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Pest survey card on Stegophora ulmea

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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 Member States in preparing data and information for surveys for the causal agent of black spot disease of elm, Stegophora ulmea. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. Stegophora ulmea is a well-defined and distinguishable fungal species of the family Sydowiellaceae. The fungus is a Union guarantine pest, currently not known to occur in the EU and present only in North America and Asia. Stegophora ulmea has two conidial stages: the first, during spring and early summer, produces infectious macroconidia dispersed by rain splash; the second, from mid-summer, produces noninfectious microconidia that act as spermatia. The pathogen mainly overwinters in perithecia in leaf debris from which airborne ascospores are released in spring. The fungus infects many elm (Ulmus) species and hybrids and also Zelkova serrata. Detection and delimiting surveys should target Ulmus species and hybrids and Z. serrata. The host species, being widely distributed in the EU territory (forests, cities, parks), and the EU climatic conditions are not considered to be limiting factors for the establishment of the pathogen, if introduced. Visual examination in spring for the symptoms on the leaves (yellow lesions and black spots), is the most effective method of detecting *S. ulmea* in the field. Identification of the pathogen is done in the lab by microscopic examination of acervuli from the leaves and/or morphological identification of the pathogen in pure culture. Molecular methods for detection and identification are not available.

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Stegophora ulmea survey card



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Introduction

The objective of this pest survey card is to provide the relevant information needed to prepare surveys for the causal agent of black spot disease of elm, *Stegophora ulmea*, in EU Member States (MSs) following the methodology described in EFSA et al. (2018). 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 Toolkit¹ 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 *Stegophora ulmea*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+ manual⁴ and video tutorial⁵.

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 *S. ulmea* (EFSA PLH Panel, 2017), the European and Mediterranean Plant Protection Organization (EPPO) datasheet on *Stegophora ulmea* present in the EPPO Global Database (EPPO, online) and the Centre for Agriculture and Bioscience International (CABI) datasheet on *Stegophora ulmea* (CABI, 2023) and other documents.

The main challenge for the surveillance of *S. ulmea* is the lack of DNA sequences for the molecular identification of the pathogen.

The main knowledge gaps concern:

- The biology of the pest, in particular the incubation period, spread capacity of ascospores, the ability of the pathogen to develop perithecia not only on leaves, but also on infected fruit and twigs (EPPO, 2002, 2005; EFSA PLH Panel, 2017; Lane et al., 2013).
- The susceptibility of hybrid elm cultivars resistant to Dutch elm disease (*Ophiostoma novo-ulmi*) planted as a consequence of the Dutch elm disease pandemic in Europe, that are known to have parents susceptible to *S. ulmea* and may therefore be susceptible to the disease.
- The distribution of *S. ulmea* in China and possibly elsewhere in East Asia.

³ <u>https://r4eu.efsa.europa.eu/app/ribess</u>

¹ <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/index</u>

² The published Pest survey cards in the story map format are available in the Plant Pests Survey Cards Gallery available online: <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/gallery</u>

⁴ <u>https://zenodo.org/record/2541541#.YkrgRyhByUm</u>

⁵ A tutorial video for the use of RiBESS+ is available online: <u>https://youtu.be/qYHqrCiMxDY</u>





1 The pest and its biology

1.1 Taxonomy

Current scientific name: Stegophora ulmea (Fries) Sydow & P. Sydow

Class: Sordariomycetes

Order: Diaporthales

Family: Sydowiellaceae

Genus: Stegophora

Species: Stegophora ulmea

Synonyms: *Asteroma ulmeum* (Miles) B. Sutton; *Cylindrosporella ulmea* (Miles) Arx; *Dothidella ulmea* (Fries) Ellis & Everhart; *Gloeosporium ulmeum* Miles; *Gloeosporium ulmicola* Miles; *Gnomonia ulmea* (Fries) von Thümen; *Lambro ulmea* (Fries) E. Müller; *Sphaeria ulmea* Fries

EPPO code: GNOMUL

Common names: black spot of elm, leaf spot of elm, twig blight, elm leaf scab, anthracnose

Taxonomic rank: species



Figure 1: Elm leaves with small, raised black spots with a fine area of white tissue surrounding them (Source: Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org)





Conclusions on taxonomy

Stegophora ulmea is a well-defined and distinguishable fungal species of the family Sydowiellaceae.

1.2 EU pest regulatory status

Stegophora ulmea is a Union quarantine pest, listed in Annex II (Part A, section 2 'Fungi and oomycetes', Point 32) of Commission Implementing Regulation (EU) 2019/2072⁶.

