PEST SURVEY CARD



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Pest survey card on *Chrysomyxa arctostaphyli*

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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 *Chrysomyxa arctostaphyli*, the causal agent of spruce broom rust. Chrysomyxa arctostaphyli is a well-defined and distinguishable fungal species of the genus Chrysomyxa (Uredinales, Coleosporiaceae) and a Union guarantine pest currently not known to occur within the EU. The pathogen is rust fungus with a two-year life cycle alternating between two systematically distinct hosts: the aecial host *Picea* spp. (spruce) and the telial host *Arctostaphylos* spp. (bearberries and manzanitas). The natural spread of C. arctostaphyli is through wind-borne aeciospores and basidiospores from the aecial to the telial host and from the telial to the aecial host, respectively. The spread can be facilitated by human activity through the movement of infected host plants. Should C. arctostaphyli be introduced into the EU, climatic conditions will not be a limiting factor to pathogen establishment, and both hosts are widely distributed (spruce occurs naturally, as an ornamental and forestry plantation tree) and often found associated in the same habitats. Detection and delimiting surveys of C. arctostaphyli in the EU should target all susceptible Picea and Arctostaphylos species located within a distance of 300 m from each other. Chrysomyxa arctostaphyli is detected in the field through visual observation of symptoms on both host plants (conspicuous, perennial brooms on Picea spp., purple-brown spots on one-year-old leaves of Arctostaphylos spp.). Nevertheless, the identification of the pathogen needs to be confirmed in the laboratory by observation and description of the morphological characteristics of the pathogen and by the use of molecular diagnostic techniques of PCR.

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Keywords: spruce broom rust, *Picea* spp., *Arctostaphylos* spp., Union quarantine pest, 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 for the causal agent of spruce broom rust, *Chrysomyxa arctostaphyli* in EU Member States following the methodology described in EFSA et al. (2018). It is part of a toolkit that has been developed to assist the Member States 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 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 *Chrysomyxa arctostaphyli*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 the EFSA pest categorisation (EFSA PLH Panel et al., 2018), the European and Mediterranean Plant Protection Organization (EPPO) datasheet on *Chrysomyxa arctostaphyli* (EPPO, 2022), the EPPO Global Database (EPPO, online), the Centre for Agriculture and Bioscience International (CABI) datasheet on *Chrysomyxa arctostaphyli* (CABI, online-a) and other documents.

The challenges for the surveillance of *C. arctostaphyli* in the EU territory are the knowledge gap on to the susceptibility of *Picea abies* and *P. sitchensis*, and the lack of a rapid, specific and sensitive molecular detection tool such as real-time PCR or LAMP assays (EFSA PLH Panel et al., 2018).

1. The pest and its biology

1.1. Taxonomy

Current scientific name: Chrysomyxa arctostaphyli Dietel

Class: Pucciniomycotina

Order: Pucciniales

Family: Coleosporiaceae

Genus: Chrysomyxa

Species: *Chrysomyxa arctostaphyli*

Synonyms: *Melampsoropsis arctostaphyli* Arthur, *Peridermium coloradense* Arthur & Kern (anamorph)

EPPO Code: CHMYAR

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 Story Maps Gallery available online: <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/gallery</u>

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

⁴ https://zenodo.org/record/2541541#.YkrgRyhByUm

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



Common name: spruce broom rust, common yellow witches' broom rust

Taxonomic rank: species

Chrysomyxa arctostaphyli is a basidiomycete fungus, causal agent of spruce broom rust (Figure 1) (EFSA PLH Panel et al., 2018).



Figure 1: Characteristic broom caused by *Chrysomyxa arctostaphyli* on white spruce (*Picea glauca* (Moench) Voss) (Source: Tom Laurent, USDA Forest Service, Bugwood.org)

Conclusions on taxonomy

Chrysomyxa arctostaphyli is a well-defined and distinguishable fungal species of the family Coleosporiaceae.

1.2. EU pest regulatory status

Chrysomyxa arctostaphyli is a Union quarantine pest, listed in Annex II (Part A 'Pest not known to occur in the Union territory', section B 'Fungi and oomycetes', Point 6 of Commission Implementing Regulation (EU) 2019/2072⁶.

