

Revised Rapid Assessment of the need for a detailed Pest Risk Analysis for Phytophthora pseudosyringae

Disclaimer: This document provides a rapid assessment (RA) of the risks posed by the pest to the UK in order to assist Risk Managers decide on a response to a new or revised pest threat. It does not constitute a detailed Pest Risk Analysis (PRA) but includes advice on whether it would be helpful to develop such a PRA and, if so, whether the PRA area should be the UK or the EU and whether to use the UK or the EPPO PRA scheme.

STAGE 1: INITIATION

1.What is the name of the pest?

Phytophthora pseudosyringae. First described as a new species of *Phytophthora* by Jung *et al.* (2003).

Synonyms: None.

Common names of the pest: None.

Taxonomic position:

Kingdom - *Chromoalveolata*; Phylum *Heterokontophyta*; Class – *Oomycetes; Order* – Peronosporales; Family – Pythiaceae; Genus – *Phytophthora.*

<u>Special notes on nomenclature or taxonomy:</u> None. However, according to Jung *et al.* (2003), *P. pseudosyringae* has probably been misidentified as *Phytophthora syringae* and possibly other species of *Phytophthora* before it was first described by the authors in 2003. See 5.

2. What is the pest's status in the EC Plant Health Directive (Council Directive 2000/29/EC) and in the lists of EPPO?

P. pseudosyringae is not listed in the EC Plant Health Directive. (Anon., 2000).

P. pseudosyringae is not recommended for listing by EPPO and is not on the EPPO Alert List or the EPPO Action List. (<u>http://www.eppo.org/QUARANTINE/quarantine.htm</u>)

3. What is the reason for the rapid assessment?

This rapid assessment was initiated as a result of the first finding of *P. pseudosyringae* on a new host, *Vaccinium myrtillus* (bilberry). As part of the official surveys for *Phytophthora kernoviae* and *Phytophthora ramorum* in England and Wales, in January 2009, diseased bilberry plants in ancient woodland in Staffordshire, England (now known to be Cannock Chase – see updated section under **5**. below) were sampled and tested. Rather than detecting *P. kernoviae* or *P. ramorum*, *P. pseudosyringae* was isolated instead. Koch's postulates were completed for this host/pathogen combination. Beales *et al.* (2010).

In May 2009, symptomatic plants of *V. myrtillus* in woodland in the south-west of England (Cornwall) were also found to be infected with this pathogen. These are the first known reports of *P. pseudosyringae* on *V. myrtillus*. Sansford (2009).

At that time, it was thought that these were the first records of the pathogen in the UK. However, subsequent investigation has revealed that the first UK records date back to 2004, or possibly as early as 1938 but as a misidentification many years before *P. pseudosyringae* was first described (see 5.). This rapid assessment was initially conducted to determine the status of the pathogen in the UK, whether or not a full PRA is required, and to determine future action.

Most information published up to and including December 2010 was documented in an earlier version of this document which was placed on the Fera website on 13 June 2011 (Sansford, 2011) for a 12-week consultation period. Comments received during the period were discussed at the interdepartmental Plant Health Risk Management Workstream Meeting at Fera in September 2011 and these have been used to produce this revised document.

STAGE 2: RISK ASSESSMENT

4. What is the pest's present geographical distribution?

P. pseudosyringae is known to occur in North America and parts of Europe including the UK. Findings recorded by host, date and location are listed in Annex 1. Some of these records refer to findings in rhizosphere soil of particular tree species only and it is not clear whether the trees were symptomatic.

In addition to records on plant and tree species; *P. pseudosyringae* has also been detected in forest soils and streams in southwest Oregon and northwest California (Reeser *et al.*, 2006) (no sampling date); forest streams in North Carolina (sampled 2005-2006: Hwang *et al.*, 2007, 2008; sampled 2007: Hwang *et al.*, 2008a); Alaska (sampled June 2008: Reeser *et al.*, 2011) and forest soils and streams in Scotland (sampled 2005-2006; Scibetta, 2007). Further details on the Scottish findings are given under 5.

North America	California, North Carolina, Oregon
Central America	No record
South America	No record
Caribbean	No record
Europe	France, Germany, Italy, Spain (nursery stock), UK
Africa	No record
Asia	No record
Oceania	No record

Table 1. Distribution of *Phytophthora pseudosyringae*

5. Is the pest established or transient, or suspected to be established/transient in the UK?

Investigations show that *P. pseudosyringae* is established in the UK (England, Wales and Scotland) but the <u>full extent</u> of its distribution is unknown.

Comments on findings on *V. myrtillus* in the UK received as a result of Fera's public consultation on the first published version of this document have been used to update the distribution information towards the end of this section.

Although the findings on *V. myrtillus* in Staffordshire in January 2009 and in Cornwall in May 2009 referred to under **3.** above were thought to be the first UK records of the pathogen, earlier records on other species (some unpublished) exist and these are described below along with some amended (since April 2011) information on findings in *Nothofagus*:

In 2009, Forest Research (FR) identified *P. pseudosyringae* as the cause of bleeding cankers on *Nothofagus obliqua* (roble beech) in Cornwall; this was the first record on this tree species In 2010, *Nothofagus procera* (rauli) (now known as *N. alpina* and referred to hereafter as such; synonym *N. nervosa*; J. Webber, FR, personal communication) was also recorded in Scotland as suffering from bleeding cankers, and was found to be infected with *P. pseudosyringae*. This was also a first record on this species. (J. Webber, FR, *personal communications*).

In 2007, FR (Denman *et al.*, 2007) reported findings of *P. pseudosyringae* on two mature trees of European beech (*Fagus sylvatica*) and one mature hornbeam (*Carpinus betulus*) in south Wales. Sampled in 2005, these trees exhibited stem symptoms including bleeding cankers and lesions on the root flares. The trees were originally suspected to be infected with *P. ramorum*. These were the first published records of *P. pseudosyringae* on trees in the UK and the first record on hornbeam.

