowry Road
PO Box 110830
Gainesville, FL 32611-0830
(352) 392-1795
(352) 392-3631 ext 254 (Carrie Harmon)

Western Region:
Melodie Putnam
Oregon State University
OSU Plant Clinic
1089 Cordley Hall
Corvallis, OR 97331-2903

Northeast Region:
Karen L. Snover-Clift
Cornell University
Plant Disease Diagnostic Clinic
334 Plant Science Building
Ithaca, NY 14853
(607) 255-7850

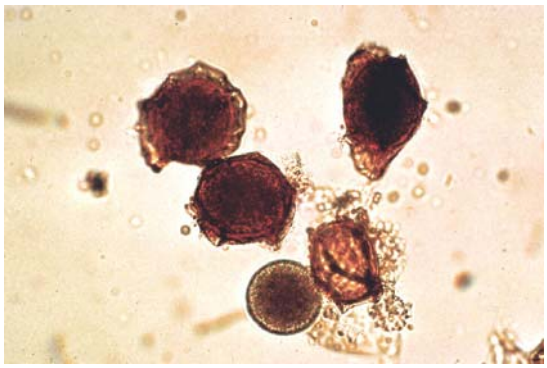
a. Initial Screening by Triage Laboratories

At this time molecular diagnostic methods are not used for this pathogen. Identification is based on microscopic observations of summer sporangia and resting spores. Plant material with warts should be examined for sporangia. Mount slices of affected tissue in water and observe at 100-400x magnification under a light microscope.

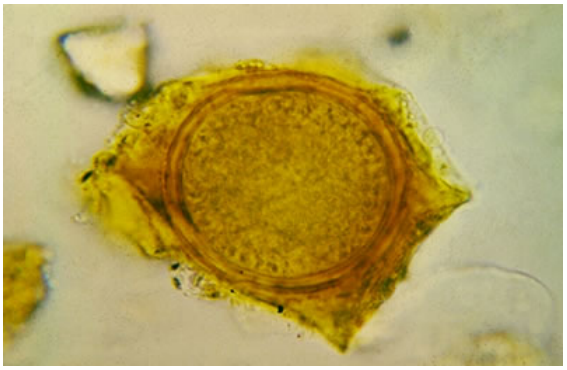
Zoospores
Zoospores are pear shaped (1.5 – 2.2 µm in diameter) with a posterior flagellum. Conjugated zoospores are biflagellate.

Summer sporangia
Summer sporangia (35-80 µm) are thin-walled, transparent, and contain many flagellate zoospores. These structures are present during the growing season.

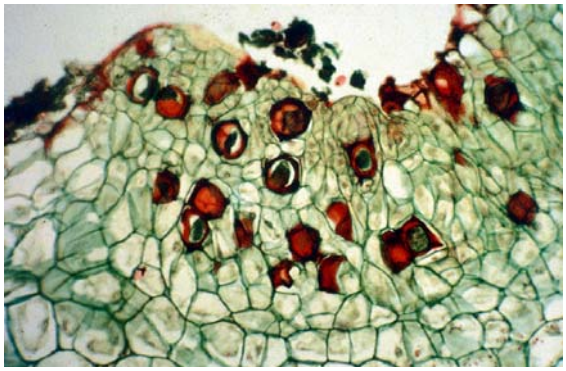
Winter sporangia
Resting spores, or "winter sporangia" are golden brown, aseptate, and have thick walls. The outer wall is furrowed with prominent ridges and contains chitin fibrils. Winter sporangia are spherical to ovoid in shape (35 – 80 µm, mean 50) and fill almost the entire host cell. As the galls decay the host cell wall disintegrates slightly changing the appearance of the outer surface of the sporangia. Eventually, the attached host cells disintegrate, and the sporangia are left with a characteristic angular appearance. These structures can be found during the growing season and in decomposing warts.



Resting sporangia of *S. endobioticum*
Photo by M. C. Hampson



Live resting (winter) sporangium of *S. endobioticum*.
Photo by Central Science Laboratory, York British Columbia



Resting spores of *S. endobioticum* in host tissue.
Photo by M.C. Hampson

Other diseases/disorders that could be confused with Potato Wart

Other *Synchytrium spp.*
Some weeds found in potato fields (ex. *Taraxacum officinale*) may be infected with other species of Synchytrium. Diagnostic features of *S. endiobioticum* to look for include winter sporangia that are golden brown, have a thick wall, and are angular in appearance with ridges (OEPP/EPPO, 2004). Sporangia size should also be measured.

