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Pest survey card on *Cryphonectria parasitica*

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Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2020-0114), at the request of the European Commission. Its purpose is to guide the EU Member States (MSs) in preparing data and information for surveys for the causal agent of chestnut blight, *Cryphonectria parasitica*. *Cryphonectria parasitica* is a protected zone quarantine pest. Therefore, the objective of this pest survey card is to provide the relevant information needed for the EU Member States that have protected zone status for this pest to prepare surveys. *Cryphonectria parasitica* is a well-defined and distinguishable fungal species of the family *Cryphonectriaceae.* The pathogen is listed as a protected zone quarantine pest in Czechia, Sweden, Ireland and Northern Ireland, and as a Union regulated non-quarantine pest (RNQP). *Cryphonectria parasitica* is native to eastern Asia and is widely present in North America and in many countries of Europe that have significant European chestnut (*Castanea sativa*) populations. The main hosts of the pathogen are *Castanea* spp. but several *Quercus* species and other tree species can be infected. *Cryphonectria parasitica* is a bark-inhabiting pathogen which only infects the above-ground parts of trees and produces cankers, causing diebacks and leading to the death of the upper parts of susceptible trees. The fungus spreads naturally via conidia and ascospores. Rain disperses conidia over short distances, while the wind disperses ascospores over longer distances. Human activities, such as the trade of infected host plants, wood and bark, also facilitate the pathogen's spread over longer distances. Climatic and ecological conditions are not to be considered as a limiting factor for the establishment and spread of *C. parasitica* in the EU Member States where the pest is not already present. The occurrence in Europe of the dsRNA hypovirus CHV-1, which acts as biological control agent, could dramatically reduce its virulence. Hypovirulent strains do not produce ascospores and do not lead to the death of *Castanea* spp. *Cryphonectria parasitica* can be detected by visual examination of symptomatic plants (cankers, dieback, epicormic shoots and characteristic fruiting structures) and morphology but needs to be confirmed by molecular analysis.

Keywords: chestnut blight, cankers, *Castanea sativa*, protected zone quarantine pests, risk-based surveillance, detection survey, delimiting survey

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Introduction

The objective of this pest survey card is to provide the relevant information needed to prepare surveys following the methodology described in EFSA et al. (2018), for the causal agent of chestnut blight, *Cryphonectria parasitica. Cryphonectria parasitica* is a protected zone quarantine pest. Therefore, the objective of this pest survey card is to provide the relevant information needed for the EU Member States (MSs) that have protected zone status for this pest to prepare surveys. It is part of a toolkit that has been developed to assist the MSs with planning a statistically sound and risk-based pest survey approach in line with the recommendations and guidelines provided by the International Plant Protection Convention (IPPC) in the various International Standards for Phytosanitary Measures (ISPM 6: FAO 2021a; ISPM 31: FAO, 2021b) and surveillance guide (FAO, 2021c). The EFSA Plant Pest Survey Toolkit[1] consists of pest-specific documents and more general documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on *Cryphonectria parasitica*[2].
ii. General documents:
   a. General guidelines for statistically sound and risk-based surveys of plant pests (EFSA et al., 2020)
   b. The statistical tools RiBESS+[3] and SAMPELATOR.
   c. The RiBESS+ manual[4] and video tutorial[5].

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2020-0114) at the request of the European Commission. The information presented in this pest survey card was summarised from EFSA's pest categorisation on *Cryphonectria parasitica*, a bark-inhabiting fungus causing blight of chestnut trees (*Castanea* spp.) (EFSA PLH Panel, 2014, 2016), the European and Mediterranean Plant Protection Organization (EPPO) datasheet on *Cryphonectria parasitica* (Murrill) Barr (EPPO, 2020) the EPPO Global Database (EPPO, online), the Centre for Agriculture and Bioscience International (CABI) datasheet on *Cryphonectria parasitica* and other documents.

The main challenge relevant for surveillance of *C. parasitica* is the lack of development of loop-mediated isothermal amplifications assays (LAMP) for in situ detections.

The main knowledge gaps concern: (i) the risk of spread by insects, birds, etc. in the natural spread of the pathogen, and (ii) the epidemiological role of conidia in soil and growing media (EFSA PLH Panel, 2014).

1. The pest and its biology

1.1. Taxonomy

Current scientific name: *Cryphonectria parasitica* (Murrill) Barr
Class: Sordariomycetes
Order: Diaporthales
Family: Cryphonectriaceae

[1] https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/index
[2] The published Pest survey cards in the story map format are available in the Plant Pests Survey Cards Gallery available online: https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/gallery
[3] https://r4eu.efsa.europa.eu/app/ribess
[4] https://zenodo.org/record/2541541#.YkrgRyhByUm
[5] A tutorial video for the use of RiBESS+ is available online: https://youtu.be/qYHqrCiMxDY

Genus: *Cryphonectria*
Species: *Cryphonectria parasitica*
Synonyms: *Diaporthe parasitica* Murrill; *Endothia parasitica* (Murrill) P.J.Anderson & H.W.Anderson
EPPO Code: ENDOPA
Common name: chestnut blight, blight of chestnut, canker of chestnut
Taxonomic rank: species

Cryphonectria parasitica is a Sordariomycete (ascomycete) fungus in the family *Cryphonectriaceae* (Order Diaporthales).

Conclusions on taxonomy

Cryphonectria parasitica is a well-defined and distinguishable fungal species of the family Cryphonectriaceae.

Figure 1: Chestnut blight induced by *Cryphonectria parasitica* on chestnut. (A) Branch wilting cause by a canker on *Castanea sativa* (Source: Andrej Kunca, National Forest Centre – Slovakia, Bugwood.org) (B) Canker on *C. dentata* that typically appears as sunken, reddish-brown bark (Source: Linda Haugen, USDA Forest Service, Bugwood.org)

1.2. EU pest regulatory status

Cryphonectria parasitica is listed as a protected zone quarantine pest in Annex III (section B 'Fungi and oomycetes') of Commission Implementing Regulation (EU) 2019/2072[6]. The protected zone status for *C. parasitica* applies for the following countries:

- Czechia
- Ireland
- Sweden
- United Kingdom (Northern Ireland).

Special requirements are laid down in Commission Implementing Regulation (EU) 2019/2072 for the introduction into or movement within these protected zones for:

- plants for planting of *Castanea* Mill. (Annex X, Points 20)
- plants for planting of *Quercus* L, other than seeds (Annex X, Point 21)
- wood and isolated bark of *Castanea* Mill. (Annex X, Points 45 and 52)
- seeds of *Castanea* Mill. (Annex XII, Points 4)
- wood of Castanea Mill., excluding wood which is bark-free (Annex XII, Point 6)
- plants, seeds, wood and isolated bark of *Castanea* Mill. (Annex XIV, Points 3, 9, 11, 12).

Cryphonectria parasitica is listed as a Union Regulated Non-Quarantine Pest (RNQP) in Annex IV (Parts D, E, J) of Commission Implementing Regulation (EU) 2019/2072[7]; as the pathogen is widely distributed in the EU and eradication and containment measures in MSs where the pathogen has been present for a long time, are no longer effective (EFSA PLH Panel, 2016). The Regulation also provides measures to prevent the presence of *C. parasitica* as RNQPs in propagating material for ornamental plants of *Castanea* L. (Annex V, Part C) and in forest reproductive material, other than seeds, of *Castanea sativa* (Annex V, Part D).

In general, the introduction into the Union of plants of *Castanea* Mill. and *Quercus* L., and isolated bark of *Castanea* Mill. from third countries is prohibited (Annex VI, Point 2 and 4). The general requirements for surveys of quarantine organisms within EU territory are laid down in Regulation (EU) 2016/2031[8] and those for protected zone quarantine pests are listed in Commission Delegated Regulation (EU) 2022/2404[9].

Overview of the EU regulatory status

Cryphonectria parasitica is a protected zone quarantine pest for Czechia, Ireland, Sweden and the United Kingdom (Northern Ireland) and a Union regulated non-quarantine pest (RNQP). Existing regulation provide for various measures to prevent its introduction and spread into the protected zones.

[6] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. OJ L 319, 10.12.2019, p. 1–279

[7] Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. OJ L 317 23.11.2016, p. 4–104.