The Regulation does not provide special requirements for *S. ulmea*, but it does provide special requirements for the introduction into the EU of the pathogen's host commodities. In fact, Annex VII (Points 32.2, 32.3, 32.6, 103, 104, 105, 106, 109, 110) of Commission Implementing Regulation (EU) 2019/2072 lists a number of special requirements for the introduction of plants for planting and plant products of *Ulmus* L., *U. parvifolia* and *Zelkova serrata* into the territory of the EU.

Phytosanitary certificates are required for the introduction into the territory of the EU of:

- plants for planting, other than seeds, from all third countries other than Switzerland (Annex XI, Part A, Point 2);
- wood of *Ulmus* L. and *Z. serrata* from third countries (Annex XI, Point 12).

Plants for planting, other than seeds, *in vitro* material and naturally or artificially dwarfed woody plants for planting of *Ulmus* L. are included in the list of high-risk plants under Commission Implementing Regulation (EU) 2018/2019⁷. Wood of *Ulmus* L. is not considered as a high-risk plant product (Commission Implementing Regulation (EU) 2020/1214⁸).

The general requirements for surveys of quarantine pests in the EU territory are laid down in Regulation (EU) 2016/2031⁹ and Commission Implementing Regulation (EU) 2020/1231¹⁰.

 $^{^6}$ 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 Commission Implementing Regulation (EU) 2018/2019 of 18 December 2018 establishing a provisional list of high risk plants, plant products or other objects, within the meaning of Article 42 of Regulation (EU) 2016/2031 and a list of plants for which phytosanitary certificates are not required for introduction into the Union, within the meaning of Article 73 of that Regulation. C/2018/8877 OJ L 323, 19.12.2018, p. 10–15.

⁸ Commission Implementing Regulation (EU) 2020/1214 of 21 August 2020 amending Implementing Regulation (EU) 2018/2019 as regards wood of Ulmus L. and certain plants for planting of Albizia julibrissin Durazzini and Robinia pseudoacacia L. originating in Israel. OJ L 275, 24.8.2020, p. 12–15.

⁹ 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.

¹⁰ Commission Implementing Regulation (EU) 2020/1231 of 27 August 2020 on the format and instructions for the annual reports on the results of the surveys and on the format of the multiannual survey programmes and the practical arrangements, respectively provided for in Articles 22 and 23 of Regulation (EU) 2016/2031 of the European Parliament and the Council. OJ L 280, 28.8.2020, p. 1–17.



Overview of the EU regulatory status

Stegophora ulmea is a Union quarantine pest. There are no special requirements specifically addressing this pest, but the introduction into the EU of plants for planting and plant products of *Ulmus* spp. and *Z. serrata* from third countries must meet special requirements and be accompanied by a phytosanitary certificate.

1.3 Pest distribution

The pathogen is considered to be native in North America (EPPO, 2005) and is currently not known to occur in the EU. The pathogen has been recorded as the most common species on *Ulmus glabra* leaves in Montenegro (Vemić A, 2022). However, there is uncertainty about the reliability of this report due to the identification methods used and also the reference to the synonymous name (C. Robin, personal communication).

Currently, EPPO (online) lists the pathogen as being present in:

- North America: Canada (Manitoba, Nova Scotia, Ontario and Québec), the USA (Alabama, California, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Mississippi, New Jersey, North Carolina, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia and Wisconsin);
- Asia: far eastern Russia and China. The presence of the pathogen in China is mainly based on its detection on bonsai imported from China to Europe (EPPO, online).

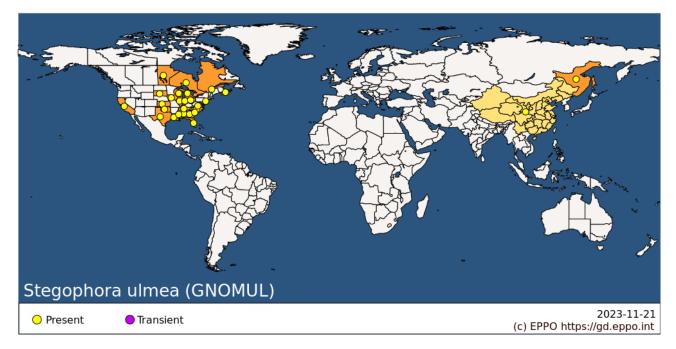


Figure 2: Global distribution of *Stegophora ulmea* (Source: EPPO Global Database, https://gd.eppo.int/, map updated on 2023-10-09, accessed on 2023-11-21)



Conclusion on pest distribution

Stegophora ulmea is currently reported present in North America and Asia and it is not known to occur in the EU.