Surveys of the Scottish environment for a range of *Phytophthora* spp. were undertaken using a (then) newly-developed molecular method; *Phytophthora* genus-specific PCR (polymerase-chain reaction) with phylogenetic analysis of ITS1 (*internal transcribed spacer*) sequences. Soil and water samples from a range of Scottish woodlands were subjected to this methodology and P. pseudosyringae was amongst the species that was detected (Scibetta, 2007). Further unpublished detail has been provided by David Cooke (Scottish Crop Research Institute, personal communication 2010): Soil samples taken in October 2004 from a 12-year old mixed woodland in Invergowrie, Dundee including from soil sampled around trees of Quercus and Acer proved positive (ITS PCR products were within a 1 base match to the sequence for Ρ. pseudosvringae pair on GenBank: http://www.ncbi.nlm.nih.gov/genbank/). Additionally water samples taken between May and July 2006 from streams on the shores of Loch Lomond and Beinn Eighe nature reserve proved positive for *P. pseudosyringae* using the same methodology. The streams flowed through woodland. Caveats that apply to the results are that the project was intended to develop the methodology and so there was very little repeat sampling. Limited baiting of the same environments did not detect *P. pseudosyringae*.

In April 2004, the Scottish Agricultural Science Agency (SASA) detected *P. pseudosyringae* on *Pieris floribunda* in a public garden in Edinburgh, Scotland. The pathogen was isolated from the plant and identified by sequencing of the ITS region but Kochs' postulates were not undertaken (A. Schlenzig, SASA, UK, *personal communication*, 2010). This was detected during the course of official surveys for *P. ramorum* and *P. kernoviae*. (J. Chard, SASA, UK, *personal communication*, 2009).

Brasier and Jung (2006) suggested that a root and collar rot of beech in the UK, described by Day (1938) as being caused by *Phytophthora cambivora* and *P. syringae* may in the latter case be *P. pseudosyringae*. Linzer *et al.* (2009) also suggested that the same early report of *P. syringae* associated with root rot of *F. sylvatica* may in fact be *P. pseudosyringae* since *P. syringae* has not been confirmed as a pathogen of *F. sylvatica* (citing evidence in Jung *et al.*, 2003). They suggested that *P. pseudosyringae* may have been in Europe for at least 70 years. Jung *et al.* (2003), state that many isolates of *P. pseudosyringae* have been identified erroneously as *P. syringae*; and that *P. syringae* has itself often been misidentified. The reasons for misidentification were described as being due to morphological and physiological similarities. Jung *et al.* (2003) state that *P. syringae* can be distinguished from *P. pseudosyringae* by differences in properties of the sporangia amongst other features but also by having only 83% similarity in ITS sequences.

Updated information on surveillance and distribution of *P. pseudosyringae* in two areas of England resulting from the Fera public consultation on the April 2011 version of this document provides more detail on the situation in bilberry (*V. myrtillus*).

- The Peak District National Park Authority (PDNPA) surveyed the Roaches Estate (south-west area of the PDNP) in the summer of 2011 following a positive finding of *P. pseudosyringae* there in *V. myrtillus* in April. (NB: See their May news release: <u>http://www.peakdistrict.gov.uk/news/current-news/roaches-remains-open-as-rare-plantdisease-is-treated</u>)
- The PDNPA surveys suggest that the pathogen is present 'across the Estate and the neighbouring landholdings'. The 'infection zone' is described as an area of 3 x 4 km.
- Staffordshire County Council (SCC) have been continuing surveillance of Cannock Chase Country Park since the pathogen was first found infecting *V. myrtillus* in January 2009. (NB: In October 2009 SCC published a report on their findings and a graph indicates that 10ha of land was treated during 8 months of the year to try to control the infection. See:

<u>http://www.staffordshire.gov.uk/Resources/Documents/p/PDFPhytophthoradiseasebriefin</u> <u>gforCannockChaseOctob.pdf</u>)

 No update was provided by SCC in the 2011 consultation on the current extent of the findings

A study of European isolates of *P. pseudosyringae* helps support the view that the pathogen may have been in Europe, including the UK, for some considerable time. AFLP analysis of 19 European (2 from Germany, 17 from Italy – 15 of which were from the Abruzzo National Park) and 29 US (California) isolates of *P. pseudosyringae* showed that US isolates were mainly contained in a subclade within the European isolates (Linzer *et al.*, 2009). (Note there had been an earlier study by Linzer *et al.*, 2005, 2005a but this only analysed US isolates of *P. pseudosyringae*; the 2009 paper is the most recent AFLP study for the RA). Evidence from the 2009 publication led the authors to suggest that *P. pseudosyringae* had been relatively recently introduced to the western US. Comparing isolates from the two continents showed that no genotypes were shared between the two continents and that there was more genetic differentiation within isolates from the same continent than between them. Both sets of isolates showed low genetic variation but it was lower in the US isolate set. Isolates from one site in Italy (the Abruzzo National Park) showed more genetic diversity than the range of isolates taken from across California. For the European isolate set there was some evidence of genetic structure being linked to location. The authors hypothesised that the

European population may be the source of that in the western US, but the pathway of entry to the forests in the western US is unproven; they are still uncertain as to whether or not it has been introduced. The origin of the population in Europe may be Europe, or it may be that the pathogen has been introduced to Europe but has been present here for a longer time than the US population. Although no UK isolates were included in this study, part of the hypothesis in this paper supports the view that *P. pseudosyringae* may have been present in the UK for many years.

Because *P. pseudosyringae* was only described as a new species in 2003 and there have been no official surveys for it, the <u>full distribution</u> of the pathogen in the UK is not well-defined. However, surveys carried-out by the PDNPA and SCC show it is clearly favoured in local areas of England in Cannock Chase and the Peak District where *V. myrtillus* has become infected. Other samples show it is established in at least Great Britain (Cornwall, Scotland, Wales).

6. What are the pest's natural and experimental host plants; of these, which are of economic and/or environmental importance in the UK?

Natural hosts are listed in Annex 1 and the results of experimental transmission studies to other plant species are shown in Annex 2. It is not always clear in publications whether Kochs' postulates have been completed for the reported natural hosts but the majority of the results in Annex 2 arise from testing plant species for Kochs' postulates.

In the UK there are very few records on six host species. Most records arise from official surveys for *P. ramorum* and *P. kernoviae*. Records on *V. myrtillus* in England arose in the first instance from official surveys but latterly from surveys conducted by the PDNPA and SCC

UK records on symptomatic deciduous hardwood trees are limited to beech (*F. sylvatica*), hornbeam (*C. betula*), roble beech (*N. obliqua*) and rauli (*N. alpina*) (all with stem cankers and root flare lesions). There has been a single record on the ornamental shrub *P. floribunda* (presumed symptomatic). Bilberry (*V. myrtillus*) was reported as a natural symptomatic host for the first time in 2009 (stem necrosis/dieback) at Cannock Chase (Staffordshire) and since then has been found infected more extensively there as well as in the Peak District.

