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Pest survey card on *Synchytrium endobioticum*

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Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), upon request by the European Commission. The purpose of this document is to assist the Member States in planning annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with the current international standards. The data requirements for such activity include the pest distribution, its host range, its biology, risk factors as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards and generic documents relevant for all pests to be surveyed, including, the general survey guidelines and statistical software such as RiBESS+.

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Table of contents

Abstract.....	1
Introduction.....	4
1. The pest and its biology	4
1.1. Taxonomy	4
1.2. EU pest regulatory status	5
1.3. Pest distribution	5
1.4. Life cycle	6
1.5. Host range and main hosts	8
1.6. Environmental suitability.....	9
1.7. Spread capacity	9
1.8. Risk factor identification	9
2. Detection and identification	11
2.1. Visual examination	11
2.2. Sampling	12
2.3. Laboratory testing and pest identification	12
3. Key elements for survey design	13
References.....	15
Glossary	18

Introduction

The information presented in this pest survey card was summarised from a recent pest categorisation by the EFSA Plant Health Panel (PLH) (2018), the European and Mediterranean Plant Protection Organization (EPPO) standard on diagnostics (PM7/28) (2017b) datasheets and other scientific documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *Synchytrium endobioticum* in EU Member States (EFSA, 2018). It is part of a toolkit that is being developed to assist Member States with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention guidelines for surveillance (FAO, 2016). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

- i. Pest-specific documents:
 - a. The pest survey card on *Synchytrium endobioticum*¹
- ii. General documents:
 - a. The general survey guidelines
 - b. The RiBESS+ manual available online²
 - c. The statistical tools RiBESS+ and SAMPELATOR which are available online³ with open access after registration.

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Synchytrium endobioticum* (Schilberszky) Percival 1909

Class: Chytridiomycetes **Order:** Chytridiales **Family:** Synchytriaceae **Genus:** *Synchytrium* **Species:** *Synchytrium endobioticum*

Synonym(s): *Chrysochlyctis endobiotica* Schilbersky; *Synchytrium solani* Masee

Common name(s) of the pathogen: Potato wart disease; black scab of potato, black wart of potato; wart disease of potato.

The chytridiomycete *S. endobioticum* is an obligate plant pathogen. One can distinguish different pathotypes based on differential reactions on defined sets of potato varieties (Baayen et al., 2006; Langerfeld et al., 1994). Initially, only one pathotype (nowadays known as pathotype 1(D1)) was known, to which most commercially grown potato varieties are resistant. Since 1941 – when wart development was reported on formerly resistant cultivars (Braun et al., 1942) – about 40 pathotypes have been reported in Europe, with pathotypes 2(G1), 6(O1), 8(F1), and 18(T1) being the most aggressive and widely distributed (Baayen et al., 2006; Obidiegwu et al., 2014; Busse et al., 2017). Pathotype identification is hampered by inconsistent nomenclature, a lack of internationally accepted differential potato varieties, the diversity of test methods and the diverse rating systems used to classify levels of resistance (Baayen et al., 2005, 2006). In addition, several pathotypes are now believed to have emerged independently and their emergence does not reflect evolutionary history (van den Vossen et al., 2018a).

¹ The content of this EFSA Supporting Publication will be made available as a live document <https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489> where it will be updated whenever relevant new information becomes available.

² <https://zenodo.org/record/2541541/preview/ribess-manual.pdf>

³ https://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response_type=code&client_id=shinyefsa&redirect_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce237f192a72d5&login=true&scope=openid

1.2. EU pest regulatory status

Synchytrium endobioticum is regulated under Council Directive 2000/29/EC⁴ in Annex I, Part A, section 2, which includes harmful organisms known to occur in the EU, whose introduction into, and spread within, all Member States shall be banned.

The import of seed potatoes from third countries (other than Switzerland) is prohibited, as laid down in Council Directive 2000/29/EC, Annex III Part A. The import of non-seed potatoes is also prohibited except from a limited number of Mediterranean and European countries. In addition, imports of plants of stolon- or tuber-forming species of *Solanum* or their hybrids are also prohibited, as is the import of soil and growing medium from most countries. Special requirements are laid down in Annex IV for tubers of *Solanum tuberosum* from those third countries, from which import is not prohibited, with the aim, among others, of preventing the introduction of *S. endobioticum* into the EU.

