

## SCIENTIFIC OPINION

### Scientific Opinion on the pest categorisation of *Cryphonectria parasitica* (Murrill) Barr<sup>1</sup>

EFSA Panel on Plant Health (PLH)<sup>2, 3</sup>

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#### ABSTRACT

The European Commission requested the EFSA Panel on Plant Health to perform a pest categorisation of *Cryphonectria parasitica* (Murrill) Barr, the fungal pathogen responsible for chestnut blight, a highly destructive disease that kills trees through bark cankers. The pathogen is listed in Annex IIAII of Directive 2000/29/EC. Its identity is clearly defined as *C. parasitica* (Murrill) Barr and methods exist for its discriminative detection. Several hosts are known, but the main hosts are species of *Castanea* and *Quercus*, particularly *C. sativa* and *Q. petraea*. These two host species are present in all the EU Member States and the disease has been recorded in most parts of the risk assessment area. *C. parasitica* is absent in Denmark, Estonia, Finland, Ireland, Malta, Iceland and Norway. No information is available on the presence of the pathogen in Latvia, Lithuania or Luxembourg. In the Czech Republic and Poland, *C. parasitica* has been eradicated. There are no recognised ecological or climatic factors limiting the potential establishment of the pathogen in the EU Member States where the pathogen is not known to occur. The pathogen can spread by propagules (mainly conidia, but also ascospores and mycelium) that are dispersed by wind, rain or vectors, as well as via the movement of infected or contaminated host plants for planting and bark, particularly asymptomatic ones. Control methods used against *C. parasitica* include exclusion and eradication, chemical control, host genetic resistance and biological control (hypovirulence). The most successful control methods of *C. parasitica* in the EU are exclusion and eradication, and hypovirulence. Potential consequences of the damage caused by *C. parasitica* include yield losses of fruit and wood, reduction in biodiversity and habitat loss for associated organisms.

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#### KEY WORDS

chestnut, *Castanea*, chestnut blight, distribution, European Union, impacts, regulated non-quarantine pest

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p. 1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

The Commission is currently carrying out a revision of the regulatory status of organisms listed in the Annexes of Directive 2000/29/EC. This revision targets mainly organisms which are already locally present in the EU territory and that in many cases are regulated in the EU since a long time. Therefore it is considered to be appropriate to evaluate whether these organisms still deserve to remain regulated under Council Directive 2000/29/EC, or whether, if appropriate, they should be regulated in the context of the marketing of plant propagation material, or be deregulated. The revision of the regulatory status of these organisms is also in line with the outcome of the recent evaluation of the EU Plant Health Regime, which called for a modernisation of the system through more focus on prevention and better risk targeting (prioritisation).

In order to carry out this evaluation, a recent pest risk analysis is needed which takes into account the latest scientific and technical knowledge on these organisms, including data on their agronomic and environmental impact, as well as their present distribution in the EU territory. In this context, EFSA has already been asked to prepare risk assessments for some organisms listed in Annex II AII. The current request concerns 23 additional organisms listed in Annex II, Part A, Section II as well as five organisms listed in Annex I, Part A, Section I, one listed in Annex I, Part A, Section II and nine organisms listed in Annex II, Part A, Section I of Council Directive 2000/29/EC. The organisms in question are the following:

Organisms listed in Annex II, Part A, Section II:

- *Ditylenchus destructor* Thome
- *Circulifer haematoceps*
- *Circulifer tenellus*
- *Helicoverpa armigera* (Hübner)
- *Radopholus similis* (Cobb) Thome (could be addressed together with the HAI organism *Radopholus citrophilus* Huettel Dickson and Kaplan)
- *Paysandisia archon* (Burmeister)
- *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al.*
- *Erwinia amylovora* (Burr.) Winsl. *et al.* (also listed in Annex IIB)
- *Pseudomonas syringae* pv. *persicae* (Prunier *et al.*) Young *et al.*
- *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye
- *Xanthomonas campestris* pv. *pruni* (Smith) Dye
- *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*
- *Ceratocystis fimbriata* f. sp. *platani* Walter (also listed in Annex IIB)
- *Cryphonectria parasitica* (Murrill) Barr (also listed in Annex IIB)
- *Phoma tracheiphila* (Petri) Kantschaveli and Gikashvili
- *Verticillium albo-atrum* Reinke and Berthold
- *Verticillium dahliae* Klebahn
- Beet leaf curl virus
- Citrus tristeza virus (European isolates) (also listed in Annex IIB)
- Grapevine flavescence dorée MLO (also listed in Annex IIB)

- Potato stolbur mycoplasma
- *Spiroplasma citri* Saglio *et al.*
- Tomato yellow leaf curl virus

Organisms listed in Annex I, Part A, Section I:

- *Rhagoletis cingulata* (Loew)
- *Rhagoletis ribicola* Doane
- Strawberry vein banding virus
- Strawberry latent C virus
- Elm phloem necrosis mycoplasma

Organisms listed in Annex I, Part A, Section II:

- *Spodoptera littoralis* (Boisd.)

Organisms listed in Annex II, Part A, Section I:

- *Aculops fuchsiae* Keifer
- *Aonidiella citrina* Coquillett
- Prunus necrotic ringspot virus
- Cherry leafroll virus
- *Radopholus citrophilus* Huettel Dickson and Kaplan (could be addressed together with IIAII organism *Radopholus similis* (Cobb) Thome)
- *Scirtothrips dorsalis* Hendel
- *Atropellis* spp.
- *Eotetranychus lewisi* McGregor
- *Diaporthe vaccinii* Shear.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a pest risk assessment of *Ditylenchus destructor* Thome, *Circulifer haematoceps*, *Circulifer tenellus*, *Helicoverpa armigera* (Hübner), *Radopholus similis* (Cobb) Thome, *Paysandisia archon* (Burmeister), *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al.*, *Erwinia amylovora* (Burr.) Winsl. *et al.*, *Pseudomonas syringae* pv. *persicae* (Prunier *et al.*) Young *et al.*, *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, *Xanthomonas campestris* pv. *pruni* (Smith) Dye, *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*, *Ceratocystis fimbriata* f. sp. *platani* Walter, *Cryphonectria parasitica* (Murrill) Barr, *Phoma tracheiphila* (Petri) Kantschaveli and Gikashvili, *Verticillium albo-atrum* Reinke and Berthold, *Verticillium dahliae* Klebahn, Beet leaf curl virus, Citrus tristeza virus (European isolates), Grapevine flavescence dorée MLO, Potato stolbur mycoplasma, *Spiroplasma citri* Saglio *et al.*, Tomato yellow leaf curl virus, *Rhagoletis cingulata* (Loew), *Rhagoletis ribicola* Doane, Strawberry vein banding virus, Strawberry latent C virus, Elm phloem necrosis mycoplasma, *Spodoptera littoralis* (Boisd.), *Aculops fuchsiae* Keifer, *Aonidiella citrina* Coquillett, Prunus necrotic ringspot virus, Cherry leafroll virus, *Radopholus citrophilus* Huettel Dickson and Kaplan (to address with the IIAII *Radopholus similis* (Cobb) Thome), *Scirtothrips dorsalis* Hendel, *Atropellis* spp., *Eotetranychus lewisi* McGregor and *Diaporthe vaccinii* Shear., for the EU territory.

In line with the experience gained with the previous two batches of pest risk assessments of organisms listed in Annex II, Part A, Section II, requested to EFSA, and in order to further streamline the preparation of risk assessments for regulated pests, the work should be split in two stages, each with a specific output. EFSA is requested to prepare and deliver first a pest categorisation for each of these 38 regulated pests (step 1). Upon receipt and analysis of this output, the Commission will inform EFSA for which organisms it is necessary to complete the pest risk assessment, to identify risk reduction options and to provide an assessment of the effectiveness of current EU phytosanitary requirements (step 2). *Clavibacter michiganensis* spp. *michiganensis* (Smith) Davis *et al.* and

*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, from the second batch of risk assessment requests for Annex IIAII organisms requested to EFSA (ARES(2012)880155), could be used as pilot cases for this approach, given that the working group for the preparation of their pest risk assessments has been constituted and it is currently dealing with the step 1 “pest categorisation”. This proposed modification of previous request would allow a rapid delivery by EFSA by May 2014 of the first two outputs for step 1 “pest categorisation”, that could be used as pilot case for this request and obtain a prompt feedback on its fitness for purpose from the risk manager’s point of view.

As indicated in previous requests of risk assessments for regulated pests, in order to target its level of detail to the needs of the risk manager, and thereby to rationalise the resources used for their preparation and to speed up their delivery, for the preparation of the pest categorisations EFSA is requested, in order to define the potential for establishment, spread and impact in the risk assessment area, to concentrate in particular on the analysis of the present distribution of the organism in comparison with the distribution of the main hosts and on the analysis of the observed impacts of the organism in the risk assessment area.

## ASSESSMENT

### 1. Introduction

#### 1.1. Purpose

This document presents a pest categorisation prepared by the EFSA Scientific Panel on Plant Health (hereinafter referred to as the Panel) for the species *Cryphonectria parasitica* (Murrill) Barr in response to a request from the European Commission.

#### 1.2. Scope

This pest categorisation is for *Cryphonectria parasitica*, which was previously named *Endothia parasitica*. The risk assessment area is the territory of the European Union (hereinafter referred to as the EU) with 28 Member States (hereinafter referred to as MSs), restricted to the area of application of Council Directive 2000/29/EC.

### 2. Methodology and data

#### 2.1. Methodology

The Panel performed the pest categorisation for *Cryphonectria parasitica* (Murrill) Barr, following guiding principles and steps presented in the EFSA Guidance on the harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in the International Standards for Phytosanitary Measures (ISPM) 11 (FAO, 2013) and ISPM 21 (FAO, 2004).

In accordance with the harmonised framework for pest risk assessment in the EU (EFSA PLH Panel, 2010), this work was initiated as a result of the review or revision of phytosanitary policies and priorities. As explained in the background of the European Commission request, the objective of this mandate is to provide updated scientific advice to the European risk managers to take into consideration when evaluating whether those organisms listed in the Annexes of Council Directive 2000/29/EC deserve to remain regulated under Council Directive 2000/29/EC, or whether they should be regulated in the context of the marketing of plant propagation material, or should be deregulated. Therefore, to facilitate the decision-making process, in the conclusions of the pest categorisation, the Panel addresses explicitly each criterion for a quarantine pest in accordance with ISPM 11 (FAO, 2013) but also for a regulated non-quarantine pest (RNQP) in accordance with ISPM 21 (FAO, 2004) and includes additional information required as per the specific terms of reference received by the European Commission. In addition, for each conclusion the Panel provides a short description of its associated uncertainty.

Table 1 presents the ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria on which the Panel bases its conclusions. It should be noted that the Panel's conclusions are formulated respecting its remit and particularly with regards to the principle of separation between risk assessment and risk management (EFSA founding regulation<sup>4</sup>); therefore, instead of determining whether the pest is likely to have an unacceptable impact, the Panel will present a summary of the observed pest impacts. Economic impacts are expressed in terms of yield and quality losses and not in monetary terms, in agreement with EFSA Guidance on the harmonised framework for pest risk assessment (EFSA PLH Panel, 2010).

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<sup>4</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31/1, 1.2.2002, p. 1–24.