The majority of natural hosts in mainland Europe are also deciduous hardwood tree species with most records being on beech (*F. sylvatica*) (Germany and Italy) (stem cankers, root infections, collar rots). Records for English oak (*Quercus robur*) (France and Germany) are from rhizosphere soil only. Other records have been on alder (*A. glutinosa*) (Germany) (collar rot as well as rhizosphere soil), Turkey oak (*Quercus cerris*) (Italy) (rhizosphere soil only), sweet chestnut (*C. sativa*) (Spain) (stem symptoms on saplings), with one record of an isolate from apple (*Malus pumila*) (Italy) but no details as to whether this was symptomatic or just isolated from soil. In Europe the pathogen has often been found in association with other *Phytophthora* spp.

In the western US the pathogen has often been isolated from plants symptomatic for infection by *P. ramorum*, along with *Phytophthora nemorosa*. Records (with symptoms) are very common on California bay laurel (*Umbellularia californica*) (foliage/twig infections), far fewer records have been made on big leaf maple (*Acer macrophyllum*) (foliage), manzanita (*Arcostaphylos* sp.) (foliage/twig), tanoak (*Lithocarpus densiflorus*) (stem cankers), and coast live oak (*Quercus agrifolia*) (stem cankers). These findings have mainly arisen during surveillance for *P. ramorum*.

In addition to the natural hosts, *Quercus petraea* (sessile oak) and *Ilex aquifolium* (English holly) were susceptible to infection by *P. pseudosyringae* when subjected to experimental testing.

To help support this RA, one-off testing of detached leaves of *Rhododendron catawbiense* 'Cunninghams White' was undertaken to determine whether rhododendron had the potential to act as a foliar host of *P. pseudosyringae*. The pathogen successfully infected wounded leaves on the upper and lower leaf surfaces and unwounded leaves on lower leaf surfaces only. (P. Beales, Fera, *personal communication*, 2009).

In terms of economic/environmental importance, beech, oak, alder and sweet chestnut are valuable species in the UK along with bilberry (*V. myrtillus*) which is an important environmental component of heathland and woodlands.

Clearly apple is of great economic importance but the single record in Annex 1 relates to an isolate used in the study with no details of the status of the host.

7. If the pest needs a vector, is it present in the UK?

Phytophthora pseudosyringae does not require a vector for dispersal. However, it has the potential to be moved as a contaminant on the feet of human and animals and possibly on animal fur.

8. What are the pathways on which the pest is likely to move and how likely is the pest to enter the UK?

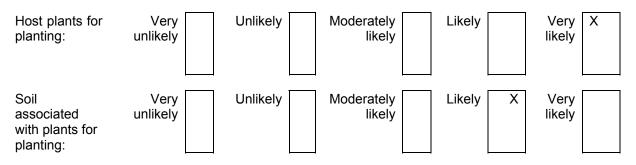
The main pathway on which *P. pseudosyringae* is likely to move is on plants for planting of known natural hosts from countries where *P. pseudosyringae* is known to occur. It may also move in soil associated with host and non-host plants in the nursery trade.

The pest has already entered the UK, further entry is very likely.

There are no <u>specific</u> phytosanitary requirements for *P. pseudosyringae* in the EC Plant Health Directive (Anon., 2000) that would directly affect further entry of the pathogen into the UK or movement within the UK.

Linzer *et al.* (2009) reviewing evidence of the origin of European and US isolates discuss the presence of the pathogen on US and European nurseries (only one European paper – Pintos-Varela *et al.*, 2007) and suggest that the body of evidence links the spread of *P. pseudosyringae* and other species of *Phytophthora* into natural forest ecosystems through nursery stock. They describe the '*invisible invasion*' of the pathogen alongside other more aggressive (and thus more easily observed) species of *Phytophthora*; an example (no citation) being in Germany with the movement of *P. pseudosyringae* with nursery plants that were also infected with *P. cambivora*, *P. citricola* and *P. quercina*.

Likelihood of entry:



9. How likely is the pest to establish outdoors or under protection in the UK?

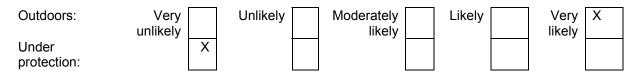
Based upon the records of findings of *P. pseudosyringae* on host plants listed under 5., the pest is already established outdoors in the UK in England (Cornwall, Staffordshire, the Peak District), south Wales and Scotland.

In addition to records on plants (including trees) *P. pseudosyringae* has also been detected in forest soils and streams in Scotland (Scibetta, 2007; D. Cooke, SASA, UK, *personal communication*, 2010).

Similar records exist in the USA. *P. pseudosyringae* has been detected in forest soils and streams in southwest Oregon and northwest California (Reeser *et al.*, 2006) (no sampling date); forest streams in North Carolina (sampled 2005-2006: Hwang *et al.*, 2007, 2008; sampled 2007: Hwang *et al.*, 2008a) as well as in Alaska (Reeser *et al.*, 2011).

The fact that *P. pseudosyringae* has been found in Scotland in the wider environment in soil and water means that its further establishment, if not already widely-established, is very likely. The US records support this.

Establishment under protection is very unlikely because *P. pseudosyringae* is not an organism that affects protected crops. The nursery finding on *C. sativa* in Spain (Pintos Varela *et al.*, 2007) is not thought to be on plants grown under protection.



10. How quickly could the pest spread in the UK?

The fact that *P. pseudosyringae* is known to occur in England in Cornwall, Staffordshire, and the Peak District as well as in south Wales and Scotland, means that it has already been distributed to a number of geographically discrete locations, possibly on plants for planting but also potentially in contaminated soil and in waterways, since rhizosphere soil associated with infected trees has been found to harbour *P. pseudosyringae* and waterways have also proved positive for the pathogen.

The distribution of *P. pseudosyringae* in UK nursery stock is unknown but if it has entered or enters the nursery trade it could be spread quickly through movement of infected plants or contaminated soil associated with planting material of hosts and non-hosts.

The biology of the pathogen is described in the literature as having an '*aerial phase*' in the western USA and a '*soil phase*' in Europe. Rizzo and Fichtner (2007) suggested that the soil phase of *P. pseudosyringae* in California forests needs additional investigation as do the aerial aspects of its biology in European forests.

