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Synchytrium endobioticum – pathotypes, resistance of Solanum tuberosum and management

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Content

Summary

Synchytrium endobioticum is the causal agent of potato wart. This quarantine potato pathogen is believed to originate in the Andean mountains of Latin America and spread to Europe and further to other parts of world at the end of the nineteenth century. The pathogen is found as different pathotypes displaying differences in virulence against different potato cultivars. Currently around 40 different pathotypes have been described in Europe. There is no clear relationship between pathotypes and genotypes and therefore isolates of the pathogen with different genotypes and origin can still display the same virulence profile in bioassays.

Several collaborative research initiatives have been undertaken and the detection and diagnostics methods available has been improved and updated. However, the EPPO standard bioassay available to describe the different pathotypes is limited to the detection of the major pathotypes 1(D1), 2(G1), 6(O1) and 18(T1). There is currently no agreed standard assay available to detect and describe other pathotypes.

The resistance breeding is complicated by the complex inheritance pattern of cultivated potato and that different genes and different alleles are involved in the resistance against different pathotypes. Nevertheless breeding for resistance in potato has been very successful against pathotype 1(D1). However, it has proven more difficult against the other pathotypes. Fewer potato cultivars that are resistant against e.g. pathotypes 2(G1), 6(O1) and 18(T1) are thus available and even fewer for the more rarely found pathotypes.

S. endobioticum is extremely resistant and the only efficient way to control the disease is to prevent the spread into new locations. The use of resistant potato cultivars is an essential part of the current control strategy to limit further spread. The level of resistance required to prevent further spread is however not known and no standard is currently available for the assessment of the level of resistance of different potato cultivars, although it is under development at EPPO. The level of resistance of the potato cultivars used is not only important in terms of preventing secondary spread of the pathogen but has also been found to affect the potential for development of new pathotypes.

Background and assignment

In 2017, pathotype 8(F1) and 40(BN1) of the quarantine pathogen *Synchytrium endobioticum*, which is the causal agent of potato wart, were found for the first time in Sweden (Swedish Board of Agriculture, 2018). Due to these findings the Swedish Board of Agriculture requested the Unit for Risk Assessment of Plant Pests at SLU to conduct a review of the available information in order to describe; the different pathotypes of *S. endobioticum* in Europe, the corresponding resistance found in different potato cultivars, and the potential consequences of the currently used management practises. Furthermore, the recent progress in the work to harmonise diagnostic methods across Europe and the development of molecular detection methods was summarised.

This final report replaces the interim report delivered May 21, 2018.

Description of *Synchytrium endobioticum*

Life cycle

Synchytrium endobioticum is an obligate biotrophic chytrid fungus, i.e. it is a species that is dependent on live hosts and belongs to a basal lineage in the fungal kingdom. It lacks hyphae and instead is characterized by the production of mobile zoospores and the development of long-lived sporangia. The fungus has two reproductive cycles (Franc, 2007). It has an asexual reproduction cycle with the production of summer sporangia and release of infectious zoospores during conducive conditions (spring and summer). The fungus also has a sexual reproduction cycle, which is initiated during unfavourable conditions (autumn and winter), where zoospore-like gametes fuse, infect the host and develop into resting or winter sporangia.

In short, the different stages in the life cycle are as follows (Weiss 1925; Hampson 1993; Franc 2007 and references therein); after winter when the environmental conditions become favourable (>8°C and high water content), zoospores are released from overwintering resting sporangia. The zoospores swim in the water film in the soil and infect the potato tuber or stolon. Infected epidermal cells starts to swell and the zoospore develops into an aggregate structure containing several zoosporangia known as a sorus. Each sporangium gives rise to hundreds of new haploid zoospores released into the soil water to further infect susceptible host tissue. This asexual reproductive cycle continues as long as the conditions are favourable. Each cycle takes about 10-12 days (Weiss, 1925).

When environmental conditions become unfavourable (*e.g.* dry or cold) a sexual reproduction cycle is initiated. The different segments of the sorus then produce gametes that resemble zoospores but are smaller in size. These gametes may fuse, forming diploid zygotes that can swim to infect a host cell. The infection develops

into a resting sporangium which is released into the soil as the host tissue decay (Franc, 2007). Germination of the resting sporangia is possible within two months but the sporangium may also remain dormant to overwinter (Weiss, 1925). The resting sporangia are extremely resistant and can survive more than 40 years in the soil (Przetakiewicz, 2015a).

Geographical distribution

Synchytrium endobioticum is believed to originate in the Andean mountains of Latin America where it probably co-evolved with potato (Hampson, 1993). At the end of the nineteenth century it spread to Europe and parts of North America and further to potato-growing countries in Asia, Africa and Oceania (Hampson 1993; Baayen et al., 2006). Recent studies show that the pathogen has been introduced to Europe at least three times (Van de Vossenberg et al. 2018a).