[8] Commission Delegated Regulation (EU) 2022/2404 of 14 September 2022 supplementing Regulation (EU) 2016/2031 of the European Parliament and of the Council by laying down detailed rules for the surveys on protected zone quarantine pests and repealing Commission Directive 92/70/EEC, OJ L 317, 9.12.2022, p. 42–53.

1.3. Pest distribution

Cryphonectria parasitica is native to eastern Asia. The pathogen has been introduced to North America (Canada and USA), Asia (China, India, Iran, Japan, Korea, Taiwan), Australia (Victoria), Tunisia and most of Europe. However, it is absent in Sweden, Ireland and Northern Ireland and present under eradication in Czechia (EPPO, online) (Figure 2).

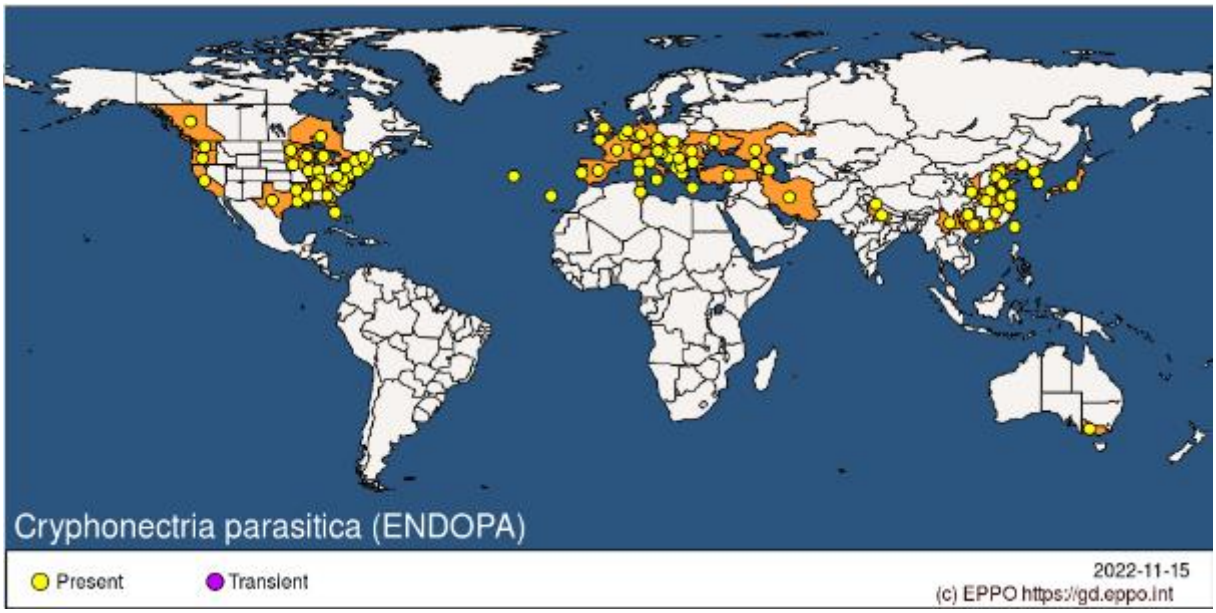

Figure 2: Global distribution of *Cryphonectria parasitica* (Source: EPPO Global Database, https://gd.eppo.int/, map updated on 2022-11-03, accessed on 2022-11-15)

Conclusion on pest distribution

Cryphonectria parasitica is native to eastern Asia. The pathogen has been introduced to North America (Canada and USA), Asia (China, India, Iran, Japan, Korea, Taiwan), Australia (Victoria), Tunisia and most of Europe. However, it is absent in Sweden, Ireland and Northern Ireland and present under eradication in Czechia.

1.4. Disease and life cycle

Cryphonectria parasitica is a bark pathogen, which only infects the above-ground parts of trees: stems, branches and twigs (Rigling and Prospero, 2018).

The pathogen enters the host plants through fresh wounds or growth cracks in the bark, facilitated by wind and rain (Rigling and Prospero, 2018). The common entry points for the spores of the pathogen are mechanically produced, weather-related, insect-generated and graft wounds (Russin, et al., 1984; Lovat and Donnelly, 2019; EPPO, 2020). The activity of the bark miner *Spulerina simploniella* (Lepidoptera: Gracilariidae) can expose the plant phloem that is then colonised by the pathogen, facilitated by rain (Diamandis and Perlerou, 2005). Galls of the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae), can be colonised by *C. parasitica* after the adult wasps emerge and abandon the galls, contributing to the pathogen's persistence in an area (Meyer et al., 2015).

The pathogen colonises the bark (CABI, 2021) and spreads into the cambium through mycelial fans, causing nutrient and water disruption (EFSA PLH Panel, 2014; EPPO, 2020), which produces

cankers and the subsequent wilting and dieback of the affected branches and stems (Anagnostakis and Aylor, 1984; EFSA PLH Panel, 2014; EPPO, 2020).

On the infected bark, the fungus produces masses of pustules (stromata) harbouring asexual (pycnidia) and sexual (perithecia) fruiting bodies (EPPO, 2005; Rigling and Prospero, 2018). Pycnidia and perithecia can co-exist on the same canker (Prospero et al., 2006) (See Section 3.1.1).

Conidia (asexual spores) are released during moist conditions in long, yellow-orange masses that ooze from the pycnidia (Rigling and Prospero, 2018) and are dispersed by rain and wind-borne dust. Birds (Heald and Studhalter, 1914; Scharf and DePalma, 1981), insects (Russin et al., 1984) and mammals (Scharf and DePalma, 1981) can passively spread conidia if they come into contact with them (Russin et al., 1984; Rigling and Prospero, 2018; EPPO, 2020). Animals are not considered to be the main means of spreading the disease (EFSA PLH Panel, 2014; EPPO, 2020). *Cryphonectria parasitica* can sporulate year-round, even in the winter months (Romon-Ochoa et al., 2022), and the conidia germinate optimally at 25–26°C (EFSA PLH Panel, 2014).

If the conidia reach the ground, they can remain viable in the soil for a long time (up to 4 months; Heald and Gardner, 1914; Rigling and Prospero, 2018). Uncertainties remain on the ability of conidia in the soil to infect host plants.

Ascospores (sexual spores) are released from the perithecia and dispersed by the wind (Rankin, 1914; Heald et al., 1915) from spring to autumn (between March and October), with a peak in spring (May), typically after rainfall (Guérin et al., 2001; EFSA PLH Panel, 2014), and germinate optimally at 21°C (Fulton, 1912; EFSA PLH Panel, 2014). In France, for example, the peak of ascospore dispersal coincides with the highest chestnut susceptibility to blight (in May–June) (Guérin and Robin, 2003).

Latent infections may occur during autumn and winter and become active in spring (Guérin and Robin, 2003). *Cryphonectria parasitica* overwinters as stromatal mycelium, harbouring pycnidia and perithecia, in bark cankers (EFSA PLH Panel, 2014). The pathogen can survive in cankers on cut trees for more than one year and it is able to live saprophytically in the bark of recently dead chestnuts and sporulate (Prospero et al., 2006).

The fungus can also survive as a saprophyte on other broad-leaved trees beyond its parasitic host range (EPPO, 2020). The pathogen can also be considered an endophyte (Bissegger and Sieber, 1994). It has been isolated from asymptomatic *C. sativa* bark up to seven months after inoculation (Guérin and Robin, 2003) and development of disease symptoms was even observed on asymptomatic imported plants 16 months after importation (Cunnington and Pascoe, 2003).

Latency may have important implications for chestnut blight detection because the pathogen can be present even when no lesions are visible and become active again when environmental conditions become favourable. (Guérin and Robin, 2003). On the fruit, the pathogen infections are confined to the nutshells and apparently do not affect seed germination or seedling growth (Jaynes and Depalma, 1984).

Hypovirulence of *C. parasitica* strains occurs in natural populations of the fungus and it is due to the infection and spread of mycovirus CHV-1 (Robin and Heiniger 2001; Gobbin et al., 2003). This hypovirus causes a reduction in virulence and pathogenicity in comparison to the uninfected fungus, thus leading to reduced damage of the host plants (Rigling and Prospero, 2018).