**Table 1:** International Standards for Phytosanitary Measures (ISPM) 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria under evaluation

<b>Pest categorisation criteria</b>	<b>ISPM 11 for being a potential quarantine pest</b>	<b>ISPM 21 for being a potential regulated non-quarantine pest</b>
<b>Identity of the pest</b>	The identity of the pest should be clearly defined to ensure that the assessment is being performed on a distinct organism and that biological and other information used in the assessment is relevant to the organism in question. If this is not possible because the causal agent of particular symptoms has not yet been fully identified, then it should have been shown to produce consistent symptoms and to be transmissible	The identity of the pest is clearly defined
<b>Presence or absence in the PRA area</b>	The pest should be <b>absent from all or a defined part of the PRA area</b>	The pest is <b>present</b> in the PRA area
<b>Regulatory status</b>	If the pest is present but not widely distributed in the PRA area, it should be under official control or expected to be under official control in the near future	The pest is under official control (or being considered for official control) in the PRA area with respect to the specified plants for planting
<b>Potential for establishment and spread in PRA area</b>	The PRA area should have ecological/climatic conditions including those in protected conditions suitable for the establishment and spread of the pest and, where relevant, host species (or near relatives), alternative hosts and vectors should be present in the PRA area	–
<b>Association of the pest with the plants for planting and the effect on their intended use</b>	–	Plants for planting are a pathway for introduction and spread of this pest
<b>Potential for consequences (including environmental consequences) in the PRA area</b>	There should be clear indications that the pest is likely to have an unacceptable economic impact (including environmental impact) in the PRA area	–
<b>Indication of impact(s) of the pest on the intended use of the plants for planting</b>	–	The pest may cause unacceptable economic impact on the intended use of the plants for planting
<b>Conclusion</b>	If it has been determined that the pest has the potential to be a quarantine pest, the PRA process should continue. If a pest does not fulfil all of the criteria for a quarantine pest, the PRA process for that pest may stop. In the absence of sufficient information, the uncertainties should be identified and the PRA process should continue	If a pest does not fulfil all the criteria for a regulated non-quarantine pest, the PRA process may stop

In addition, in order to reply to the specific questions listed in the terms of reference, three issues are specifically discussed only for pests already present in the EU: the analysis of the present EU distribution of the organism in comparison with the EU distribution of the main hosts; the analysis of



the observed impact of the organism in the EU; and the pest control and cultural measures currently implemented in the EU.

The Panel will not indicate in the conclusions of the pest categorisation whether to continue the risk assessment process as it is clearly stated in the terms of reference that at the end of the pest categorisation the European Commission EC will indicate if further risk assessment work is required following its analysis of the Panel's scientific opinion.

## 2.2. Data

### 2.2.1. Literature search

An extensive literature search on *Cryphonectria parasitica* (Murrill) Barr and *Endothia parasitica* was conducted at the beginning of the mandate. Further references and information were obtained from experts and from citations within the references.

### 2.2.2. Data collection

To complement the information concerning the current situation of the pest provided by the literature and online databases on pest distribution, damage and management, the PLH Panel sent a short questionnaire on the current situation at country level based on the information available in the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Retrieval (PQR) database to the National Plant Protection Organization (NPPO) contacts of the 28 EU Member States, and of Iceland and Norway. Iceland and Norway are part of the European Free Trade Association (EFTA) and are contributing to EFSA data collection activities, as part of the agreements EFSA has with these two countries. A summary of the pest status based on EPPO PQR and MSs replies are presented in Table 3.

Information on distribution of the main host plants was obtained from the EUROSTAT database, EUFORGEN and JRC database. Relevant information was also obtained from Europhyt database, Plantwise (2014) and CABI CPC (2013).

## 3. Pest categorisation

### 3.1. Identity and biology of *Cryphonectria parasitica*

#### 3.1.1. Taxonomy

**Name:** *Cryphonectria parasitica* (Murrill) Barr.

**Synonyms:** *Endothia parasitica* (Murrill) P.J. Anderson & H.W. Anderson.

**Taxonomic position:** Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes, Diaporthales; Cryphonectriaceae; *Cryphonectria*; *Cryphonectria parasitica*.

**Common names:** The common names used in English-speaking countries are chestnut blight, blight of chestnut, canker of chestnut, blight of oak (EPPO PQR, 2014).

#### 3.1.2. Biology of *Cryphonectria parasitica*

The life cycle of *C. parasitica* is typical of a filamentous ascomycete; it is predominantly haploid and lives as hyphae to form a mycelium. The pathogen is heterothallic and sexual reproduction occurs in populations where both mating types are present; however, some homothallic individuals exist within certain populations (McGuire et al., 2004).

Ascstromata, stromatal mycelium that bear perithecia (the sexual fruiting bodies), are gregarious or single, and are pulvinate, semi-immersed in bark, orange, typically 200–350 µm high, 300–1 200 µm

and linear to globose in shape, and their necks emerge at the stromatal surface as black ostioles. Asci ( $30\text{--}60 \times 7\text{--}9 \mu\text{m}$ ) are oblong ellipsoidal to sub-clavate and contain eight hyaline ascospores, ellipsoidal to fusoid, with rounded ends and one median septum ( $7.5\text{--}9.5 \times 3.5\text{--}4.5 \mu\text{m}$ ). The ascospore dimensions, which are distinctive for the species, are  $8\text{--}9 \times 3.5\text{--}4.5 \mu\text{m}$ . Conidial locules can be found within ascostromata or as separate structures with the following characteristics: pulvinate, semi-immersed, orange, uni- to multilocular globose in shape,  $120\text{--}390 \mu\text{m}$  high and  $270\text{--}390 \mu\text{m}$ . Conidiophores are cylindrical or flask-shaped bearing at the apex conidia that are hyaline, cylindrical, aseptate ( $3.5\text{--}4 \times 1\text{--}1.5 \mu\text{m}$ ), and exuded as orange droplets or cirri (Gryzenhout et al., 2009).

The cultural morphology of *C. parasitica* varies according to the growing media used. On potato dextrose agar, cultures are orange with abundant pycnidia (Shear et al., 1917; Kobayashi, 1970). Cultures infected by *Cryphonectria hypovirus 1* (CHV-1) (Hillman and Suzuki, 2004) can be easily recognised in culture by the strong reduction in pigment production and absence of asexual structures, making them a simple diagnostic character in the laboratory (Milgroom and Cortesi, 2004).

*C. parasitica* can be found on all the species of the genus *Castanea* and on other species, particularly several oak (*Quercus*) species, and, occasionally, on other genera, e.g. *Acer*, *Fagus* and *Eucalyptus*. However, on species other than *Castanea* spp., only virulent isolates of the fungus are able to form cankers, which are occasionally lethal, but the pathogen can survive on those species as a saprobe (Roane et al., 1986; Minervini et al., 1993; Gryzenhout et al., 2009).

*C. parasitica* overwinters as stromatal mycelium, harbouring pycnidia and perithecia, in bark cankers. After cutting of chestnut trees, the pathogen can survive in cankers for more than one year and it displays considerable saprophytic activity and sporulation on the bark of recently dead chestnuts (Hepting, 1974; Prospero et al., 2006). The pathogen can also be considered an endophyte (Bisseger and Sieber, 1994); it was isolated from symptomless inoculated stems three months after inoculation (Guérin and Robin, 2003) and disease symptoms developed on symptomless imported plants after 16 months of quarantine (Cunnington and Pascoe, 2003). On fruits, the fungus is associated with only the nutshell and apparently does not affect seed germination or seedling growth (Jaynes and Depalma, 1984).

*C. parasitica* infects the host through wounds of woody tissue or growth cracks, and quickly colonises the bark to the depth of the cambium, thus forming the bark canker. Dying tissues might also function as entry points for the pathogen (Roane et al., 1986; Prospero et al., 2006). Hail storms (Cortesi, unpublished data), forest fires and drought conditions can enhance the occurrence of infections (Prospero and Rigling, 2013). As the fungus continues to grow, the cankers expand, girdling and killing the trees.

Conidia are rain dispersed and germinate optimally at  $25\text{--}26 \text{ }^\circ\text{C}$ , whereas ascospores are wind dispersed and germinate optimally at  $21 \text{ }^\circ\text{C}$  (Fulton, 1912). Following infection, the pathogen colonises bark and cambium with its typical fan-shaped, buff-coloured mycelium, which can be easily observed at the canker margin beneath the bark. The mycelium grows intercellularly. Wound periderm formation inhibits infections, and wounds older than four days are resistant to infection (Bazzigher and Schmid, 1962). In susceptible host species, wound periderm formation is inhibited by mycelium growth, which kills host tissue through the production of toxins, cell wall-degrading enzymes and oxalic acid (Havir and Anagnostakis, 1983; Hebard et al., 1984; Roane et al., 1986).

When growing in bark, the fungus invades and forms a compact mass of mycelium, in which asexual and sexual spores are produced. The mycelium of *C. parasitica* grows in a broad range of temperatures; its growth rate changes very little at temperatures between  $21$  and  $32 \text{ }^\circ\text{C}$ , with minimum growth rate changes at  $4$  and  $35 \text{ }^\circ\text{C}$  (Anagnostakis and Aylor, 1984). The greatest rate of canker development occurred for inoculations made in the spring and summer (Guérin and Robin, 2003).

Under field conditions, infections by conidia in May and July resulted in the greatest disease incidence, whereas inoculations in the autumn and winter did not reveal any visible disease symptoms (Guérin and Robin, 2003). Ascospore discharge is associated with rainfall and temperatures above 11 °C for at least three days. When cankers were incubated at a constant temperature for a week, ascospores were discharged between 15 and 25 °C, with the maximum discharge at 20 and 25 °C, whereas, at lower or higher temperatures, only a few cankers released a small number of ascospores (Guérin et al., 2001). The temporal patterns of ascospore dispersal in the field in Europe showed seasonality. Most ascospores were trapped between March and October, with a peak in May, probably due to rain events triggering the discharge of ascospores from large numbers of mature perithecia, rather than the direct effect of the daily weather conditions on ascospore production or discharge (Guérin et al., 1998). However, many factors may influence the relative importance of ascospores versus conidia and mycelia as the primary inoculum for initiating new cankers or for disease epidemics, such as the viability and pathogenicity of ascospores and the availability of susceptible host tissue and infection sites (i.e. presence of wounds), which is maximal in May, since this is when *C. sativa* growth and susceptibility to the pathogen is the highest (Bazzigher, 1981) and favourable weather conditions occur.

### 3.1.3. Intraspecific diversity

*C. parasitica* has a sexual recognition system controlled by the mating type locus. Studies of mating in natural populations in the USA have shown that this fungus has a mixed mating system with both self-fertilisation and self-incompatibility occurring in the same population with different frequencies (Marra et al., 2004). The fungus also has a vegetative (self–non-self) recognition system controlled by several vegetative incompatibility (*vic*) loci, each with two alleles, and six loci have been fully characterised (Anagnostakis, 1982; Cortesi et al., 1996; Huber and Fulbright, 1996; Cortesi and Milgroom, 1998; Cortesi et al., 1998; Milgroom and Cortesi, 1999). Individuals with different alleles at one or more *vic* loci are vegetatively incompatible, and incompatibility is expressed as localised cell death after anastomosis (Newhouse and Macdonald, 1991; Biella et al., 2002), thus preventing the exchange of nuclei and transmission of CHV-1 viruses, reducing virulence (Cortesi et al., 2001; Papazova-Anakieva et al., 2008).

Vegetative incompatibility has been used as a phenotypic marker to test for variability within and among natural populations (Cortesi et al., 1996; Cortesi and Milgroom, 1998; Milgroom and Cortesi, 1999) and as an estimate of outcrossing in natural populations of *C. parasitica* (Milgroom and Cortesi, 1999; Marra et al., 2004). In North America, ascospores are an important source of inoculum (Anagnostakis and Kranz, 1987; Milgroom and Lipari, 1991), and their wind dispersal (Heald et al., 1915) results in long-distance spread of the disease and in high population diversity (Milgroom and Cortesi, 1999). In contrast, in Europe, the overall population diversity is lower than in North America, China and Japan, and the pathogen is geographically sub-divided into sub-populations with different genetic and vegetative compatibility (*vc*) types, the diversity of which is higher in south-eastern France, northern Italy and southern Switzerland (where individuals of EU-1, EU-2 and EU-5 *vc* types dominate) than in northern France, central and southern Italy and northern Switzerland (Cortesi et al., 1996; Cortesi and Milgroom, 1998; Robin and Heiniger, 2001). In Portugal, northern Spain and south-western France, the populations are quite different from those in other countries in Europe, and are dominated by *vc* types EU-11, EU-33, EU-66 and EU-72, which gives evidence for recurring introductions of the pathogen of an origin different from those introduced in Italy (Robin et al., 2000; Montenegro et al., 2008; Robin et al., 2009) and substantial absence of long-distance spread of the disease. In Greece, southern Italy, Slovakia, the former Yugoslav Republic of Macedonia, Romania and Bulgaria, populations are dominated by the *vc* type EU-12 (Sotirovski et al., 2004; Perlerou and Diamandis, 2006; Milgroom et al., 2008; Erincik et al., 2011), whereas, in Turkey, *vc* types EU-12 and EU-1 coexist (Gurer et al., 2001; Akilli et al., 2009). In Georgia, the *C. parasitica* population has a higher diversity of *vc* types than those of neighbouring countries, with many new *vc* types unknown in Europe which have emerged locally through sexual recombination (Prospero et al., 2013).

