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Organisation Européenne et Méditerranéenne pour la Protection des Plantes
European and Mediterranean Plant Protection Organization

Normes OEPP
EPPO Standards

Diagnostic protocols for regulated pests
Protocoles de diagnostic pour les organismes réglementés

PM 7/28

Organization Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations

Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

Scope

EPPO Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are written according to a 'common format and content of a diagnostic protocol' agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use, etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all diagnostic protocols:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable
- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

References

EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.

FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).

IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).

IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).

OEPP/EPPO (2003) EPPO Standards PM 1/2 (12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris.

Definitions

Regulated pest: a quarantine pest or regulated non-quarantine pest.
Quarantine pest: a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

Outline of requirements

EPPO Diagnostic Protocols for Regulated Pests provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

Existing EPPO Standards in this series

Nineteen EPPO standards on diagnostic protocols have already been approved and published. Each standard is numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:

PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44

PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51

PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60

PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69
PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77
PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253
PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259
PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265
PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275
PM 7/10 (1) *Cacyreus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279
PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292
PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298
PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310
PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256
PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264
PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270
PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280
PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288
PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296

Several of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four 'contractor' diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 'intercomparison' laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostic protocols for regulated pests[1]
Protocoles de diagnostic pour les organismes réglementés

Synchytrium endobioticum

Specific scope
This standard describes a diagnostic protocol for *Synchytrium endobioticum.*

Specific approval and amendment
Approved in 2003-09.

Introduction

Synchytrium endobioticum causes potato wart disease (Langerfeld, 1984). At the end of the nineteenth century, the disease spread from its original range in the Andean region of South America to parts of North America and Europe. Eventually, the fungus was found in potato-growing countries all over the world, including Asia, Africa and Oceania. Under conditions favourable to disease development, it is a highly destructive disease; infected tubers may then be largely converted to warts. Although a number of solanaceous crops can be infected experimentally, potato is the principal host. Favourable conditions for the development of the fungus are cool summers, with an average temperature of 18 °C or less, and an annual precipitation of at least 700 mm. The capacity for natural spread of *S. endobioticum* is limited. The fungus may be carried in international trade on infected potato tubers, or in soil, alone or accompanying plants, from land on which potato wart has occurred in the past. Due to the limited capacity for natural spread, the disease has been controlled effectively by statutory means in many countries. Strict phytosanitary control and obligatory cultivation of resistant cultivars have allowed the eradication of the pathogen in some countries. Eradication requires very many years, because the fungus survives in soil for decades. Because of this, and the destructive nature of the disease, the fungus is considered worldwide as an important quarantine pest.

The fungus is an obligate parasite which does not produce hyphae, but sporangia containing motile zoospores. Summer sporangia are thin-walled and short-lived. They are formed in the affected potato tissue and give rise to new zoospore infections. Winter sporangia are thick-walled and remain viable for extremely long periods of time. They are released from decomposing warts into the soil. Diagnosis of *S. endobioticum* concerns both the plant, on which warts may have formed, and the soil, which may carry winter sporangia. Infected fields should not be used for potato production for 20 years (OEPP/EPPO, 1999). Nevertheless, if requirements for partial descheduling (OEPP/EPPO, 1999) are satisfied, a shorter scheduling period may be applied, after which resistant ware potatoes may be grown. Descheduling or partial descheduling of fields requires direct analysis of the soil for the presence of remaining winter sporangia (if any) and/or a bioassay with a highly susceptible cultivar, all in order to ensure that descheduled fields no longer pose a phytosanitary risk. A method for direct analysis of winter sporangia in soil has been published previously (OEPP/EPPO, 1999). A description of the descheduling bioassay is included in the present protocol.