Following the introduction to Europe the pathogen caused outbreaks in many European countries (Hampson 1993). Today, the pathogen has been reported from most countries in Europe, although it has been eradicated in some of them (EPPO, 2018a). The distribution is fragmented and localised, probably as a result of the strict regulatory strategies in place (Obidiegwu et al. 2014).

Different pathotypes

Synchytrium endobioticum is found in different variants displaying differences in virulence against different potato cultivars classified as different pathotypes, also referred to as different races. A standard differential set of potato cultivars is used in bioassays to define the four major pathotypes, i.e. 1(D1), 2(G1), 6(O1) and 18(T1) (Baayen et al. 2006; EPPO 2017a). To define other pathotypes other cultivars needs to be added to the bioassay.

For a long time the first pathotype described, 1(D1), was the only one found in Europe (Baayen et al. 2006). However, in the early 1940's many new pathotypes were discovered as symptoms developed on formerly resistant potato cultivars (Baayen et al. 2006). Currently, around 40 different pathotypes have been described in Europe, but some has not been found for many years and others are considered to be of minor importance (Table 1)(Baayen et al. 2006; Cakir et al. 2009; Przetakiewicz 2015b). New pathotypes are, nevertheless, still reported, e.g. the pathotype 38(Nev) and 39(P1) found in Turkey and Poland, respectively, (Cakir et al. 2009; Przetakiewicz 2015b).

Table 1. Described pathotypes of *Synchytrium endobioticum*, their importance and reported distribution, i.e. presence in different countries. The information is mainly based on the summary table provided in Baayen et al. (2006) but additional information has been added from other sources.

Pathotype	Importance	Distribution	References[d]
1 (D1)	+	multinational	1
2 (G1)[a]	+	Germany, The Netherlands, Czech Republic, Canada, Turkey	1; 2
2(Ch1)	+	Poland	1; 3; 4
3(SB)	Eradicated	Czech Republic, Canada	1
3(M1)	+	Poland	3; 4; 12
4(P1), 5(K1), 7(S1), 9(R1), 10(E1), 12(B1)	Eradicated	Germany	1
6(O1)[b]	+	Germany, The Netherlands, Czech Republic, Canada, Turkey	1; 5
8(F1)	+	Germany, Canada, Sweden, Bulgaria, Denmark	1; 6; 7; 8
14(Newfoundland)	-	Canada	1
15(P2), 16(N1), 17(M2), 26(S3), 27(MS1)	+	Czech Republic	1
19(V1), 23(NR1), 24(R3), 28(T2), 29(K2), 30(M3), 31(OL1), 32(V2), 33(K3) 34(K4), 35(L1), 36(ZZ1), 37(K5)	-	Czech Republic	1
11(M1), 13(R2), 16(Sinevik), 18(Yasinya), 20(Sheshory), 21(Sokolovka), 22(Bystrets)	+	Ukraine	1
18(T1)	+	Germany, The Netherlands, Sweden, Turkey, Poland, Greece	1; 5; 9; 14
38(Nev)	?	Turkey	5; 10
39(P1)	+	Poland	11; 12
40 (BN1)[c]	?	Poland, Sweden	4; 13

[a]Including a pathotype earlier described as 19(Haag) (Baayen et al. 2006).
[b]Including a pathotype earlier described as 20(Innernzell) (Baayen et al. 2006).
[c]The virulence profile of this pathotype has not yet been published.
[d]**References**: (1) Baayen et al. 2006, (2) Cakir & Demirci 2017, (3) Przetakiewicz 2010, (4) European Commission 2014, (5) Cakir et al. 2009, (6) Busse et al. 2017, (7) Nielsen, 2017, (8) Laginova 2017, (9) Przetakiewicz 2014, (10) Cakir 2005, (11) Przetakiewicz 2015b, (12) Plich et al. 2018, (13) Swedish Board of Agriculture, 2018 and (14) van de Vossenberg et al. 2018a.

The pathotypes 1(D1), 2(G1), 6(O1), and 18(T1) are considered to be the most widespread and important pathotypes in the western part of the EPPO region (EPPO, 2017a). These are also the pathotypes included in the EPPO standard (EPPO, 2017a), i.e. the only pathotypes that can be distinguished from each other based on the susceptibility of the set of differential potato cultivars in the EPPO standard. There are nevertheless also other pathotypes with a more limited distribution that potentially are economically important, e.g. 2(Ch1) and 3(M1) in Poland (Plich et al. 2018).