In Europe, new and expanding *C. parasitica* populations are mainly established by just one or a few genotypes and often the mating is limited because of the absence of individuals of the other mating type or because of the skewed ratio between individuals of the opposite mating type (Hoegger et al., 2000; Gurer et al., 2001; Milgroom et al., 2008; Dutech et al., 2010). However, it is important to highlight that any new introduction of genetically different individuals in an existing population can contribute to increased population genetic diversity (Jezic, 2012; Prospero and Rigling, 2012, 2013), although, so far, in most populations in Europe, random mating has been ruled out, and, even in populations where mating is occurring, ascospores are not likely to be the primary inoculum (Milgroom and Cortesi, 1999).

### 3.1.4. Detection and identification of *Cryphonectria parasitica*

Early symptoms on *C. sativa* vary according to the age of the tree, the infected organ and the virulence of the pathogen. The site of infection turns light brown, yellowish brown or orange-red brown and as the canker grows the margin retains the colour, while the centre dies and the bark eventually cracks. Cankers girdling the stem or the branches cause death of the distal parts of the tree, and leaves wilt and typically remain hanging on the tree, while, below the canker, epicormic shoots may develop. Beneath the bark, typical fan-shaped, buff-coloured mycelium can be easily observed at the canker margin. Infections by hypovirulent strains of the pathogen can initially cause the same symptoms, but the cankers are smaller, superficial, swollen and calloused, generally without fan-shaped, buff-coloured mycelium beneath the bark. Stromatal mycelium, possibly harbouring pycnidia and perithecia, is visible in bark cankers (or under a dissecting microscope) or following incubation in a moist chamber. Two-celled ascospores are distinctive for the species if their dimensions are  $8\text{--}9 \times 3.5\text{--}4.5 \mu\text{m}$ . In contrast, conidia dimensions are not informative (EPPO, 2005; Gryzenhout et al., 2009).

Following isolation, cultural morphology of *C. parasitica* is typical if the fungus is grown on potato dextrose agar. The mycelium of virulent isolates is initially white and then turns yellow, followed by orange, and has abundant pycnidia after 10–14 days of incubation at 20–24 °C in the light (EPPO, 2005; Gryzenhout et al., 2009). Cultures of CHV-1 infected isolates remain white with or without a few scattered pycnidia. A reduction in pigment production and the absence of asexual structures are simple diagnostic characters in the laboratory for CHV-1 infected isolates (Milgroom and Cortesi, 2004).

Vegetative compatibility characterisation of single conidial pure cultures of *C. parasitica* can be done according to the method described by Cortesi et al. (1996), in which each strain is paired with the genetically characterised tester strains (EU-1 to EU-64) (Cortesi and Milgroom, 1998) and, when necessary, with additional testers strains (Robin et al., 2000; Montenegro et al., 2008; Robin et al., 2009).

DNA-based identification of pure cultures of *C. parasitica* relies on the sequence analysis of DNA regions, such as the internal transcribed spacer regions of the ribosomal DNA operon or the  $\beta$ -tubulin gene region (details can be found in Hoegger et al., 2002; Gryzenhout et al., 2009; Braganca et al., 2011).

The mating-type allele carried by each strain of *C. parasitica* at the *MAT* locus can be identified using idiomorph-specific PCR) primer pairs (details in Marra and Milgroom, 1999; McGuire et al., 2001; McGuire et al., 2004).

The CHV-1 hypoviruses can be identified using the reverse transcription (RT)-PCR restriction fragment length polymorphism method and partial sequencing of the viral genome (details in Allemann et al., 1999; Gobbin et al., 2003; Hillman and Suzuki, 2004).

### 3.1.5. Similarities to other diseases and disorders

In the bark of *C. sativa*, many saprophytes and weak pathogens can be found. Among them, *Melanconis modonia* (syn. *Coryneum modonium*), *Cryphonectria radicalis* and *Diplodinia castaneae*,

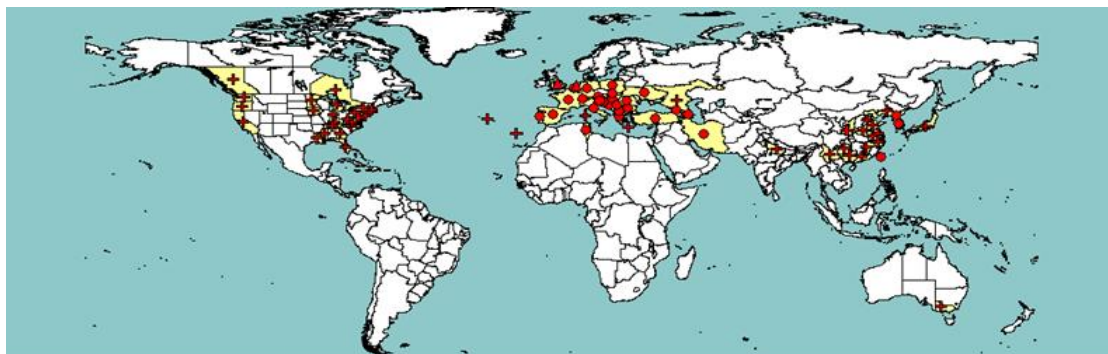
which are generally considered saprophytes or weak pathogens, can occasionally cause cankers on weakened trees. Cankers are generally smaller than those caused by *C. parasitica* and, when the fruiting bodies of the above-mentioned fungi are present, they differ significantly from those of *C. parasitica* (Bissegger and Sieber, 1994; Hoegger et al., 2002; EPPO, 2005; Gryzenhout et al., 2009; Adamcikova et al., 2013).

### 3.2. Current distribution of *Cryphonectria parasitica*

#### 3.2.1. Global distribution of *Cryphonectria parasitica*

According to the EPPO PQR (2014), *C. parasitica* occurs in:

- Europe: Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Macedonia, the Netherlands, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Ukraine;
- Asia: Azerbaijan, China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hebei, Henan, Hubei, Jiangsu, Jiangxi, Liaoning, Shaanxi, Shandong, Yunnan, Zhejiang), Democratic People's Republic of Korea, Georgia, India (Uttar Pradesh, Uttarakhand), Iran, Japan (Honshu), Republic of Korea, Taiwan, Turkey;
- Africa: Tunisia;
- North America: Canada (British Columbia, Ontario), USA (Arkansas, California, Connecticut, Florida, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Tennessee, Texas, Virginia, Washington, West Virginia, Wisconsin);
- Oceania: Australia (Victoria) (Figure 1).



**Figure 1:** Global distribution map of *Cryphonectria parasitica*, as extracted from EPPO PQR (2014), version 5.3.1, accessed on 17 June 2014. Red circles and crosses represent national and sub-national pest records, respectively

#### 3.2.2. Distribution of *Cryphonectria parasitica* in the EU

As indicated by EPPO PQR (2014) and the answers to the EFSA questionnaire received from the EU MSs, Iceland and Norway, the presence of *C. parasitica* is reported in 15 MSs (Table 2). In five MSs (Croatia, Hungary, Italy, Portugal, and Slovenia), the pathogen is present in all (or almost all) of the areas where the host plants occur; in eight MSs (Austria, Belgium, Bulgaria, France, Germany, Greece, Slovak Republic, and Spain), it has restricted distribution; and, in two MSs, it is under eradication (the Netherlands and the UK). In the Czech Republic, the pathogen was eradicated.

In the above-mentioned information sources there are no data concerning Romania. However, based on Plantwise (2014), *C. parasitica* is present in some areas of Romania (Tarcali and Radócz, 2006; Milgroom et al., 2008). Concerning Greece, past and current literature (Xenopoulos, 1982; Holevas et

al., 2000; Sotirovski et al., 2004; Perlerou and Diamandis, 2006, 2009; Milgroom et al., 2008; Tsopelas, 2008) confirms the presence of *C. parasitica* as stated in the EPPO PQR (2014).

Based on the EPPO PQR database (2014) and Plantwise (2014), *C. parasitica* is also present in the following European non-EU countries, which are more or less at the borders of the risk assessment area: Albania, Bosnia and Herzegovina, Georgia, Macedonia, Russia, Serbia, Switzerland, Turkey and Ukraine (Table 3).

**Table 2:** Current distribution of *Cryphonectria parasitica* in the 28 EU MSs, Iceland and Norway, based on the answers received via email from the NPPOs or, in absence of reply, on information from EPPO PQR database (EPPO PQR, 2014; version 5.3.1, accessed on 17 June 2014) (and other sources if relevant)

Member State	Pest status according to the responses to the EFSA questionnaire received from the NPPOs of the EU Member States	Other sources
Austria	Present, restricted distribution	
Belgium	Present, no details	Previous status was kept by the NPPO in 2007 because of a lack of survey data. This status might go back to an old publication (1924, Verplancke G. In Journal Bulletin de la Société Royale de Botanique de Belgique, 1930, XII (2nd Ser. XII), 105–107) and there is doubt about its accuracy. However, for the moment, given the situation in the neighbouring countries and the lack of specific survey data, the status is kept as before.
Bulgaria	Present, restricted distribution	
Croatia	Present, widespread	Present, widespread, in natural sweet chestnut stands. The first notice of chestnut blight in 1955 was near Opatija (Primorsko-goranska County), Croatia. From the 1950s to the 1980s, <i>C. parasitica</i> spread to almost all sweet chestnut stands. Natural hypovirulence is determined in isolates from different areas.
Cyprus	–	
Czech Republic	Absent, confirmed by survey, previous occurrences eradicated	
Denmark	Not known to occur	
Estonia	Absent, no pest records	
Finland	Absent, no pest records	
France	Present	
Germany	Present, only in some areas	

Member State	Pest status according to the responses to the EFSA questionnaire received from the NPPOs of the EU Member States	Other sources
Greece <sup>(a)</sup>	–	Present, restricted distribution
Hungary	Present: nearly in all parts of the area where the host crop grown	
Ireland	Absent, no pest record	
Italy	Widespread where <i>Castanea</i> occurs. Biological control with hypovirulent strains. Never reported on <i>Quercus</i> .	The infection of <i>Quercus</i> by <i>C. parasitica</i> (Biraghi, 1950; Turchetti et al., 1991; Dallavalle and Zambonelli, 1999).
Latvia	–	
Lithuania	–	
Luxembourg	–	
Malta	Absent, no pest records	
Poland	Absent, pest no longer present	In 2009–2013, in total, 6 640 visual inspections were carried out by the State Plant Health and Seed Inspection Service (SPHSIS) on host plants. In addition 3 490 samples were tested in the laboratory. All samples tested gave negative results.
Portugal	Present, widespread, no details for Azores and Madeira	
Romania	–	
Slovak Republic	Present only in some areas where host crop(s) are grown	
Slovenia	Present in all parts of Slovenia on <i>Castanea</i> and <i>Quercus</i>	
Spain	Present, restricted distribution	<i>C. parasitica</i> is widely spread in the north of the Iberian Peninsula in Galicia, Asturias, País Vasco, Cantabria, Navarre, Cataluña, Castilla and León, and its presence has also been registered in Andalucía. It causes significant economic losses in chestnut plantations (Aguin Casal et al., 2005; Benavides and Vázquez, 2005).
Sweden	Absent, confirmed by survey	
The Netherlands	Transient, incidental findings, under eradication	
United Kingdom	Present, under eradication (limited outbreak sites where no further evidence of the pest has been detected since 2011)	
Iceland	–	
Norway	–	

(a): When no information was made available to EFSA, the pest status in the EPPO PQR (2012) was used  
 – no information is available.