Commission Implementing Regulation (EU) 2019/2072 contains general measures that target *Picea* spp. (Pinaceae) and *Arctostaphylos* spp. (Ericaceae), the two alternate hosts for this rust fungus. The

www.efsa.europa.eu/publications

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.



Regulation prohibits the introduction into the EU of *Picea* plants (other than fruits and seeds) from some non-EU countries (Annex VI, point 1). The introduction into the EU of fresh plant parts (other than fruits and seeds) of *Picea* and *Arctostaphylos* spp. from non-EU countries (excluding Switzerland) and the USA, respectively (Annex XI, Part A, point 3), need to be accompanied by a phytosanitary certificate.

The general requirements for surveys of quarantine organisms within EU territory are laid down in Regulation (EU) 2016/2031⁷ and Commission Implementing Regulation (EU) 2020/1231⁸.

Overview of the EU regulatory status

Chrysomyxa arctostaphyli is a Union quarantine pest. Import of plants of *Picea* from certain non-EU countries is prohibited and fresh plant parts of *Picea* from non-EU countries and *Arctostaphylos* spp. from the USA need to be accompanied by a phytosanitary certificate.

1.3. Pest distribution

Chrysomyxa arctostaphyli is only present in native North America where the two host genera, *Picea* and *Arctostaphylos*, occur together (Crane, 2000). In Canada, the pathogen is widespread (Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland, Northwest Territories, Nova Scotia, Ontario, Quebec, Saskatchewan and Yukon Territory) (EFSA PLH Panel et al., 2018; EPPO, online). In the USA, the pathogen is present in the northern and western states (Alaska, Arizona, Colorado, Idaho, Maine, Michigan, Montana, New Mexico, New York, Oregon, South Dakota, Utah, Washington, Wisconsin and Wyoming) (EPPO, online). The pathogen has not been reported in Europe and the EU territory (EFSA PLH Panel et al., 2018).





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 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.



Conclusion on pest distribution

Chrysomyxa arctostaphyli is known to occur in North America and it is has not been reported in the EU territory.

1.4. Disease cycle

Chrysomyxa arctostaphyli, the causal agent of spruce broom rust, is a heteroecious rust with a twoyear life cycle alternating between the aecial host *Picea* spp. and the telial host *Arctostaphylos* spp. (EFSA PLH Panel et al., 2018; Crane, 2000) (Figure 3).



Figure 3: Disease cycle of *Chrysomyxa arctostaphyli* (Source: Paul Wray, Iowa State University, Bugwood.org (aecial host); Mary Ellen (Mel) Harte, Bugwood.org (telial host); FIDS, Natural Resources Canada, Canadian Forest Service (symptoms on *Arctostaphylos uva-ursi*); John W. Schwandt, USDA Forest Service, Bugwood.org (symptoms on spruce)). Dotted lines indicate life cycle stages developing on the aecial host while continuous lines indicate life cycle stages developing on the telial host. (1) First year of the life cycle, (2) second year of the life cycle

On *Picea* spp. (aecial host)

The pathogen overwinters as mycelium in the twig and bud tissues of the brooms (Crane, 2000; EFSA PLH Panel et al., 2018) and infects the current year's needles in the spring (EFSA PLH Panel et al., 2018).



In spring (Anon, 2011), subepidermal spermogonia develop on needles from which hyaline spermatia are released (EFSA PLH Panel et al., 2018). Spermogonia have a strong characteristic odour that attracts insects favouring cross-fertilisation of the fungus (EFSA PLH Panel et al., 2018).

In summer (Anon, 2011), July–August (Crane, 2000), spermogonia are followed by the development of aecia, bright orange pustules, from which numerous yellow-orange aeciospores are released. The wind-borne aeciospores infect the leaves of the telial host (presumably during late summer and autumn (Cannon, 2007)) on which purple-brown spots develop. The rust overwinters on these leaves as mycelium (Feau et al., 2011; EFSA PLH Panel et al., 2018).

In autumn the infected needles shed, giving the broom a dead appearance over the winter, but each spring the broom produces new chlorotic needles that are systematically infected (Hennon and Trummer, 2001; Cannon, 2007).