Although Rizzo and Fichtner (2007) appear to describe a different biology for this pathogen in the different continents (from observed behaviours), *P. pseudosyringae* produces aerial spores known as sporangia with 10 to >80 % being deciduous (*'caducous'*) depending upon the isolate tested (all European isolates). This is therefore a semi-caducous species (Jung *et al.*, 2003), i.e. it has the ability to shed its spores aerially. Every caducous *Phytophthora* species has both an aerial and a soilborne phase (Jung, *personal communication*, 2011) and so the lifecycle of the pathogen is likely to include an element of both wherever it is found.

With respect to aerial spread, *P. pseudosyringae* needs a host on which it can sporulate in order to generate aerial inoculum. In California, Wickland and Rizzo (2005) reported in their study on the predominance of three species of *Phytophthora* (including *P. ramorum*) in mixed evergreen forests, that bay laurel (*U. californica*) was the predominant species for foliar infection by both *P. pseudosyringae* and *P. nemorosa*. They surmised that bay laurel could be acting as the driver of spread and survival for these species in coastal Californian forests.

In the UK it is not known which species might act as producer(s) of aerial inoculum since until the bilberry (*V. myrtillus*) findings most records have been on trees with root/stem symptoms. However, FR are investigating the potential for above-ground plant parts of known natural tree hosts of *P. pseudosyringae* to sporulate (J. Webber, FR, *personal communication*, 2011). Until the results are known it is not possible to say whether trees generate aerial inoculum in the UK.

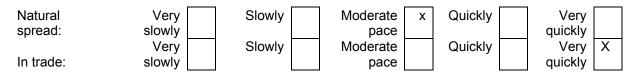
Bilberry (*V. myrtillus*) has the potential to produce aerial inoculum since infection described by Beales *et al.* (2010) was of above-ground parts. Also, in a Defra-funded investigation commissioned to investigate *P. ramorum* and *P. kernoviae* in bilberry (Turner *et al.*, 2009; not included in the first published version of this RA), a short period of aerial monitoring for spores of *P. pseudosyringae* was also undertaken. This was done using rain traps in an area of infected plants in Staffordshire, however, no spores were detected. Sporulation on aerial parts of bilberry has not been observed in the field (personal communication from P. Jennings, Fera, October 2011), but stem lesions in the 2009 study yielded spores (sporangia) in the laboratory. The route of spread in bilberry is currently not clear but the authors of this study suggested that deer may be implicated in spread at this particular site. Although *Phytophthora* spp. do not have true vectors (i.e. they are not dependent upon other species for spread) they can move as contaminants associated with people, animals and machinery (etc.).

There has been no published investigation of the potential of other species to act as sporulators in the natural environment in the UK/Europe. One small laboratory study showed detached leaves of rhododendron were susceptible to infection by *P. pseudosyringae* but other than the work on bilberry (Turner et al., 2009) no other work has been published on sporulation potential. (P. Beales, Fera, *personal communication*, 2009).

To date in Europe there have been no records of any of the other known hosts (trees) suffering from foliar infection; all symptoms have been of root and collar rots as well as stem cankers. The pathogen has been isolated from rhizosphere soil and this would allow limited local spread by movement in soil water, and possibly by entry into waterways.

Overall, because of its biology, the pathogen has the potential to move aerially and in soil or water; in addition to movement in infected planting material and with people or animals as a contaminant of footwear, feet and fur.

Natural spread is likely to be moderate across the area for which this RA has been conducted (the UK); however, it is considered to be rapid in local areas of infected bilberry (*V. myrtillus*) in the Peak District. Spread in trade is likely to be quickly with infected host material. The scores for spread given below relate to the UK rather than to local spread.



11. What is the area endangered by the pest?

Climatically-favourable areas where the known hosts occur in woodlands, gardens, and heathlands. Geographically the west of the UK may be more favourable than the east but not necessarily exclusively so and in all cases this depends upon the presence of natural hosts and a favourable climate. Particularly at risk are areas of bilberry (*V. myrtillus*) in natural habitats such as heathland or where it occurs as an understorey plant in woodland; this is commented on in more detail below. FR consider that the pathogen is likely to be locally damaging in favourable areas to species of *Nothofagus*. Presence of waterways may favour the pathogen. Human activity may help introduce the pathogen and could affect soil structure making roots/stem bases of trees susceptible to infection. Large mammal activity may have a similar effect. The following information supports this:

- *P. ramorum* and *P. kernoviae* have been favoured in the west and south-west of the UK. The findings of *P. pseudosyringae* in Scotland have been in the west as well as the east but near the coast. The findings in England have been in the west. Findings have also been made Wales. Although the west of the UK appears to be favourable for the pathogen this is not necessarily exclusive.
- Jung *et al.* (2003) found *P. pseudosyringae* to be a low temperature species with optimum and maximum temperatures of 20 and 25°C for *in vitro* growth. Thus the temperate climate in the UK is not limiting.
- Denman *et al.* (2007) described the site in south Wales where beech and hornbeam were found infected in 2005, stating that the trees were close to pathways and a source of water; and that the first reports in Italy '*emphasise this observation*'. The authors suggest that disease developed through introduction of the pathogen during recreation or because compaction affected drainage, thus stressing the trees, making them susceptible to infection of roots and stem bases.
- Turner *et al.* (2009) consider that spread in bilberry in Staffordshire may have been supported by deer as the first symptoms were at the edges of deer paths.

Following comments received during the Fera consultation on the first public version of this document the pathogen is considered to have the potential to spread more widely in areas where bilberry is currently infected in the Peak District and Staffordshire thus endangering these special environments.

The PDNPA responded thus:

'In the Peak District the potential area of endangerment is very extensive as there is a contiguous distribution of upland heathlands and blanket bog habitats with a bilberry component to the north and east of the Roaches Estate across the entire Peak District'.

And:

'Bilberry is extensive across the Peak District National Park so potential dieback could potentially be very extensive. In a classification of 514 km2 of moorland using colour and infrared aerial photographs (images from 2005) into seven dominant land-cover classes, 11.5% of the area was classified as bilberry-dominated or ~60 km2 – however, bilberry is also present within heather dominated vegetation and so in reality is even more extensive. Therefore the current P. pseudosyringae infection in the Park has the potential to significantly affect the vegetation composition over a very large area'.

At Cannock Chase (Country Park and Special Area of Conservation (SAC)) the pathogen also has potential to spread more widely thus endangering a wider area.

SCC responded thus:

'It appears that we will lose the bilberry component of the heathy understorey of all of the woodlands in the SAC, and there is scattered infection on open heathland. This is a significant threat to the 'favourable condition' status of parts of the SAC, because the presence of bilberry is one of the cited reasons for designation. We are also concerned that the 600 or so veteran sessile oaks comprising an important surviving fragment of medieval wood pasture are at risk of infection'.