Since the fungus is known to occur in the EU, the measures in Section II of Annex IV are of particular relevance. These refer to the Union provisions listed in Council Directive 69/464/EEC⁵. This Directive includes phytosanitary measures for controlling potato wart disease and preventing its further spread in the EU, including destruction of the infected potato crop and a prohibition on the growing of potatoes or the growing or storing of other plants intended for transplanting in the infested field. In addition, a safety zone is delimited around infested fields in which only resistant potato varieties are allowed to be grown. The respective Council Directive does not include specific regulations on surveillance.

1.3. Pest distribution

Synchytrium endobioticum has a fragmented distribution (Figure 1). The pest originates in the Andean region of South America (Hampson, 1993; EPPO, 2017b). *Synchytrium endobioticum* was introduced into Europe in the 1880s and into North America in the 1900s. Since then, the pest has also spread to Africa, Asia and Oceania, but to a limited number of countries and often to limited parts of those countries. The strict regulatory measures imposed in infested countries have contributed significantly to the prevention of further spread (EPPO, 2017b), particularly given the limited natural dispersal capacity of *S. endobioticum*. Note that the actual distribution of *S. endobioticum* might be wider than reported given the difficulty of detecting the extremely long-lived winter sporangia.

By historic account, potato wart disease entered England in 1876 or 1878 (Franc, 2007; Obidiegwu et al., 2014). Soon afterwards the pest had spread to several other European countries. At present, *S. endobioticum* occurs in 16 EU Member States (Bulgaria, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Poland, Romania, Slovakia, Sweden and the UK) (Figure 1). Each Member State reports the distribution of *S. endobioticum* as being restricted (EPPO global database). Outside the EU, *S. endobioticum* also occurs in other European countries, including Armenia, Belarus, Faroe Islands, Georgia, Montenegro, Norway, the European part of Russia, Switzerland, Turkey, and Ukraine (Figure 1).

⁴ 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. OJ L 169, 10.7.2000, p. 1–112. Consolidated version of 01/04/2018

⁵ Council Directive 69/464/EEC of 8 December 1969 on control of Potato Wart Disease. OJ L 323, 24.12.1969, p. 1–2.

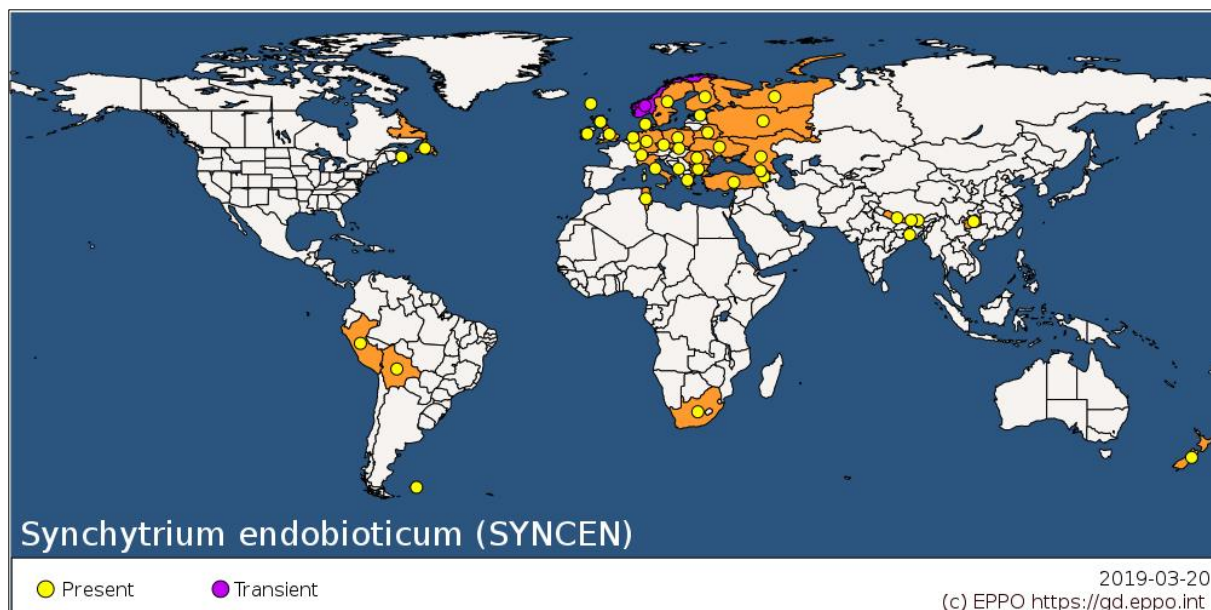


Figure 1: *Synchytrium endobioticum* distribution map (Source: EPPO global database, <https://gd.eppo.int>)