Numerous pathotypes (races) have been described in *S. endobioticum*. These are defined by their virulence on differential potato cultivars. Pathotype 1 is the commonest in Europe, but few potato cultivars are susceptible to it, apparently due to the availability of dominant resistance in the host. Other pathotypes occur more infrequently in western Europe, and are particularly found in the rainy mountainous areas of central and eastern Europe. They also occur outside Europe (e.g. in Newfoundland, CA). Resistance to these pathotypes is rare, so control is more difficult. Pathotypes 2 and above are distinguished through differential virulence to specific potato cultivars (Stachewicz, 1980; Langerfeld & Stachewicz, 1993; Langerfeld *et al*., 1994; Stachewicz *et al*., 2000), but otherwise seem to be more closely related to each other than to pathotype 1. The phytosanitary import requirements of the European Union (EU, 2000) do not require any further distinction within this group. However, the EU internal directive for control of potato wart (EU, 1969) requires that potatoes grown in the buffer zone of an infested field be resistant to the specific pathotype present in that field.

[1]The Figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

Identity

Name: *Synchytrium endobioticum* (Schilbersky) Percival
Synonym: *Chrysophlyctis endobiotica* Schilbersky, *Synchytrium solani* Massee
Taxonomic position: Fungi; Chytridiomycota; *Chytridiales*
Bayer computer code: SYNCEN
Phytosanitary categorization: EPPO A2 list no. 82; EU Annex designation I/AII

Detection

Disease symptoms

The typical symptoms (Web Fig. 1A–D) of potato wart disease on tubers are the proliferating warts which may vary markedly in form but are primarily spherical to irregular. The infection invariably originates in eye tissue, but may expand to engulf the whole tuber. Warts are formed that vary in size from pea-sized proliferations to the size of a fist. Above-ground warts (which are only occasionally formed) are green but later become black, and subterranean warts are white to brown, becoming black on decay. Early infection of young developing tubers results in their becoming so distorted and spongy as to be scarcely recognizable. In older tubers, the eyes are infected and develop into characteristic, warty, cauliflower-like protuberances. These are initially whitish (or green if exposed to light), but gradually darken and eventually rot and disintegrate. The whole tuber may be entirely replaced by the warty proliferation. Similar warts occur on stolons. Roots are not known to be infected. Small greenish warts may form in the position of the aerial buds at the stem bases. Leaves may also be attacked. The disease does not kill the host and, in the case of subterranean symptoms, may not be evident until harvest.

Detection of sporangia in soil

Several methods are available for the detection of winter sporangia in soil. The procedure recommended by EPPO (OEPP/EPPO, 1999) is a modification of the method developed by Pratt (1976). This procedure is currently under investigation with the aim of improving reliability and reducing costs.

Bioassay for descheduling of fields

Soil samples are collected from fields that are to be descheduled according to the appropriate EPPO Standard (OEPP/EPPO, 1999). Depending on the sampling intensity, this will result in single 2-L soil samples per 0.1 ha or per 0.33 ha. Soil volumes for direct analysis of winter sporangia (2 × 100 mL) are taken from the sample. The remaining soil is put in a 2.5-L plastic pot (i.e. one pot per sample). Three tubers of a susceptible potato cultivar (e.g. Maritiema or Deodara) are planted 3 cm under the soil surface in each of the pots. The potato cultivar should be susceptible to the race of *S. endobioticum* found in the investigated field at time of scheduling; alternatively, a cultivar may be used that is susceptible to all races. The pots are placed on separate dishes in a quarantine glasshouse at a constant 16–18 °C (higher temperatures are not recommended) and 90% humidity with a light regime of 16 h light/8 h darkness. The soil should be carefully kept moist (but not wet) by daily watering or irrigation, and cross-contamination between pots should be prevented. Sprouts are cut back when 60 cm high, so as to induce sprouting of additional eyes and stolon formation. After 70–100 days, tubers are removed from the pots and inspected for wart formation. For descheduling, the tested soil should not have induced wart formation, while a positive control with known infected soil (> 5 sporangia per g) should have induced wart formation on the same potato cultivar. Tubers of this cultivar planted in noninfected soil should not have produced warts.

In a test by the Dutch NPPO, 50% of tubers of cv. Maritiema developed warts with races 1 and 6 at an inoculum density of 1 sporangium per g soil, and > 90% of tubers developed warts at a density of 5 sporangia per g soil.