Should *P. pseudosyringae* be introduced to other areas where bilberry is common these too may be further endangered.

12. What is the pest's economic, environmental or social impact within its existing distribution?

Economic:	Very small	Small	х	Medium		Large	Very large	
Environmental:	Very small	Small		Medium	х	Large	Very large	
Social:	Very small	 Small	х	Medium		Large	Very Large	

The ratings given above relate to the impacts that the pathogen is thought to cause overall across North America (west coast of the USA) and Europe, including the UK. <u>Locally</u> the pathogen is having greater impacts as described below.

P. pseudosyringae causes a range of symptoms on a narrow range of hosts in Europe and in the USA. Sometimes it is only found in rhizosphere soil. It is often found in combination with other *Phytophthora* species. However, in England, following the 2011 Fera consultation on the first published version of this document the PDNPA and SCC consider that it is acting in isolation from other *Phytophthora* species in damaging and in some cases killing the bilberry (*V. myrtillus*) component in the Peak District and at Cannock Chase. Also, FR consider that it is locally damaging to species of *Nothofagus*.

In Europe, P. pseudosyringae is associated with declining oak, beech and alder (Linzer et al., 2009). Beech seems to be the most commonly affected tree host. Brasier and Jung (2006) report that subsequent to the 1938 UK report of a collar and root rot of beech in the UK (Day, 1938) now possibly attributable at least in part to P. pseudosyringae, there were few additional records of *Phytophthora* spp. on beech between the 1930s and 1995. They suggest that from 2000 there has been a marked increase of collar rots and aerial bleeding lesions on beech with a range of *Phytophthora* spp. involved including *P. pseudosyringae* as well as P. cambivora, P. citricola, P. cactorum and P. gonapodyides, as well as P. ramorum From these reports, the impact of the pathogen by itself on trees is and P. kernoviae. difficult to gauge. Symptoms attributed to it include damage to the root systems of deciduous hardwood trees as well as stem cankers and foliar symptoms. In their report of a disease on seedlings of C. sativa in Spain suffering from stem lesions, Pintos-Varela et al. (2007) describe the pathogen as causing stem necrosis and collar rot of deciduous species of oak, beech and alder. Non-tree species that have been found infected include bilberry (V. myrtillus) in the UK. This plant is an ecologically important species in heathland and woodland and has been extensively damaged and killed locally in England in the Peak District and at Cannock Chase. In the latter case, in addition to the direct damage caused to the plants, following the 2011 Fera consultation on the first version of this document, SCC

stated that 'The cost of control operations to date has been about £180,000, exclusive of the opportunity costs of deploying several senior staff on the programme. We have a statutory duty to maintain the conservation interest of the SAC, so we do not regard this spending as optional. Natural England takes a similar view, and helps to fund the operations through Higher Level Stewardship'.

Pieris (*P. floribunda*) was the first known host in the UK but this is a one-off record (in Scotland in 2004) and there is no description of the effect that the pathogen had. Denman *et al.* (2007) consider that in Europe *P. pseudosyringae* is regarded as a pathogen causing fine root rot and bleeding stem cankers of native tree species. However they acknowledge that it is often associated with other species of *Phytophthora*, both in Europe and the USA.

In California, surveys for *P. ramorum* based upon isolation from symptomatic plants often yield P. ramorum, P. nemorosa and P. pseudosyringae (e.g. Murphy and Rizzo, 2005). Murphy et al. (2007 and 2008), describe these three pathogens as causing similar disease symptoms but emphasise that P. pseudosyringae (and P. nemorosa) do not cause landscape mortality of oaks, in contrast to *P. ramorum*, despite occupying similar geographic locations and ecological niches at least in coastal Californian forests. P. pseudosyringae and P. nemorosa have a smaller host range and extend over a broader area than P. ramorum in California (Murphy et al., 2008). Earlier Wickland and Rizzo (2005) studied the distribution and host association of all three of these species of *Phytophthora* in mixed evergreen forests in California and stated that 'the low incidence and mortality of coast live oak and tanoak attributed to both P. pseudosyringae and P. nemorosa suggests that although these are common foliar pathogens of mixed evergreen forests, at this time they are causing low levels of change and are essentially background mortality agents'. Understory species in US woodlands, such as U. californica (California bay laurel), infected with P. pseudosyringae suffer from foliar and twig symptoms; this is described by Martin and Tooley (2003), as being in contrast to the description of the pathogen by Jung et al. (2003) as a root and collar pathogen of trees. Rizzo and Fichtner (2007) contrast the pathogen as being a soil inhabitant causing root and crown rot in European oak and beech forests stating that although this species was occasionally isolated from crown rot on coast live oak in California, it was primarily isolated from foliage on bay laurel. Denman et al. (2007) state that in the USA it is isolated from necrotic leaves and twig cankers in tree canopies specifically bay laurel (U. californica), with only a few records of it being associated with oak bleeding stem cankers.

<u>Overall</u> the impact of *P. pseudosyringae* is very hard to gauge because at least in the case of tree infections the pathogen is often found in association with other *Phytophthora* spp. However, the comments received following the 2011 Fera consultation on the first published version of this document show that the pathogen is considered to be extremely damaging, indeed lethal to bilberry (*V. myrtillus*) in the Peak District and Cannock Chase and costs have been incurred in trying to bring it under control. It is also locally damaging to species of *Nothofagus*.

Linzer *et al.* (2009) describe it as being of '*low virulence*'. It may have been present in Europe for much longer than 2003 when it was first described, but ignored, because it was not known to be causing significant damage, at least to trees. More recently since its description it has been found to be locally very damaging in England on bilberry (*V. myrtillus*) and locally on some standing trees especially *Nothofagus*.

13. What is the pest's potential to cause economic, environmental or social impacts in the UK?

See 11 and 12. The impact on trees is likely to be minor in isolation from other species of *Phytophthora* but it could be locally damaging to species of *Nothofagus*. The Forestry Commission for Scotland commented in the 2011 Fera public consultation on the first public version of this document that this 'suggests that the use of N. obliqua and N. procera (= *N. alpina;* syn. *N. nervosa*) as suitable species for climate change adaptation could be limited'. With respect to non-tree hosts the pathogen has the potential to spread further in areas where it is currently infecting bilberry (*V. myrtillus*) or where it is introduced there and this is likely to have a knock-on effect in the first instance on biodiversity (see Conyers *et al*, 2011).