**Table 3:** Current distribution of *Cryphonectria parasitica* (Murrill) Barr in European non-EU countries, based on the EPPO PQR database (2014) (version 5.3.1, accessed on 28 July 2014) and Plantwise (2014)

Country	Pest status according to EPPO PQR (2014)	Pest status according to Plantwise (2014)
Albania		Present, no details
Bosnia and Herzegovina	Present, no details	
Georgia	Present, no details	
Macedonia	Present, no details	
Russia	Present, restricted distribution	
Serbia	Present, widespread	
Switzerland	Present, widespread	
Turkey	Present, restricted distribution	
Ukraine	Present, no details	

In the Europhyt database (accessed on September 2014) there were 14 interception records of *C. parasitica* on wood and bark from the NPPOs of Italy (12 interceptions) and Germany (2): 1 in 1998, 8 in 2000, 1 in 2001, 3 in 2002, 1 in 2003 and 1 interception record in 2010 on host plants for planting from the NPPO of Ireland (Table 4).

**Table 4:** : List of notification of *Cryphonectria parasitica* interceptions in the EU extracted from the Europhyt database (September 2014)

No	EC reference	Country of origin	Point of entry	Class of commodity	Plant	Year
1	11724	RU (Russia)	Italy	Wood and bark	<i>Castanea sativa</i>	2000
2	14317	RU	Germany	Wood and bark	<i>C. sativa</i>	2002
3	7683	RU	Italy	Wood and bark	<i>C. sativa</i>	1998
4	11725	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
5	14599	RU	Italy	Wood and bark	<i>C. sativa</i>	2001
6	11705	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
7	11726	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
8	17798	RU	Germany	Wood and bark	<i>C. sativa</i>	2003
9	11721	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
10	11736	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
11	58083	FR (France)	Ireland	Plants for planting	<i>C. sativa</i>	2010
12	11722	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
13	14231	RU	Italy	Wood and bark	<i>C. sativa</i>	2002
14	11723	RU	Italy	Wood and bark	<i>C. sativa</i>	2000
15	14232	GE (Georgia)	Italy	Wood and bark	<i>C. sativa</i>	2002

### 3.3. Regulatory status

#### 3.3.1. Council Directive 2000/29/EC

**Harmful organism:** *Cryphonectria parasitica* (Murrill) Barr

This species is a regulated harmful organism in the EU and listed in Council Directive 2000/29/EC in the Annex II as follows: Annex II, Part A, Section II, (c) Fungi, point 3 (Table 5). It is also listed in Annex II Part B for the protected zones of Czech Republic, Ireland, Sweden and United Kingdom (except the Isle of Man).



**Table 5:** *Cryphonectria parasitica* (Murrill) Barr in Council Directive 2000/29/EC.

<b>Annex II, Part A</b>	Harmful organisms whose introduction into, and spread within, all Member States shall be banned if they are present on certain plants or plant products		
<b>Section II</b>	Harmful organisms known to occur in the community and relevant for the entire community		
<b>(c) Fungi</b>	<b>Species</b>	<b>Subject of contamination</b>	
<b>3.</b>	<i>Cryphonectria parasitica</i> (Murrill) Barr	Plants of <i>Castanea</i> Mill and <i>Quercus</i> L., intended for planting, other than seeds	
<b>Part B</b>	Harmful organisms whose introduction into, and whose spread within, certain protected zones shall be banned if they are present on certain plants or plant products		
<b>(c) Fungi</b>	<b>Species</b>	<b>Subject of contamination</b>	<b>Protected zone(s)</b>
<b>0.1.</b>	<i>Cryphonectriaparasitica</i> (Murrill.) Barr.	Wood, excluding wood which is bark-free, isolated bark and plants intended for planting of <i>Castanea</i> Mill.	CZ, IRL, S, UK

**Regulated hosts for *Cryphonectria parasitica*:**

*C. parasitica* is a polyphagous pest and has many more potential hosts than those for which it is regulated in Annex IIAII (see section 3.4.1 Host range). In addition, it is important to mention that other specific commodities could also be a pathway of introduction of the pest in the risk assessment area.

Below, specific requirements of Annex III, Annex IV and Annex V of the Council Directive 2000/29/EC are presented only for the host plants and commodities regulated for *C. parasitica* in Annex IIAII (Table 6).

**Table 6:** *Cryphonectria parasitica* (Murrill) Barr host plants in Council Directive 2000/29/EC.

<b>Annex III Part A</b>	Plants, plant products and other objects the introduction of which shall be prohibited in all Member States	
	Description	Country of origin
<b>2.</b>	Plants of <i>Castanea</i> Mill., and <i>Quercus</i> L., with leaves, other than fruit and seeds	Non-European countries
<b>5.</b>	Isolated bark of <i>Castanea</i> Mill.	Third countries
<b>Annex IV Part A</b>	Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states	
<b>Section I</b>	Plants, plant products and other objects originating outside the community	
<b>11.2</b>	Plants of <i>Castanea</i> Mill. and <i>Quercus</i> L., intended for planting, other than seeds	Without prejudice to the provisions applicable to the plants listed in Annex III(A)(2) and IV(A)(I)(11.1), official statement that: (a) the plants originate in areas known to be

		free from <i>Cryphonectria parasitica</i> (Murrill) Barr; or (b) no symptoms of <i>Cryphonectria parasitica</i> (Murrill) Barr have been observed at the place of production or its immediate vicinity since the beginning of the last complete cycle of vegetation.	
<b>Annex IV, Part A</b>	Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states		
<b>Section II</b>	Plants, plant products and other objects originating in the Community		
	Plants, plant products and other objects	Special requirements	
<b>7.</b>	Plants of <i>Castanea</i> Mill. and <i>Quercus</i> L., intended for planting, other than seeds	Official statement that: (a) the plants originate in areas known to be free from <i>Cryphonectria parasitica</i> (Murrill) Barr or (b) no symptoms of <i>Cryphonectria parasitica</i> (Murrill) Barr have been observed at the place of production or in its immediate vicinity since the beginning of the last complete cycle of vegetation.	
<b>Part B</b>	Special requirements which shall be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within certain protected zones		
	Plants, plant products and other objects	Special requirements	Protected zone(s)
<b>6.3.</b>	Wood of <i>Castanea</i> Mill.	(a) The wood shall be bark-free or  (b) Official statement that the wood: (i) originates in areas known to be free from <i>Cryphonectria parasitica</i> (Murrill.) Barr. or (ii) has undergone kiln-drying to below 20 % moisture content, expressed as a percentage of dry matter, achieved through an appropriate time/temperature schedule. There shall be evidence thereof by a mark 'Kiln-dried' or 'KD' or another internationally recognised mark, put on the wood or on any wrapping in accordance with current usage.	CZ, IRL, S, UK
<b>14.9.</b>	Isolated bark of <i>Castanea</i> Mill.	Official statement that the isolated bark: (a) originates in areas known to be free from <i>Cryphonectria parasitica</i> (Murrill.) Barr. or (b) has been subjected to fumigation or other appropriate treatment against <i>Cryphonectria parasitica</i> (Murrill.) Barr. to a specification approved in accordance with the procedure laid down in Article 18.2. There shall be evidence of the fumigation by indicating on the certificates referred to in Article 13.1.(ii), the active ingredient, the minimum bark temperature, the rate (g/m <sup>3</sup> ) and the exposure time (h)	CZ, IRL, S, UK

<b>19.1</b>	Plants of <i>Castanea</i> Mill., intended for planting	Without prejudice to the provisions applicable to the plants listed in Annex III(A)(2) and IV(A)(I)(11.1), and (11.2), official statement that: (a) the plants have been grown through their life in places of production in countries where <i>Cryphonectria parasitica</i> (Murrill) Barr is known not to occur;  or (b) the plants have been grown throughout their life in an area free from <i>Cryphonectria parasitica</i> (Murrill) Barr, established by the national plant protection organisation in accordance with relevant International Standards for Phytosanitary measures  or (c) the plants have been grown throughout their life in the protected zones listed in the right-hand column	Cz, IRL, S, UK
<b>Annex V</b>	Plants, plant products and other objects which must be subject to a plant health inspection (at the place of production if originating in the Community, before being moved within the Community—in the country of origin or the consignor country, if originating outside the Community) before being permitted to enter the Community		
<b>Part A</b>	Plants, plant products and other objects originating in the Community		
<b>Section I</b>	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community and which must be accompanied by a plant passport		
<b>2</b>	Plants, plant products and other objects produced by producers whose production and sale is authorised to persons professionally engaged in plant production, other than those plants, plant products and other objects which are prepared and ready for sale to the final consumer, and for which it is ensured by the responsible official bodies of the Member States, that the production thereof is clearly separate from that of other products.		
<b>2.1.</b>	Plants intended for planting other than seeds of the genera <i>Castanea</i> Mill., <i>Quercus</i> L		
<b>Section II</b>	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for certain protected zones, and which must be accompanied by a plant passport valid for the appropriate zone when introduced into or moved within that zone		
	Without prejudice to the plants, plant products and other objects listed in Part I.		
<b>1.10.</b>	Wood within the meaning of the first subparagraph of Article 2(2), where it (a) has been obtained in whole or part from [...] — <i>Castanea</i> Mill., excluding wood which is bark-free; and (b) meets one of the following descriptions laid down in Annex I, Part two to Council Regulation (EEC) No 2658/87		
<b>1.11.</b>	Isolated bark of <i>Castanea</i> Mill, and conifers (Coniferales).		
<b>Part B</b>	Plants, plant products and other objects originating in territories, other than those territories referred to in part a		

<b>Section I</b>	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community
<b>1</b>	Plants, intended for planting, other than seeds [...].
<b>2.</b>	Parts of plants, other than fruits and seeds of: — <i>Castanea</i> Mill., [...],
<b>Section II</b>	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for certain protected zones
	Without prejudice to the plants, plant products and other objects listed in I.
<b>7.</b>	Wood within the meaning of the first subparagraph of Article 2(2), where it: (a) has been obtained in whole or part from conifers (Coniferales), excluding wood which is bark-free originating in European third countries, and <i>Castanea</i> Mill., excluding wood which is bark-free

### 3.3.2. Marketing Directives

Host plants of *C. parasitica* that are regulated in Annex IIAII of Council Directive 2000/29/EC are explicitly mentioned in the following Marketing Directives:

- Council Directive 2008/90/EC<sup>5</sup>
- Council Directive 1999/105/EC<sup>6</sup>

### 3.4. Elements to assess the potential for establishment and spread in the EU

#### 3.4.1. Host range

The detailed host range is shown in Table 7. The three main hosts of *C. parasitica* (Murrill) Barr are American sweet chestnut (*Castanea dentata*), European sweet chestnut (*C. sativa*) and Durmast oak (*Quercus petraea*) (Anderson et al., 2013; CABI CPC, 2013).

**Table 7:** Host range of *Cryphonectria parasitica* following either natural or experimental inoculations with the pathogen (see text for further details)

Host	Common name
<i>Acer</i> spp.	Maple
<i>Alnus cordata</i>	Italian alder
<i>Carpinus</i> spp.	Hornbeam
<i>Carya ovata</i>	Shagbark hickory
<i>Castanea dentata</i>	American sweet chestnut
<i>C. sativa</i>	European sweet chestnut
<i>C. mollissima</i>	Chinese chestnut
<i>C. crenata</i>	Japanese chestnut
<i>C. davidii</i>	Père David's chestnut

<sup>5</sup> Council Directive 2008/90/EC of 29 September 2008 on the marketing of fruit plant propagating material and fruit plants intended for fruit production. OJ L 267, 8.10.2008, p. 8-22.

<sup>6</sup> Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material. OJ L 11, 15.01.2000, Volume 43, p. 17-40.