Identification

Identification of sporangia

Plant material with warts can be examined for the presence of sporangia with a stereo microscope (Web Fig. 1E–G) (Walker, 1983). When necessary, microscopic slices of wart tissue are made, placed in water and examined under a normal light microscope at 100–400 × magnification. Thin-walled, transparent summer sporangia are formed in young warts during the growing season. Thick-walled, golden-brown winter sporangia are found during the growing season and in decomposing warts; they are embedded in the host tissue, filling the host cell almost completely.

Winter sporangia are aseptate, golden brown, thick-walled (triple wall) with the outer wall furrowed, prominently ridged and irregularly thickened, 25–75 (mean 50) µm in diameter. They are spherical to ovoid in shape. When warts decay, the sporangia initially carry some attached host tissue. Eventually, the attached host cells disintegrate, and the sporangia are left with a characteristic angular appearance in median view, as the last remainders of host cell wall structure. In surface view, the remaining host tissue typically appears as characteristic ridging. This, and other morphological characters, distinguishes the winter sporangia of *S. endobioticum* from those of other terrestrial *Synchytrium* species that could be encountered in potato fields as a result of weed infection (Pratt, 1976). Summer sporangia are of similar size to winter sporangia but transparent and thin-walled, and contain numerous flagellate zoospores.

According to the internal wart control requirements of the European Union (EU, 1969), descheduling of infected fields is allowed only if the presence of *S. endobioticum* can no longer be demonstrated. Winter sporangia are responsible for survival and infection of new potato crops, and therefore descheduling procedures rely on methods to demonstrate the presence or absence of winter sporangia. In its Standard on descheduling, EPPO has introduced the distinction between live and dead

sporangia (OEPP/EPPO, 1999), on the basis that dead sporangia should not pose any risk to potato cultivation. The assumption is that live and dead sporangia can be distinguished from one another in a reliable manner. Fresh winter sporangia of *S. endobioticum* generally have homogeneous, granular, greyish contents. Upon germination, sporangia become empty and disintegrate. There is considerable dispute whether viability of winter sporangia can be assessed by light microscopic examination of sporangial contents and/or plasmolysis of these contents. It is generally agreed that sporangia with incomplete, heterogeneous contents may be difficult to identify as dead or alive. It is also agreed that the use of vital staining and UV fluorescence techniques does not solve this difficulty. Following Pratt (1974; 1976), diagnosticians in the UK consider sporangia to be dead if they are obviously plasmolysed (plasma membrane disrupted and cell contents clumped and not completely filling the sporangium). In their experience, germinating winter sporangia may also appear plasmolysed due to a rounding-off of the cytoplasm. However, the plasma membrane is intact, thereby aiding the assessment of spore viability. In contrast, the experience of diagnosticians in the Netherlands and Germany is that too many intermediate conditions exist between live and assumedly dead sporangia, that seemingly dead, empty-looking or plasmolysed sporangia can recover overnight to a normal condition under laboratory examination. Distinguishing between live and dead sporangia should therefore be restricted to cases where the features observed allow for unambiguous discrimination, and to experts with many years of experience with *S. endobioticum*. In case of doubt, winter sporangia should be considered viable. A PCR test for the presence of sporangia of *S. endobioticum*, developed by P.H.J.F. van den Boogert (Plant Research International, Wageningen, NL), will become available in the near future.

Pathotype identification

Pathotype identification of new findings of *S. endobioticum* is possible using the so-called Spieckermann method (Spieckermann & Kothoff, 1924), the Glynne-Lemmerzahl method (Glynne, 1925; Lemmerzahl, 1930; Noble & Glynne, 1970), and field tests. The Spieckermann method requires composting of warts to liberate the winter sporangia, and can be carried out no earlier than the spring following the finding of warted tubers. The test takes 8 weeks, and results become available in early summer. This method can be applied using winter sporangia and is therefore suited for pathotype identification of tubers with old warts or rotting warts. An advantage of the Spieckermann method is the possibility of using reference compost of the various pathotypes. In the method of Glynne-Lemmerzahl, fresh wart tissue is placed in close association with sprouts on entire tubers or eye fields cut out from these tubers. This method is rapid (warts are formed within a few weeks) but requires, as inoculum, warts containing summer sporangia. It is also possible to perform pathotype identification in the field by planting of differential cultivars. This can be done in the year following the finding of the infestation.