1.4 Life cycle

Stegophora ulmea is the causal agent of black spot disease of elm (EFSA PLH Panel, 2017).

In spring, airborne ascospores (the primary inoculum), produced in overwintered perithecia in leaf debris, are released. This usually occurs under conditions of alternating wetness and drying after several days with temperatures of at least 7°C and usually in synchrony with the elm's leaf development (Sinclair et al., 1987; EFSA PLH Panel, 2017). Following release, the airborne ascospores commonly infect the lower leaves and twigs of elms (*Ulmus* spp.) (EFSA PLH Panel, 2017). 'An additional, unidentified, source of inoculum, thought to originate from overwintered buds, can result in severe infection' (McGranahan & Smalley, 1984b, quoted in EPPO, 2005). *S. ulmea* may also overwinter in persistent leaves (EFSA PLH Panel, 2017).

Following infection by the airborne ascospores, yellow lesions develop on the upper surface of the leaves. Subsequently, acervuli develop in the centre of the lesions which are followed by the formation of black stroma (Sinclair et al., 1987; EPPO, 2005; EFSA PLH Panel, 2017).

Two conidial stages develop successively in the acervuli (McGranahan and Smalley, 1984b; EFSA PLH Panel, 2017). The first conidial stage (*Gloeosporium ulmicolum*) produces a white mass of infectious unicellular macroconidia (EPPO, 2005), which are liberated when the acervuli is split open. These macroconidia act as the secondary inoculum during spring and early summer and represent the asexual stage of *S. ulmea* reproduction (McGranahan and Smalley, 1984a; McGranahan and Smalley, 1984b). Rain splash is presumed necessary for dispersal of macroconidia from the lower to the upper leaves (EPPO, 2005). The macroconidia mature 10–20 days after primary infection (Sinclair et al., 1987; EPPO, 2005). Besides the leaves, the petioles, succulent stems and green fruit of some elms can also be infected by the fungus. This can cause leaf and twig blight and fruit curling (Sinclair et al., 1987; EPPO, 2005).

The second conidial stage (*Cylindrosporella ulmea*) produces non-infectious microconidia in the lesions during mid-summer (McGranahan and Smalley, 1984a; EPPO, 2005; EFSA PLH Panel, 2017). This suggests that microconidia act as spermatia in the sexual cycle of the fungus (McGranahan and Smalley, 1984a, b; EPPO, 2005).

Perithecia begin to develop in lesions during late summer and autumn and development continues in senesced leaves, where the perithecia overwinter (McGranahan and Smalley, 1984a; EPPO, 2005).

The ability of the pathogen to develop fruiting bodies not only on leaves, but also on infected fruit and twigs remains uncertain.





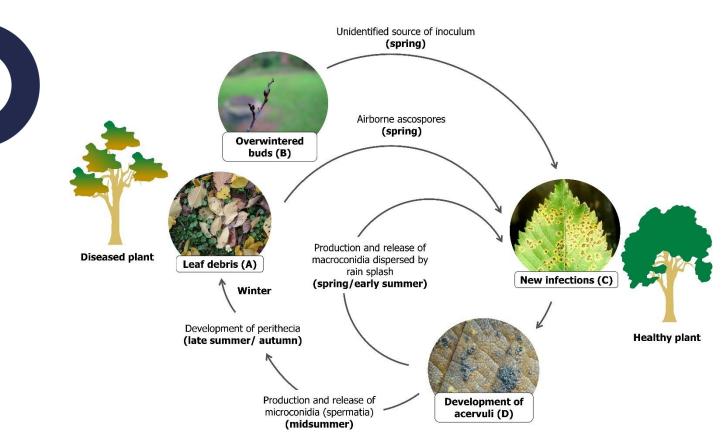


Figure 3: Life cycle of *S. ulmea.* (A) Overwintered perithecia in leaf debris; (B) unidentified source of inoculum from overwintered buds; (C) yellow lesions following infection; (D) acervuli on an elm leaf. (Source: (A) Robert Vidéki, Doronicum Kft., Bugwood.org; (B) Chris Evans, University of Illinois, Bugwood.org; (C, D) Bruce Watt, University of Maine, Bugwood.org)

Conclusion on life cycle

Stegophora ulmea is the causal agent of black spot disease of elm. New infections of leaves and twigs occur in spring from airborne ascospores and from overwintering buds. The pathogen has two conidial stages: macroconidia cause new leaf infections (asexual and secondary inoculum), and microconidia act as spermatia.