Synchytrium endobioticum in Sweden

In Sweden, the first observation of the potato wart disease was made in 1912 on potato from a garden plot on Ljusterön in Stockholm County (Hammarlund, 1915). Tracing the origin of the seed potatoes used led to further findings of infected fields in Södermanland County. Hammarlund (1915) arrived to the conclusion that the disease arrived during the years 1910-1911, most likely from Germany.

Only pathotype 1(D1) had been reported until 2004 when a second pathotype 18(T1) was found (Nordin and Kvist, 2008). Then in 2017, two new isolates displaying the resistant pattern corresponding to those of pathotype 8(F1) and 40(BN1) were found (Swedish Board of Agriculture, 2018). While isolates of pathotype 1(D1) has been found in locations ranging from the most southern part of Sweden up to the provinces of Dalarna and Hälsingland (Melin and Nordin, 1996), the distribution of the findings of other pathotypes are restricted to a much smaller region between the eastern parts of Skåne and western part of Blekinge (Swedish Board of Agriculture, 2018).

Here follows a short description of each of the four pathotypes which has been found in Sweden:

Pathotype 1(D1) has a multinational distribution but the current impact of pathotype 1(D1) has been questioned and in Germany, for example, pathotype 1(D1) is responsible for less than 1% of all outbreaks (Schlenzig 2008). Breeding for resistance against this pathotype was very successful and this created an abundance of potato cultivars resistant to pathotype 1(D1) which effectively controlled the disease (Baayen et al. 2006). Due to extensive use of resistant potato cultivars, Baayen et al. (2005) considers pathotype 1(D1) to be eradicated in most European countries.

Pathotype 18(T1) is also reported from Germany, the Netherlands, Turkey, Poland and Greece (Baayen et al. 2006; Cakir et al. 2009; Przetakiewicz 2014; van de Vossenberg et al. 2018a). The pathotype was first described in Germany in the 1970's (Stachewicz 1978). It is considered to be more virulent than other pathotypes (Busse et al. 2017).

Pathotype 8(F1) is considered to be important, has previously been reported from Germany, Canada (Newfoundland), Denmark, and it has probably also recently been found in Bulgaria (Baayen et al. 2006; Nielsen, 2017; Laginova 2017). It has however caused less damage than the other main pathotypes, i.e. pathotypes 2(G1), 6(O1) and 18(T1) (Nielsen (2017) citing personal communication with Flath in 2017). There is currently a discussion about pathotype 8(F1) among the institutions that perform diagnostics since the pathotypes 8(F1) and 18(T1) display very similar virulence pattern (Nielsen 2017). In the EPPO-standard from 2003 pathotype 8(F1) was included but in the revised 2017 version it is stated that ”In particular, it should be noted that pathotype 8(F1) has not been included in the table as distinction from pathotype 18(T1) is difficult. In case of doubt, specialist laboratories should be contacted (see section 8, Further information).”. From a practical point of view, it has been questioned whether it is relevant to distinguish pathotype 8(F1) from pathotype 18(T1). Pathotype 8(F1) is not included in the official tests that are done in the Netherlands and in Germany they stopped testing for pathotype 8(F1) when pathotype 18(T1) was found there (Nielsen (2017) citing personal communication with Flath in 2017).

Pathotype 40(BN1) is a new pathotype for which the virulence profile has not yet been published. It was first found in Poland in 2010 and later, in the same region as the first finding, both in 2011 and 2012 (European Commission, 2014). The pathotype is reported to be present to a limited extent in the southern parts of Poland (European Commission, 2014). The identification of this pathotype, requires additionally differential potato cultivars to be included in the bioassay, in addition to the standard set included in the EPPO standard (EPPO 2017a). If the bioassay is performed using the differential potato cultivars included in the EPPO standards (EPPO, 2004; EPPO, 2017a), the resulting virulence profile of isolates of pathotype 40(NB1) appear to match that of other pathotypes (Fitopatologii 2016).

Genetic components of virulence

Few studies have been conducted to unravel the genetic components for virulence in *S. endobioticum*. The difficulty of obtaining pure cultures on artificial media due to *S. endobioticum* being an obligate biotroph may be one main reason (Obidiegwu et al. 2014; Bonants et al. 2015). Consequently the knowledge about the genetic basis for virulence of the pathogen and the genetic difference between the pathotypes is limited. Nevertheless, recent studies conducted with the aim to develop molecular methods to differentiate between pathotypes and to determine the intraspecific variation of the pathogen has provided some insights.