On Arctostaphylos spp. (telial host)

At the beginning of the following spring (Crane, 2000), telia develop on the underside of one-year-old leaves of *Arctostaphylos* spp. (but sometimes also on the upper surface as in *A. patula* (Crane, 2000)), from which teliospores are released. Teliospores germinate without a dormant period (Cannon, 2007) and produce wind-borne basidiospores that in spring (May–June (Ziller, 1974)) infect young spruce needles restarting the cycle (EFSA PLH Panel et al., 2018). *Chrysomyxa arctostaphyli* does not produce uredinia (Anon, 2011). Microclimate strongly influences disease development, with low temperature and damp conditions reported as predisposing factors for disease development (Bega and Scharpf, 1993; EFSA PLH Panel et al., 2018).

Conclusion on disease cycle

Chrysomyxa arctostaphyli is a heteroecious rust that infects *Picea* spp. (aecial host) and *Arctostaphylos* spp (telial host) to complete a two-year life cycle through four stages and four types of fructification and spores.

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 for 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 units⁹ 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.

⁹ Defined as 'homogeneous subdivisions where the interactions between the pest and the environment would result in similar epidemiology'.



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. arctostaphyli* within the EU is proposed in Section 2.5 (Figure 6).

2.1. Host range and main hosts in the EU

Chrysomyxa arctostaphyli is a rust with a heteroecious biological cycle alternating on two specific hosts that are systematically separated but ecologically closely associated (Crane, 2000) (Table 1).

The aecial hosts of *C. arctostaphyli* are *Picea engelmannii* (Parry ex Engelm), *Picea abies* (L.) H. Karst. and *Picea sitchensis* (Bongard) Carrière (Peterson, 1961a, 1961b; EPPO, 2022). It has also been inoculated successfully with *Picea glauca* (Moench) Voss and *Picea mariana* (Mill.) Britton, Sterns & Poggenb (EPPO, 2022). *Picea pungens* Engelm (EPPO, online) and *Picea rubens* (Sargent) have also been reported as hosts of the pathogen (Peterson, 1961a, 1961b; Sinclair et al., 1987; EPPO, 2022; EFSA PLH Panel et al., 2018).

The major telial host is *Arctostaphylos uva-ursi* (L.) which is present both in North America and in Europe (EFSA PLH Panel et al., 2018). *Arctostaphylos nevadensis* (A. Gray) and *A. patula* (Greene) have also been recorded as telial hosts of the pathogen (Peterson, 1961a, 1961b). The latter are only present in western North America (EFSA PLH Panel et al., 2018; EPPO, online). There are no reports on possible experimental hosts in the literature. *Arctostaphylos alpina* (L.) Spreng. and *Arctostaphylos rubra* (Rehder & Wilson) Fernald, close relatives of *A. uva-ursi*, may also be susceptible to the fungus (Hennon and Trummer, 2001) (Table 1).

Detection and delimiting surveys of *C. arctostaphyli* in the EU should target all susceptible *Picea* spp. (aecial hosts) and *Arctostaphylos* (telial hosts) species located within 300 m of each other, which corresponds to the dispersal range of basidiospores that infect *Picea* hosts (see Section 2.3) (Hennon and Trummer, 2001; EFSA PLH Panel et al., 2018).



Table 1:	Plants reported to be aecial	and telial hosts of Chi	rysomyxa arctostaphyli
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Host status	Species	Common name	Reference	Native in the EU	Ornamental or forest
	<i>Picea engelmannii</i> (main aecial host)	Engelmann's spruce	Peterson, 1961a, 1961b; Sinclair et al., 1987; EFSA PLH Panel et al., 2018	No	O/F
	Picea abies	Norway spruce	Sinclair et al., 1987	Yes	-
	Picea glauca	White spruce	Peterson, 1961a, 1961b; Sinclair et al., 1987	No	0
Aecial host	Picea mariana	Black spruce	Sinclair et al., 1987	No	0
	Picea pungens	Blue spruce	Peterson, 1961a, 1961b; Sinclair et al., 1987	No	O/F
	Picea rubens	Red spruce	Sinclair et al., 1987	No	-
	Picea sitchensis	Sitka spruce	Sinclair et al., 1987	No	F
Telial host	<i>Arctostaphylos uva-ursi</i> (main telial host)	Bearberry/kinnikinnik	EFSA PLH Panel et al., 2018	Yes	-
	Arctostaphylos nevadensis	Pinemat manzanita	EFSA PLH Panel et al., 2018	No	-
	Arctostaphylos patula	Greenleaf manzanita	EFSA PLH Panel et al., 2018	No	-
	Arctostaphylos alpina	Alpine bearberry	Hennon and Trummer, 2001	Yes	-
	Arctostaphylos rubra	Red fruit bearberry	Hennon and Trummer, 2001	No	-