Hypovirulence will not lead to the death of infected trees (Rigling and Prospero, 2018). Hypovirulent isolates produce conidia and just some of them are infected by the virus (Rigling and Prospero, 2018). Hypovirulent isolates cannot produce ascospores (Rigling and Prospero, 2018).

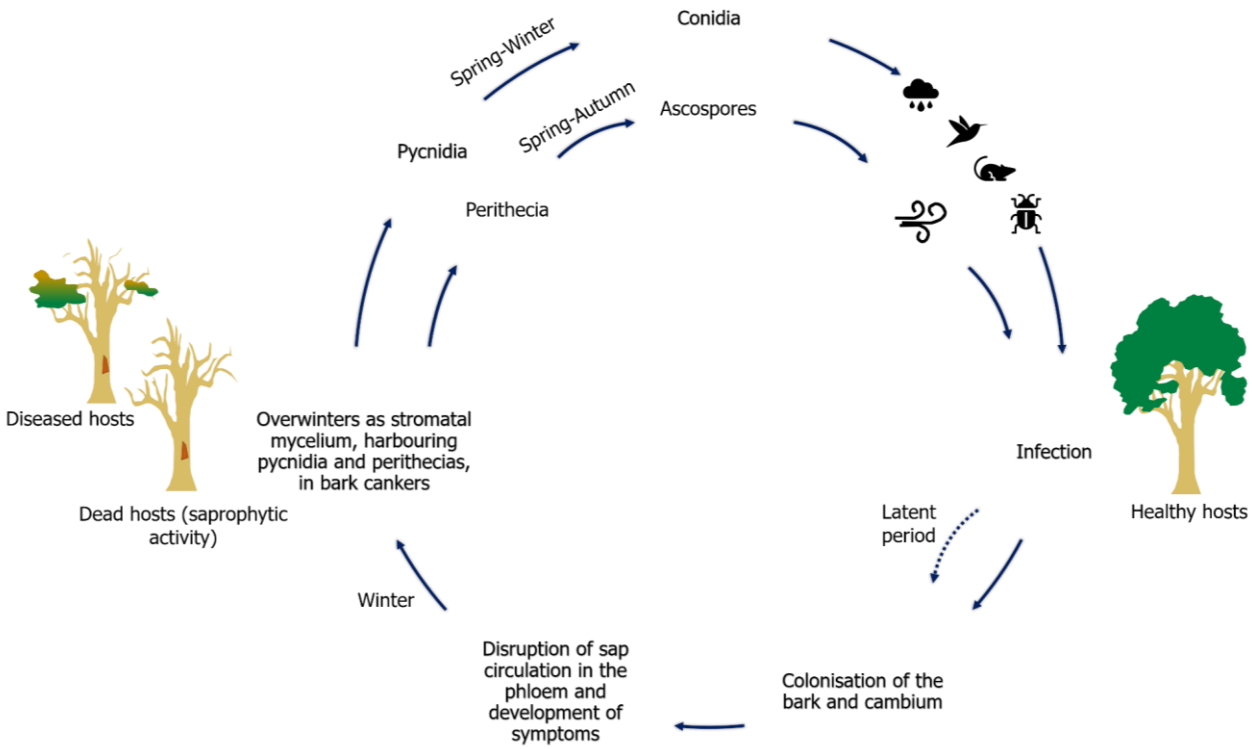

Figure 3: Disease cycle of virulent strains of *Cryphonectria parasitica*

Conclusion on disease and life cycle

Cryphonectria parasitica is a bark-inhabiting pathogen that infects only the above-ground parts of trees, spreading from tree to tree via ascospores (carried by the wind) and conidia (spread by rain, mammals, insects and birds). The pathogen shows considerable saprophytic activity and can also be considered an endophyte.

2. Target population

This section provides the information needed to characterise the population of host plants to target in a survey, as described in the 'General guidelines for statistically sound and risk-based surveys of plant pests' (EFSA et al., 2020). This includes the pest's host range and main hosts in the EU (Section 2.1), the suitability of EU environments to the pest's establishment (Section 2.2), the ability of the pest to spread (Section 2.3), and the identification of risk factors associated with an increased probability of presence (Section 2.4).

Once the above parameters have been defined, the target population can be structured in multiple levels. At level 1 is the survey area, which corresponds to the entirety or part of the Member State. At levels 2 and 3 are the epidemiological units that can be distinguished within the survey area. Epidemiological units can be chosen as administrative regions (e.g. EU NUTS areas or Member State-level regions) if they are homogeneous, further subdivided into the

environments where host plants are present using a land-use categorisation (e.g. urban, agricultural and natural areas, nurseries). At level 4, if risk factors are identified, the risk areas are defined around the risk locations. At level 5 are the inspection units, the elementary subdivisions of the target population that are inspected for the detection of the pest (e.g. host plants), depending on the pest detection method (Section 3). For the definitions of the target population, epidemiological units and inspection units, see the glossary of terms available at the end of this document.

The hierarchical structure of the target population should be tailored to the situation in each Member State. A possible structure of the target population for surveys of *C. parasitica* within the EU is proposed in Section 2.5 (Figure 5).

2.1. Host range and main hosts in the EU

The American chestnut *C. dentata* and the European chestnut *C. sativa* are the main hosts of *C. parasitica* (EFSA PLH Panel, 2014) (Table 1).

The Japanese chestnut (*C. crenata*), Henry's chestnut (*C. henryi*), Chinese chestnut (*C. mollissima*) and Séguin's chestnut (*C. seguinii*) can be considered resistant to *C. parasitica*, perhaps due to their co-evolution with the pathogen (Rigling and Prospero, 2018; Lovat and Donnelly, 2019). These hosts are able to exclude or overcome, completely or to some degree, the effect of the pathogen (Agrios, 2004). These species can hybridise with *C. sativa* and several hybrids are present in Europe which can also host *C. parasitica.*

The American chinkapin (*Castanea pumila*) (Rigling and Prospero, 2018; EPPO, 2020) and several oak species (in Europe mostly *Quercus petraea* and less often *Quercus robur*, *Quercus ilex* and other oaks) have occasionally been infected by *C. parasitica* (EPPO, 2005; Adamčíková et al., 2010; EFSA PLH Panel, 2016; EPPO, 2020). On these hosts, infections progress slowly and develop perennial 'healing', swollen and callusing cankers that do not usually kill the attacked plant part (EPPO, 2005; Rigling and Prospero, 2018). The pathogen is able to complete its life cycle on *Quercus* (Adamčíková et al., 2010).

The pathogen has also been found in nature on *Ostrya carpinifolia* and *Alnus cordata* (Turchetti et al., 1991) *Cryphonectria parasitica* can be found, occasionally, on other genera e.g. *Acer* and *Fagus* (Table 1) (EFSA PLH Panel, 2014).

Detection surveys for *C. parasitica* in protected zones should mainly focus on *C. sativa* trees and hybrids, as these are considered to be the major hosts (EPPO, online), while delimiting surveys following a detection should focus on all symptomatic host species (Table 1) (EPPO, 2020).

Table 1: Main and rare host plants of *Cryphonectria parasitica.* *A rare host is a host on which the disease is sporadically present and/or is uncommonly reported in literature

Host status	Species	Common name	Reference
Main hosts	*Castanea dentata* (Major host)	American chestnut	Rigling and Prospero, 2018; Dennert et al., 2020; EPPO, 2020
	Castanea sativa (Major host)	European chestnut	Rigling and Prospero, 2018; Dennert et al., 2020; EPPO, 2020
Main co-evolved hosts	*Castanea mollissima*	Chinese chestnut	Rigling and Prospero, 2018; EPPO, 2020
	Castanea crenata	Japanese chestnut	Rigling and Prospero, 2018; EPPO, 2020
	Castanea henryi	Henry's chestnut	Diller, 1965; EPPO, 2020
	Castanea seguinii	Séguin's chestnut	Diller, 1965; EPPO, 2020
***Rare hosts**	*Quercus petraea*	Sessile oak	Diller, 1965; Adamčíková et al., 2010; EPPO, 2020
	Quercus alba	White oak	Rigling and Prospero, 2018; EPPO, 2020
	Quercus coccinea	Scarlet oak	Rigling and Prospero, 2018; EPPO, 2020
	Quercus frainetto	Hungarian oak	Tziros et al., 2015; Rigling and Prospero, 2018; EPPO, 2020
	Quercus ilex	Evergreen oak	Diller, 1965; EPPO, 2020
	Quercus pubescens	Downy oak	Dallavalle and Zambonelli, 1999; Rigling and Prospero, 2018; EPPO, 2020