Proliferation of eyes
Physiological causes or varietal factors can cause otherwise healthy tubers to germinate all the buds in one eye, forming wart like growths. These growths are made up of abundant shoots and do not start rotting, as potato wart galls would.

Powdery scab
Disease caused by *Spongospora subterranea* f.sp. *subterranea* causes scab like outgrowths. Inside the lesions spore balls can be found. These spores are much smaller (3.5-4.5 µm) than sporangia of potato wart.

Pollen grains
Pollen grains can be found in soil, do not confuse these with sporangia of *S. endobioticum.*

b. Advanced Screening by Expert Laboratories

If the sample is determined to be *Synchytrium endobioticum*, the sample must be submitted to USDA-APHIS-PPQ-CPHST for confirmation to species level. Dr. Mary Palm has all necessary authorizations to receive samples submitted for identification to species level.

Write the responsible diagnostician's name and contact information on the APHIS Form 391. Follow the shipping protocol (#1) when sending plant material to another diagnostic facility.

5-Communication:

If the sample is determined to be *S. endobioticum*, follow this communications protocol. If the sample is not identified to be *S. endobioticum*, no further communications are necessary.

a. Notify Drs. Mary Palm (301) 504-5327 or John McKemy at (301) 504-5280 of the suspect sample.

b. Once Drs. Mary Palm or John McKemy have been consulted, samples need to be documented on PPQ form 391 and properly secured. They are then to be sent by overnight freight to:

Dr. Mary Palm
USDA, APHIS, PPQ
Bldg. 011A, Room 329, BARC-West
10300 Baltimore Blvd.
Beltsville, MD 20705-2350

Keep a record of all tracking numbers. Be sure to complete the attached "Transfer Form" and indicate if it was a complete or partial transfer of sample materials. This form is to be retained for university records.

c. Contact the State Plant Health Director (SPHD) and the State Plant Regulatory Official (SPRO) in the sample state of origin,

State Plant Health Director: _____
Address:_____
Address:_____
Phone Number:_____
Fax Number:_____
Email:_____

State Plant Regulatory Official: _____
Address:_____
Address:_____
Phone Number:_____
Fax Number:_____
Email:_____

and fax the SPHD at the PPQ regional office:

1. A copy of the updated form 391 with the preliminary diagnosis and the responsible diagnostician's contact information,
2. a copy of the overnight delivery form used to submit the sample to Dr. Mary Palm,
3. and a copy of the collection information submitted with the sample.

d. Notify your Institution's Environmental and Health Safety Official.

EHS Official:_____
Address:_____
Address:_____
Phone Number:_____
Fax Number:_____
Email: _____

e. Notify your Regional Center and other diagnosticians within your state, of your findings to this point. Please be very clear with the extent of the diagnosis. eg. "*S spp.* was detected on potato. Identification was based on morphological characteristics. The sample has been sent to Beltsville for confirmation to species level." Do not include the submitter's name or contact information

**National and regional network members will be notified by their Regional Center when confirmation is received from Beltsville.

6-Confirmation:

a. Diagnosticians will be notified of the results by Dr. Mary Palm's laboratory.

b. Notify your Regional Center of the confirmation. The Regional Center will notify other Regional Centers and NAPIS.

Regional Center Contact: _____
Contact Phone Number:_____

c. States officials will be notified of the results by the PPQ regional office. Once confirmation is made, state and federal regulatory officials will handle any actions dealing with containment and eradication.

d. Notify your Institution's Environmental and Health Safety Official.

e. In compliance with the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331), if a diagnostic laboratory held back part of a sample or culture that was later shown to be *S. endobioticum* by USDA, the diagnostic laboratory is required to notify,

   APHIS, PPQ, Biological and Technical Services (Permit Unit) in Riverdale, MD at 301-734-7211, 6828, or 5055.

   as soon as possible that the sample exists. According to the regulation, this notification must take place within seven (7) days of the lab being notified their sample or culture was positive. A PPQ Officer must have the opportunity to witness the destruction of the sample or culture. At that time, or if the sample/culture has already been destroyed, the responsible laboratory manager/plant pathologist must complete the **APHIS form 2040** (Guidance Document for Reporting the Identification of a Select Biological Agent or Toxin in a Clinical or Diagnostic Laboratory) and sent to the permit unit in Riverdale, MD. This form is available on the Permits website at: http://www.aphis.usda.gov/ppq/permits/agr_bioterrorism/index.html

7-Sample Destruction:

Plant material, cultures and/or supplies used in the examination and isolation of the suspect sample must be destroyed using a biologically monitored autoclave. The autoclave must be set at a minimum of 15 psi, 121 C for 20 minutes.