Conclusion on host range and main hosts

Chrysomyxa arctostaphyli utilizes *Picea engelmannii* and other *Picea* species as an aecial host, and *Arctostaphylos uva-ursi as well as* other *Arctostaphylos* species as a telial host. Detection and delimiting surveys of *C. arctostaphyli* in the EU should target all susceptible *Picea* and *Arctostaphylos* species.



2.2. Environmental suitability in the EU

Climatic suitability

Climatic conditions in EU territories are not a limiting factor to the establishment of *C. arctostaphyli* if introduced (EFSA PLH Panel et al., 2018). Climate types suitable to pathogen survival also overlap to a large extent with the distribution of potential aecial and telial hosts (EFSA PLH Panel et al., 2018).

Host availability

Picea abies and *P. sitchensis* are the main susceptible aecial hosts of the pathogen that are found in Europe (EFSA PLH Panel et al., 2018). *Picea abies* is widely distributed in Europe (from the mountainous area of central Europe to northern and eastern Europe up to the Ural Mountains (Figure 4) (Caudullo et al., 2016)) and is also extensively cultivated outside its natural range (e.g. central and north-western parts of Europe) (EFSA PLH Panel et al., 2018) for timber and pulpwood production (Caudullo et al., 2016) and in urban areas as amenity trees. *Picea sitchensis* is widely planted in some EU countries (e.g. Ireland) for timber production (Houston Durrant et al., 2026; Brus et al., 2019). The North American species *P. engelmannii*, *P. glauca*, *P. mariana* and *P. pungens*, in Europe, are commonly used as ornamental trees in urban areas, in a variety of cultivars (Gellini and Grossoni, 1996; Bernetti, 1995). In Switzerland, *P. engelmannii* has been planted to reduce the risk of avalanches (Gellini and Grossoni, 1996) and *P. pungens* is used in reforestation programmes and Christmas tree production (Černý et al., 2016; Pettersson et al., 2019).

The alternate host *A. uva-ursi* is a circumpolar evergreen shrub species (Krpata et al., 2007) widely distributed throughout the EU territory (mainly Scandinavia, Scotland, the Pyrenees, the Massif Central, the Alps, the Carpathian Montains and the Balkans) (EFSA PLH Panel et al., 2018). It is also used as an ornamental plant. *Arctostaphylos alpina* is a deciduous shrub species with edible berries (Fromard, 1982; Linderborg et al., 2011) that grows in the circumpolar areas of northern Scandinavia and in the high altitudes of the Pyrenees and the Alps (Linderborg et al., 2011).



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





Figure 5: Distribution map for *Arctostaphylos uva-ursi* (Source: EFSA PLH Panel et al., 2018, courtesy of Swedish Museum of Natural History)

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.

2.3. Spread capacity

Natural spread

Natural spread of *C. arctostaphyli* is via wind-borne spores across aecial and telial hosts (see Section 1.4) (EFSA PLH Panel et al., 2018; Hennon and Trummer, 2001):

- Aeciospores are wind-borne from *Picea* spp. (aecial host) and reach *Arctostaphylos* spp. (telial host). Aeciospores cannot reinfect spruce trees but their ability to be dispersed over long distances and survive storage for several months can facilitate intercontinental spread (Hennon and Trummer, 2001; Cannon, 2007; EPPO, 2022).
- Basidiospores are wind-borne from the *Arctostaphylos* spp. (telial host) and reach *Picea* spp. (aecial host). Basidiospores spread over a shorter range, and it has been suggested that infection on nearby spruce can be reduced by the removal of *Arctostaphylos* spp. plants from within 300 m (EFSA PLH Panel et al., 2018).