In a recent study the DNA of several pathotypes of *S. endobioticum* was sequenced and revealed tree distinct genotype groups (Busse et al. 2017). The pathotypes 1(D1), 39(P1) and 2(Ch1) was classified into one group, pathotypes 2(G1), 3(M1) and 6(O1) formed a second group, while 8(F1) and 18(T1) were classified together into a third group (Busse et al. 2017).

The different pathotypes appear to be genotypically very similar to each other and the development of new pathotypes has been suggested to most likely arise from small changes in avirulence factors, i.e. molecules involved in the host-pathogen interaction, rather than through recombinations of divergent genotypes (Bonants et al. 2015; Busse et al. 2017). Still, genetic variation has been found among isolates of pathotype 1(D1) (Bonants et al. 2015). Some isolates of pathotype 1(D1) from Sweden and United Kingdom for example appear to lack a genetic marker found in isolates from Germany and the Netherlands (Bonants et al. 2015; EPPO 2017a; van de Vossenberg et al. 2018b).

Recent studies on the intraspecific variation of the pathogen have shown that there are in fact clear genotypic differences between isolates within the *S. endobioticum* community. Gagnon et al. (2016) found that European isolates included in the study were genetically different and did not cluster according to geographical origin of the isolates. This is corroborated by Van de Vossenberg et al. (2018a) that investigated the population dynamics by studying the mitochondrial genome of the pathogen.

Both studies further indicate that there is no clear association between the genotypes and the pathotypes (Gagnon et al. 2016; van de Vossenberg et al. 2018a). In the study by van de Vossenberg et al. (2018a), the pathotypes 2(G1), 6(O1) and 8(F1) were found in different mitochondrial lineages. That is, the same pathotype was derived within different mitochondrial groups or, in other words, isolates of the pathogen with different genotypes and origin can still display the same virulence profile. Although this observation may be the results of selective processes it may also be partly ascribed to the difficulty of determining the virulence profile using bioassays (Gagnon et al. 2016). Linked to the recent finding of pathotype 40(BN1) in Sweden; even though the same pathotype has previously been found in Poland it cannot directly be interpreted as the origin of the Swedish isolate.

Recent progress of detection and diagnostic methods

Several collaborative research initiatives have been done during the last decade to improve the detection and diagnostics methods available, e.g.;

- Euphresco project SENDO (Diagnostic methods for *Synchytrium endobioticum*, especially for pathotype identification, 2012-2015)(https://www.euphresco.net/projects/portfolio)
- Euphresco project SENDO D1 (Development of pathotype-specific PCR tests for the identification of *Synchytrium endobioticum* pathotype 1(D1), 2013-2014) (https://www.euphresco.net/projects/portfolio)
- CORNET project SynTest (Establishment of a harmonized methodology for testing the resistance of potato cultivars to potato wart disease (*Synchytrium endobioticum*) in the EU, 2013-2015) (http://www.ihar.edu.pl/cornet2.php)

As a result of these efforts the standard describing the bioassays for pathotype identification has been revised and different molecular methods has been developed and validated as briefly described below.

Pathotype identification

The robustness and reproducibility of the Glynne-Lemmerzahl method for pathotype identification was evaluated in five laboratories in Germany, Poland and the Netherlands by Flath et al. (2014). For some pathotypes and potato cultivars different results were found between laboratories and specific protocols. Further interlaboratory tests of differential potato cultivars has also been performed within the Euphresco-SENDO project and in the CORNET project SynTest (Flath et al. 2014; www.ihar.edu.pl/cornet2.php).

In 2017, the EPPO diagnostic standard for *Synchytrium endobioticum*, originally published in 2003, was revised and the list of differential potato cultivars used for pathotype identification was updated (EPPO, 2017a). This standard is, however, still limited to distinguish between the pathotypes 1(D1), 2(G1), 6(O1) and 18(T1). There is currently no agreed standard assay available to detect and describe other pathotypes than the four most important ones. A method able to differentiate all relevant pathotypes may facilitate both the detection and management of less common pathotypes as well as the detection of new pathotypes.

Resistance screening using bio-assays are both labour and time intensive and efforts have been placed into the development of molecular methods. A real-time Taqman PCR assay was developed that can discriminate between pathotype 1(D1) and other pathotypes (Bonants et al. 2015). The method is currently the only molecular method described that can differentiate between the different pathotypes. Still, the method is limited to discriminate pathotype 1(D1) from other pathotypes and has not been validated on other pathotypes than 2(G1), 6(O1), 18(T1). In addition, the method can only be used for diagnostic purposes if a positive result is obtained since isolates of pathotype 1(D1), identified by a bioassay, from different geographic origin display some genetic variation in the target marker and negative results obtained may thus be false (Bonants et al. 2015; van de Vossenberg et al. 2018b). This has for example been found for an isolate of pathotype 1(D1) from Sweden (van de Vossenberg et al. 2018b). Thus, the identification of pathotype 1(D1) isolates lacking the marker and the identification of other pathotypes is still dependent on bioassays.