Table 1 Differential potato cultivars for the identification of pathotypes of *Synchytrium endobioticum*

	Pathotype				
Cultivar	1	2	6	8	18
Deodara	S	S	S	S	S
Tomensa	S	S	S	S	S
Eersteling	S	S	S	S	S
Producent	R	S	S	S	S
Combi	R	S	S	S	S
Saphir	R	S	R	R	R
Delcora	R	R	R	S	S
Miriam	R	R	R	R	S
Karolin	R	R	R	R	R
Ulme	R	R	R	R	R
Belita	R	R	R	R	–

Differential cultivars

The most important pathotypes in the EPPO region are 1, 2, 6, 8 and 18. These can be differentiated using differential potato cultivars[2] (Table 1).

Spieckermann test

To prepare compost, fresh warts should be used within 14 days and, in the meantime, stored at 4 °C to prevent rotting. Warts are cleaned from leaves, stems and normal tuber parts (adhering soil need not be removed) and are cut into 1 cm pieces or slices. The pieces and slices are well mixed with clean river sand (3 kg of sand per kg of warts) and incubated at a temperature of 18–25 °C. The mixture is moistened daily with distilled or tap water, but should not be allowed to become too wet as it will then start to acidify and rot. The mixture is mixed daily during the first 2 months, and then at weekly intervals during the next two months. After 4 months, the mixture is no longer stirred or moistened but is slowly air-dried at the same temperature for two further months. After a total of six months, the compost is ready and can be used for pathotype identification, although the aggressiveness of the inoculum generally increases in the second year. When stored at 10–18 °C, the compost can be used for 10–30 years.

Before the compost is used, the sporangium density should be determined using the EPPO method (OEPP/EPPO, 1999). The aggressiveness and vitality of the compost is determined in

[2]Cultivars are available as follows: Deodara – Plant Protection Service, Wageningen (NL); Tomensa and Miriam – H. Böhm, Nordkartoffel Zuchtgesellschaft, Postfach 1380, 21303 Lüneburg (DE); Producent – Kweekbedrijf Prummel, Zuiderdiep 252, Tweede Exloermond (NL); Combi – Firlbeck KG, Johann Firlbeck Straße 20, 94348 Atting (DE); Saphir – Dr K. Schüler, IPK GenBank Außenstelle Nord, Parkweg 1, D-18190 Groß Lüsewitz (DE); Delcora – Agrico Research, Burchtweg 17, 8314PP Bant (NL); Eersteling – Fédération nationale de producteurs de plants de pomme de terre, 9 rue d'Athènes, Paris (FR); Ulme – Bayerische Pflanzenzuchtgesellschaft, Elisabethstrasse 38, 80796 München (DE); Karolin – NORIKA Nordring-Kartoffelzucht- und Vermehrungs, Parkweg 4, 18190 Groß Lüsewitz (DE).

the Spieckermann test using 54 tuber blocks of a universally and highly susceptible cultivar such as Deodara. From tubers stored at 4 °C, blocks of tissue (2 × 2 cm) with at least one main eye are cut out. It is advisable to disinfect the blocks with pencycuron or maneb in order to prevent infection with *Thanatephorus cucumeris* (anamorph *Rhizoctonia solani*). The blocks are placed in rows of nine at a distance of 2 mm from each other in wooden boxes (24.0 × 37.5 × 4.5 cm) disinfected (e.g. with steam, 100 °C, 2 h) and containing a moistened, 1 cm-thick, formalin-free, softboard plate. The blocks are dried in a cool environment (4–18 °C) for 24 h. They are then moistened with a fine mist, after which 1.0–1.5 g inoculum compost per block is placed on top of the eye. The inoculated blocks are again moistened, and placed in a controlled environment at 16–18 °C in the dark. Relative humidity in the controlled environment should be high (e.g. initially set at 80% and brought to 90% after 14 days). In the meantime, the boxes with the blocks are moistened daily with a fine water mist, just sufficient to keep them moist (not wet). After 8–10 days, the main sprouts (by then 6–8 cm long) are cut down to 1 cm. The side sprouts are cut down to 3.5 cm after another 8–10 days, and twice more after sprouts have again attained a length of 8–10 cm.