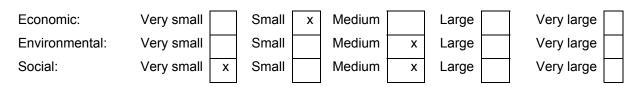
In addition to mentioning the potential for impacts on biodiversity the PDNPA commented:

'The bilberry is also one of the features for which compartments within the Roaches Estate is designated as a SSSIs*. Therefore potential loss of the bilberry component will have significant repercussions on the condition status of infected SSSI units. This will have economic implications as the downgrading of the condition status of SSSIs will require action, at economic cost, to address. Natural England can provide more detailed information on this. There are also social implications of the loss of bilberry as certainly within the Peak District there is an established bilberry picking culture that will be lost if the P. pseudosyringae outbreak spreads across the Park. There are also millions of visitors to the Peak District moorlands every year'.

*Site of Special Scientific Interest.

In addition to the comments quoted above (see 11). The SCC also stated: *'With respect to the environmental impact I believe that it is similar, with respect to heathland, to those of* P. ramorum *and* P. kernoviae'.

Thus the pathogen has the potential to have a negative environmental impact on a localscale as well. Whether it is affecting the visitor numbers to the Peak District, Cannock Chase or has the potential to do so is difficult to gauge but with the death of bilberry plants already occurring it has further potential to do so. The ratings below reflect this.



14. What is the pest's potential as a vector of plant pathogens?

P. pseudosyringae is a plant pathogen with no capacity to act as a vector of other pathogens.

STAGE 3: PEST RISK MANAGEMENT

15. What are the risk management options for the UK?

Action for keeping the pest out of the UK

P. pseudosyringae is already present in the wider environment so no action is considered necessary.

Options for control if the pest became established

P. pseudosyringae is already established in the UK although the extent of its distribution is unknown.

16. Summary and conclusion of rapid assessment.

This rapid assessment shows:

Likelihood of entry is: Likely

Likelihood of establishment is: Established

- *Economic impact is expected to be:* Difficult to separate the impact from other *Phytophthora* spp. with which it appears to be associated, but the impact on trees appears minor in isolation, albeit with some uncertainty. The impact on bilberry and the environment in which it occurs is likely to be locally damaging.
- *Endangered area:* Woodland and heathland *a*reas of the UK where known natural hosts are present; possibly favoured by the presence of waterways and human activity such as walking, mountain biking, etc. Managed gardens may also be favourable to the pathogen. It is possible that western and coastal areas may be more favourable but this is not proven.

Risk management: No statutory action is recommended.

17. Is there a need for a detailed PRA?

No. All of the information that has been published was used to produce the first version of this document and although more information on the status of *P. pseudosyringae* in bilberry in England has been used to revise this document (contributed by the PDNPA and SCC) the pathogen is already established in parts of the UK (as well as elsewhere in Europe). No action has been taken against it by any National or Regional Plant Protection Organisation. The process of PRA is to '*evaluate biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it'.* (FAO, 2010). Whilst *P. pseudosyringae* is a pest and although there is potential for further movement of the pathogen in plants in trade, and further spread within the UK, taking statutory action would not prevent further spread.

Yes No X

If yes, select the PRA area (UK or EU) and the PRA scheme (UK or EPPO) to be used.

PRA area: UK or EU?	Not	PRA scheme: UK or EPPO?	Not applicable
	applicable		

Date of production: January 30th 2012.

Version no.: 4.

Revision of version 3, November 3rd 2011. : Done in response to comments received from Joan Webber, Forest Research, UK on version 3. Version 3 was prepared in response to Fera's 2011 public consultation on version 2 (version dated 18th April 2011).

Author: Dr Claire Sansford, Fera, Sand Hutton, UK, YO41 1LZ

Reviewers: The concept of the revision of this document post-public consultation was agreed by Fera's Plant Health Risk Management Workstream. The document was reviewed by Dr Joan Webber, Forest Research, UK (whose suggested revisions were factual changes to names of tree species only).

References

Anon. (2000) (*as amended*). 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.* 43, no. L 169, 1 - 112.

Beales PA, Giltrap PM, Webb KM, Ozolina A (2010). A further threat to UK heathland bilberry (*Vaccinium myrtillus*) by *Phytophthora pseudosyringae*. *Plant Pathology*, **59**, 406

Brasier CM, Jung T (2006). Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe. In: Brasier, C.M.; Jung, T.; Osswald, W., eds. Progress in Research on *Phytophthora* Diseases of Forest Trees. Proceedings of the 3rd IUFRO Working Party SO7.02.09 Meeting, *Phytophthoras* in Forests and Natural Ecosytems, Freising, Germany, September 11–17, 2004. 5–16.

Cacciola SO, Diana G, Pane A, Chimento A, Raudino F (2005). Un focolaio di cancro basale gommoso del faggio causato da *Phytophthora pseudosyringae* nel parco Nazionale d'Abruzzo. *Informatore Fitopatologico*, **4**, 52-57.

Cacciola SO, Motta E, Raudino F, Chimento A, Pane A, Magnano di San Lio G (2005a). *Phytophthora pseudosyringae* the causal agent of bleeding cankers of beech in central Italy. *Journal of Plant Pathology*, **87**, 289.

Conyers S, Somerwill K, Ramwell C, Hughes J, Laybourn R, Jones N (2011). Review of the known and potential biodiversity impacts of *Phytophthora* and the likely impact on ecosystem services'. Defra-funded project report for PHO601. 113pp. <u>http://randd.defra.gov.uk/Document.aspx?Document=PH0601_10149_FRP.pdf</u>.

Day WR (1938). Root-rot of sweet chestnut and beech caused by species of *Phytophthora* I. Causes and symptoms of disease: its relation to soil conditions. *Forestry*, **12**, 101-116.

Denman S, Rose J, Slippers B (2007). *Phytophthora pseudosyringae* found on European beech and hornbeam trees in the United Kingdom. Proceedings of the 4th IUFRO Working Party SO7.02.09 Meeting, *Phytophthoras* in Forests and Natural Ecosytems, August 26-31, Monterey, California, USA, 273-280.

FAO (2010). Glossary of Phytosanitary Terms. International Standards for Phytosanitary Measures no. 5. 27pp.

Fleischmann F, Gottlein A, Rodenkirchen H, Lutz C, Osswald W (2004). Biomass, nutrient and pigment content of beech (*Fagus sylvatica*) saplings infected with *Phytophthora citricola*, *P. cambivora*, *P. pseudosyringae* and *P. undulata. Forest Pathology*, **34**, 79-92.