Development and validation of molecular methods for detection and identification

Several molecular methods are available to identify and detect *S. endobioticum*, e.g. conventional PCR amplification of the ITS region (Niepold and Stachewicz, 2004; van den Boogert et al. 2005), a real-time PCR approach to amplify ITS2 from soil and plant tissue (van Gent-Pelzer et al. 2010) and a real-time PCR assay amplifying in tandem the SSU and the ITS1 from soil samples (Smith et al. 2014).

Recently, van de Vossenberg et al. (2018b) published the results of a test performance study aimed to generate validation data for molecular assays. All the methods evaluated (van den Boogert et al. 2005; van Gent-Pelzer et al. 2010; Bonants et al. 2015) performed equally on DNA extracted from warted potato tissue. On samples of resting spore suspension, the assay by Gent-Pelzer et al. (2010) was the best in terms of overall accuracy and analytical sensitivity and the authors conclude this as the method of choice for identification of resting spores.

The EPPO diagnostics was revised in 2017 taking into account the development of the molecular detection and diagnostics methods (EPPO, 2017a; van de Vossenberg et al. 2018b).

Resistance in potato against different pathotypes

Genetic components of resistance

Several studies have been devoted to elucidate the genetic basis of the resistance of potato against *S. endobioticum*. The progress in understanding has, however, been limited both by the fact that different studies have used potato material with different genetic background for the analysis, for example with different ploidy level (i.e. the number of chromosomes), and because the resistance is based on several genes (Obideigwu et al. 2014). Cultivated potato is normally tetraploid, having four sets of each chromosome, but can also be diploid, having two sets of chromosomes. The cultivated tetraploid potato also has a very complex inheritance pattern making breeding for disease resistance complicated (Muthoni et al. 2015).

The resistance in potato against pathotype 1(D1) has been linked to one or two dominant genes (e.g. *Sen 1*) (Hehl et al., 1999; Brugmans et al., 2006). It was thus suggested that only one gene mainly control the resistance against this pathotype (Groth et al. 2013). The resistance in potato against pathotype 1(D1) has thus been regarded as a mainly qualitative trait resulting in either complete resistant or susceptible reactions (Flath et al. 2014). However, later studies suggest that other genes may also be involved in the resistance against pathotype 1(D1) (Obideigwu et al. 2015). The conflicting results have been attributed to differences in ploidy level of the potato genotypes used in the studies (Obidiegwu et al. 2015).

The resistance in potato against the new pathotypes observed after the 1940's, e.g. pathotype 2(G1), 6(O1) and 18(T1) appear nevertheless to be clearly linked to multiple genes (Groth et al. 2013). No individual marker loci in itself could explain the variation in resistance, but combined together they were found to result in a significant reduction in disease severity (Groth et al. 2013). Some loci were only linked to certain pathotypes while others were linked to several (Groth et al. 2013). The involvement of multiple alleles for resistance results in a quantitative trait with

varying levels of resistance and susceptibility depending on the genes present in the potato cultivar (Flath et al. 2014).

Whether the main resistance locus found (i.e. *Sen1*), clearly linked to the resistance of pathotype 1(D1), is also involved in the resistance against the other pathotypes has not been unanimously reported in the literature. Groth et al. (2013) state that this locus does not seem to be effective against the pathotypes 2(G1), 6(O1) and 18(T1) while Obidiegwu et al. (2015), on the other hand, observed the opposite. Again this is suggested to be a result of the tetraploid nature of potato and that there are multiple resistance alleles at the same locus that differ between potato families thus giving rise to different results in the studies conducted (Obidiegwu et al. 2015). Recently however, Plich et al. (2018) found resistance gene *sen1* to only be linked to resistance against pathotype 1(D1).

The study by Plich et al. (2018) further found another broad spectrum resistance gene (*sen2*), which provided resistance against all pathotypes tested (1(D1), 2(G1), 6(O1), 8(F1), 18(T1), 2(Ch1), 3(M1) and 39(P1)). The study was made on diploid potato clones and the efficiency of the resistance gene needs to be confirmed in tetraploid cultivated potato (Plich et al. 2018).

Breeding and resistant potato cultivars

Breeding for resistance in potato against *S. endobioticum* started some 100 years ago and was successful in developing resistant cultivars against pathotype 1(D1) (Obidiegwu et al. 2014), presumably due to the presence of a dominant resistance gene and the dominance of one pathotype (e.g. Lellbach and Effmert, 1990). More than 450 cultivars of potato resistant against this pathotype are available as listed by the NPPO in the Netherlands (NVWA, 2018).