After 8 weeks, the blocks are cut from the boxes, washed with water to remove the compost, and evaluated under a stereo microscope for their reaction to the sporangia. The blocks are classified and counted according to the following categories:

- Early defence necrosis (type 1; in Germany: Resistenzgruppe 1; in the Netherlands: type -) (Web Fig. 1H): disrupted dark brown scales of dead epidermal cells, often in stripes or 'ladder-shaped', longitudinally distributed over the sprout. No sorus (sporangium) formation; invading fungus, as such, no longer detectable
- Late defence necrosis (type 2; in Germany: Resistenzgruppe 1; in the Netherlands: type P [named after the ancient variety Preussen which produced typical late defence necrosis responses]) (Web Fig. 1I): necrotic areas larger, containing scattered, immature or dark brown, necrotic sori (sporangia)
- Very late defence necrosis (type 3; in Germany: Resistenzgruppe 2; in the Netherlands: type F [after the variety Furore]) (Web Fig. 1J): single ripe sori or sorus fields with sporangia developed, but completely surrounded by necrosis. Defence reactions predominate, but not always faster than sorus or field maturation; up to 5 non-necrotic ripe sori with sporangia, clear necrosis in other zones of the same tuber piece
- Weakly susceptible (type 4; in Germany: 'schwach anfällig'; in the Netherlands: type R [after the variety Roland]) (Web Fig. 1K): Scattered infections; scattered necrosis in other zones of the tuber pieces. The sprout can be slightly malformed (thickened)
- Susceptible (type 5; in Germany: 'stark anfällig'; in the Netherlands: wart types I, II, III, IV, V, and X): small to large, non-necrotic infection areas and tumours. Dense infection fields occur on sprouts and tumours. Sprout formation is suppressed. According to size, warts may be classified as I (2–3 mm diam; Web Fig. 1L), II (4–5 mm), III (6–7 mm), IV (8–10 mm), V (11–15 mm), and X (16–20 mm and bigger).

Glynne-Lemmerzahl method

In order to prevent infection with *T. cucumeris*, the tubers are first treated with pencycuron. Sprouts 1–2 mm in length on entire tubers or eye fields (27 × 27 mm) cut out from these tubers are ringed with warm vaseline or vaseline-paraffin, using a syringe without needle, and pieces of fresh wart tissue are placed inside the rings, together with some water. After 48 h incubation at 8–12 °C, the wart tissue is removed, tubers are placed in moist peat at 16–18 °C, and results are recorded after 25 days. This method requires the availability of fresh warts with summer sporangia. Such fresh warts can be cultivated with the Spieckermann method using compost, or can be collected directly in the field. When too few fresh warts are available, wart tissue should be multiplied on highly susceptible cultivars (e.g. Deodara or Tomensa) following the Glynne-Lemmerzahl method. With this method, multiple series of tests can be carried out in relatively short periods of time, under optimal conditions for infection and incubation, guaranteeing reliable results. It is recommended to perform at least three independent tests per identification, using 15 eye fields per cultivar in each test.