Hartmann G, Blank R (1998). Buchensterben auf zeitweise nassen Standorten unter Beteiligung von *Phytophthora*-Wurzelfäule. *Forst und Holz*, **53**, 187-193.

Hwang J, Jeffers SN, Oak SW (2007). Occurrence and distribution of *Phytophthora pseudosyringae* in forest streams of North Carolina. *Phytopathology* **97**, S49.

Hwang J, Oak SW, Jeffers SN (2008). Detecting *Phytophthora ramorum* and other species of *Phytophthora* in streams in natural ecosystems using baiting and filtration methods.

Proceedings of the 3rd Sudden Oak Death Science Symposium, March 5-9 2007, Santa Rosa, California, USA, 55-58.

Hwang J, Oak SW, Jeffers SN (2008a). Variation in population density and diversity of Phytophthora species in streams within a forest watershed. *Phytopathology* **98**, S70

Jung T, Nechwatal J, Cooke DEL, Hartmann G, Blaschke M, Oβwald WF, Duncan JM, Delatour C (2003). *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research*, **107**, 772-789.

Jung T (2005). Wurzel-und Stammschäden an Buchen (*Fagus sylvatica* L.) durch bodenbürtige *Phytophthora*-Arten in Bayern. Schadbilder, Verbreitung und Standortbezüge. Forst und Holz. **60**, 131-139.

Jung T, Hudler GW, Jensen-Tracy SL, Griffiths HM, Fleischmann F, Osswald W (2005). Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. *Mycologist*, **19**, 159-166.

Linzer R, Rizzo D, Cacciola S, Garbelotto M (2005). AFLP analysis of *Phytophthora nemorosa* and *P. pseudosyringae* genetic structure in North America and Europe. *Phytopathology* **95**, S62.

Linzer R, Rizzo D, Cacciola S, Garbelotto M (2005a). AFLP analysis of *Phytophthora nemorosa* and *P. pseudosyringae* genetic structure in North America and Europe. Proceedings of the 2nd Sudden Oak Death Science Symposium, January 18-21 2005, Monterey, California, USA, 148-151.

Linzer R, Rizzo D, Cacciola S, Garbelotto M (2009). AFLPs detect low genetic diversity for *Phytophthora nemorosa* and *P. pseudosyringae* in the US and Europe. *Mycological Research* **113**, 298-307.

Martin FN, Tooley PW (2003). Phylogenetic relationships of *Phytophthora ramorum*, *P. nemorosa*, and *P. pseudosyringae*, three species recovered from areas in California with sudden oak death. *Mycological Research*, **107**, 1379-1391.

Martin FN, Tooley PW, Blomquist C (2004). Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. *Phytopathology*, **94**, 621-631.

Motta E, Annesi T, Pane A, Cooke DEL, Cacciola SO (2003). A new *Phytophthora* sp. causing a basal canker on beech in Italy. *Plant Disease*, **87**, 1055.

Murphy SK, Rizzo DM (2005). Incidence of *Phytophthora ramorum*, *P. nemorosa* and *P. pseudosyringae* in three coastal California forest communities. Proceedings of the 2nd Sudden Oak Death Science Symposium, January 18-21 2005, Monterey, California, USA, 69-71.

Murphy SK, Wickland AC, Lynch SC, Jensen CE, Maloney PE, Rizzo DM (2007). Ecological niche patterns of three exotic forest pathogens in California coastal forest communities. Abstract COS 6-9. ESA/SER Joint meeting, August 5-10 2007, San Jose, California, USA.

Murphy SK, Wickland AC, Lynch SC, Jensen CE, Maloney PE, Rizzo DM (2008). Distribution of *Phytophthora ramorum*, *P. nemorosa*, and *P. pseudosyringae* in native

coastal California forest communities. Proceedings of the 3rd Sudden Oak Death Science Symposium, March 5-9 2007, Santa Rosa, California, USA, 51-54.

Pintos Varela C, Mansilla Vázquez JP, Aguín Casal O, Rial Martínez C (2007). First report of *Phytophthora pseudosyringae* on chestnut nursery stock in Spain. *Plant Disease*, **91**, 1517.

Reeser PW, Hansen EM, Hesse C, Rizzo DM, Sutton WC (2006). Estimating diversity of *Phytophthora* in forest soils and streams in southwest Oregon and northwest California. *Phytopathology*, **96**, S96.

Reeser PW, Sutton W, Hansen EM (2008). *Phytophthora* species causing tanoak stem cankers in southwestern Oregon. *Plant Disease*, **92**, 1252.

Reeser PW, Sutton W, Hansen EM, Remigi P, Adams GC (2011) (*in press*). *Phytophthora* species in streams. *Mycologia*, **103**, 22-35.

Rizzo DM, Fichtner E (2007). Phytophthoras in forests and natural ecosystems of the Americas. Proceedings of the 4th IUFRO Working Party SO7.02.09 Meeting, *Phytophthoras* in Forests and Natural Ecosytems, August 26-31, Monterey, California, USA, 35-44.

Sansford C (2009). *Phytophthora pseudosyringae* – first findings on bilberry in the UK. <u>http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/phytophthoraPseudosyringae0609.pdf</u>

Sansford C (2011). Rapid assessment of the need for a detailed Pest Risk Analysis for *Phytophthora pseudosyringae*. April 18th 2011. http://thefera.org.uk/plants/plantHealth/pestsDiseases/documents/phytophthoraPseudosyrin gae.pdf

Schena L, Duncan JM, Cooke DEL (2008). Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology*, **57**, 64-75.

Scibetta S (2007). A molecular method to assess *Phytophthora* diversity in natural and semi-natural ecosystems. *Journal of Plant Pathology*, **89**, S4.

Tooley PW, Martin FN, Carras MM, Frederick RD (2006). Real-time fluorescent polymerase chain reaction detection of *Phytophthora ramorum* and *Phytophthora pseudosyringae* using mitochondrial gene regions. *Phytopathology*, **96**, 336-345.

Turner, J.A., Jennings, P., Thorp, G.L., Taylor, M.C., Kitchingman, L (2009). Studies to evaluate risks posed by *Phytophthora kernoviae* and *Phytophthora ramorum* to UK heathlands. Defra Project code PHE/2467 (PROV7). http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/research/vacciniumSiteStudy.pdf

Wickland AC, Rizzo DM (2005). Ecology of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in mixed-evergreen forests. Proceedings of the 2nd Sudden Oak Death Science Symposium, January 18-21 2005, Monterey, California, USA, 73-75.