Autoclaves are required to be tested periodically for their effectiveness. This can be achieved using a biological monitoring product. Information on one such product can be found at: http://cms.3m.com/cms/US/en/2-21/cirFFFQ/view.jhtml.

Appendix 1: Documentation and Specimen Submission Forms

University Documentation

Inactivation of Significant Biological Agents

Name of Significant Biological Agent:_____

Accession number and description of diagnostic case:_____

_____

_____

Date(s) agent was isolated:

_____

Amount of agent on site prior to inactivation:

_____

Significant agent was:

o Inactivated on site

Date:_____

Method and description of inactivation:_____

_____

If using an autoclave, provide location and cycle conditions (e.g., temperature, pressure, time):_____

_____

Witness to the inactivation:

Print name:_____Signature:_____

o Other (provide detailed explanation):_____

_____

_____

I certify that all biological agents isolated by this facility have been inactivated or transferred to a registered facility pursuant to 7 CFR 331, and that all information on this form is true and correct to the best of my knowledge.

Print name:_____Signature:_____

Date:_____

Received by Environmental Health and Safety:

Print name:_____Signature:_____

Date:_____

University Documentation

Transfer of Significant Biological Agents

Name of Significant Biological Agent:_____

Accession number and description of diagnostic case:_____

_____

_____

Date(s) agent was isolated: Amount of agent on site prior to transfer:

_____ _____

Significant agent was:

o Transferred to a registered entity (give name, date, and USDA/APHIS confirmation number):_____

_____

_____

o All related material was transferred

o All plant material was transferred

o A portion of the plant material was transferred

o All cultures were transferred

o A portion of the cultures were transferred

o Other (provide detailed explanation):_____

_____

_____

I certify that all biological agents isolated by this facility have been inactivated or transferred to a registered facility pursuant to 7 CFR 331, and that all information on this form is true and correct to the best of my knowledge.

Print name:_____Signature:_____

Date:_____

Received by Environmental Health and Safety:

Print name:_____Signature:_____

Date:_____

Specimen Submission Form that must accompany specimens submitted to the USDA/APHIS National Mycologist.

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make an accurate record of plant pest conditions.

FORM APPROVED
OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTOIN SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001. Pest Data Section - Complete Items 14, 15 and 16 or 19 or 20 and 23 as applicable. Complete Items 17 and 18 if a trap was used.

FOR USDA USE
LOT No.
PRIORITY

1. COLLECTION NUMBER

2. DATE MO | DA | YR

3. SUBMITTING AGENCY ☐ State Cooperator ☐ PPQ ☐ Other .....

4. NAME OF SENDER

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*

6. ADDRESS OF SENDER

INTERCEPTION SITE

7. NAME AND ADDRESS OF PROPERTY OR OWNER

ZIP

COUNTRY/ COUNTY

8. REASON FOR IDENTIFICATION *("X" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)
B. ☐ Damaging Crops/Plants
C. ☐ Suspected Pest of Regulatory Concern (Explain in remarks)
D. ☐ Stored Product Pest
E. ☐ Livestock, Domestic Animal Pest
H. ☐ Possible Immigrant (Explain in remarks)
J. ☐ Survey (Explain in remarks)
L. ☐ Other (Explain in remarks)

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

10. HOST INFORMATION
NAME OF HOST *(Scientific name when possible)*

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
PLANTS AFFECTED *(Insert figure & indicate number or percent)* ☐ Number ☐ Percent

12. PLANT DISTRIBUTION
☐ LIMITED
☐ SCATTERED
☐ WIDESPREAD

13. PLANT PARTS AFFECTED
☐ Leaves, Upper Surface ☐ Trunk/Bark ☐ Bulbs, Tubers, Corms ☐ Seeds
☐ Leaves, Lower Surface ☐ Branches ☐ Buds
☐ Petiole ☐ Growing Tips ☐ Flowers
☐ Stem ☐ Roots ☐ Fruits or Nuts

14. PEST DISTRIBUTION
☐ FEW
☐ COMMON
☐ ABUNDANT
☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD

17. TYPE OF TRAP AND LURE

18. TRAP NUMBER

19. PLANT PATHOLOGY - PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION

24. DETERMINATION AND NOTES *(Not for Field Use)*

FOR USDA USE
DATE RECEIVED
NO. LABEL SORTED PREPARED
DATE ACCEPTED
BY

PPQ FORM 391
(JUL 86)

Previous editions are obsolete.