The breeding for resistance against the newer pathotypes reported after the 1940's has proven to be more difficult (Baayen et al. 2005 and references therein). Only 15-29 cultivars are listed as resistant against the new pathotypes 2(G1), 6(O1) and 18(T1) and only five potato cultivars are listed as resistant against all four of the pathotypes by the Dutch NPPO (NVWA, 2018).

Studies indicate that resistance against pathotypes 2(G1), 6(O1) and 18(T1) may frequently be inherited together, i.e. genetic markers for resistance against these pathotypes frequently occur in combination (Ballvora et al. 2011; Groth et al. 2013). Markers for resistance against pathotype 1(D1), on the other hand, appear to be independent from the other pathotypes, although some exceptions exist (Ballvora et al. 2011; Groth et al. 2013; Obidiegwu et al. 2015). This pattern is, however, not clearly observed in the potato cultivars listed by the Dutch NPPO and for the pathotypes 2(G1), 6(O1) and 18(T1), about half of the listed resistant potato cultivars were only demonstrated to be resistant against one of these pathotypes (NVWA, 2018). Although, this lack of a pattern could potentially also be the result of an uneven resistance screening intensity for the different pathotypes.

In a report to the Danish NPPO, Nielsen (2017) has collected information regarding the reported resistance of different potato cultivars to different pathotypes. Following the resistant classification of Langerfeld & Stachewicz (1994) for cultivar testing the list includes; 162 cultivars resistant against pathotype 1(D1), 22 cultivars resistant against pathotype 6(O1), 18 cultivars resistant against pathotype 2(G1), 15 cultivars resistant against pathotype 18(T1) and only two cultivars resistant against pathotype 8(F1) (i.e. Otolia and Tivoli; Nielsen, 2017). None of the potato cultivars is listed as resistant against all 5 pathotypes but more than half of the cultivars resistant against the new pathotypes are resistant against more than one of them.

However, not all cultivars have been tested against all pathotypes, e.g. the low number of potato cultivars reported to be resistant against pathotypes 8(F1) may also be due to the low number of potato cultivars specifically tested against this pathotype (Nielsen, 2017). Nevertheless, Nielsen (2017) further states that potato varieties described as resistant to pathotype 18(T1) are also generally considered to be resistant to pathotype 8(F1) (Nielsen (2017) citing personal communication with Leeuwen, Flath, and Przetakiewicz) or the difference between them may be very small (Nielsen (2017) citing personal communication with Boerma).

Potato cultivars verified to be resistant against pathotype 40(BN1) are Megusta, Otolia, Saphir, Ulme and Nicola (J. Przetakiewicz, pers.comm.).

Management options and perspectives

Epidemiology and management

S. endobioticum is an obligate biotroph only able to extract nutrients from living host tissues. Potato (*Solanum tuberosum*) is the main host, although other *Solanum* species (e.g. *S. dulcamara* and *S. nigrum*), tomato (*Lycopersicon esculentum*) as well as species from other genera have been successfully infected in laboratory experiments (Weiss, 1925; Hampson, 1993). Any contribution of other species than potato in the epidemiology of the disease has yet to be confirmed (Arora and Sharma, 2014).

The main means of spread of the pathogen is considered to be via seed potato tubers (Franc 2007). Symptoms may be small and can thus be overlooked in infected fields or may take weeks to month to become visible on infected potato tubers. The pathogen can also spread via the dispersal of winter sporangia in soil attached to tubers and machinery or in soil of potted ornamental plants (Franc 2007; Obidiegwu et al. 2014). Other means of spread listed in the literature includes spread via manure, earth worms, windblown dust and run off water (Obidiegwu et al. 2014 and references therein). In Denmark, the pathogen was suggested to have been spread by wash water from a starch potato industry

(Nielsen, 2017). The local dispersal capacity is very limited and the zoospores themselves for example are only capable of limited spread in the soil, up to 5 cm, and survival is limited to 1-2 h (Weiss, 1925; Franc, 2007).

The pathogen is however extremely resilient due to its durable winter sporangia. Winter sporangia have been found to survive composting for 12 days at 60-65°C and for longer time periods at lower temperatures (Steinmöller et al. 2012). Winter sporangia were also found to survive 90 min at 70°C (i.e. pasteurisation) and 8 h at 80°C in a water bath and at 90°C in a dry oven (Steinmöller et al. 2012). Furthermore, investigations of the survival of *S. endobioticum* in sewage sludge using quantitative examinations with isolated and dried resting sporangia and different types of sewage sludge showed that viable resting sporangia (around 30-40%) was found after a 2h treatment at 140°C (4bar) (UBA, 2015). Storage of the sewage sludge reduced the proportion of viable resting sporangia, but viability rates differed between sewage sludge type and storage conditions and viable sporangia were still found after 5 month storage (UBA, 2015).