Wickland AC, Jensen CE, Rizzo DM (2008). Geographic distribution, disease symptoms and pathogenicity of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in California, USA. *Forest Pathology*, **38**, 288-298.

Annex 1. Natural hosts of *Phytophthora pseudosyringae* including isolation from rhizosphere soil

Host		Family	Symptom or location of detection	Location	Date sample taken	Reference	
Scientific name	Common name	-					
Acer macrophyllum	Big leaf maple	Aceraceae	Foliage	California	<u>></u> 2000	Wickland <i>et al.</i> , 2008	
Alnus glutinosa	Alder	Betulaceae	Collar rot Rhizosphere	Germany	1999, 2000 2001	Jung <i>et al</i> ., 2003	
Arctostaphylos sp.	Manzanita	Ericaceae	soil ?Leaf/twig infection	California	-	Martin and Tooley, 2003; Martin <i>et al.</i> , 2004; Tooley <i>et al.</i> , 2006	
			Foliage	California	<u>></u> 2000	Wickland <i>et al.</i> , 2008	
Castanea sativa	Sweet chestnut	Fagaceae	Necrosis of inner bark, cambium. Reddish sunken lesions on bark surface – stem base and higher.	Spain (nursery stock)	2006	Pintos Varela <i>et al.</i> , 2007	
Carpinus betulus	Hornbeam	Betulaceae	Stem cankers and root flare lesions	UK (south Wales)	2005	Denman <i>et al.</i> , 2007	
Fagus sylvatica	European beech	Fagaceae	Stem cankers and root flare lesions	UK (south Wales)	2005	Denman <i>et al</i> ., 2007	
			Necrosis of fine root	Germany	1996	Jung <i>et al.</i> , 2003	
			Collar rot	Germany	1997, 2001		
			-	Germany	1997	Linzer <i>et al.</i> , 2009	
			Collar rot	Germany	2003- 2004	Jung 2005; Jung <i>et</i> <i>al.</i> , 2005	
			Cankers (stem base), isolated from roots	Germany	Unknow n	Hartmann & Blank, 1998 (in Fleischmann <i>et al</i> ., 2004)	
			Bleeding canker (base of trunk)	Italy	2001	Motta <i>et al.</i> , 2003	
			Bleeding canker (base of trunk)	Italy	2003	Cacciola <i>et al.,</i> 2005.	
			-	Italy	2001, 2003, 2004	Linzer <i>et a</i> l., 2009	
Lithocarpus densiflorus	Tanoak	Fagaceae	Bleeding cankers	California	-	Reeser <i>et al.</i> , 2008; Wickland <i>et al.</i> , 2008	
Malus pumila	Apple	Rosaceae	-	Italy	-	Schena <i>et al</i> ., 2008	

Nothofagus obliqua	Roble beech	Fagaceae	Bleeding cankers	UK (Cornwall)	2009	Webber J, personal communication, UK,
Nothofagus alpina (formerly N. procera. Syn. N. nervosa)	Rauli	Fagaceae	Bleeding cankers	UK (Cornwall)	2010	Webber J, personal communication, UK,
Pieris floribunda	Pieris	Ericaceae	Unknown	UK (Scotland)	2004	Schlenzig A, personal communication, UK, 2010
Quercus agrifolia	Coast live oak	Fagaceae	Cankers	California	-	Wickland & Rizzo, 2005
			Cankers	California	<u>></u> 2000	Wickland <i>et al.</i> , 2008
			-	California	2001, 2002	Linzer <i>et al.</i> , 2009
Quercus cerris	Turkey oak	Fagaceae	Rhizosphere soil	Italy	1998	Jung <i>et al.</i> , 2003
Quercus robur	English oak	Fagaceae	Rhizosphere soil	Germany	1997, 1998	Jung <i>et al</i> ., 2003
				France	1998, 1999, 2000	
			Rhizosphere soil	Germany	1997	Linzer <i>et al.</i> , 2009
Umbellularia californica	Californian bay laurel	Lauraceae	?leaf/twig infection	California	-	Martin and Tooley, 2003; Martin <i>et al.</i> , 2004; Tooley <i>et al.</i> , 2006; Wickland & Rizzo, 2005
			Leaf necrosis	California	<u>></u> 2000	Wickland <i>et al.</i> , 2008
Vaccinium myrtillus	Bilberry	Ericaceae	Stem necrosis and dieback	UK (Staffordshire, Cornwall)	2009	Beales et al., 2010

Annex 2. Results	of inoculation	experiments	with Phytophthora	a pseudosyringae

Isolate source	Experimental host	Common name	Family of experimental host	Plant material	Inoculation method	Resulting symptom	Reference
Castanea sativa Castanea sativa	Sweet chestnut		Seedlings Detached leaves	Wound inoculation of stem with agar plug	Red, sunken bark, extended stem lesions	Pintos Varela <i>et al.</i> , 2007	
					Zoospore dip – wounded and unwounded leaves	Foliar lesions	
Fagus sylvatica Quercus sp.	Fagus sylvatica	European beech	Fagaceae	Containerised saplings	Soil inoculation	Root rot, reduction in fine root length/ no. of root tips	Jung <i>et al.</i> , 2003
	Quercus petraea						
	Quercus robur						
Fagus sylvatica	Fagus sylvatica	European beech	Fagaceae	Containerised saplings	Soil inoculation	Reduction in fine root length/ no. of root tips	Fleischmann et al., 2004
Fagus sylvatica	Fagus sylvatica	European beech	Fagaceae	Saplings	-	-	Cacciola <i>et</i> <i>al</i> ., 2005a
Fagus sylvatica (Wales) (x 2) Carpinus betula (Wales) Quercus robur (Germany)	Fagus sylvatica	European beech	Fagaceae	Wounded logs	Agar plug	Lesions on inner bark; size of lesion greatest with beech isolates (i.e. more aggressive than those caused by <i>C. betula</i> and <i>Q. robur</i> isolates)	Denman <i>et</i> <i>al.</i> , 2007
Various	llex aquifolium	English holly	Aquifoliaceae	Wounded detached leaves	Floated in soil extract water containing mycelia plug	Oak and beech isolates caused necrosis in wounded and unwounded areas; alder plus one beech isolate non- pathogenic	Jung <i>et al.</i> , 2003
Umbellularia californica	llex aquifolium	English holly	Aquifoliaceae	Detached leaves	Agar plug	Leaf lesions	Martin and Tooley, 2003