Host	Common name
<i>C. henryi</i>	Henry's chestnut
<i>C. segunii</i>	Seguin's chestnut
<i>C. pumila</i>	Chinquapin
<i>Castanopsis chrysophylla</i>	Giant chinkapin
<i>Eucalyptus</i> spp.	Eucalyptus tree
<i>Fagus</i> spp.	Beech
<i>Liriodendron tulipifera</i>	Tulip tree
<i>Malus domestica</i>	Apple
<i>Ostrya carpinifolia</i>	Hop hornbeam
<i>Quercus coccinea</i>	Scarlet oak
<i>Q. ilex</i>	Holm oak
<i>Q. petraea</i>	Durmast oak
<i>Q. pubescens</i>	Downy oak
<i>Q. rubra</i>	Northern red oak
<i>Q. stellata</i>	Post oak
<i>Q. virginiana</i>	Live oak
<i>Rhus typhina</i>	Staghorn sumac

A review of the host range of *C. parasitica* (CABI CPC, 2013) identified certain *Castanea* spp. and *Quercus* spp. as the most important susceptible taxa.

The American chestnut (*C. dentata*) is considered one of the most susceptible species, and it has nearly been eradicated in central and eastern USA by *C. parasitica*, destroying what was previously a major component of hardwood forests in the region (Anagnostakis, 1987). Other North American sweet chestnut species are also affected: *C. pumila*, *C. alnifolia*, *C. ashei*, *C. floridana* and *C. paupispina*. The pathogen causes significant damage on *C. sativa*, but this species is considered to be less susceptible to *C. parasitica* than is *C. dentata*. Other non-European *Castanea* species referred to in CABI CPC (2013) were reported to have a range of susceptibilities to *C. parasitica*. The Asian species of *Castanea*, including the Chinese chestnut (*C. mollissima*), the Japanese chestnut (*C. crenata*), Père David's chestnut (*C. davidii*), Henry's chestnut (*C. henryi*) and Seguin's chestnut (*C. seguinii*), are all much less susceptible to the pathogen than the European or American sweet chestnuts, but none of these species is immune, despite having co-evolved with the pathogen.

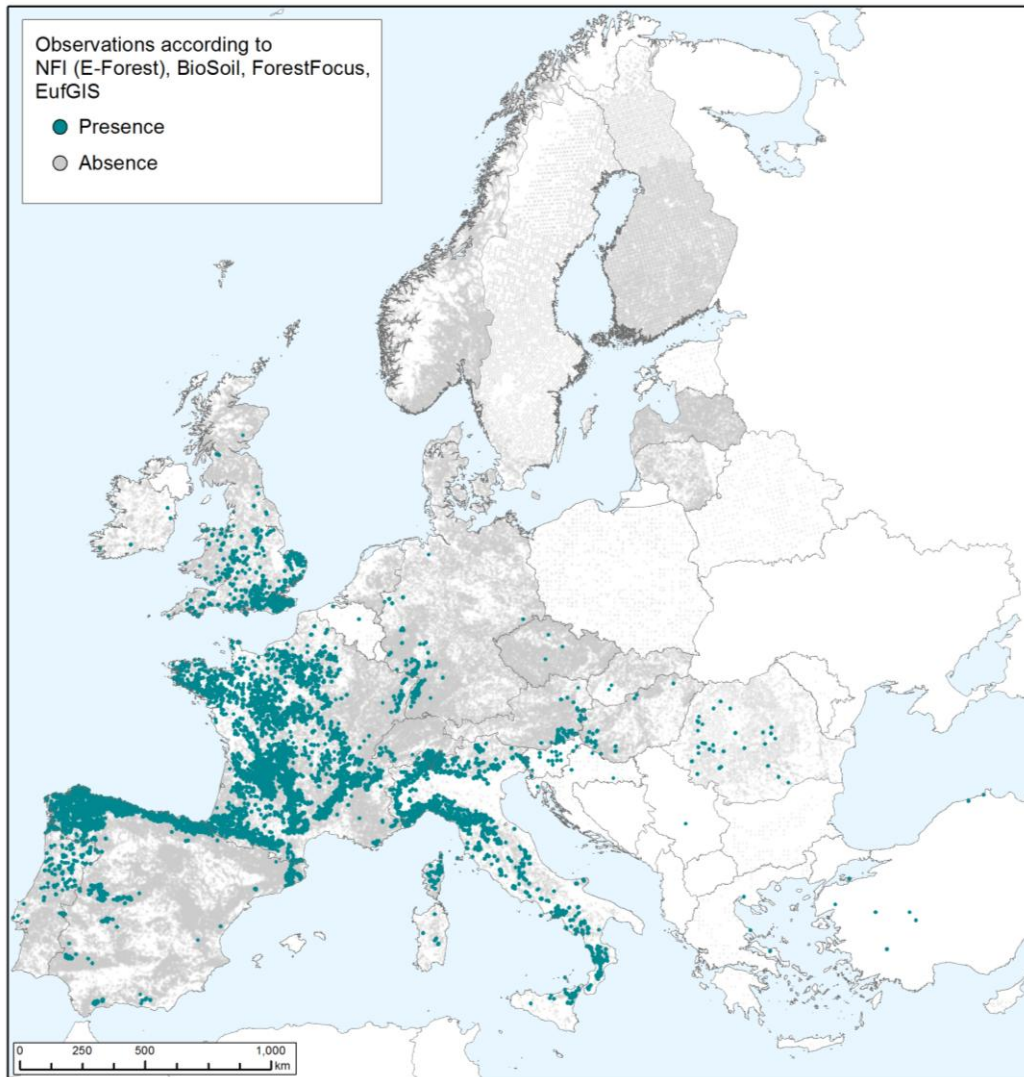
*C. parasitica* has also been reported on *Q. petraea* in Switzerland (Bissegger and Heiniger, 1991), Hungary (Szabó et al., 2009) and Slovakia (Adamcikova et al., 2010). Other oaks, such as *Q. virginiana* (live oak) and *Q. stellata* (post oak), are often affected by *C. parasitica* in North America and some trees may be killed. *Q. coccinea* (scarlet oak) is often infected by *C. parasitica* (Roane et al., 1986; Nash and Stambaugh, 1989; Torsello et al., 1994). The pathogen has also been found in nature on *Ostrya carpinifolia* and *Alnus cordata* (Turchetti et al., 1991).

*Eucalyptus* spp. are also hosts as are *Castanopsis chrysophylla*, *Q. rubra*, *Malus × domestica*, *Acer* spp., *Fagus* spp., *Rhus typhina*, *Carpinus* spp., *Carya ovata* and *Liriodendron tulipifera* (Table 7). Some of these hosts are documented based on experimental inoculations made on parts of cut branches (Shear et al., 1917; Baird, 1991) and have not been found to be infected in nature.

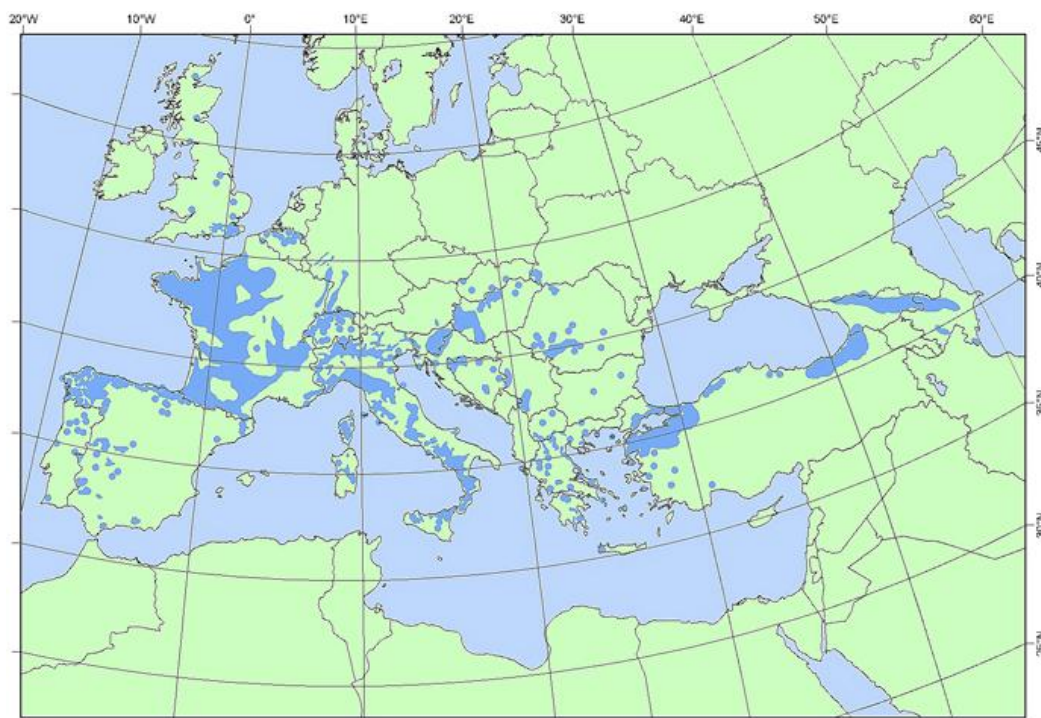
### 3.4.2. EU distribution of main host plants

Two out of the three main hosts of *C. parasitica* (*C. sativa* and *Q. petraea*) together almost cover all the risk assessment area (Figures 2, 3 and 4). For *C. sativa*, JRC and EUFORGEN distribution maps were both inserted in the opinion as they complement each other. Natural and naturalised occurrences of *C. sativa* include western, eastern, central and southern Europe. According to the two maps, *C. sativa* is not reported as present in the Czech Republic, Denmark, Estonia, Latvia, Lithuania, Poland, Finland, Sweden, Iceland and Norway. However, it cannot be excluded that, in these countries (or in some of them), small populations of *C. sativa* were undetected.

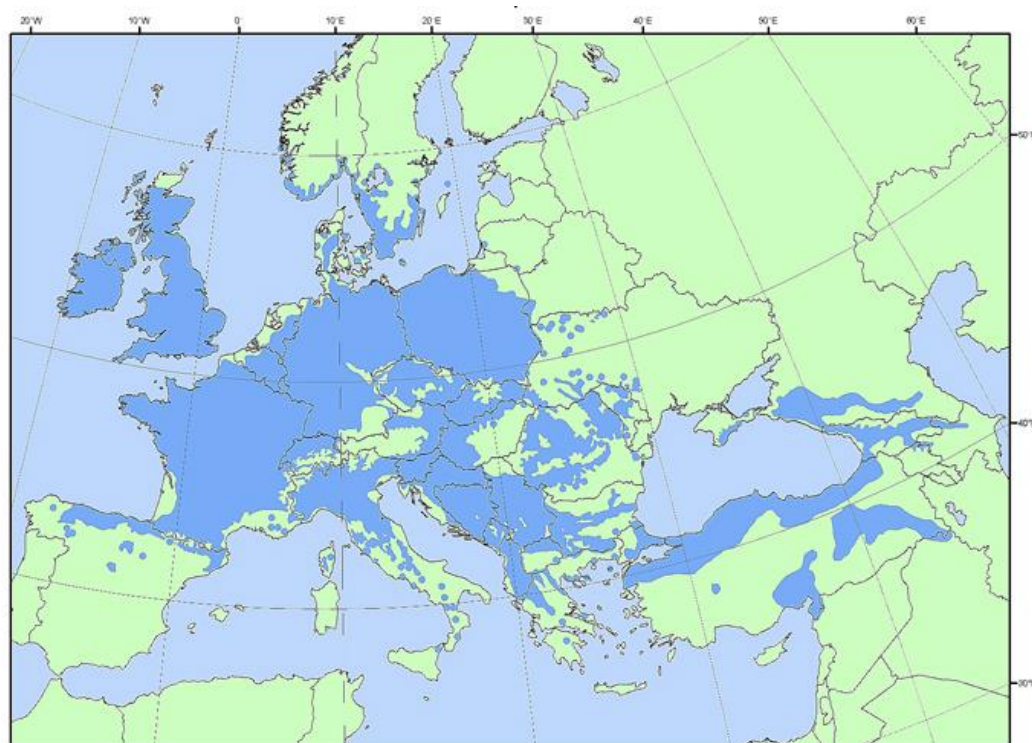
According to the EUFORGEN map, *Q. petraea* has a wider distribution than *C. sativa*. Moreover, the distribution of *Q. petraea* overlaps that of *C. sativa*, except in Portugal (Figure 4). Based on the EUFORGEN map, *Q. petraea* is present in all MSs with the exception of Estonia and Latvia, Portugal, Finland and Iceland (Figure 4).