Human-assisted spread

The spread of *C. arctostaphyli* over long distances is facilitated by the import and trade of infected host plants (both aecial and telial hosts) and other *Picea* spp. commodities that can contain the pathogen (e.g. cut branches, including cut Christmas trees without roots or soil (EPPO, 2018)) (EFSA PLH Panel et al., 2018).



Conclusion on spread capacity

Natural spread of *Chrysomyxa arctostaphyli* occurs by the wind-borne dispersal of aeciospores and basidiospores from aecial to telial hosts and from telial to aecial hosts, respectively. The spread of the pathogen over long distances can also be facilitated by human activity of importing and trading infected host plants.

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. arctostaphyli* 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 fungus. 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.

For the identification of risk areas, it is first necessary to identify the activities that could contribute to introduction or spread of *C. arctostaphyli*. 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 Interceptions, 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: The import and trade of Picea spp. plants

The main pathway of entry of *C. arctostaphyli* into EU territory is through the import and trade of both host species from non-EU countries (EPPO, 2018; EFSA PLH Panel et al., 2018). The risk of entry of *C. arctostaphyli* via the *Picea* pathway is a restricted pathway, as the import of *Picea* of plants for planting into EU territory is banned and the import of fresh parts of plants of *Picea* spp. from non-EU countries needs to be accompanied by a phytosanitary certificate. However, activities involved in the import of fresh parts of plants of plants could still be considered risk activities.

Example 2: The import and trade of Arctostaphylos spp. plants

The import of plants of *Arctostaphylos* spp. is a potential pathway for the introduction of *C. arctostaphyli* in the EU, since import is currently unregulated. Nurseries, garden centres and parks where imported plants of Arctostaphylos spp. are stored, traded or planted as ornamental trees can be considered as risk locations. Taking into consideration the fact that the spread of basidiospores from the telial hosts is potentially less than 300 m, this distance can be considered the risk area surrounding risk locations where aecial hosts (*Picea* spp.) of *C. arctostaphyli* are present.



Table 2: Examples of risk activities and the corresponding risk locations that are relevant for the surveillance of *C. arctostaphyli*

Risk activity	Risk locations	Risk areas	
Import and trade of plants and plant parts of <i>Picea</i> spp. from areas where the fungus is present	Nurseries, garden centres and parks where imported plants are stored, traded or planted as ornamental trees	Areas surrounding risk locations where the aecial host (<i>Picea</i> spp.) is present (forests, plantations, urban areas as ornamental trees)	
Import and trade of plants of <i>Arctostaphylos</i> spp. from areas where the fungus is present	Nurseries, garden centres and parks where imported plants are stored, traded or planted as ornamental trees	Areas surrounding risk locations where the telial host (<i>Arctostaphylos</i> spp.) is present (forests, urban areas as ornamental trees)	

2.5 Structure of the target population



Figure 6: Example of the hierarchical structure of the target population *Chrysomyxa arctostaphyli* in the EU (Sources: Eurostat, 2018 (levels 1–2), Gil Wojciech, Polish Forest Research Institute, Bugwood.org (level 3, top (*P. abies* stand); Dave Powell, USDA Forest Service (retired), Bugwood.org (level 3, bottom (forest scene showing extensive carpet of *A. uva-ursi*)); Chris Schnepf, University of Idaho, Bugwood.org (level 4, top (private nursery)); John Ruter, University of Georgia, Bugwood.org (level 4, bottom); WG Ziller, Bugwood.org (level 5, top); Rob Routledge, Sault College, Bugwood.org (level 5, bottom))

3. Detection and identification

Surveys of *C. arctostaphyli* should be based preferentially on examination of symptoms on *Picea* spp. and *Arctostaphylos* spp. plants, followed by lab identification of the pathogen from symptomatic plant samples (both hosts).