	Quercus rubra	Northern red oak	Haltofová et al., 2005; EPPO, 2020
	Quercus stellata	Post oak	Diller, 1965; EPPO, 2020
	Quercus virginiana	Live oak	Rigling and Prospero, 2018; EPPO, 2020
	Acer spp.		Diller, 1965; EPPO, 2020
	Castanea pumila	American chinquapin	Rigling and Prospero, 2018; EPPO, 2020
***Rare and asymptomatic hosts**	*Ostrya carpinifolia*	Hop hornbeam	EPPO, 2020
	Alnus cordata	Italian alder	EPPO, 2020
	Carya ovata	Shagbark hickory	Diller, 1965; EPPO, 2020
	Castanopsis chrysophylla	Giant chinkapin	EPPO, 2020
	Fagus spp.	Beech	EPPO, 2020
	Liriodendron tulipifera	Tulip tree	EPPO, 2020
	Malus × *domestica*	Apple	EPPO, 2020
	Rhus typhina	Staghorn sumac	EPPO, 2020

Conclusion on host range and main hosts

Cryphonectria parasitica is mainly a pathogen of the chestnut tree (*Castanea* spp.). The pathogen can also occasionally be found on oaks (*Quercus* spp.) and other broadleaved trees. Detection surveys in the EU should focus on *Castanea sativa* and its hybrids, while delimiting surveys should be conducted on all symptomatic hosts.

2.2. Environmental suitability

There are no obvious ecological or climatic factors limiting the establishment and spread of *C. parasitica* in the EU Member States that are protected zones for *C. parasitica* and where the main host species, *C. sativa* is present, both naturally occurring and planted (EFSA PLH Panel, 2014, 2016).

The distribution area of European chestnut (natural and naturalised), ranges from southern Europe (the Iberian Peninsula, Italy, the Balkans, the Mediterranean Islands) and North Africa (Morocco), to north-western Europe (England, Belgium) and eastward to western Asia (north-east Turkey, Armenia, Georgia, Azerbaijan, Syria) (Conedera et al., 2016). In Europe the main chestnut forests are concentrated in a few countries such as Italy and France and on the Iberian Peninsula (Figure 4).

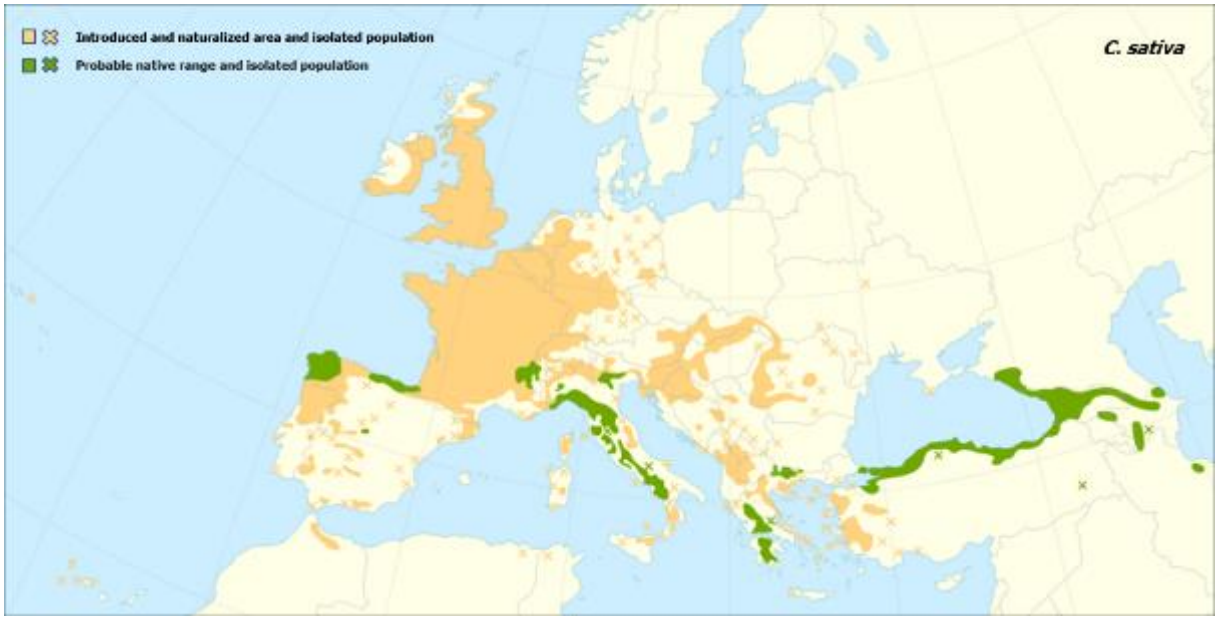

Figure 4: Distribution map of *Castanea sativa* (Source: modified from Caudullo et al., 2017)

Chestnut 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 (EFSA PLH Panel, 2014). It is an economically important species (Fernández-López and Alía, 2003) and is managed for timber production as well as for fruit production (orchards) (Conedera et al., 2016). It is also widely planted as ornamental trees in parks and cities (EFSA PLH Panel, 2014).

In the protected zones, *C sativa* is an introduced species and is mainly used for ornamental purposes in parks, gardens and urban areas.

In Czechia, *C. sativa* is mainly used for ornamental purposes (parks, gardens, urban vegetation) (Jankovský et al., 2004). It is found sporadically in woods and within a limited nut production area (Haltofová and Jankovský, 2003; Kupka, 2021; Selina Wamuci, 2023).

In Ireland, *C. sativa* occurs mainly in the woodlands of the southern and south-eastern counties (Jarman et al., 2019) (Figure 5), while in Sweden, it is mainly used as an amenity tree.

Conclusion on environmental suitability

Environmental conditions are not to be considered a limiting factor for the establishment and spread of *C. parasitica* in the EU Member States that are protected zones for this pest.

2.3. Spread capacity

Natural spread

Cryphonectria parasitica spreads naturally through conidia (asexual) and ascospores (sexual). Conidia are 'mainly dispersed by rain over short distances (a few metres) or washed down the stem and branches' (Rigling and Prospero, 2018). Therefore, conidia generate new cankers more frequently within the same tree (EFSA PLH Panel, 2014). Mammals, insects (e.g. *S. simploniella* and *D. kuriphilus*) and birds that come into contact with the spores, can passively disseminate them across large areas (EPPO, 2020). Insects carrying the pathogen have been found up to 32 m from the nearest source of inoculum (EFSA PLH Panel, 2014). Wind-borne dust may also transport them over long distances (Rigling and Prospero, 2018).

Ascospores are wind-dispersed up to a few hundred metres from a perithecial source (CABI, 2021; EFSA PLH Panel, 2014; Rigling and Prospero, 2018).

Human-assisted spread

Spread of the pathogen to greater distances is facilitated by human activity, primarily through trade within the EU of infected host plants for planting (*Castanea* spp.) (such as rootstocks, scions, grafted plants, self-rooted plants, ornamental plants) (EFSA PLH Panel, 2016) and asymptomatic and infected wood with bark, including wood chips with bark fragments (EFSA PLH Panel, 2014, 2016; EPPO, 2020).

Cryphonectria parasitica could potentially spread via the movement of infected fruit (nuts). However, since the pathway of infection, from the traded nuts to the orchard and seedlings, has not been demonstrated, this pathway is considered to be of minor importance (Jaynes and DePalma, 1984; EFSA PLH Panel, 2016; EPPO, 2020). Uncertainty remains on the risk of spread related to vertical transmission (CABI, 2021).

The pathogen could also spread to new environments through the movement of infested soils and growth substrates (including isolated chestnut bark). Conidia of *C. parasitica*, once they reach the soil, can remain viable for a long time (Rigling and Prospero, 2018). However, as there is no evidence that *C. parasitica* has been introduced to a new country or area via infested soil, this can be considered a minor pathway (EFSA PLH Panel, 2016).