1.4. Life cycle

In spring, the winter sporangia (resting spores) of *S. endobioticum* that have survived in the soil germinate to produce about 200–300 motile zoospores each (Curtis, 1921), which can move in soil water using a tail-like single flagellum up to a distance of 50 mm (Hampson, 1986). The fungus is an obligate biotrophic pathogen and the short-lived zoospores need to find a suitable host plant within 1–2 hours after their formation. Once zoospores reach young tubers or potato stolons, they encyst and infect the host (Curtis, 1921). The infected host cells enlarge greatly, and haploid sori form inside the cells while neighbouring cells proliferate, resulting in the characteristic wart-like outgrowths that have given the disease its common name. Figure 2 illustrates the life cycle of *S. endobioticum*.

The secondary disease cycle is initiated when mature summer sporangia release haploid zoospores. In contrast to the winter sporangia, these summer sporangia are thin-walled and short-lived, and do not have a resting stage. Each sorus contains 1 to 9 short-lived summer sporangia (Curtis, 1921) that contain several hundred uniflagellate zoospores. These zoospores infect new susceptible host tissues and these rapidly repeating secondary disease cycles ultimately result in an extensive invasion of host cells and rapid onset of wart formation (Curtis, 1921). Young warts are a nutrient sink and expand rapidly at the expense of other plant tissues (Weiss, 1925). In the presence of favourable environmental conditions, this process continues throughout the growing season (Hampson, 1993).

Under stress (e.g. water shortage) or in senescing wart tissue, the zoospores can act as isogametes and fuse to form uninucleate, diploid, biflagellate zygotes, which infect the host tissue in the same way as the zoospores to form winter sporangia (Curtis, 1921). Following infection by zygotes, the host cell in which winter sporangia form does not swell but divides to form warts. As these warts mature, they decay and disintegrate, releasing the winter sporangia into the soil (Curtis, 1921). These winter spores are thick-walled and can remain dormant and infectious for at least 40 years (Hampson, 1993) even in the absence of host plants. Minimal survival has been recorded for various periods of time, but what stands out in all studies is the long period of survival (Hampson, 1981; McDonnell and Kavanagh, 1980; Rintelen et al., 1983; Laidlaw, 1985; Putnam and Sindermann, 1994; Przetakiewicz, 2015). Przetakiewicz (2015) showed that, after 43 years, under favourable conditions, disease recurrence may occur even from a single winter sporangium of *S. endobioticum*. Laidlaw (1985) even reports that winter sporangia survived in the soil for 70 years. Given the long survival, EPPO (2017a) recommends that 'a plot that has previously been infested with *S. endobioticum* can be completely descheduled after a minimum of 20 years since the last detection, provided that it is sampled, tested and found free from viable resting spores'.

Crop inspection to detect potato wart disease is not recommended given the ambiguity or absence of symptoms on potato plants (Section 2.1.1). The recommended tuber inspections are best undertaken after harvest, because this allows for easy inspection of large numbers of tubers.