2 Target population

This section provides the information needed to characterise the population of host plants to target in a survey, as described in '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 its 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, or 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 also 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 *Stegophora ulmea* within the EU is proposed in Section 2.5 (Figure 5).

2.1 Host range and main hosts

Stegophora ulmea causes a serious foliar disease on many elm species (Ulmus spp.) (McGranahan and Smalley, 1984a). Zelkova serrata is also susceptible to the pathogen (EPPO, 2005).

In North America, *Ulmus americana* (American white elm) is the major host of *S. ulmea* (EPPO, 2005).

The European species *Ulmus minor*, *U. laevis* and *U. glabra* were found to be susceptible to the disease (McGranahan and Smalley, 1981).

The susceptibility of hybrid elm cultivars resistant to Dutch elm disease (*Ophiostoma novo-ulmi*), planted as a consequence of the Dutch elm disease pandemic in Europe, which are known to have parents susceptible to *S. ulmea*, is unknown (EPPO, 2005; EFSA PLH Panel, 2017; Lane et al., 2013). Table 1 lists the known hosts of *S. ulmea*.

Detection and delimiting surveys in the EU should focus on *Ulmus* species and hybrids and *Zelkova serrata*.





Table 1: Host species of *Stegophora ulmea*, their presence in the EU and level of susceptibility (if known) (McGranahan and Smalley, 1981)

Species and hybrids	Common name	Presence in the EU	Susceptibility	References
U. glabra	Wych elm	Native	High	McGranahan and Smalley, 1981
U. laevis	European white elm	Native	High	McGranahan and Smalley, 1981
U. minor*, U. carpinifolia	European field elm	Native	Moderate to low	McGranahan and Smalley, 1981; Benet et al. 1995
U. x hollandica	Dutch elm	Native	Unknown	EPPO, 2005; EFSA PLH Panel, 2017
U. americana	American white elm	Introduced	High (major host)	McGranahan and Smalley, 1981
U. japonica	Japanese elm	Introduced	Moderate to low	Mittempergher and Santini, 2004; McGranahan and Smalley, 1981
U. parvifolia	Lacebark elm	Introduced	Moderate to low	Mittempergher and Santini, 2004; McGranahan and Smalley, 1981
U. pumila	Siberian elm	Introduced	Moderate to low	McGranahan and Smalley, 1981; McGranahan and Smalley, 1984b
Zelkova serrata	Japanese zelkova	Introduced	Moderate to low	EPPO, 2005; EFSA PLH Panel, 2017
Interspecific <i>Ulmus</i> hybrids	-	Introduced	Moderate to low	Benet et al. 1995
U. alata	Winged elm	Not present	Unknown	EPPO, 2005; EFSA PLH Panel, 2017
U. crassifolia	Cedar elm	Not present	Unknown	EPPO, 2005; EFSA PLH Panel, 2017
U. laciniata	-	Not present	Moderate to low	McGranahan and Smalley, 1981; McGranahan and Smalley, 1984b
U. rubra	Slippery elm	Not present	Unknown	EPPO, 2005; EFSA PLH Panel, 2017
U. serotina	Red elm	Not present	Unknown	EPPO, 2005; EFSA PLH Panel, 2017
U. thomasii	Rock elm	Not present	Moderate to low	McGranahan and Smalley, 1981; EPPO, 2005

* The English elm, named *U. procera*, is a clone of *U. minor*.



Conclusion on host range and main hosts

Stegophora ulmea infects many elm species (Ulmus spp.) and also Zelkova serrata. Detection and delimiting surveys in the EU should focus on Ulmus species and hybrids and Zelkova serrata.

2.2 Environmental suitability

Climatic conditions in the EU territory are not a limiting factor for the establishment of *S. ulmea*, if introduced (EFSA PLH Panel, 2017). Climate types suitable to pathogen survival also overlap to a large extent with the distribution of *Ulmus* species in Europe (EFSA PLH Panel, 2017).

Ulmus is a genus consisting of medium-sized species with a predominantly arboreal habit. These are mainly widespread in the temperate and boreal regions of the northern hemisphere (Gellini and Grossoni, 1997).