Pruning and grafting tools, if unsterilised, can also be a source of contamination and potentially spread the disease locally (EFSA PLH Panel, 2014; EPPO, 2020). However, there is lack of evidence on the formation of new cankers from the use of tools carrying the inoculum (EFSA PLH Panel, 2014).

Conclusion on spread capacity

Natural spread of *C. parasitica* is through conidia and ascospores. The former are dispersed mainly by rain over short distances, the latter by the wind over longer distances. The spread of the pathogen over longer distances can also be facilitated by the human activity of trading infected host plants, especially asymptomatic ones, for planting and isolated bark, and to a lesser extent by infected fruit, infested soil or the use of unsterilised pruning tools.

2.4. Risk factor identification

Identification of risk factors and their relative risk estimation are essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for surveillance need to be characterised by their relative risk (should there be more than one level of risk for the target population) and the proportion of the overall target population to which they apply. The identification of risk factors needs to be tailored to the situation in each Member State. This section presents examples of risk factors for *C. parasitica* but they are not necessarily exhaustive.

For the identification of risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of the bacterium and the insects. These activities should then be connected to specific locations. Around these locations, risk areas can be defined, bearing in mind that their size depends on the spread capacity of the target pest and the availability of host plants around these locations.

The Member States can opt to utilise the information available on the EU Platforms of TRACES NT, EUROPHYT Interceptions and EUROPHYT Outbreaks. The information available, in particular, relating to the country of origin, type of commodity and hosts of intercepted or outbreak reports can be extracted from such platforms for specific harmful organisms. This information can allow Member States to identify potential pathways of introduction from previous historical findings. Thus, Member States might consider focusing their surveillance efforts around activities and locations related to previous interceptions and outbreaks.

Such information should only be considered as indicative and given the possible dynamic changes, it should be reviewed and analysed periodically.

EXAMPLE 1: TRADE OF *CASTANEA* SPP. COMMODITIES AND INFESTED SOIL

The activity most associated with the introduction of *C. parasitica* into new areas is the trade of plants for planting of *Castanea* spp. from the EU (EFSA PLH Panel, 2016). The pathway of entry via plants for planting, seeds, wood and isolated bark of *Castanea* Mill., from the EU is mitigated by the special requirements in place (see Section 1.2). The pathway of entry from third countries, is virtually closed since the current legislation prohibits the introduction into the EU of plants and isolated bark of *Castanea* Mill.

Another potential pathway of introduction of the pathogen is through the movement of infected soil, where conidia of *C. parasitica* can remain viable for a long time (Rigling and Prospero, 2018), and the import of the fruit of *Castanea*. However, the risk associated with these pathways remains uncertain and should thus be considered to be of minor importance (Jaynes and DePalma, 1984; EFSA PLH Panel, 2016; EPPO, 2020).

Nurseries, garden centres and markets where the hosts of *C. parasitica* are present should be considered as the risk locations, while the surrounding area with the host plants present and planted should be considered as risk areas to be surveyed.

EXAMPLE 2: ORCHARD OR FOREST MANAGEMENT OPERATIONS DURING THE PERIOD OF PEAK SPORE DISCHARGE

Orchard or forest management operations such as pruning, grafting and logging, which may cause injury to plants, conducted when the fungus is more likely to infect trees (May–June) can

be considered as a risk activity. This can be associated with a higher probability of finding the pest.

Consequently, orchards or forests subject to pruning, grafting and logging in May–June can be considered as both risk locations and risk areas to be surveyed.

Table 2: Examples of risk activities and the corresponding risk locations and risk areas that are relevant for the surveillance of *Cryphonectria parasitica*

Risk activity	Risk locations	Risk areas
Import and trade of plants for planting, wood with bark, isolated bark, fruit and infested soil of *Castanea* spp.	Nurseries, garden centres and markets where such imported plants are stored, propagated, traded or planted	Areas surrounding risk locations where the hosts of *C. parasitica* are present and planted
Orchard or forest management operations during the period of peak spore discharge	Orchards and forest stands, where *C. sativa* plants are cultivated and present with other host plants	*C. sativa* orchards and forest stands, where host plants of *C. parasitica* are present

2.5. Structure of the target population

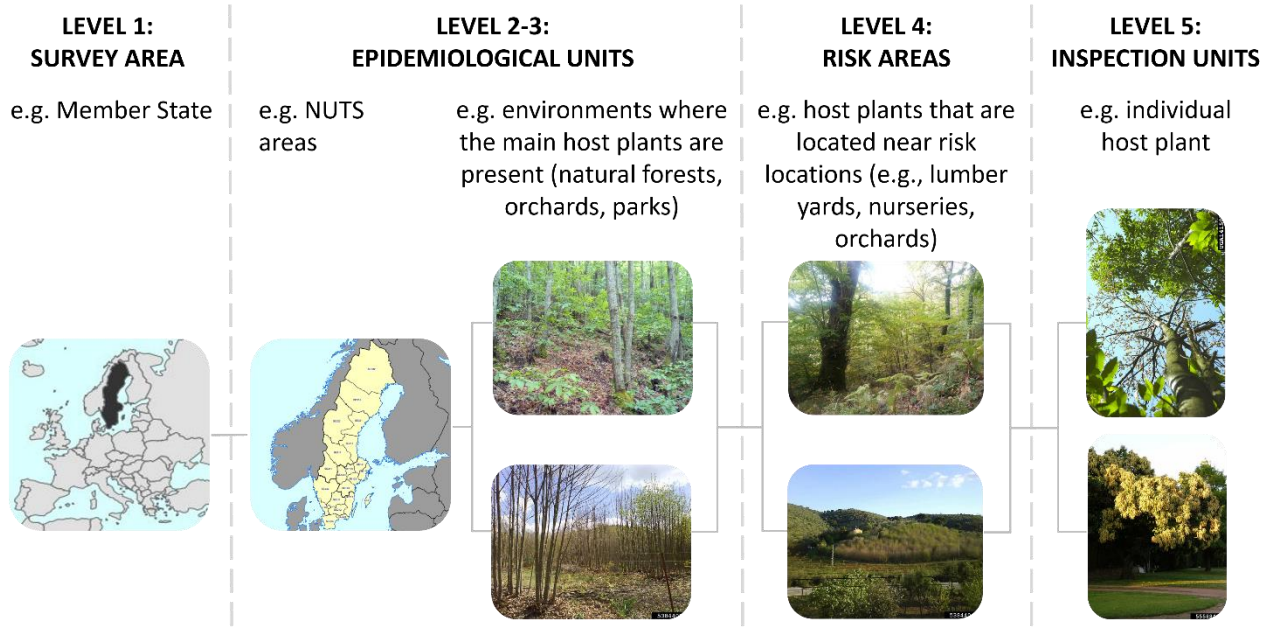

Figure 5: Example of the hierarchical structure of the target population for *Cryphonectria parasitica* in the EU (Sources: Eurostat, 2022 (levels 1–2); Alessandra Gionni (level 3, top); Fernando Gallardo, Huelva Forestry University, Bugwood.org (level 3, bottom); Alessandra Gionni (level 4, top); Fernando Gallardo, Huelva Forestry University, Bugwood.org (level 4, bottom); Andrej Kunca, National Forest Centre - Slovakia, Bugwood.org (level 5, top), Juan Campá, MGAP, Bugwood.org (level 5, bottom))

3. Detection and identification

Cryphonectria parasitica can be detected in the field by visual examination of the symptoms (cankers, epicormic shoots, dieback, characteristic fruiting structures).

Morphological identification of *C. parasitica* isolates needs to be confirmed by molecular analysis. In addition, *C. parasitica* can be detected directly from bark samples using real-time PCR.

3.1. Detection and identification in the field

3.1.1. Visual examination

The occurrence of early symptoms on *C. sativa* varies according to the age of the tree, the infected organ and the virulence of the pathogen (EFSA PLH Panel, 2014).

The appearance of cankers greatly depends on the virulence of the strain. Considering that hypovirulence is widely present in European *C. parasitica* populations, being able to identify all types of cankers is very important for the detection of the pathogen in a new area.

The detection of virulent strains of *C. parasitica* on chestnut trees should be based mainly on the presence of cankers on the stems or branches and by the symptoms in the canopy and epicormic shoots (CABI, 2021).