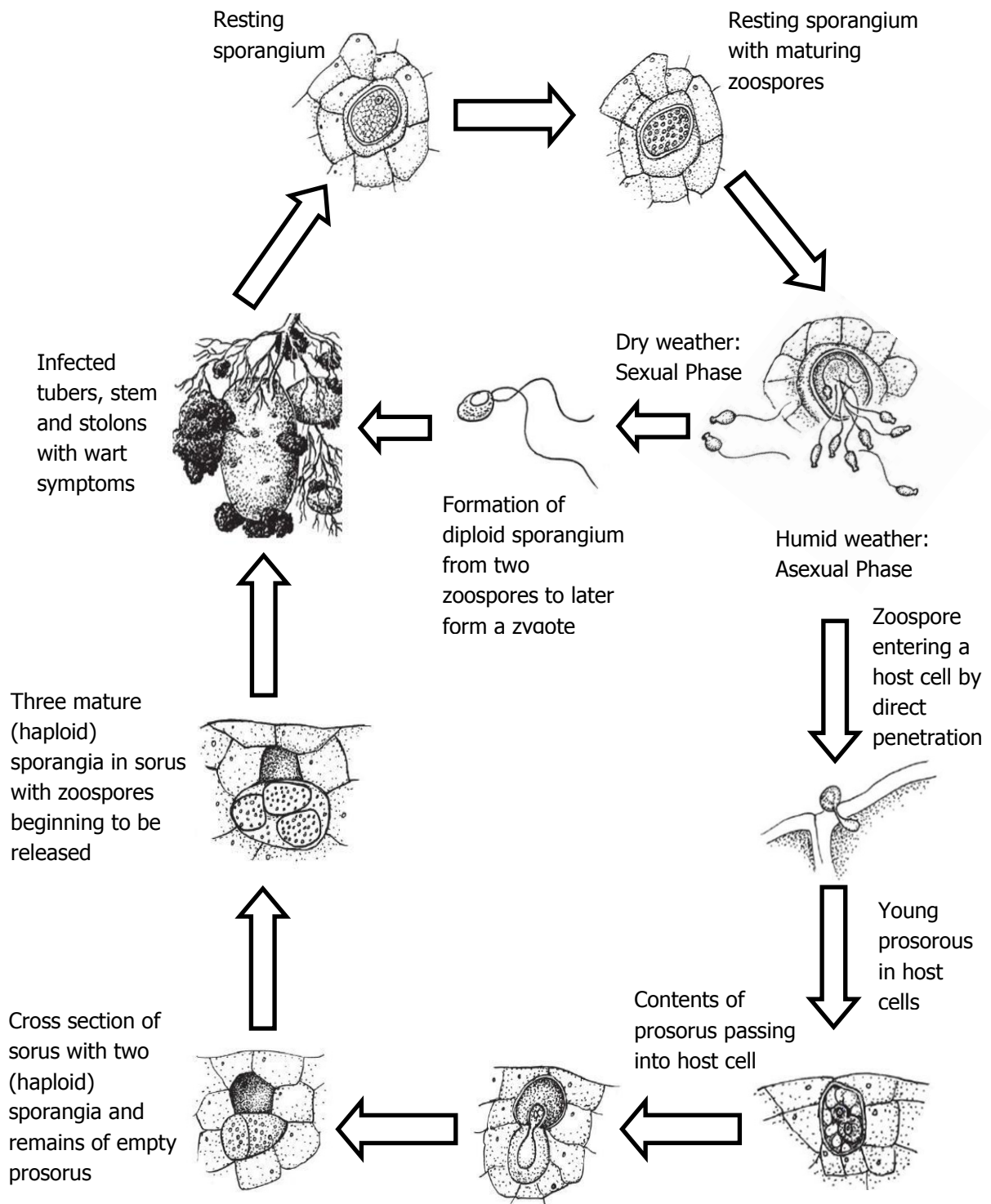


Figure 2: Life cycle diagram of *Synchytrium endobioticum*. (Adapted from Franc, 2001; illustrations after Walker, 1957)

1.5. Host range and main hosts

Potato (*Solanum tuberosum*) is the main natural host of *S. endobioticum*. In the EU, potato is widely cultivated (Figure 3).