In Europe, the elms, whose populations have already been extensively depleted by Dutch elm disease, are found in fresh mixed deciduous forests and are used as ornamental plants on roadsides and in parks and gardens (EPPO, 2002; Caudullo and de Rigo, 2016). The elm species native to Europe are *Ulmus minor*, *Ulmus glabra* (and their natural hybrid *U*. *x hollandica*) and *Ulmus laevis*. The field elm, *U. minor*, has a more southern range (Figure 4A). The English elm, *U. procera*, is a clone of *U. minor* and is widespread in the western part of central and southern Europe (Gellini and Grossoni, 1997). Like other elm species, it has been planted as a multifunctional plant to meet a wide range of needs (e.g. for fodder, firewood and timber production, and ornamental purposes (CABI, 2023)). The Wych elm, *U. glabra*, has a more northerly range, occurring only in the mountain forests of southern Europe (Collin, 2002; Caudullo and de Rigo, 2016) (Figure 4B). The European white elm, *U. laevis*, has a more eastern range with sporadic spots in the Mediterranean area (Torre et al., 2022) (Figure 4C).

These three native species are part of the traditional rural landscape as trees with multiple purposes such as working and firewood, for fodder and as a living grapevine support. Elms are also planted as ornamental and roadside trees in cities and parks and are a component of fresh mixed deciduous forests (Santini et al., 2010; Caudullo and de Rigo, 2016). The Dutch elm, *U.* x *hollandica* is a natural hybrid between *U. glabra* and *U. minor*. It is present in mixed forests (e.g. in Lithuania) (Petrokas and Baliuckas, 2012) and is widely used for urban greenery (in Holland and Belgium) (Mittempergher and Santini, 2004; Lawalrée, 1952; Solla et al., 2005).

The North American species *U. americana* was introduced to Europe, where it is occasionally planted as an ornamental tree in a variety of cultivars. The Siberian elm, *U. pumila*, was introduced from Asia to southern Europe (e.g. Spain and Italy) and planted as an ornamental plant or to replace field elms heavily impacted by Dutch elm disease epidemics (Solla et al., 2005, Brunet et al., 2013). *Ulmus parvifolia* and *U. japonica* were introduced to Europe for ornamental purposes and as a source of genes resistant to Dutch elm disease (Mittempergher and Santini, 2004).

Zelkova serrata was introduced from Asia to Europe where it is planted as an ornamental tree in streets, parks and arboreta (EPPO, 2005; CABI, 2019). It is also used for bonsai (Lane et al., 2013; CABI, 2019).



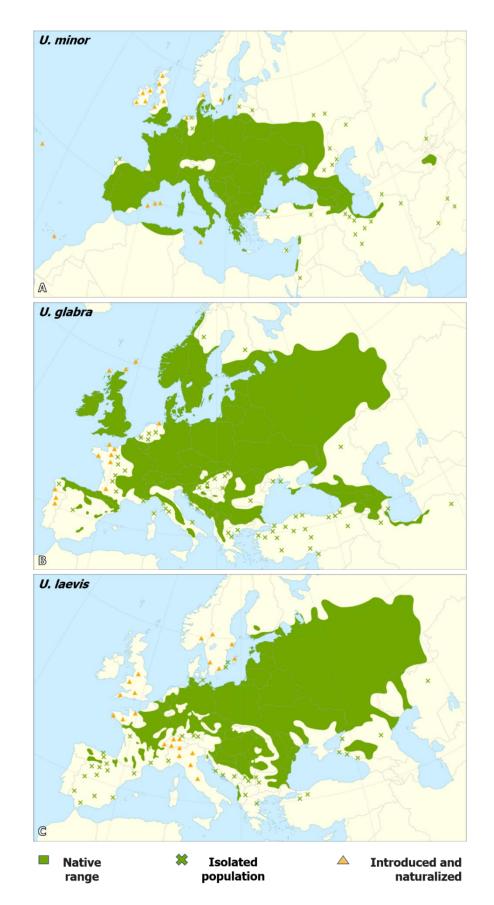


Figure 4: Distribution map of (A) *U. minor*, (B) *U. glabra* and (C) *U. laevis* (Source: modified from Caudullo et al., 2017)



Conclusion on environmental suitability

Climatic conditions are not to be considered as a limiting factor for the establishment of the pathogen in the EU territory if introduced. The host species are widely distributed in the EU territory (forests, cities, parks) and the climate types suitable to pathogen survival overlap to a large extent with the distribution of both native and introduced host species.

2.3 Spread capacity

Natural spread

Ascospores are forcibly expelled from perithecia and are airborne over long distances (EPPO, 2005). Estimating the distance for ascospore spread capacity remains a challenge as it can vary from a few metres to a few hundred metres and the median distance is not easy to know without precise experiments, but the end-of-distribution events can be very significant in terms of disease spread. Spread from ascospores can occur within the same field plot or the same nursery, but further spread (depending on wind) should also be taken into account (C. Robin, personal communication).