SYMPTOMS

Cankers caused by virulent C. parasitica strains

- Below the canker the branches have healthy foliage (EPPO, 2020) and the tree reacts by producing numerous epicormic shoots (Rigling and Prospero, 2018) (Figure 8).
- Cankers (necrotic lesions) are produced on the bark of stems and branches of susceptible host trees. These typically appear either as sunken or swollen (Lovat and Donnelly, 2019) reddish-brown bark lesions (Rigling and Prospero, 2018; EPPO, 2020) (Figure 7). A canker can become visible on a tree within three to five weeks (EPPO, 2020).
- Bark cankers on young, smooth-barked branches and stems are orange to reddish-brown on the surface. On older stems and branches, the discoloration is less obvious (Rigling and Prospero, 2018; EPPO, 2020). As the canker grows, the margin retains the colour while the centre dies and the bark eventually cracks (EFSA PLH Panel, 2014).
- Cracks and new vertical fissures (EPPO, 2020) might be visible when colonisation by the pathogen is slow (depending on the weather conditions) and the tree will produce new layers of bark under the affected area.

Figure 6: (A) Grafted chestnut seedling infected by *C. parasitica* (Source: Daniel Rigling, WSL Switzerland) (B–F) Various virulent chestnut blight cankers. Cankers typically appear as sunken, reddish-brown bark lesions (Sources: (B) Linda Haugen, USDA Forest Service, Bugwood.org; (C) USDA Forest Service – Region 8 – Southern, USDA Forest Service, Bugwood.org; (D–E-F) Daniel Rigling, WSL Switzerland)

Figure 7: Epicormic shoots below the canker (Source: Ministry of Agriculture and Regional Development, Bugwood.org)

Cankers caused by hypovirulent C. parasitica *strains*

- The cambium is not colonised and killed by the fungus, the regions above the cankers survive and no epicormic shoots are produced below it (Rigling and Prospero, 2018).
- Cortical lesions are initially similar to those caused by the virulent strain (EFSA PLH Panel, 2014).
- Cankers are usually smaller, non-lethal, superficial, swollen or callused and swollen (EFSA PLH Panel, 2014; Rigling and Prospero, 2018; CABI, 2021) (Figure 9).
- Stromata are rarely formed in the cracks of the bark. Conidiomata are usually produced; ascomata are almost never formed (EPPO, 2005).
- Hypovirus infections can only be confirmed by laboratory analysis (Rigling and Prospero, 2018; Lovat and Donnelly, 2019).

Figure 8: (A–B) Cankers caused by hypovirulent *Cryphonectria parasitica* strains. Cankers appear typically swollen, superficial or callused (Sources: (A) Daniel Rigling, WLS Switzerland; (B) EPPO Global Database, courtesy of Daniel Rigling)

Mycelial fans

- Pale white-brownish mycelial fans are a common sign of the disease (Rigling and Prospero, 2018). Mycelial fans form in the inner bark and may be exposed by cutting away the outer bark (EPPO, 2020). Some annual rings of sapwood can also be infected, although mycelial fans do not form there (EPPO, 2005) (Figure 9).
- Mycelial fans developed by hypovirulent *C. parasitica* strains are not easily found and are smaller, paler and thinner than in the virulent form of the disease (EPPO, 2005).

Fruiting bodies (perithecia and pycnidia)

- Masses of yellow-orange to reddish-brown pustules (stromata), harbouring sexual (perithecia) or asexual (pycnidia) fruiting bodies (Rigling and Prospero, 2018; EPPO, 2020), are produced on the surface of infected bark, in cracks and crevices (CABI, 2021) (Figure 10).
- Perithecia have ostioles that can be observed using a hand magnifying lens and appear as black dots on papillate protuberances on the surface of the stroma (EPPO, 2005).
- Perithecia are not produced by hypovirulent strains (C. Robin, personal communication).
- Pycnidia release conidia in long (up to more than 1 cm), orange-yellow, twisted and distinctive tendrils of spores in moist weather (Rigling and Prospero, 2018; EPPO, 2020).

Figure 9: (A–D) Typical pale white-brownish mycelial fans of *Cryphonectria parasitica* under the bark of *Castanea sativa* (Sources: (A) Andrej Kunca, National Forest Centre – Slovakia, Bugwood.org; (B) Daniel Rigling, WLS Switzerland; (C) Ignazio Graziosi, University of Kentucky, Bugwood.org; (D) Ignazio Graziosi)

Figure 10: (A) Orange coloured stroma of *Cryphonectria parasitica* on a main stem of *Castanea dentata* around the base of a dead branch (Source: Linda Haugen, USDA Forest Service, Bugwood.org). (B) Reddish-brown pustules (stromata) on the infected bark of *Castanea sativa* (Source: Félix TENG, Walloon Agricultural Research Centre (CRA-W), Bugwood.org). (C–D) Long, orange-yellow, twisted and distinctive tendrils of spores of *Cryphonectria parasitica* exuding from pycnidia on chestnut (*Castanea sativa*) tree bark (Source: Andrej Kunca, National Forest Centre – Slovakia, Bugwood.org; Ministry of Agriculture and Regional Development, Bugwood.org). (E) masses of reddish-brown pustules (stromata) in the crevices of the infected bark of *Castanea sativa* (Source: Andrej Kunca, National Forest Centre – Slovakia, Bugwood.org)

Symptoms in the canopy

- The leaves above the canker wilt and turn brown and remain hanging on the tree even after leaf fall, within 1 or 2 years after the cankers are apparent (Rigling and Prospero, 2018). This produces the so-called flag which is the most pronounced early symptom of chestnut blight in the crown of adult trees (Rigling and Prospero, 2018; EPPO, 2020) (Figure 12).
- Girdling and death of the stem or branch part distal to the canker can be observed (Rigling and Prospero, 2018).
- Crown dieback is not observed in the case of infection by hypovirulent strains (Rigling and Prospero, 2018).