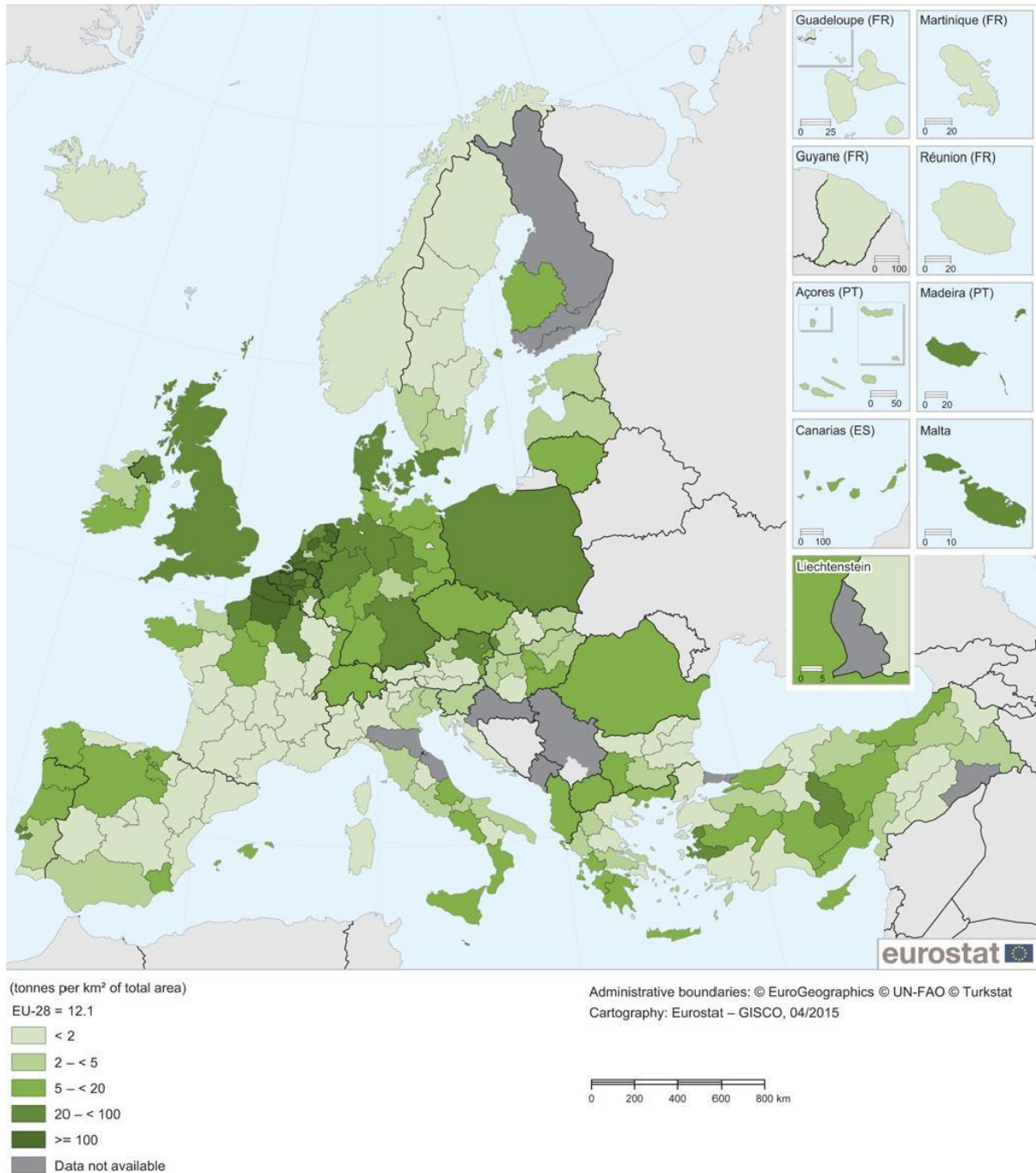


Figure 3: Harvested production of potatoes in 2013 (by NUTS level 2 region in tonnes per km² of total area). Germany: only available for NUTS level 1 regions. Czech Republic, Denmark, Poland, Romania, United Kingdom, Norway, Switzerland and Albania: only available at national level. Croatia: ratio calculated using land area and not total area. Norway, Albania and Turkey: 2012. Bulgaria: 2011. Source: Eurostat Regional Yearbook 2015 (ec.europa.eu/eurostat/documents/3217494/7018888/KS-HA-15-001-EN-N.pdf; Accessed 16 July 2018)

In Mexico, unconfirmed reports suggest that the pest affects wild species of the genus *Solanum* (Obidiegwu et al., 2014; CABI, 2018). Under experimental conditions, *S. endobioticum* can infect the roots of *Solanum lycopersicum* (tomato) and other solanaceous species, such as *Capsicastrum nanum*, *Datura* sp., *Schizanthus* sp., *Physalis franchetii* and *Solanum dulcamara*, without inducing wart formation (Hampson, 1979; CABI, 2018). Given the lack of clear symptoms and lack of information on whether these plants are natural hosts, solanaceous species other than potato are currently not targeted for surveillance activities.

1.6. Environmental suitability

Soil temperatures of at least 8°C and soil water are required for the germination of both winter and summer sporangia and for the dispersal of zoospores (Hampson, 1993). Potato wart disease is favoured by cool summers with average temperatures of 18°C or less and wet soils during tuber development (Weiss, 1925; Bojňanský, 1960). This does not mean the disease is in any way limited to wet and moderate conditions given that outbreaks have been reported from areas in south-eastern Europe with higher summer temperatures (EPPO, 2017b). The EFSA PLH Panel (2018) concluded that the abiotic factors (climate suitability) in the EU suggest that the pest can potentially become established wherever potato is cultivated. The potential for *S. endobioticum* to become established is also related to the fact that the pathogen is soil-borne and that potatoes are commonly irrigated, so that soil moisture may be sufficient for the development of the pathogen.

1.7. Spread capacity

Natural spread

In fields that are infested with the winter sporangia of *S. endobioticum*, natural dispersal by wind or water is limited (Hampson, 1993; Franc, 2007). Nevertheless, winter sporangia can be dispersed within a field or between neighbouring fields by irrigation water runoff, wind and windblown soil particles.