Macroconidia are dispersed by rain splashes from the lower to the upper leaves (local spread) (EPPO, 2005).

Human-assisted spread

Stegophora ulmea can survive on leaves, twigs, fruit and dormant buds (EPPO, 2005; EFSA PLH Panel, 2017). Therefore, the spread of the pathogen over greater distances can be facilitated by human activity, primarily through the importation of infected plants for planting, cut branches and bonsai plants of *Ulmus* spp. and hybrids and *Z. serrata* (mainly from China (Lane et al., 2013)). The ability of the pathogen to develop perithecia even on infected fruit and twigs is uncertain, so the role of infected fruit and twigs as a means of spread/entry is also uncertain.

In the Netherlands, the pathogen was found on bonsai plants from China (EPPO, 2002). The infected plants were destroyed and, to date, the pathogen has been eradicated (EPPO, online).

Conclusion on spread capacity

Stegophora ulmea spreads naturally through the dispersal of airborne ascospores and conidia by rain splash. Human-assisted spread is facilitated by the import of infected plants for planting, cut branches and bonsai plants of *Ulmus* spp. and *Zelkova serrata*.

2.4 Risk factor identification

Identification of risk factors and their relative risk estimation are essential for performing riskbased 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 have more than one level of risk for the target population) and the proportion of the overall target population on which they apply. The



identification of risk factors needs to be tailored to the situation of each Member State. This section presents examples of risk factors for *Stegophora ulmea* and is 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 *Stegophora ulmea*. These activities should then be connected to specific locations. Around these locations, risk areas can be defined, knowing 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 on 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: Import of plants for planting and cut branches of *Ulmus* **spp. and** *Z. serrata* **from countries where** *S. ulmea* **is known to occur**

The main pathway of introduction of *S. ulmea* into new areas is through the importation of infected plants for planting and cut branches of *Ulmus* spp. and *Z. serrata* (EPPO, 2002; EPPO, 2005; EFSA PLH Panel, 2017; EPPO, 2020), especially given the pathogen's ability to survive on leaves and overwinter in dormant buds (EPPO, 2003; EFSA PLH Panel, 2017).

Nurseries, garden centres, markets and new plantations where imported *Ulmus* spp. and *Z. serrata* plants are stored, propagated, traded or planted should be considered as the risk locations, while surrounding areas, where host plants are present, should be considered as risk areas to be surveyed.

Example 2: Import of bonsai plants of *Ulmus* spp. and *Z. serrata* from counties where *S. ulmea* is known to occur

As the pathogen has been repeatedly intercepted in the European territory on bonsai plants (*Ulmus* spp. and *Z. serrata*) imported from China or in transit via the Netherlands (EPPO, 2002; Lane et al., 2013; EFSA PLH Panel, 2017), the import and trade of bonsai plants from countries where *S. ulmea* is present, is a pathway for entry and spread of the pathogen (EPPO, 2005; EFSA PLH Panel, 2017).

Thus, nurseries and garden centres importing *Ulmus* spp. and *Z. serrata* bonsai plants from areas where *S. ulmea* is known to occur should be considered risk locations. The nurseries and garden centres themselves, and the surrounding areas where *S. ulmea* host plants are present should be considered as risk areas (EPPO, 2005; Lane et al., 2013).

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Table 2: Examples of risk activities and corresponding risk locations and risk areas relevant for the surveillance of Stegophora ulmea

Risk activity	Risk locations	Risk areas
and cut branches of <i>Ulmus</i> spp. and <i>Z. serrata</i> from	Nurseries, garden centres, markets and new plantations where imported host plants are stored, propagated, traded or planted.	themselves and surrounding
	Nurseries and garden centres	5

Ulmus spp. and *Zelkova* importing bonsai host plants. serrata from areas where S. ulmea is known to occur.

themselves and surrounding areas where S. ulmea host plants are present.