3.1. Detection and identification in the field

3.1.1. Visual examination

Symptoms on *Picea* spp. (aecial host)

Brooms and current year's needles

- Characteristic conspicuous, perennial and dense brooms that can develop on the main stem or branches over time and can become up to 2 m tall (EPPO, 2022; Anon, 2011; EFSA PLH Panel et al., 2018; Hennon and Trummer, 2001). Internodes and needles on brooms are shorter than normal (EFSA PLH Panel et al., 2018; Hennon and Trummer, 2001).
- In spring, spermogonia develop on the yellowish current year's needles (Cannon, 2007).
- In summer, spermogonia are followed by aecia development, bright orange pustules that can be so abundant that they give the broom a yellow-orange appearance, which makes it more obvious in contrast to the adjacent healthy foliage (EPPO, 2022; Anon, 2011; Cannon, 2007); the first symptom of spruce broom rust is etiolation of needles in summer (EPPO, 2022).
- In autumn, the infected needles in the broom die and fall (EFSA PLH Panel et al., 2018). This gives the broom a dead appearance over the winter, but each spring the broom produces new chlorotic needles that are systematically infected (Cannon, 2007; Hennon and Trummer, 2001).

Branches and stems

• Branches and stems at the base of the brooms become swollen due to infection and can form a cancer or gall (EFSA PLH Panel et al., 2018).

Symptoms on *Arctostaphylos* spp. (telial host)

Leaves

- Rust is most noticeable in late spring when the telia form on the underside of one-year-old leaves and become orange-brown, (Ziller, 1974; EPPO, 2022; Hennon and Trummer, 2001; EFSA PLH Panel et al., 2018).
- The pathogen causes annual and localised infections which, if severe, may result in leaf drop (Cannon, 2007).





Figure 7: Symptoms of *Chrysomyxa arctostaphyli* on spruce (*Picea* spp., aecial host): (A) young *C. arctostaphyli* broom on spruce (Source: Forestry Canada, Ottawa, Bugwood.org); (B) witches' broom in early summer, when the aecia are still immature (Source: WG Ziller, Bugwood.org); (C) witches' broom on *P. glauca* in late summer, covered with spermogonia and mature aecia (Source: WG Ziller, Bugwood); (D) broom on *P. pungens* after infected current year's needles have shed (Source: USDA Forest Service – Rocky Mountain Research Station – Forest Pathology, Bugwood.org); (E) twigs of a witches' broom with live current-year needles covered with aecia (centre) and dead needles from the previous year still attached (Source: WG Ziller, Bugwood.org)



Figure 8: Telia of *Chrysomyxa arctostaphyli* visible on the lower surface of bearberry *Arctostaphylos uva-ursi* leaves (Source: FIDS, Natural Resources Canada, Canadian Forest Service)



Risk of misidentification

Many *Chrysomyxa* species infect spruce species (*Picea* spp.) both in Europe and North America. Both the aecial stage of the heteroecious species and the telia of the autoecious species develop on the needles, buds and cones of spruce, so the morphological characteristics of the spores are always necessary to discriminate and identify the causal agent (Crane, 2000).

- *Chrysomyxa abietis* (Wallroth) Unger (type species of the genus) and *C. rhododendri* de Bary are both widely distributed in Europe, where they cause limited damage to *Picea* spp. (Crane, 2000; CABI, online-b,c). Should *C. arctostaphyli* be introduced into EU territory, the risk of misidentification with the European species is high since they share the same aecial host and cause similar early symptoms.
- *Chrysomyxa abietis* is an autoecious rust that produces only the telial stage on *Picea* spp. (aecia and uredinia are lacking) (Crane, 2000). The disease is visible year-round, either by the presence of golden yellow pustule-like telia (Butin, 1995) on the abaxial surface of one-year-old needles (immature telia may be visible in late autumn, but their development is interrupted in winter and resumes the following spring (Crane, 2000)) or by the transverse yellowish bands on infected needles of the current year (Crane, 2000; CABI, online-b).
- *Chrysomyxa rhododendri* is a heteroecious rust with an alternating life cycle on *Picea* spp. (aecial host) and *Rhododendron* spp. (telial host) (CABI, online-c). On *Picea* spp. spermogonia and aecia occur on the yellowish needles of the year causing premature defoliation. On *Rhododendron* spp. uredinia and telia occur on the underside of the leaves of the previous year (uredinia also appear on leaf petioles, fruit pedicels and twigs) (Crane, 2000).
- Identification keys based on the aecial states on spruces and morphological characteristics of the uredinal and telial states are available to identify the *Chrysomyxa* species occurring in North America and Europe (Crane, 2000).