**Figure 2:** Map of the observed distribution of the sweet chestnut *Castanea sativa* in Europe. JRC, (2014)



**Figure 3:** Distribution map of European sweet chestnut (*Castanea sativa*) in Europe (prepared by EUFORGEN, 2009). This map refers to both natural and naturalised occurrences of *C. sativa*



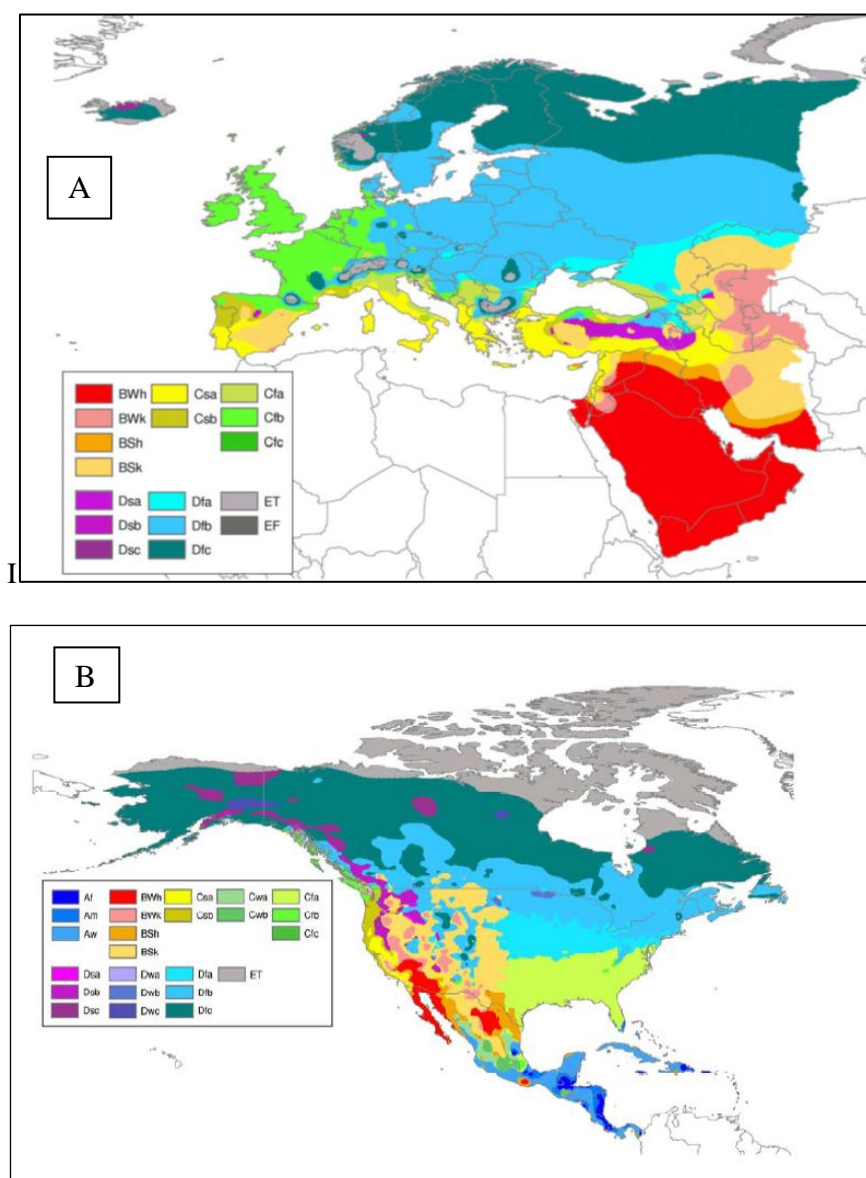
**Figure 4:** Map of sessile oak *Quercus petraea* in Europe (prepared by EUFORGEN, 2009). This map refers to both natural and naturalised occurrences of *Q. petraea*

### 3.4.3. Analysis of the potential distribution of *Cryphonectria parasitica*

*C. parasitica* is known to be present in a large part of the risk assessment area where susceptible hosts are grown.

Analysis of the distribution of *C. parasitica* based on the climate map of Europe (Peel et al., 2007) (Figure 5) leads to the conclusion that the pathogen is present:

- in all the areas of the Mediterranean basin characterised by Mediterranean climates; in 2008, *C. parasitica* was also found in Andalusia (southern Spain), one of the few areas in south-western Europe which was free of chestnut blight until then (Bascón et al., 2014);
- in central Europe and in the north of the Iberian Peninsula areas with temperate climates;
- in eastern Europe, in Austria and Switzerland, and in the Caucasian countries with cold climates;
- in parts of northern Europe and Iceland, where the climate is cold, with cold summers without dry periods (Dfc); this climate is present in parts of North America (e.g. in Canada) (Figure 5) where *C. parasitica* is known to occur (see section 3.2.1).



**Figure 5:** Köppen–Geiger climate maps of Europe and western Asia (A) and North America (B) (from Peel et al., 2007)



Based on the above data and the biology of *C. parasitica* (see section 3.1.2), the Panel concludes that there are no obvious ecological or climatic factors limiting the potential establishment and spread of the pathogen in the EU MSs where the pest is not known to occur.

#### 3.4.4. Spread capacity

##### 3.4.4.1. Spread by natural means

*C. parasitica* propagules (ascospores and conidia), which are produced in abundance on blight-susceptible chestnut trees, and, to a lesser extent, on blight-tolerant (asymptomatic) chestnut trees and oaks (CABI, 2014), can spread locally by wind and/or rain, but might also occasionally be carried by other agents, such as arthropods and birds.

Conidia are mostly dispersed short distances, thus generating new cankers more frequently within the same tree (Milgroom et al., 1991), whereas ascospores can be dispersed longer distances—up to 120 m from an inoculum source—and generate new cankers (Anagnostakis and Kranz, 1987; Guérin et al., 2001).

Conidia may also adhere to the bodies, feet, fur or feathers of insects, birds or mammals, thus spreading the disease over longer distances (Craighead, 1912; Heald and Studhalter 1914; Studhalter and Ruggles, 1915; Scharf and DePalma, 1981; Sinclair et al., 1987; Smith, 2012). Russin and Shain (1983) found *C. parasitica* to be associated with 75 insect species, most of which belong to the order Coleoptera. Insects carrying the pathogen were found up to 32 m from the nearest source of inoculum. In addition, other insects, such as the bark miner, *Spulerina simploniella*, occurring in *C. sativa* coppices in Greece and in Italy (Diamandis and Perlerou, 2005) can create several infection courts for *C. parasitica*.

Although animal vectors are not considered to play a very important role in disease transmission, it is noteworthy that chestnut blight cankers have a very large and diverse fauna. In trapping experiments in the USA, 495 arthropod species were captured on old blight cankers. A considerable number of insects spent parts of their life cycle on cankers and nearly 69 species were found to carry inoculum of *C. parasitica* (Russin et al., 1984). More recently, mites and nematodes have been reported to carry *C. parasitica* inoculum too (Nannelli et al., 1998; Griffin et al., 2009). However, to our knowledge, there are no epidemiological studies using molecular markers that unequivocally identified the relative role of the different propagules and vectors.

##### 3.4.4.2. Spread by human assistance

*C. parasitica* can spread over long distances via the movement of infected host plants for planting (rootstocks, scions, grafted plants, self-rooted plants, etc.), particularly asymptomatic (i.e. either latently infected or tolerant to infection) and infected wood with bark (CABI/EPPO, 2003), because *C. parasitica* mycelium can survive in bark, even if it has been air dried for more than one year (Prospero et al., 2006). The first hypothesis that *C. parasitica* was introduced into the USA on imported Japanese chestnut trees (*C. crenata*) (Anagnostakis, 1987) was later confirmed with the use of molecular markers and through genetic diversity analysis (Milgroom et al., 2008). It should be also noted that, by 1900, many mail-order nurseries in the USA offered Japanese chestnut trees for sale throughout the country, thus contributing to the spread of the disease (Rellou, 2002). Hunter et al. (2013) reported that the disease was first recorded in the UK in 2011 on *C. sativa* trees grown on a farm in Warwickshire (England) and originating in a French nursery.

*C. parasitica* could potentially spread via the movement of infected fruit/seeds of host plants. Fruit (i.e. nuts plus the husk) of European and American chestnut have been found to be naturally infected by the pathogen (Collins, 1913, 1915; Gravatt et al., 1935; Jaynes and DePalma, 1984); however, the infection of seedlings has not been demonstrated so far. Pruning and grafting tools or other equipment used in chestnut nurseries, orchards or forests may potentially spread the disease locally (Bragança et

al., 2009); however, no molecular evidence of new cankers originating from the use of tools carrying inoculum has been documented.

### Spread rate

In the USA, the disease was first detected in 1904 in the Bronx Zoo in New York City. Over the following 40 years, it rapidly spread throughout the range of the American chestnut (*C. dentata*) and, by 1940, 3.5 billion American chestnut trees had been killed by the pathogen (Hepting, 1974; Cock, 2003). The spread of *C. parasitica* in the USA was documented as proceeding at a rate of 30–37 km per year (Anagnostakis, 1987; Sinclair et al., 1987). In Europe, *C. parasitica* was reported for the first time in Italy in 1938, although there is a suggestion that it may have been introduced earlier in France and remained unnoticed (Guérin and Robin, 2003). Within Italy, the disease spread at almost at the same rate as in the USA (Biraghi, 1950), but the Alps and the Adriatic sea probably reduced the spread of the disease to neighbouring countries north of Italy (Heiniger and Rigling, 1994; Robin and Heiniger, 2001).

The relatively slow spread of the pathogen through Europe, as well as the sub-division of the population of the fungus in several genetically diverse sub-populations, is probably the result of the negative influence of natural barriers (i.e. mountains, sea or the discontinuity in *Castanea* distribution within and among counties) or of the pathogen's prevalent short-distance dispersal (Milgroom and Lipari, 1991; Milgroom and Cortesi, 1999), but also of the legislative measures enforced by several countries (France, Italy, Turkey and so on) soon after the appearance of the pathogen in the USA and Italy. Climate change may have contributed to the spread and establishment of the pathogen further north in France (Desprez-Loustau et al., 2007).

## 3.5. Elements to assess the potential for consequences in the EU

### 3.5.1. Potential effects of *Cryphonectria parasitica*

*C. parasitica* primarily attacks chestnut trees (*Castanea* spp.) but can also cause damage to oak trees (*Quercus* spp.) and other hardwood tree species (FAO, 2005). It occurs in natural forests and orchards, where it attacks bark tissue producing cankers that can develop as sunken regions due to tissue collapse; damage to vascular tissues produces wilts and diebacks distal to the canker (Hebard et al., 1984). Chronic infections can girdle major branches or the trunk, thus killing the tree.

In eastern USA, the disease has had a devastating impact on the American sweet chestnut (*C. dentata*) where it has killed 3.5 billion American chestnut trees, an overstorey species that dominated in the hardwood forests of eastern USA prior to the introduction of chestnut blight (Day and Monk, 1974; Hepting, 1974; Karban, 1978; Russell, 1987; Cock, 2003). The areas affected included Maine, Alabama, south-eastern Michigan, Indiana and Ontario.