2.5 Structure of the target population

LEVEL 1: SURVEY AREA	LEVEL 2-3: EPIDEMIOLOGICAL UNITS		LEVEL 4: RISK AREAS	LEVEL 5: INSPECTION UNITS
e.g. Member State	e.g. NUTS areas	e.g. forests, urban areas and parks where host plants are present	e.g. host plants that are located near risk locations (nurseries and garden centres)	e.g. individual host plant (<i>Ulmus laevis</i> and <i>Zelkova serrata</i>)

Figure 5: Example of the hierarchical structure of the target population for Stegophora ulmea in the EU (Source: Eurostat, 2022 (levels 1-2); Norbert Frank, University of West Hungary, Bugwood.org (level 3, top); Richard Webb, Bugwood.org (level 3, bottom); Rachel McCarthy, Cornell University, Bugwood.org (level 4, top); Robert L. Anderson, USDA Forest Service, Bugwood.org (level 4, bottom); Robert Vidéki, Doronicum Kft., Bugwood.org (level 5, top); Richard Webb, Bugwood.org (level 5, bottom))



3 Detection and identification

Stegophora ulmea can be detected in the field by the observation of black spot disease symptoms and signs, whose sampling is consequently required. Identification is based on its morphological and microscopic characteristics. *Stegophora ulmea* can also be successfully isolated by the surface-sterilised disc-leaf method. Molecular methods for detection and identification are unavailable.

3.1 Detection and identification in the field

3.1.1 Visual examination

Symptoms and signs

- Initial symptoms appear early in the spring as small yellow spots (1 mm diameter) on the upper surfaces of the leaves when they are unfolding from buds (Miles, 1921; Sinclair et al., 1987; EFSA PLH Panel, 2017).
- Within several days, the centres of the yellow spots turn black as the stromata develop (Figure 6A) (McGranahan and Smalley, 1984b; EPPO, 2005). The black spots can increase to occupy the entire chlorotic area (Miles, 1921). In most cases, the black spots are surrounded by a narrow band of whitish dead leaf tissue (Miles, 1921; Stipes and Campana, 1981; EPPO, 2005) (Figure 6B,C).
- Premature shedding of leaves may occur during mid-summer (EPPO, 2005).
- Severe infections cause blight of young leaves and shoots and complete defoliation in early August (Sinclair et al., 1987; EPPO, 2005).
- The green fruit of some elm species can become infected and crumpled (EPPO, 2005).



Figure 6: (A–C) black spots of elm (Sources: (A) Bruce Watt, University of Maine, Bugwood.org; (B) Elizabeth Bush, Virginia Polytechnic Institute and State University, Bugwood.org; (C) Olson, 2015)



3.1.2 Sample collection

Symptomatic leaves with yellow lesions and/or black spots should be collected (McGranahan and Smalley, 1984a; EPPO, 2005) and stored in paper bags for transport from the field to the laboratory.

3.1.3 Timing of detection and identification

Visual examination of leaves in the spring for yellow lesions and/or black spots would be the most effective method of early detection of the disease (EPPO, 2005). However, disease symptoms (yellow lesions and/or black spots) can be observed throughout the year on species of evergreen elm (e.g. *U. parvifolia*) (EPPO, 2005).

Conclusion on detection and identification in the field

Stegophora ulmea can be detected in the field through visual examination of symptoms on the leaves (yellow lesions and/or black spots) and premature defoliation. Symptomatic leaves should be sampled and sent to the laboratory for confirmation. The best time for visual examination of the leaves is in spring, but disease symptoms can be observed throughout the whole year on evergreen species.

3.2 Detection and identification in the laboratory

3.2.1 Morphological identification

Microscopic inspection of acervuli can be used to detect and confirm the identification of the fungus (EPPO, 2005) (Figure 7A–C).



Figure 7: (A) close-up of infected spots with blister-like, brown spore-producing bodies (acervuli) within the spots; (B) acervuli on elm leaf; (C) asexual spores of *Stegophora ulmea* (Source: (A) Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org; (B,C) Bruce Watt, University of Maine, Bugwood.org)

The pathogen can also be isolated from leaf spot with fresh sporulating lesions (McGranahan and Smalley, 1984a). *Stegophora ulmea* is isolated by the surface-sterilised leaf-disc method (McGranahan and Smalley, 1984a). Growth on solid media is usually not visible for at least 3 weeks and sometimes even for 6 weeks (McGranahan and Smalley, 1984a). The pathogen is in fact a very slow growing ascomycete (McGranahan and Smalley, 1984a). Identification of *S. ulmea* after culturing is based on the observation of specific morphological characteristics of the



colonies and microscopic characteristics, which are provided in the EPPO datasheets on quarantine pests: *Stegophora ulmea* (EPPO, 2005).

Colonies are initially white, raised and callus-like. Then a dark margin of acervuli develops and eventually the entire culture turns dark brown (McGranahan and Smalley, 1984a). Further details on the pathogen isolation technique are available in McGranahan and Smalley (1984a).

Confirmation by microscopic observations of the acervuli from collected leaf samples is quicker, and gives more information because colony aspect is not a good indicator. The challenge for identification is due to the fact that molecular identification by internal transcribed spacer sequencing is not possible, since there is no sequence available in Genbank.