Crop rotation decreases the risk for build-up of sporangia in the field and thereby reduces the risk for infections. However, crop rotation can also fail to efficiently control *S. endobioticum* since it has dormant structures that can survive in the soil for very long periods and a very low inoculum density is sufficient to induce disease (Fiers et al. 2012). Under ideal conditions very low levels of inoculum have been found to induce disease and <1 spores/g soil may be enough for infection (Hampson 1992; Browning, 1995). Even though survival of winter sporangia has been found after more than 40 years, the infection rates have also been found to decrease rapidly if susceptible potato is not grown in the soil. Field tests in Pennsylvania showed a reduction already after 5 years and after 10 years no infection was found in plots under fallow or cultivated with resistant potato cultivars (Hartmann 1955).

No fungicides or other chemicals have been found to be effective for managing *S. endobioticum*. Many chemical compounds have been tested, but was either not able to eliminate the pathogen or was phytotoxic or sterilised the soil (Obidiegwu et al. 2014 and references therein). Other studies using different biocontrol measures or other treatments were likewise not fully effective (Obidiegwu et al. 2014 and references therein).

In summary, the only efficient way to control the disease is to prevent the introduction of *S. endobioticum* into new locations, to prevent further spread if introduced and to grow resistant potato varieties.

Resistant potato cultivars and Council Directive 69/464/EEC

Strict regulations are in place in the EU to prevent further spread of the pathogen (Council Directive 2000/29/EC). Seed potato production must be free from the disease and no trade of infected potato is allowed. When *S. endobioticum* is found,

measures to prevent further spread must be implemented as outlined in Council Directive 69/464/EEC on control of potato wart disease. In short, cultivation of potato is prohibited in fields found to be infested by *S. endobioticum* and in a safety zone surrounding the infested field, potato cultivation is allowed only if cultivars used are sufficiently resistant to the pathotype present to prevent secondary infection.

In many ways the current measures appear to have been effective. Although the pathogen has been present in Europe for more than a hundred years the geographical distribution of the pathogen is still restricted in most countries where it is found. Yet, the pathogen is still discovered in new countries and locations and new pathotypes that have broken the resistance of some of the cultivated potato varieties used are discovered (e.g. Çakir et al. 2005, 2009; Przetakiewicz, 2015b).

Prevention of spread

In Council Directive 69/464/EEC it is stated that;

"A potato variety shall be regarded as being resistant to a particular race of *Synchytrium endobioticum* when it reacts to contamination by the pathogenic agent of that race in such a way that there is no danger of secondary infection.".

As argued in Baayen et al. (2005) this means that complete resistance is not required but rather that no further spread of the infection should be possible in the current crop with summer sporangia or the following crop with surviving winter sporangia. Such secondary spread in effect will also depend on the build-up of sporangia, the disease threshold, i.e. density of sporangia needed for infection of a certain cultivar, and different abiotic factors (Baayen et al. 2005).

This gives rise to two aspects to consider; i) what level of resistance is required and ii) how can it be determined.

The resistance against the different pathotypes is often displayed as a quantitative trait with varying levels of resistance and susceptibility (Flath et al. 2014). It is however not clearly defined where the level of resistance in potato cultivars should be placed in order to ensure the elimination of further spread (Langerfeld & Stachewicz 1994; Baayen et al. 2005). Further, there is currently no agreed guidance on how to assess the level of resistance of different potato cultivars. The resistance tests described in the EPPO standard PM 7/28 is for pathotype identification and the revised version published in 2017 does not include any details regarding the classification of the degree of resistance (c.f. EPPO, 2004). A new standard providing guidance on testing of potato varieties to assess the resistance to *S. endobioticum* is, however, under development at EPPO with the goal to finalize a draft in 2019 (EPPO 2017c; EPPO 2018b).

Nevertheless, most resistance screening appear to be done following the scheme by Langerfeld & Stachewicz (1994) where five reaction types are described (as summarized by Obidiegwu et al 2014):

- Extremely resistant (R1) = early defence necrosis; no visible sorus[1] formation
- Resistant (R1) = late defence necrosis; single necrotic sori visible
- Weakly resistant (R2) = very late defence necrosis; up to five non-necrotic sori
- Slightly susceptible (S1) = scattered infections; sorus fields, sprout can be malformed
- Extremely susceptible (S2) = dense infection fields, numerous ripe sori and sorus fields, predominant tumor formation

Potato cultivars categorised as either R1 or R2 appear to generally be considered as resistant cultivars (e.g. Nielsen, 2017). Cultivars with this level of resistance are assumed to fulfil the requirement of Council Directive 69/464/EEC.