The loss of these great trees had a profound impact on forest composition, nature and visual amenity of woodland in the affected regions as well as on biodiversity throughout most of the USA. In southern Appalachian forests, following the disappearance of *C. dentata* as a result of the *C. parasitica* epidemic, oak (*Quercus* spp.), red maple (*Acer rubrum*) and hickory (*Carya* spp.) became the dominant overstorey tree species (Keever, 1953; Stephenson et al., 1991). The American chestnut survives at present as mostly non-flowering, small understorey trees on which *C. parasitica* is endemic. Infected sprout clusters exhibit reductions in survival and size, particularly when in competition with other hardwoods (Griffin et al., 1991; Parker et al., 1993).

Before the chestnut blight epidemic, American chestnut also played an important role in the ecosystem: by growing in rich woods to heights of 30 m or more, and with a canopy diameter of over 30 m, the trees provided cover, shade and nutrients for most other lower tree species and shrubs (Rellou, 2002). The threats to the ecosystem created by the loss of *C. dentata* as a result of the *C. parasitica* epidemic are evident: the pathogen disrupted the habitat and food web of the abundant and diverse plant and animal life that thrived in the American chestnut's shade and depended on its food

source (nuts) and rich soil for subsistence (Rellou, 2002). Although *C. dentata* was the cornerstone tree species and made up to 25 % of eastern USA forest, the ecological impact of the loss of the species has been poorly recorded. Seven species of moth which depended on *C. dentata* became extinct (Opler, 1978; Orwig, 2002). The American chestnut tree has been replaced in forests by hickory (*Carya glabra*) and species of oak (McCormick and Platt, 1980).

Habitat loss as a result of forest clearing and damage to trees during logging operations were additional consequences of blight disease (Anonymous, 2014).

In addition to the ecological consequences, the pathogen has also had serious impacts on the timber industry, since chestnut wood—which is highly resistant to decay and rot, and has been used extensively for poles, fencing and building materials—is no longer available (Rellou, 2002).

Although Chinese chestnut (*C. mollissima*) is considered to be highly blight resistant, variation in resistance has been found among Chinese chestnut cultivars and wild chestnut trees, and *C. parasitica* is currently considered to be the most important pathogen affecting the genus *Castanea* in China (Qin et al., 2002). The pathogen is also sometimes destructive in Japan on the relatively blight-resistant Japanese chestnut (*C. crenata*) (Uchida, 1977). The pathogen has also been an important pathogen on live oak and post oak in the USA (Roane et al., 1986).

### 3.5.2. Observed impacts of *Cryphonectria parasitica* in the risk assessment area

#### 3.5.2.1. Direct pest effects

Since its introduction in 1938 into Italy (Biraghi, 1946), chestnut blight has spread throughout EU MSs and neighbouring countries (see Tables 2 and 3 for details). Disease incidences ranging from 67 to 99 % and 17 to 65 % have been reported in Italy (Amorini et al., 2001) and Portugal (Bragança et al., 2007), respectively. Dallavalle and Zambonelli (1999) reported a high incidence of chestnut blight on oak in mixed forests in southern and central Italy, where the pathogen caused severe damage on chestnut. Although the disease did not cause damage on hosts other than chestnut, those hosts could play a role in the epidemiology of the disease. Based on a survey of 185 sites in Portugal, the disease had become widespread by 2005 (Bragança et al., 2005). The first report of chestnut blight in Spain dates back to 1947 (Elorrieta Artaza, 1949). Based on a recent study in the El Bierzo region (north-western Spain), where 8 000 tonnes of nuts are produced per year, the disease incidence was 78.5 %, with the *C. parasitica* population in that region having a low incidence of hypovirulent strains (found on only 3 % of infected trees) (Tizado et al., 2012). According to the information collected through the EFSA questionnaire sent by the Spanish NPPPO, currently, the pathogen causes significant losses in chestnut plantations in north-western Spain (Aguín Casal et al., 2005; Benavides and Vázquez, 2005). In south-western Germany, where there is an increasing utilisation of *C. sativa* for high-grade timber (Mettendorph, 2007), the disease was first reported in 1992 and has been monitored since then. According to Peters et al. (2012), from 2003 to 2010, the area affected by the disease in the Rhine valley had increased six-fold from 0.5 to 3 %. The authors considered that the hot dry summer of 2003 could explain the rapid spread of the disease and the increase in symptom expression.

In general, however, the disease has been less destructive in Europe than in North America, killing fewer trees, even at the early stages of the epidemic (Biraghi, 1950; Robin and Heiniger, 2001).

The lower level of disease severity in Europe than in the USA may be the result of a higher level of blight resistance in the European chestnut than in the American chestnut, and of the natural occurrence and spread of hypovirulence within the *C. parasitica* population that actively reduces the virulence of the pathogen and that is also used for biological control of the disease (Anagnostakis, 1982; Heiniger and Rigling, 1994; Grente, 1965; Grente and Sauret, 1969; Milgroom and Cortesi, 2004). Hypovirus CHV-1 transmission to new virulent individuals of the pathogen on other host trees is mediated through hyphal fusion (anastomosis) between infected and non-infected strains. Survival of fused hyphae is mediated by vegetative compatibility (vc) and the rate of successful transmission of the virus, ranging from 0 to 100 %, is regulated by the type of *vic* alleles that are interacting between

individuals (Cortesi and Milgroom, 1998; Cortesi et al., 2001; Biella et al., 2002). Therefore, the natural spread of hypovirulence occurred to variable extents in different populations of the fungus, but, in general, it is negatively correlated with the diversity of vc types in each population of the pathogen (Liu et al., 2000; Robin and Heiniger, 2001; Milgroom and Cortesi, 2004).

Hypovirulence is widespread in most areas of the EU where the pathogen is present (Bryner et al., 2012); however, hypovirulence is still absent in some recently infested areas, such as northern France, northern Switzerland and Portugal, or its incidence is very low in some areas, for example in the chestnut fruit-producing region of El Bierzo (north-western Spain) (Tizado et al., 2012), where chestnut blight is having a negative impact on chestnut fruit and/or wood production.

If the pathogen is introduced into the chestnut-growing EU areas that are, so far, not infested (Table 2), impacts are expected to be moderate to high during the early stages of the epidemic, whether or not hypovirulent strains are introduced at the same time as the virulent one. Finally, as the success of natural spread of hypovirulence is negatively correlated with the diversity of vc types in the pathogen population, an increase in the diversity of vc types within the risk assessment area would lower the natural spread of hypovirulence and increase the disease severity and damage in forests and orchards.

#### 3.5.2.2. Indirect effects of *Cryphonectria parasitica*

Sweet chestnut (*C. sativa*) is native to the deciduous woodlands of southern Europe and is a tree species that has been intensively cultivated for centuries as a monoculture (coppices and orchards), even at the limits of its potential ecological range (Pitte, 1986; Bernetti, 1987). In the EU, sweet chestnut is grown commercially mainly for fruit (nut) and wood production. However, since the early 1950s, changes in the socio-economic structure of rural areas, as well as the spread of chestnut diseases such as chestnut blight (*C. parasitica*) and ink disease (*Phytophthora* spp.), have caused a decline in the cultivation of sweet chestnut forests in many European regions (Pitte, 1986). However, chestnut forest ecosystems still represent an important landscape component in the mountainous regions around the European Mediterranean basin and in the southern Alps, covering more than 2.2 million hectares (Conedera et al., 2004). Sweet chestnut is also a substitute for ash (*Fraxinus* spp.) on certain non-calcareous sites in lowland England and Wales. It is also widely planted for aesthetic reasons in arboreta, parks and private gardens, where it is highly valued by the public who may also forage for the nuts in autumn. As a consequence, the loss of this species would also reduce the enjoyment of these areas by the public.

Therefore, and in addition to the direct impacts, *C. parasitica* may have indirect impacts in the EU, including environmental ones. Although there are no data available on the observed indirect impacts of the pathogen in the already infected areas of the EU, the introduction of the pathogen into new EU areas is expected, at least during the first stages of the epidemic, to negatively affect the chestnut fruit and timber industries, as well as ornamental tree nurseries.

Potential environmental impacts include reductions in biodiversity, food resources and habitats for associated organisms.

### 3.6. Currently applied control methods in the EU

Control methods used against *C. parasitica* include exclusion and eradication, chemical control, host genetic resistance and biological control. One of the most successful control methods is biocontrol based on the natural spread of hypovirulence or the therapeutic application of hypovirulent *C. parasitica* strains into cankers, which will heal within one year after successful transmission of hypovirulence.

#### 3.6.1. Exclusion and eradication

The exclusion of infected plants can be one of the most important methods for preventing the introduction of the pathogen into new areas; however, it is a hard task to perform, as there is evidence of *C. parasitica* having a long latency period on plants of the genus *Castanea*. In post-entry quarantine

in Australia, symptomless plants showed symptoms 16 months after import (Cunnington and Pascoe, 2003), which indicated a long latency period (Prospero et al., 2006). In a report of CABI CPC (2013) prepared in England, it was concluded that, in theory, post-entry quarantine could be used to allow the import of host plants for planting that originated in infested areas. However, host plants would need to be maintained in quarantine for a minimum period of two years (CABI CPC, 2013) to ensure that any latent infections were detected. Plants would need to be maintained inside enclosed glasshouses or growth chambers to prevent possible escape of the pathogen. This would be expensive, regular inspections would be required and contained conditions may not be conducive to symptom development (CABI CPC, 2013). Eradication could be achieved, if infection was detected soon after import of the plants, via the destruction of infected plants and all other plants from the same lot. There would also need to be a survey of hosts in the surrounding area over a minimum period of two years to ensure that spread had not occurred prior to the identification of infection (Anderson et al., 2013).

### **3.6.2. Cultural practices**

One of the physico-mechanical methods that can be used for control is the felling of infested chestnut trees and burning of all infected wood material to prevent the spread of *C. parasitica* (Petto et al., 2013). Mechanical control (e.g. removal of infected plants and plant parts) can delay the spread of the disease, but it seldom eliminates inoculum sources (Tarcali and Radócz, 2006).

### **3.6.3. Chemical control**

Some preliminary data showed that phosphite-based fungicides, such as Agri-Fos used with the organosilicate surfactant Pentra-Bark in trunk bark wetting applications, were effective in controlling chestnut blight in American chestnut (Anderson et al., 2013). However, widespread use is not reported in practice. In general, preventative fungicide treatments against chestnut blight do not appear to be used in countries where the disease occurs (Anderson et al., 2013).

### **3.6.4. Host genetic resistance**

In the long term, breeding for resistance may help manage the disease on *C. sativa* in Europe, as promising selections of canker-resistant hybrid *C. dentata* are undergoing field trials (Thompson, 2012). The crosses between the American species *C. dentata* and the Chinese species *C. mollissima*, which carries resistance genes from the Chinese parent, have shown resistance to the pathogen while maintaining growth characteristics of the American chestnut. A back-cross breeding system was launched, with resistant Japanese and Chinese chestnut trees crossed with susceptible American trees, and with the partially resistant hybrids crossed several times with native American trees. This repeated back-crossing increased the percentage of both American genes and resistance genes in the hybrids (Anderson et al., 2013).

In some European countries, such as France, ink disease-resistant hybrids (*C. sativa* × *C. crenata*) have been used to relaunch chestnut cultivation (Salesses et al., 1993). However, some countries, such as Italy and Greece, are not using hybrids in orchards, but fruit varieties such as “Marroni” types, which have better fruit quality than the hybrids (Warmund et al., 2011) but lower resistance to chestnut blight.

### **3.6.5. Biological control**

Hypovirulence contributes to a long-term strategy to manage the disease in Europe (Heiniger and Rigling, 1994). The presence of a virus that can infect strains of *C. parasitica* has been shown to reduce disease and promote canker healing (Robin and Heiniger, 2001).