Human-assisted spread

Human-assisted spread of *S. endobioticum* may occur via transport and subsequent planting of seed potatoes. In addition, soil adhering to potato tubers or the roots of non-host plants intended for planting can result in long-distance spread, while equipment, vehicles, machinery or footwear may transfer the sporangia of the pest to other fields at the regional level (EFSA PLH Panel, 2018; Hampson et al., 1996; Hampson, 1996). Once a non-resistant potato variety is planted, the pest multiplies rapidly and inoculum builds up.

Potato tubers intended for consumption or processing (ware potatoes) may pose a risk, particularly those with inconspicuous warts, as they may be planted (especially in smallholdings and private gardens), discarded (whole potatoes or peelings) or used for livestock feed. Animal manure may spread the disease given that winter sporangia have also been shown to survive the digestive system of animals fed on infected potato tubers or grazed in infested fields (Hartman and McCubbin, 1924; Weiss and Brierley, 1928). Soil and plant material from potato processing industries that is used as fertiliser – even after composting (Steinmüller et al., 2012) – or processing water used for irrigation may result in the spread of the pest.

1.8. Risk factor identification

Identification of risk factors and their relative risk estimation is essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for the surveillance are those that have more than one level of risk for the target population. The identification process needs to be tailored to the situation of each Member State. To allow for sample size calculations, the proportion of the target population for each risk factor needs to be estimated as well as the relative risk associated with the

risk factor. This section presents examples of risk factors and is not necessarily exhaustive. The four examples below are related to the main potential pathways for the introduction and spread of *S. endobioticum*.

With regard to seed potatoes, the major pathways of entry are closed based on the current legislation (see Annex III of Council Directive 2000/29/EC).

Example 1: Soil conditions

Soil conditions are a potential risk factor given that the disease is favoured by both wet soils and relatively cool soil temperatures (Weiss, 1925; Bojňanský, 1960). According to Weiss (1925), the most favourable conditions for the appearance of the disease are periodic flooding of the potato field, followed by draining and aeration. In general, regions with a lot of rainfall during the growing period of the crop offer the most suitable conditions for disease development. Bojňanský (1968) found that peat and well-aerated sandy soils provide conditions favourable for potato wart disease. When reliable information on soil types and soil wetness is available in a Member State, this information can be used as a risk factor when designing the survey.

Example 2: Proximity to infested fields

The proximity to infested fields – or fields with historical findings – is another risk factor that can be useful for performing a risk-based survey, because of the possibility of human-assisted spread via infected vehicles or equipment or natural spread by wind or runoff water, for example. Note that surveillance in the infested fields themselves is not useful prior to ‘descheduling’ given that potato tubers are not allowed to be grown in infested fields. Surveillance in the surrounding buffer/safety zone is of limited use given that only potato cultivars that are resistant to the pathotype in the infested field are allowed to be grown in that zone. Still, surveillance in the area surrounding the buffer zone might increase the likelihood of detection. After the demarcation has been lifted – and non-resistant varieties are allowed to be grown again – the historical safety zone would be a target for risk-based surveillance.

Example 3: Growing of susceptible varieties

Initially, only pathotype 1(D1) of potato wart disease occurred in Europe, and the use of resistant varieties provided a good level of control. Since new pathotypes have emerged, such as the aggressive and widely distributed pathotypes 2(G1), 6(O1), 8(F1) and 18(T1) (Baayen et al., 2006; Obidiegwu et al., 2014; Busse et al., 2017), the effectiveness of varietal resistance depends on the pathotypes of *S. endobioticum* present in the soil. Only a few potato varieties are resistant to all the pathotypes that are widespread in Europe (Langerfeld et al., 1994; Ballvora et al., 2011). Cultivation of non-resistant varieties can be considered a risk factor, but this needs to be tailored to the situation in each Member State, depending on which pathotypes are present locally and can be expected during surveillance.

Example 4: Proximity to potato processing plants

Mismanagement of waste (plant material, soil or processing water) derived from the potato processing industry may result in the spread of *S. endobioticum*. Proximity to these processing sites is another risk factor that can be useful for performing a risk-based survey, as well as the nature of the processing that is used on this waste material. Various ways of utilising waste material from potato starch factories (such as potato juice, sludge and pulp), and how it is treated, may modify this risk.

2. Detection and identification

2.1. Visual examination

Disease symptoms on plants

Symptoms of *S. endobioticum* generally appear on the stolons and tubers of infected potato plants. Infected plants occasionally show general symptoms of reduced vigour. As a consequence, the disease is often not noticed before harvest. Field inspections of plants are therefore not recommended for general surveillance.