3.2.2 Laboratory testing and other methods of identification

Molecular methods for detection and identification are unavailable (EFSA PLH Panel, 2017) because there is no sequence available in Genbank. This presents one of the challenges for identification.

Conclusion on detection and identification in the laboratory

Identification is based on its morphological and microscopic characteristics. *Stegophora ulmea* can also be successfully isolated by the surface-sterilised disc-leaf method. Molecular methods for detection and identification are not available.



4 Conclusion

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

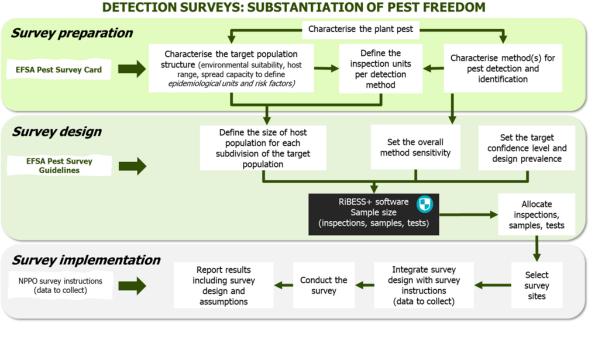
Table 3: Preparation of surveys for *Stegophora ulmea* included in Sections 1, 2 and 3

Survey question	Sections	Key information
What?	1. The pest and its biology	<i>Stegophora ulmea</i> is the causal agent of black spot disease of elm (<i>Ulmus</i> spp.) and Japanese Zelkova (<i>Zelkova serrata</i>).
		Epidemiological units: homogeneous areas that contain at least one individual host plant of <i>S. ulmea</i> (elm and Zelkova serrata plants).
Where?	2. Target population	Risk areas: areas surrounding risk locations (nurseries, garden centre markets and new plantations) where hosts of <i>S. ulmea</i> are stored, traded or planted.
		Inspection unit: individual host plants (<i>Ulmus</i> spp. and <i>Zelkova serrata</i>) examined for <i>S. ulmea.</i>
How?	3. Detection and identification	Recommended method: detection of symptoms by visual examination of leaves (black spots/yellow lesions). Sampling of symptomatic leaves and identification of the pathogen is done in the lab by microscopic examination of acervuli from the leaves and/or morphological identification of the pathogen in pure culture.
When?		The best period for visual examination is during the spring months or throughout the year in species of evergreen elm (e.g. <i>U. parvifolia</i>). Samples of infected leaves for laboratory identification (isolation and morphology) can be collected during the growing season.



5 Survey framework

Figure 8 shows the next steps after the survey preparation for designing statistically sound and risk-based detection and delimiting surveys for *Stegophora ulmea*. Guidance on selecting the type of survey, related survey preparation and design, is provided in the EFSA general guidelines for pest surveys (EFSA et al., 2020).



DELIMITING SURVEYS: DELIMITATION OF INFESTED ZONES

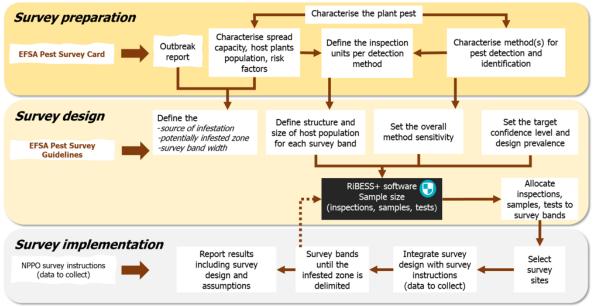


Figure 8: Steps required for the preparation, design and implementation of detection and delimiting surveys, in accordance with the methodology for statistically sound and risk-based surveillance (EFSA et al., 2020)



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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: <u>https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2020.EN-1919</u>
- Pest survey card on *Stegophora ulmea*: <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/stegophora-ulmea</u>
- Pest categorisation of *Stegophora ulmea*: <u>https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2017.5105</u>
- Index of the EFSA Plant Pest Survey Toolkit: <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/index</u>
- Plant pest survey cards gallery: <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/gallery</u>
- Pest survey cards: what, when, where and how to survey? <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/video-pest-survey-card</u>
- The statistical tool RiBESS+: <u>https://r4eu.efsa.europa.eu/app/ribess</u>
- The RiBESS+ manual: <u>https://zenodo.org/record/2541541#.Ys7G5HZByUn</u>
- The RiBESS+ video tutorial: <u>https://youtu.be/qYHqrCiMxDY</u>