The inoculation of eye fields on the tubers (each field with at least one sprout 1–2 mm in length) is done with fresh, cauliflower-like warts (white to light brown in colour) containing large numbers of summer sporangia. Inoculum can be taken on 3–4 successive occasions from warts of 2–3 g or large warts can be cut up. Within the ring of vaseline or vaseline-paraffin, a water bridge is essential between the inoculum and the sprout, to ensure movement of zoospores. Only the uncut surface of the wart inoculum should be put into contact with the water. After 48 h infection at optimal temperatures (8–12 °C), the eye fields are moistened with distilled water and immediately covered with a moist, sterile soil/peat mixture of 2 cm thickness. The cover mixture should be frequently moistened with distilled water during the entire incubation period (23–25 days) so as to promote wart formation. The evaluation of the reaction types produced is done after 25 days. The sprouts are carefully cleaned of soil, and the susceptible and resistant reactions observed with a stereo microscope at 40–80 × magnification. Reaction types are classified according to the scheme of Hille (1965) given above for the Spieckermann method, or the related, simplified scheme of Langerfeld & Stachewicz (1994) which is summarized as follows (for illustrations, see the original publication):

- Extremely resistant (resistance group 1): early defence necrosis; no visible sorus formation
- Resistant (resistance group 1): late defence necrosis; sorus formation partially visible, sori immature or necrotic before maturity
- Weakly resistant (resistance group 2): very late defence necrosis; single ripe sori or sorus fields developed, but completely surrounded by necrosis; defence reactions are dominant, but not always faster than sorus or sorus field maturation; scattered infections, up to five non-necrotic sori, clear necrosis in other zones of the same tuber piece, high degree of attack of the control cultivar (essential!). The present class 3 includes the old class 4 of Hille (1965)

- Slightly susceptible: scattered infections; sori or sorus fields non-necrotic, few in number; late necrosis can be present on other infection sites on the sprout; the sprout can be slightly malformed (thickened)
- Extremely susceptible: dense infection fields, numerous ripe non-necrosed sori and sorus fields, fields with dense non-necrotic infection sites, predominant tumour formation.

Field tests
Pathotype identification is also possible in the field in the year following the finding of the infestation. In the centre of the infested area, the complete test assortment is planted. Prior to the assay, all tubers left over from previous years should be carefully removed. At least 5 × 6 tubers per differential cultivar are planted in a random pattern. When the plants have matured, the tubers are harvested and wart development is evaluated for the stem base, stolons and tubers. Even when only a single wart with winter sporangia has been formed, the cultivar should be rated as susceptible. When no such reaction has occurred and exclusively resistant responses have developed, the cultivar should be rated as resistant. The disease severity can also be evaluated for each plant separately according to the number and size of the warts. Rating can, for instance, be done according to the following scale:
1 not affected
2 single proliferation (< 5 mm)
3 2 or 3 proliferations (< 5 mm) or a single larger one (5–10 mm)
4 several small warts (5–10 mm)
5 several medium-sized warts (> 10 mm)
6 several large warts, at lest one of these being > 10 mm, and beginning deformation of the tuber
7 large warts with a diameter of > 10 mm and disruption of tuber formation
8 very large warts, but individual tubers still recognizable
9 very large warts, no normal tubers present.
Other scales may be used for evaluating so-called field resistance levels of potato cultivars. Such partial resistance is also assessed in the field, and is employed in the Netherlands as a precautionary measure in non-infested fields in regions where some fields have been found infected with wart disease.

Reference material

Reference compost of pathotypes 1, 2, 6 and 18 can be obtained from Dr H. Stachewicz (see Further information below).

Possible confusion with similar species

Other Synchytrium spp.

Some wild plant species found in potato fields such as *Taraxacum officinale* may be infected by *Synchytrium* species other than *S. endobioticum*. None of these combines the unique features of winter sporangia of *S. endobioticum* (golden brown sporangia with a thick wall, an angular appearance and strong ridges, sized 25–75 µm). Pollen grains found in the soil should not be confused with sporangia of *Synchytrium*.

Proliferation of eyes ('pseudo-wart')

Simultaneous germination of all buds in one eye results in wart-like outgrowths very similar to those caused by potato wart disease. However, these pseudo-warts consist of abundant pointed shoots compacted together and do not start rotting when ripe. No winter sporangia are present in the affected tissue. The individual apices of the shoots are somewhat less swollen and more recognizable as buds than are the outgrowths of wart. The cause of this symptom is not clear, but is physiological or varietal and it is thought to be stimulated by contamination of tubers by various chemicals.