Hypovirulent strains of *C. parasitica* are less virulent and typically form superficial cankers without killing the trees. Four hypovirus species have been described from *C. parasitica*, but CHV-1 has relevance to Europe (originally found in Italy and France and has since been found throughout southern and eastern Europe). CHV-1 contains two open reading frames (ORFs) encoding multifunctional polyproteins (Ghabrial and Suzuki, 2009). Based on the variation found within both

ORFs, five different sub-types have been characterised with sequence divergence levels ranging from 11 to 19 % (Gobbin et al., 2003). Subtype I is widespread in southern and south-eastern Europe from south-eastern France to Turkey (Sotirovski et al., 2006; Robin et al., 2010; Krstin et al., 2011).

Very soon after the discovery of hypovirulent isolates, Grente and Berthelay-Sauret (1978) demonstrated that inoculation of cankers with compatible hypovirulent isolates resulted in canker healing. They suggested using a yearly release of hypovirulent strains as a treatment against chestnut blight in orchards. This biological control method has been successfully applied in southern France for 40 years. Biocontrol with CHV-1 still needs to be improved for reducing the density and impact of *C. parasitica* populations without continuous human assistance. There is also a need to expand biological control to forest plantations and coppices and to European areas where chestnut blight recently emerged but where CHV-1 has not yet established, for example northern France (De Villebonne, 1998), Portugal (Bragança et al., 2007), northern Switzerland (Hoegger et al., 2000) and part of south-western Germany (Peters et al., 2012). In these regions, chestnut blight impact is high and the development of a sustainable biological control method has been requested by stakeholders.

In each infested area, the *C. parasitica* population and hypovirus have to be characterised by experts in order to plan the release of hypovirulent strains for biological control. The movement of strains outside their own population must be avoided because of the risk of increasing vc type diversity (Heiniger and Rigling 1994; Milgroom and Cortesi, 2004).

### 3.6.6. Integrated control

An integrated control system is critically needed to stem the course of the blight fungus and reduce death of chestnut populations. The combined use of hypovirulence (through inoculation) and blight resistance (through grafting) may produce effective blight control.

### 3.7. Uncertainty

The main sources of uncertainties of this pest categorisation are listed below:

- Uncertainty on the current distribution of the pest in the risk assessment area. No information is available in the literature or in the EPPO PQR database on the pest status in Cyprus, Latvia, Lithuania, Luxembourg, Iceland or Norway, and no replies to the EFSA questionnaire were provided by the NPPOs of these countries. As climatic conditions in these countries may be suitable for the establishment and spread of *C. parasitica* (see section 3.4.3), and as host plants are present (see section 3.4.2), there is uncertainty with respect to the pest status in these countries. However, this uncertainty may only partially affect the conclusions of the pest categorisation.
- Uncertainty about the role of ascospores in disease epidemiology. Ascospores are produced on infected host plants and are dispersed by air currents; therefore, high genetic variability would be expected to exist within the European population of the pathogen. However, in many sub-populations of the pathogen, the genetic diversity is lower than expected based on the hypothesis of random mating. Therefore, it seems that ascospores do not play an important role in disease epidemiology in the EU. However, in Georgia, the *C. parasitica* population has higher vc type diversity than the diversities in neighbouring countries, with many new vc types unknown in Europe that have emerged locally through sexual recombination. The reasons for these differences are not known.
- Uncertainty on natural spread by arthropods, birds, etc. Arthropods and birds have been reported to be carriers of the pathogen's propagules, but there is no scientific evidence that propagules carried by arthropods or birds can cause new infections. The relative role of the carriers of the pathogen is unknown.
- Uncertainty on spread rate. The disease is spreading more slowly through Europe than through the USA. Many factors may contribute to this (phytosanitary measures, level of host

susceptibility, host discontinuity, presence of hypovirulence, role of ascospores in the epidemiology, etc.), but the relative contribution of each of these factors to the spread rate is not clear.

- Uncertainty on the spread of the pathogen through soil and growing media. This uncertainty exists because of a lack of data concerning the use of *Castanea* bark and residues as components of growing media.
- Uncertainty on the current status of biocontrol in the EU MSs. In the 1980s, the disease was managed in some restricted areas through the release of hypovirulent isolates.
- Uncertainty about the distribution of minor hosts. There are no data available on the distribution of the pathogen's minor hosts (see section 3.4.1) in the risk assessment area. However, as the two main host genera, *Castanea* and *Quercus*, cover most of the risk assessment area, this uncertainty does not affect the conclusions on pest categorisation.
- Uncertainty on the current impact of the disease in the risk assessment area. For both forests and orchards, numerical documented data on disease incidence and severity are seldom reported in the literature; therefore, only fragmented information is available, which cannot adequately represent the current situation in the MSs.

## CONCLUSIONS

The Panel summarises in Table 8 below its conclusions on the key elements addressed in this scientific opinion in consideration of the pest categorisation criteria defined in ISPM 11 and ISPM 21 and of the additional questions formulated in the terms of reference.

**Table 8:** The Panel's conclusions on the pest categorisation criteria defined in the International Standards for Phytosanitary Measures (ISPM) No 11 and No 21 and on the additional questions formulated in the terms of reference (ToR)

Criterion of pest categorisation	Panel's conclusions against ISPM 11 criterion	Panel's conclusions against ISPM 21 criterion	List of main uncertainties
<b>Identity of the pest</b>	<i>Is the identity of the pest clearly defined? Do clearly discriminative detection methods exist for the pest?</i>		
	<i>Cryphonectria parasitica</i> is a clear taxonomic entity and sensitive and reliable methods exist for its detection and identification, as well as for its discrimination from other related fungal plant pathogens		
<b>Absence/presence of the pest in the risk assessment area</b>	<i>Is the pest absent from all or a defined part of the risk assessment area?</i>	<i>Is the pest present in the risk assessment area?</i>	Uncertainty exists on the current distribution of the pest in the risk assessment area (see details in section 3.7)
	The pest is absent in the Czech Republic (eradicated), Denmark, Estonia, Malta, Poland (eradicated) and Finland, and no information is available for Cyprus, Latvia, Lithuania, Luxembourg, Iceland and Norway	The pest is present in the risk assessment area	
<b>Regulatory status</b>	<i>Mention in which annexes of 2000/29/EC and the marketing directives the pest and associated hosts are listed without further analysis.</i>		
	<i>C. parasitica</i> and/or its hosts are listed in Annexes II, III, IV and V of Council Directive 2000/29/EC		
<b>Potential establishment and spread</b>	<i>Does the risk assessment area have ecological conditions (including climate and those in protected conditions) suitable for the establishment and spread of the</i>	<i>Are plants for planting a pathway for introduction and spread of the pest?</i>	Uncertainty exists about the role of ascospores in disease epidemiology; on
		Plants for planting are a	

	<p><i>pest?</i></p> <p><i>And, where relevant, are host species (or near relatives), alternate hosts and vectors present in the risk assessment area?</i></p> <p>There are no obvious eco-climatic conditions limiting the establishment and spread of <i>C. parasitica</i> in the risk assessment area. The main host species are present in the risk assessment area</p>	<p>pathway for the introduction and spread of <i>C. parasitica</i></p>	<p>natural spread by arthropods, birds, etc.; on the spread rate; on the spread of the pathogen through soil and growing media; and about the distribution of minor hosts (see details in section 3.7)</p>
<p><b>Potential for consequences in the risk assessment area</b></p>	<p><i>What is the potential for consequences in the risk assessment area?</i></p> <p><i>Provide a summary of impact in terms of yield and quality losses and environmental consequences</i></p> <p><i>C. parasitica</i> primarily attacks chestnut trees (<i>Castanea</i> spp.) but can also cause damage to oak trees (<i>Quercus</i> spp.) and other hardwood tree species in forests and orchards. It produces cankers, causing wilting and diebacks, eventually killing the trees or relevant tree portions</p> <p>Disease incidence ranges from less than 1% in the recently infested areas (such as Germany) to more than 90% in the countries where the pathogen has existed for a long time (e.g. Italy, France, Switzerland, Portugal, etc.). However, there is no direct relationship between disease incidence and disease severity (and therefore between disease incidence and impact) because of several factors, including hypovirulence. In areas where the fungal population has a low diversity of vegetative compatibility (vc) types, the natural spread of hypovirulence lowers the disease severity (and impact). In these populations, the introduction of new vc types may increase vc type population diversity, therefore lowering the hypovirulence efficacy</p> <p>Potential environmental impacts of damage caused by <i>C. parasitica</i> include reductions in biodiversity, food and wood resources and</p>	<p><i>If applicable is there indication of impact(s) of the pest as a result of the intended use of the plants for planting?</i></p> <p>The pathogen was introduced into the USA in 1876 on imported Japanese chestnut trees (<i>Castanea crenata</i>). By 1900, many mail-order nurseries in the USA offered Japanese chestnut trees for sale throughout the country, thus contributing to the spread of the disease</p> <p>In eastern USA, the disease has had a devastating impact on the American sweet chestnut (<i>C. dentata</i>) since its first detection in 1904. By 1940, 3.5 billion American chestnut trees had been killed by the pathogen throughout the natural range of <i>C. dentata</i></p>	<p>Uncertainty exists on the current status of biocontrol in the EU MSs and on the current impact of the disease in the risk assessment area (see details in section 3.7)</p>



Conclusion on pest categorisation	habitat for associated organisms	Provide an overall summary of the above points	Uncertainty exists on the current distribution of the pest in the risk assessment area; about the role of ascospores in disease epidemiology; on natural spread by arthropods, birds, etc.; on the spread rate; on the spread of the pathogen through soil and growing media; about the distribution of minor hosts; on the current status of biocontrol in the EU MSs; and on the current impact of the disease in the risk assessment area (see details in section 3.7)
	<p data-bbox="421 230 807 288"><i>Provide an overall summary of the above points</i></p> <p data-bbox="421 315 807 584"><i>C. parasitica</i> is a clear taxonomic entity and reliable methods exist for its detection and identification. <i>C. parasitica</i> is reported to be present in 15 MSs and absent in six MSs; its status in Cyprus, Latvia, Lithuania, Luxembourg, Iceland and Norway is unknown because of a lack of information</p> <p data-bbox="421 600 807 808">There are no obvious eco-climatic conditions limiting the establishment and spread of <i>C. parasitica</i> in the non-infested part of the risk assessment area, where the main hosts (<i>Castanea</i> spp. and <i>Quercus</i> spp.) are present</p> <p data-bbox="421 824 807 1301"><i>C. parasitica</i> causes cankers, wilt and diebacks, resulting in the death of its hosts. No direct relationship exists between disease incidence and disease severity or impact because of several factors, including hypovirulence. In areas where low vc type diversity exists within the pathogen's population, the natural spread of hypovirulence decreases the disease severity and, thus, the impact. However, the introduction of new vc types into those areas may increase the diversity of vc types resulting in lower hypovirulence efficacy</p> <p data-bbox="421 1317 807 1503">Potential environmental impacts of <i>C. parasitica</i> in the risk assessment area include reductions in biodiversity, food and wood resources and habitat for associated organisms</p>	<p data-bbox="831 230 1171 288"><i>Provide an overall summary of the above points</i></p> <p data-bbox="831 315 1171 1055"><i>C. parasitica</i> is a clear taxonomic entity and reliable methods exist for its detection and identification. The pest is present in the risk assessment area and is listed in Council Directive 2000/29/EC. The pathogen is present in part of the risk assessment area. Plants for planting are a pathway for the introduction into and spread within new areas of <i>C. parasitica</i>. The first introduction of the pathogen in the USA was on host plants for planting that had been imported from infested areas. Since then, <i>C. parasitica</i> has had a devastating impact on the American sweet chestnut (<i>C. dentata</i>), killing 3.5 billion American chestnut trees in the natural range of <i>C. dentata</i> within approximately 40 years</p>	

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## ABBREVIATIONS

EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
EPPO-PQR	European and Mediterranean Plant Protection Organization Plant Quarantine Retrieval system
EU	European Union
ISPM	International Standard for Phytosanitary Measures
MS(s)	Member State(s)
NPPO	National Plant Protection Organization
ORF	open reading frame
PCR	polymerase chain reaction
PLH Panel	Plant Health Panel
PRA	Pest Risk Analysis
RNQP	regulated non-quarantine pest