Disease symptoms on tubers

Typical symptoms of *S. endobioticum* (Figure 4) are proliferating warts produced on tubers (Hampson, 1981; Franc, 2007; EFSA PLH Panel, 2018; EPPO, 2017b). Warts vary markedly in shape but are mostly spherical, with their diameter ranging from less than 1 cm to more than 8 cm (Hampson, 1981). They also vary in size from less than 1 g to more than 50 g fresh weight (Hampson and Coombes, 1985; Hampson, 1993). Infection of tubers originates in eye tissue, but the warts may expand to engulf the whole tuber. Early infection of young developing tubers results in distortions and sponginess and makes them unrecognisable. In older tubers, the infected eyes develop into characteristic, warty, cauliflower-like protuberances. Similar warts may occur on stolons, while roots are not affected. In severely infected plants or very susceptible potato varieties, warts also form on the lower leaves and the aerial buds located at the stem bases (Hampson, 1981). The roots of potato plants are not infected (in contrast to the roots of tomato plants).

Above-ground warts are green, because of their exposure to light, while subterranean warts are white to brown (Hampson, 1981). At maturity, all warts become dark brown to black. The warts eventually rot and disintegrate, sometimes prior to harvest. Typically, the disease is not noticed until the tubers are lifted (Franc, 2007). As the disease may continue developing after harvest, small warts hardly noticed at harvest may become evident during prolonged storage of tubers.



Figure 4: Tubers with potato wart symptoms (Central Science Laboratory, Harpenden, British Crown, Bugwood.org)

Risk of misidentification

Symptoms associated with potato wart may appear similar to those caused by powdery scab (*Spongospora subterranea* f. sp. *subterrenea*), common scab (*Actinomyces scabies*), potato smut (*Thecaphora solani*) or a non-parasitic disease named 'proliferation of eyes' or 'pseudo-wart'. Especially in severe cases of powdery scab, knob-like protuberances are covered by scab tissue which then closely resembles potato wart symptoms. Powdery scab can, in contrast to wart disease, attack the roots of potato plants. Laboratory examination is required for accurate identification of *S. endobioticum* on potato plant material (EPPO, 2017b).

Limitations of surveillance activities

Inconspicuous warts present on potato tubers may be overlooked during visual inspection. Tubers of resistant potato varieties do not show symptoms, but their tubers may carry soil contaminated with winter sporangia, thus reducing the effectiveness of visual inspection. The aggregated distribution of the winter sporangia in the soil of infested fields (Hampson et al., 1996) makes soil sampling for the detection of the pest difficult, particularly when inoculum levels are very low.

2.2. Sampling

A representative sample from each lot should be checked and suspicious looking tubers should be examined in more detail, while including any suspicious-looking tubers.

2.3. Laboratory testing and pest identification

Synchytrium endobioticum can be detected and identified on potato plant material based on symptomatology and morphology of the sporangia formed in warts (EPPO, 2017b). The identity of sporangia – or potato wart material – can be further confirmed by conventional PCR or real-time PCR methods (Boogert et al., 2005; van Gent-Pelzer et al., 2010; Smith et al., 2014; EPPO, 2017b). A test performance study of the molecular methods including the method for identification of the 1(D1) pathotype is also available (van de Vossenbergh et al., 2018b). Details of the morphological characteristics of summer sporangia and winter sporangia have been described by EPPO (2017b). Additional methods can be used to determine the viability of these winter sporangia (EPPO, 2017b), but given the difficulties surrounding these methods, the distinction between live and dead winter sporangia is recommended to be restricted to cases where the features observed enable unambiguous discrimination, and to highly experienced experts. In case of doubt, winter sporangia should be considered viable (EFSA PLH Panel, 2018).

The most common pathotypes that are present in the EU can be identified using a biosassay in which a set of differential potato varieties is tested. Molecular methods are available to distinguish pathotype 1(D1) from the other three most common pathotypes present in the EU (Bonants et al., 2015).

In addition, EPPO standard PM 7/28 (EPPO, 2017b) also describes a sampling and diagnostic protocol for the detection and identification of resting spores of *S. endobioticum* in soil. This method is particularly useful for evaluating the disease status of a demarcated field and less used for surveillance activities.