Powdery scab

On tubers, enlargement and division of host cells, due to infection by *Spongospora subterranea* f.sp. *subterranea* forces the periderm to rupture, resulting in scab-like outgrowths which, in very wet soil, can develop into hollowed-out areas or very large cankers. Inside fresh lesions, ovoid, irregular, or elongate spore balls (3.5–4.5 µm in diameter) can be observed, consisting of an aggregate of closely associated resting spores. Powdery scab can, in contrast to *S. endobioticum*, attack roots. Milky-white galls, 1–10 mm in diameter or more, develop on roots and stolons. In the severe canker form of powdery scab, the tuber is induced to form knob-like protuberances which often become totally covered by scab tissue, thus closely resembling wart symptoms.

Potato smut

Tubers infected by *Thecaphora solani* become deformed and can have warty swellings on the surface. When sectioned, numerous brown-black specks (sori) can be observed in the flesh, filled with spore balls. Spore balls are usually 2–8-spored and when teased apart, single spores (7.5–20 × 8–18 µm) are smooth on the contiguous side and densely verrucose on the free side. This is a quarantine pest absent from the EPPO region.

Requirements for a positive diagnosis

The procedures for detection and identification described in this protocol should have been followed. For diagnosis of symptomatic potato material, wart tissue should be present and should contain winter or summer sporangia. The morphology of the sporangia should be in accordance with the characteristics given in this protocol. For detection of sporangia in soil, winter sporangia should have been detected in the soil extract. The morphology of the sporangia should be in accordance with the characteristics given in this protocol. For the soil bioassay used as a test for descheduling, tubers planted in known wart-infested soil should have produced warts (positive control). Tubers planted in non-infected control soil should not

have produced warts (negative control). Temperature and humidity conditions in the glasshouse should have been recorded. Any warts produced in test samples should be examined microscopically for sporangia (see item 2). For pathotype identification, warts with winter sporangia should have formed on the reference cultivars susceptible to the identified pathotype, but not on those known to be resistant to that pathotype. Using the Spieckermann method, at least 20% of the tested pieces (11 out of 54) of the susceptible reference cultivar should have formed warts.

Report on the diagnosis

A report on the execution of the protocol should include:

- results obtained by the recommended procedures
- information and documentation on the origin of the infected material or soil
- information on the potato cultivar
- for diagnosis of symptomatic potato material, a description of the disease symptoms (with photographs if possible)
- for detection of sporangia in soil, a statement how many sporangia per g of soil were found in the soil extract
- for the soil bioassay for descheduling, a record of the temperature and humidity conditions in the glasshouse and of the performance of control samples
- for pathotype identification, a table with the reaction types formed on the various potato cultivars, how many pieces or tuber eyes were tested, and how many of these had formed the various reaction types
- measurements and drawings or photographs (as relevant) of sporangia
- an indication of the magnitude of infection on the commodity or of the infestation of the soil (how many sporangia per g of soil)
- comments as appropriate on the certainty or uncertainty of the identification.

Further information

Further information on this organism can be obtained from: R.P. Baayen, Plant Protection Service, PO Box 9102, 6700 HC Wageningen, the Netherlands (E-mail: r.p.baayen@minlnv.nl); H. Stachewicz, Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Institut für Pflanzenschutz in Ackerbau und Grünland, Außenstelle Kleinmachnow, Stahnsdorfer Damm 81, D-14532 Kleinmachnow, Germany (E-mail: H.Stachewicz@BBA.DE).

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References

EPPO/CABI (1997) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

EU (1969) Council Directive 69/464 of 8 December 1969 on control of potato wart disease. *Official Journal of the European Communities* L323, 561–562.

EU (2000) Council Directive 2000/29 of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.

Glynne MD (1925) Infection experiments with wart disease of potatoes. *Synchytrium endobioticum. Annals of Applied Biology* **12**, 34–60.

Hille M (1965) [Classification of potato cultivars for their relationship with *Synchytrium endobioticum*.]. *Nachrichtenblatt des deutschen Pflanzenschutzdienstes* **17**, 137–142 (in German).