The Netherlands also appear to use another scale to identify the resistance level required for cultivation of so called "prevention areas", i.e. areas outside the safety zone, and for such areas a lower level of resistance is allowed (Baayen et al. 2005; Nielsen 2017). In the Netherlands, studies indicate that a field resistance ≥7, on a scale to 9, would adequately prevent secondary infection in tests done on pathotype 1(D1) and 6(O1) (Baayen et al. 2005).

Resistance screening of different potato cultivars against the pathotypes is reported to currently be done mainly in the laboratory using the same methods applied for pathotype description, i.e. the Glynne-Lemmerzahl method or the Spieckermann method (Obidiegwu et al. 2014; Nielsen, 2017). Field tests does not appear to be commonly used as environmental variation may affect the reliability (Obidiegwu et al. 2014). Baayen et al. (2005), nevertheless, showed that field tests could provide stable results in tests done on pathotype 1(D1) and 6(O1) when environmental variation was statistically corrected.

Emergence of new pathotypes

The emergence of new pathotypes is difficult to manage (Obidiegwu et al. 2014). According to Bojnansky (1984) new pathotypes frequently originate and occur in gardens and small size plots where potatoes are commonly grown for own needs or in areas where there is a high probability of direct transmission by e.g. seed potatoes and contaminated tools. Many of the new pathotypes has also been associated with areas with favourable climatic conditions (Bojnansky 1984; Przetakiewicz 2015b). Pathotype 39(P1) was, for example, found in a small garden potato plot in a rainy mountainous area where potatoes were cultivated without crop rotations (Przetakiewicz 2015b).

[1] A sorus (plural sori) is a clusters of sporangia.

It is also hypothesised that new virulent pathotypes develop on potato cultivars that are not completely resistant (Melnik 1998; Plich et al. (2018) citing Malec (1974)). Studies suggest that if the pathogen is able to complete its life cycle, including the sexual reproduction cycle, on partially resistant cultivars the virulence may increase (Melnik 1998 and references therein). Cultivation of cultivars with incomplete resistance may select for new pathotypes that display slightly different resistant profiles, e.g. the development of pathotype 39(P1) and 40(NB1) has for example been suggested to originate from the Polish pathotype 2(Ch1) (Przetakiewicz 2015b; European Commission 2014).

Such a shift in phenotype is further supported by the recently published article Van de Vossenberg et al. (2018a). This study also shows that this shift from one pathotype to another can be a very rapid process as an isolate with a pathotype 1(D1) phenotype changed into a pathotype 6(O1) phenotype after only two multiplications on a partially resistant potato cultivar. The authors also observed an increased diversity of the isolate and suggest that the changed virulence profile was the result of a selection of virulent genotypes already present among the genotypes present in the isolate (Van de Vossenberg et al. 2018a).

Such potential change in virulence due to the selection of the pathogen population needs to be considered when developing control strategies (Van de Vossenberg et al. 2018a). Melnik (1998) state the necessity to define the resistance of new cultivars and to breed cultivars with high resistance to prevent the development of the pathogen. When breeding potato for resistance for example it may be advisable to combine several resistance genes to enhance the durability of the resistance (Plich et al. 2018).

Descheduling of previously infected fields

For a general background of the descheduling procedure of previously infected fields see McNamara and Smith (1998).

In Council Directive 69/464/EEC, Article 6, it is stated that; "The Member States shall revoke the measures taken to control Potato Wart Disease or to prevent it from spreading only if *Synchytrium endobioticum* is no longer found to be present.". The specific requirements for revoking the demarcation of the infected fields or 'descheduling' previously scheduled plots are not given in the Council Directive.

However, a guidance is given in the EPPO standard describing the procedure for descheduling plots previously infested by *S. endobioticum* (PM 3/59) which was revised in 2017 (EPPO, 2017b). The standard includes a procedure to support a complete or partial descheduling of plots previously infested by *S. endobioticum*. Plots where the last detection of the pathogens was done > 50 years ago and where no susceptible crops have been grown can be completely descheduled without any further tests. Plots where the last detection was done > 20 years ago can be

completely descheduled if bioassays performed on specific number of soil samples are negative.

There is also a procedure described to support a *partial* descheduling after >10 years or after >5 years (if soil are treated or not conducive) also based on negative bioassays of a specific number of soil samples. Partially descheduled plots may then be used for cultivation of resistant potato cultivars.

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