3. Key elements for survey design

A survey of the causal agent of potato wart disease, *S. endobioticum*, should focus on inspections of the potato tubers. Because the above-ground parts of potato plants may be asymptomatic for *S. endobioticum*, field inspections are not recommended for general surveillance. Instead, the focus should be given to the detection of symptoms in tubers during harvest because the disease is typically not noticed until the tubers are lifted (Franc, 2007). In practice, it is difficult to perform surveillance activities at harvest; therefore surveillance could target the harvested lots and waste heaps. To design a survey of *S. endobioticum* in potato one will need to follow a step-by-step process:

1/ choose the type of survey depending on the objectives of the survey. For *S. endobioticum* the type of survey in potato (pest freedom or pest prevalence) will depend on the pest status in the specific Member State or on the current presence of the pest. *S. endobioticum* is present in several EU countries, where it generally has a fragmented and limited distribution (Figure 1). In the larger part of the Member States where the pest is not known to occur, a statistically sound and risk-based sample size could be calculated using RiBESS+ for confirming the pest status.

2/ describe the surveillance components required to determine statistically sound sample sizes. Each Member State should identify the different units needed based on their specific situation. Note that the number of epidemiological units in the defined target population needs to be known in order to calculate the appropriate sample size. Tables 1 and 2 show examples of the definitions of the units needed to design a survey in seed potatoes or in ware and starch potatoes, respectively. A clear distinction between both types of components is that a lot of seed potato can be traced back to the field in which it was grown at any time, while harvested lots of ware and starch potatoes can be mixed with other lots or may no longer be traceable once they leave the field in which they were grown. This implies that a survey in ware or starch potatoes should be performed at the place of production. In view of the survey design it is necessary to be explicit on the objectives of the survey and to clearly describe its components. In the case of potato wart disease, the survey components are (i) seed potatoes, and (ii) ware and starch potatoes. Thus, for these, specific information is required regarding:

- the target population of the survey and its size
- the epidemiological units making up the target population
- the inspection units.

These survey parameters should be harmonised among the different pests affecting the same host plants. This would optimise field inspections since they are organised per crop visit and not by pest.

Table 1: Example of definitions of the target population, epidemiological unit and inspection unit for a survey for *Synchytrium endobioticum* in seed potatoes

	Definition	Unit
Target population	All lots of seed potatoes produced in a Member State	Total number of lots
Epidemiological units	Seed potato lot	A single lot
Inspection units	Individual tuber	Number of tubers

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for a survey for *Synchytrium endobioticum* in fields that produce ware or starch potatoes

	Definition	Unit
Target population	All field plots in a Member State that are used for the production of lots of ware or starch potatoes	Total number of field plots (or lots)
Epidemiological units	A field plot that is used for the production of a lot of starch or ware potatoes	One single field plot
Inspection units	Individual tuber	Number of tubers

3/ establish the appropriate confidence level (e.g. 95%) and corresponding design prevalence (e.g. 1%) that are the target of the survey and calculate the desired sample size. By including the risk factors identified in Section 1.8, the survey should focus mainly on those fields that are more likely to be infested by the target species.

4/ choose the survey sites from the list of available locations.

5/ develop a sampling procedure within the epidemiological units. When examining potato lots, a representative number of tubers should be examined in more detail, while including any suspicious-looking tubers. Of particular interest are those tubers that are discarded during harvest and end up on waste heaps. Sampling can be directed to those heaps instead of the actual lot. The same procedure can of course be applied to seed potatoes as well.

6/ consider which data are needed and how these data will be reported.

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Glossary

TERM	DEFINITION*
Component (of a survey)	A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruits). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.
Confidence	Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).
Design prevalence	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence. (EFSA, 2018)
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).
Epidemiological unit	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology, should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2018).
Inspection unit	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place. (EFSA, 2018)
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2018).
Method sensitivity	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will give a positive result and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2018).
Pest freedom	An area in which a specific pest is absent as demonstrated by

	scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2018).
Population size	The estimation of the number of plants in the region to be surveyed (EFSA, 2018).
Potato lot	A potato crop identifiable by its homogeneity of composition (same cultivar), origin (same field), etc., or A number of potato tubers identifiable by their homogeneity of composition (same cultivar), origin (same field, same crop) and with traceability to the field in which they were produced.
Relative risk	The ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (Cameron et al., 2014).
RiBESS+	An online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of freedom from disease. Free access to the software with prior user registration is available at: https://shiny-efsa.openanalytics.eu/
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2018).
Risk factor	A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared to a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2018).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are: <ul style="list-style-type: none"> • Definition of the target population – the target population has to be clearly identified • Target population size and geographic boundary. (EFSA, 2018)
Test	Official examinations, other than visual, to determine whether pests are present or to identify pests (ISPM 5: FAO, 2018).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity (D _{Sp}) is the probability that a truly negative epidemiological unit will give a negative result and is related

	to the analytical specificity. In freedom from disease it is assumed to be 100%.
Visual examination	The physical examination of plants, plant products, or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2018).

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