Langerfeld E (1984) [*Synchytrium endobioticum*. A comprehensive account of the potato wart pathogen from literature reports.]. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft* no. 219, 1–142 (in German).

Langerfeld E & Stachewicz H (1993) [Pathotypes of *Synchytrium endobioticum* in the old and new Federal Länder.]. *Gesunde Pflanzen* **45**, 9–12 (in German).

Langerfeld E & Stachewicz H (1994) Assessment of varietal reactions to potato wart (*Synchytrium endobioticum*) in Germany. *Bulletin OEPP/EPPO Bulletin* **24**, 793–798.

Langerfeld E, Stachewicz H & Rintelen J (1994) Pathotypes of *Synchytrium endobioticum*. Germany. *Bulletin OEPP/EPPO Bulletin* **24**, 799–804.

Lemmerzahl J (1930) [A new simplified method for inoculation of potato cultivars to test for wart resistance.]. *Züchter* **2**, 288–297.

Noble M & Glynne MD (1970) Wart disease of potatoes. *FAO Plant Protection Bulletin* **18**, 125–135.

OEPP/EPPO (1999) EPPO Standards PM 3/59 *Synchytrium endobioticum*: soil tests and descheduling of previously infested plots. *Bulletin OEPP/EPPO Bulletin* **29**, 225–231.

Pratt MA (1974) Studies on the effect of biotic and abiotic factors on the survival of *Synchytrium endobioticum*. Thesis presented for membership of the Institute of Biology. CSL, Sand Hutton, York (GB).

Pratt MA (1976) A wet-sieving and flotation technique for the detection of resting sporangia of *Synchytrium endobioticum* in soil. *Annals of Applied Biology* **82**, 21–29.

Spieckermann A & Kothoff P (1924) [Testing potatoes for wart resistance.]. *Deutsche Landwirtschaftliche Presse* **51**, 114–115 (in German).

Stachewicz H (1980) [Identification of pathotypes of *Synchytrium endobioticum* by use of test cultivars.]. *Archiv für Phytopathologie und Pflanzenschutz, Berlin* **16**, 1–11 (in German).

Stachewicz H, Larsen J & Schulz H (2000) [Pathotype determination of *Synchytrium endobioticum* from Denmark.]. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **52**, 116–118 (in German).

Walker JC (1983) *Synchytrium endobioticum. CMI Descriptions of Pathogenic Fungi and Bacteria* no. 755. CAB International, Wallingford (GB).

Fig. 1. Symptoms of infection of potato (*Solanum tuberosum*) by *Synchytrium endobioticum*.

A-D. Macroscopic symptoms. **A.** Warts formed on the tubers in the soil may surface during the growing season. **B.** Warts may be formed on emerging sprouts which will not develop into shoots but obtain an irregular, warty cauliflower-like appearance. **C.** Warted tubers as seen at harvest. **D.** A heavily infected plant showing yellowish warts on subsoil tubers and greenish warts at soil level.

E-G. Microscopic symptoms. **E.** Winter sporangia in young wart tissue. **F.** Older wart tissue with sporangia in sori. **G.** Winter sporangia as seen under the light microscope.

H—L. Responses formed on sprouts in Spieckermann tests. **H.** Early defense necrosis (Resistenzgruppe 1 in Germany; Type – in The Netherlands). **I.** Late defense necrosis (Resistenzgruppe 1 / Type P). **J.** Very late defense necrosis (Resistenzgruppe 2 / Type F). **K.** Weakly susceptible (Schwach anfällig / Type R). **L.** Susceptible response consisting of a small wart (Anfällig / Type I).

Source of the illustrations: Fig. 1A, 1E, 1F: courtesy of Central Science Laboratory, York, U.K.; Fig. 1C: courtesy of Biologische Bundesanstalt für Land- und Forstwirtschaft, Kleinmachnow, Germany; Fig. 1B, 1D, 1G: courtesy of HLB B.V., Wijster, The Netherlands; Fig. 1H—1L: courtesy of Plant Protection Service, Wageningen, the Netherlands.