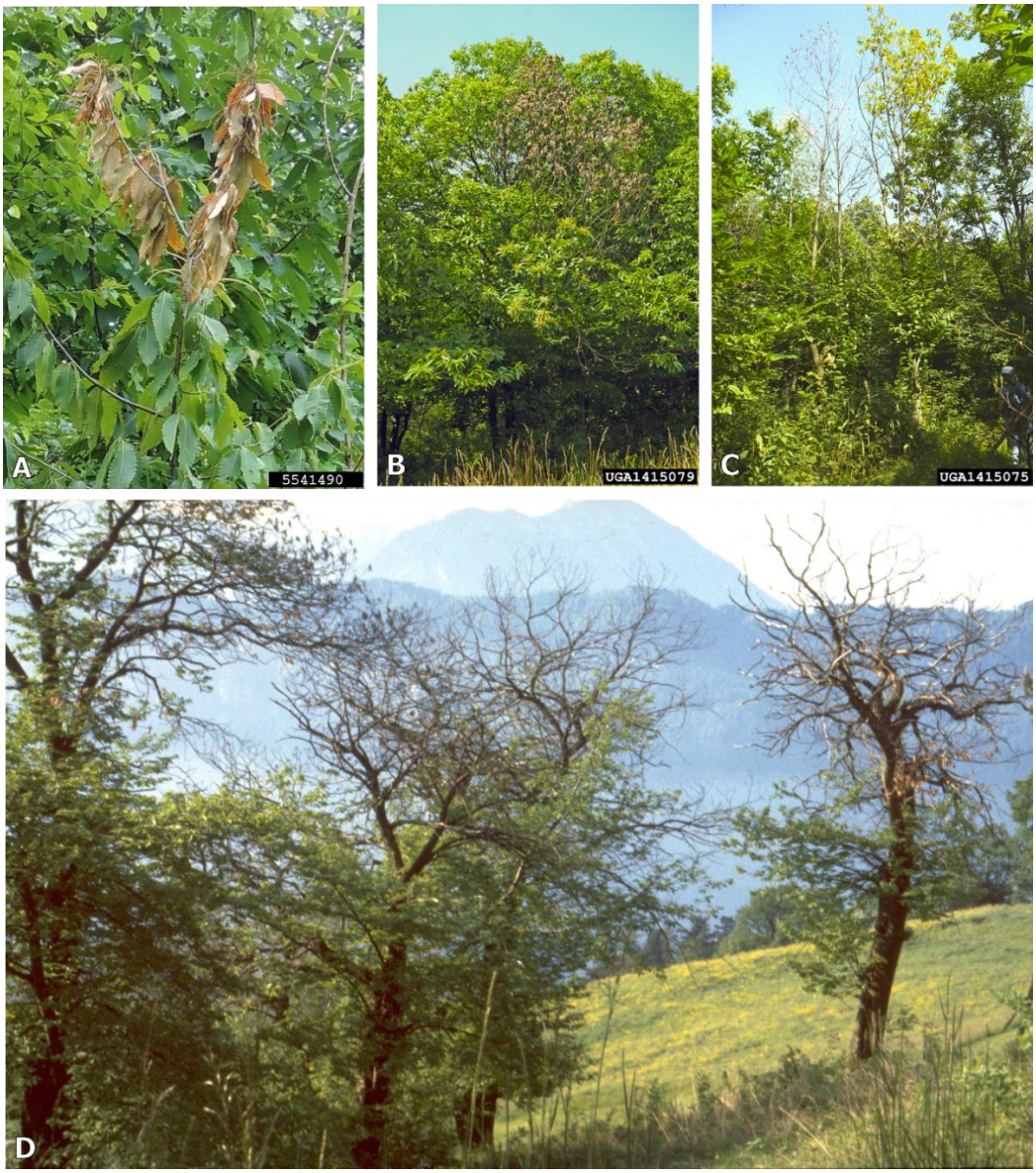

Figure 11: Symptoms of *Cryphonectria parasitica* infections on the tree canopy: (A) wilting on *C. dentata* causing the 'flag' symptom (Source: Richard Gardner, Bugwood.org); (B) wilting on *Castanea sativa* (Source: Andrej Kunca, National Forest Centre – Slovakia, Bugwood.org); (C–D) tree dieback (*Castanea sativa*) after several year of infection (Sources: (C) Andrej Kunca, National Forest Centre – Slovakia, Bugwood.org; (D) Daniel Rigling, WLS Switzerland)

RISK OF MISIDENTIFICATION

- *Cryphonectria parasitica* cankers can be confused with those caused by saprophytes and weak pathogens that may be present in the bark of *C. sativa* and those caused by *Cryphonectria radicalis* (Schweinitz) Barcan. (Bissegger and Sieber, 1994; EPPO, 2005; Adamčíková et al.,2013; EFSA PLH Panel, 2014). In order to distinguish *C. parasitica* from *C. radicalis*, a detailed characterisation of the latter was provided in a study by Hoegger et al. (2002).
- Canopy wilting and dieback could be confused with ink disease caused by *Phytophthora cambivora* and *P. cinnamomi*. However, while *C. parasitica* causes cankers, on the stem or trunk, below which the branches have healthy foliage and numerous epicormic shoots are produced, with ink disease the tree will be dead down to ground level and below (EPPO, 2020). However, this risk is more associated with seedlings or young trees.

3.1.2. Sample collection

Following an observation of cankers, samples are preferably collected from infected bark tissue, with or without stromata (preferably with), with or without mycelial fans (not always easy to observe).

Samples can also be collected from infected wood (sapwood only, sampling of wood is only necessary when the sample is debarked wood) (EPPO, 2005)).

The margin of the necroses is the best area to sample, although isolates of the fungus are also readily obtained from any visible mycelial mat (EPPO, 2005) and from the area of the canker where stroma have developed (Chandelier et al., 2019).

Samples can be taken from symptomatic plants or from cankers on trees cut more than a year before (Prospero et al., 2006). *Cryphonectria parasitica* can live up to 10 months in dried bark (Hepting, 1974; EPPO, 2020).

3.1.3. Timing of detection and identification

The best period for visual examination of dieback and cankers is in summer after spring initiation of new cankers and when latent infections may be active (Guérin and Robin, 2003). The best period for visual examination of cankers or epicormic shoots visible on stems is in winter, after leaf fall (Romon-Ochoa et al., 2022). Samples of infected plant parts for laboratory identification can be collected year-round.

Conclusion on detection and identification in the field

Cryphonectria parasitica is detected in the field by visual examination of cankers and production of epicormic shoots, canopy dieback, mycelium and characteristic fruiting structures.

3.2. Detection and identification in the laboratory

3.2.1. Morphological identification

The fungus can be identified from its characteristic fruiting structure following incubation of infected bark in a moist chamber or by isolation in culture. The isolation methods and morphological characteristics of *C. parasitica* are detailed in the EPPO diagnostic protocol (EPPO, 2005).

The fungus has a characteristic fruiting structure following incubation of infected bark in a moist chamber (EPPO, 2005; Rigling and Prospero, 2018; CABI, 2021) and it can be easily isolated on potato dextrose agar (PDA) from:

- fragments collected from the edge of lesions;
- mycelial fans under the bark;
- fruiting bodies (EPPO, 2005; Romon-Ochoa et al., 2022).

Morphological identification, after culture on PDA medium, allows virulent strains to be visually differentiated from hypovirulent strains (EPPO, 2020). The mycelium of the former is initially white and then turns yellow-orange (EPPO, 2005), while the culture of the hypovirulent strain remains white (Figure 12).

However, morphological identification needs to be confirmed using molecular tools.

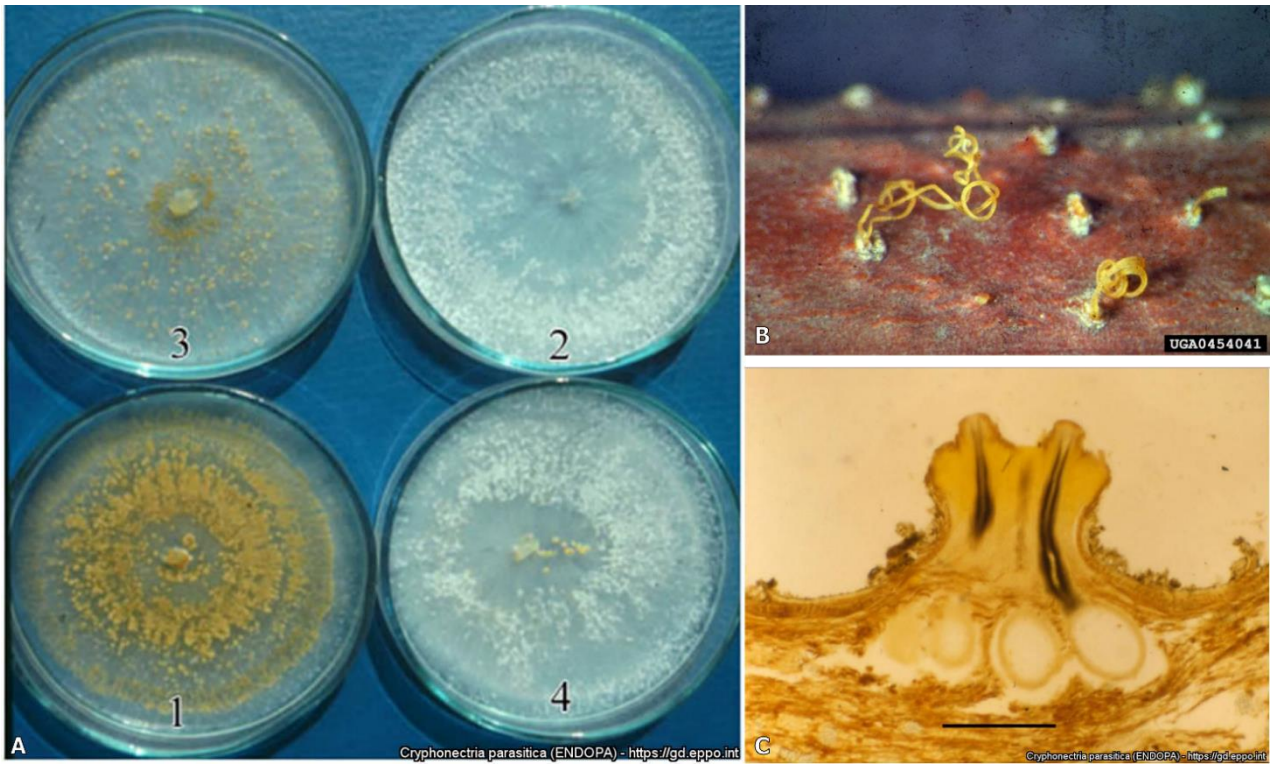

Figure 12: (A) Cultures of *Cryphonectria parasitica* isolates on PDA (1 – virulent, 2 – hypovirulent, 3 and 4 – intermediate virulence) (Source: EPPO, courtesy of SFI, Ljubljana (SI)). (B) Orange-yellow pycnidia of *Cryphonectria parasitica* on chestnut tree bark (*Castanea sativa*) (Source: Ministry of Agriculture and Regional Development, Ministry of Agriculture and Regional Development, Bugwood.org). (C) Perithecia in a stroma, necks and ostioles in papillate protuberances of the stroma (bar = 500 ìm) (Source: EPPO, courtesy of SFI, Ljubljana (SI))

3.2.2. Laboratory testing and other methods of identification

DNA-based identification of pure cultures of *C. parasitica* relies on amplification of the ITS and β-tubulin gene regions by PCR (Gryzenhout et al., 2009; Bragança et al., 2011; EFSA PLH Panel, 2014).

Real-time PCR can be used for the rapid and accurate detection of *C. parasitica* directly from bark samples (Rubio et al., 2017). The assay is capable of detecting up to 2 fg of genomic DNA (Chandelier et al., 2019).

The presence of CHV-1 (*Cryphonectria* hypovirus) can be identified by RT-PCR and sequencing of isolates, exhibiting white or intermediate (between white and orange) culture morphology (Rigling et al., 2018; Romon-Ochoa et al., 2022).

No information is currently available on the method sensitivity of the above-mentioned molecular tests.

Conclusion on detection and identification in the laboratory

Morphological identification of *C. parasitica* isolates needs to be confirmed by molecular analysis. In addition, *C. parasitica* can be detected directly from bark samples using real-time PCR.

4. Conclusion

Information on *what*, *where*, *how* and *when* to conduct survey activities for *C. parasitica* is summarised in Table 3. The identification of the target population needs to be tailored to the situation in each Member State.

Table 3: Preparation of surveys for *C. parasitica* included in Sections 1, 2 and 3

Survey question	Section	Key information
What?	1. The pest and its biology	*Cryphonectria parasitica* is a bark-inhabiting fungus and causal agent of chestnut blight (*Castanea* spp.).
Where?	2. Target population	Epidemiological units: homogeneous areas that contain at least one individual host plant for *C. parasitica* (natural forests, orchards).
		Risk areas: areas surrounding risk locations (nurseries, garden centres and markets where hosts of *C. parasitica* are traded, *C. sativa* orchards, forest stands, parks and gardens) where host plants of *C. parasitica* are present and also *C. sativa* orchards and forest stands.
		Inspection units: individual host plants (chestnut plants) examined for *C. parasitica*.
How?	3. Detection and identification	*Cryphonectria parasitica* is detected by visual examination of symptomatic plants, morphological identification and confirmed by molecular analysis. Morphological identification of *C. parasitica* needs to be confirmed by molecular analysis.
When?		The best period for visual examination is in the summer after spring initiation of new cankers and when latent infections may be active. Field surveys can also be carried out in winter, after leaf fall, when cankers or epicormic growth might be visible on stems.

5. Survey framework

Figure 13 shows the next steps after the survey preparation for designing statistically sound and risk-based detection and delimiting surveys. Guidance on the selection of the type of survey, related survey preparation and design is provided in the EFSA general guidelines for pest surveys (EFSA et al., 2020).

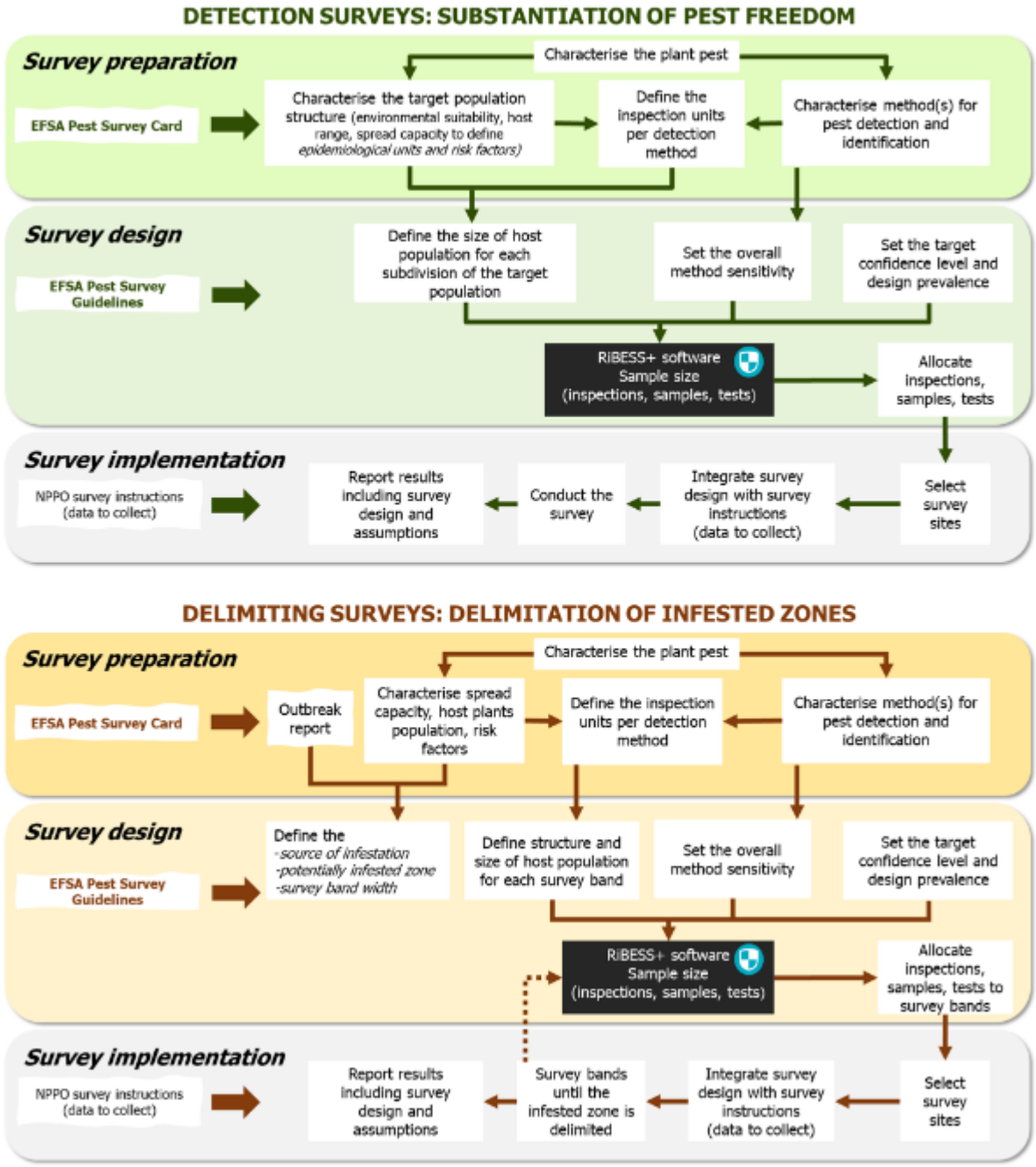

Figure 13: Steps required for the preparation, design and implementation of detection and delimiting surveys, in accordance with the methodology for statistically sound and risk-based surveillance (EFSA et al., 2020)

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General glossary for surveys of quarantine organisms

Click on the following link to access the general glossary for surveys of quarantine organisms: https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/glossary

Relevant EFSA outputs

- General guidelines for statistically sound and risk-based surveys of plant pests: https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2020.EN-1919
- Pest survey card on *Cryphonectria parasitica*: https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/cryphonectria-parasitica
- Index of the EFSA Plant Pest Survey Toolkit: https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/index
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- The statistical tool RiBESS+: https://r4eu.efsa.europa.eu/app/ribess
- The RiBESS+ manual: https://zenodo.org/record/2541541#.Ys7G5HZByUn
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