Figure 9: Telia of *C. abietis* on spruce needles (A) (Source: Dan Aamlid, Bugwood.org); aecia of *C. rhododendri* on spruce needles (B) (Source: Fabio Stergulc, Università di Udine, Bugwood.org)

3.1.2. Sample collection

To identify *C. arctostaphyli*, fresh samples of twigs and the current year's needles should be collected from infected *Picea* spp. plants and fresh samples of one-year-old leaves should be collected from infected *Arctostaphylos* spp. plants during the periods when symptoms and signs occur. Active and passive spore samplers are useful for detecting peak spore production (Gu et al., 2018).



3.1.3. Timing of detection and identification

The best period to investigate the presence of *C. arctostaphyli* is during the spring and summer when symptoms on specific hosts are most evident and when fructifications and spores occur.

On the aecial host (*Picea* spp.)

- In spring, spermogonia develop on the infected needles (Anon, 2011) and spermatia are released from them.
- In summer, bright orange aecia develop on the infected needles and release aeciospores.

On the telial host (*Arctostaphylos* spp.)

- In spring, orange-brown and waxy telia develop on the leaves of the telial host. Teliospores released from fructification germinate without a dormant period and produce basidiospores that start new infections on *Picea* spp. (Feau et al., 2011; EFSA PLH Panel et al., 2018; Hennon and Trummer, 2001; Anon, 2011).
- Spore samplers for collecting both airborne spores of *C. arctostaphyli* should be placed from spring (basidiospores) to summer (aeciospores).

Conclusion on detection and identification in the field

Chrysomyxa arctostaphyli can be detected in the field in spring and summer, through host specificity and observation of symptoms on both host plants (conspicuous, perennial brooms on *Picea* spp., purple-brown spots on the one-year-old leaves of *Arctostaphylos* spp.), and sampling of plant material for confirmation in the laboratory.

3.2. Detection and identification in the laboratory

3.2.1. Morphological identification

Because rust fungi are obligate parasites, only a few species have been successfully grown on artificial media (Crane, 2000). Therefore, identification of *C. arctostaphyli* needs to be confirmed in the laboratory by observation of the morphological characteristics of spores using a light microscope and a scanning electron microscope. Morphological characteristics of aeciospores (dimensions of aeciospore, spore ornamentation) and the aecial peridium are used to distinguish between species (Crane, 2000; You et al., 2019).

The microscopic morphological characteristics of the pathogen are provided in the EPPO data sheet on the quarantine pest *Chrysomyxa arctostaphyli* (EPPO, 2022) and in the studies of Crane (2000) and Cannon (2007). There are also identification keys to distinguish *C. arctostaphyli* from other tree rusts in western Canada based on symptoms and morphological characteristics of the pathogen (Ziller, 1974).

3.2.2. Laboratory testing and other methods of identification

Chrysomyxa arctostaphyli can be distinguished from other rust fungi of the genus *Chrysomyxa* using a PCR protocol that is available for amplification and sequencing of the ITS region and the large subunit (28S) of the rDNA (extracted from needles and uredinia) (Feau et al., 2011). Recently, to resolve the phylogenetic position of *Chrysomyxa* from China, protocols were developed to amplify and sequence the ITS region of rDNA extracted from urediospores or aeciospores (You et al., 2019; Wang et al., 2022).

A protocol for the amplification of three loci (nuclear large subunit (28S) and small subunit (18S) rDNA, and cytochrome-c-oxidase subunit 3) using PCR was developed by Aime and McTaggart (2021) for higher rank classification of rust fungi.



A rapid, specific and sensitive detection tool, such as real-time PCR or LAMP assays, for *C. arctostaphyli* is still lacking.

Conclusion on detection and identification in the laboratory

The identification of *Chrysomyxa arctostaphyli* needs to be confirmed in the laboratory by observation of the morphological characteristics of each spore stage of the pathogen (surface morphology of aeciospores is considered taxonomically useful at both genus and species level). Nevertheless, given that identification at species level is often difficult, pathogen identification needs to be confirmed by molecular diagnostic techniques (PCR) to avoid misidentification.

4. Conclusion

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

Survey question	Section	Key information
What?	1. The pest and its biology	The fungus <i>C. arctostaphyli</i> is the causal agent of spruce broom rust
	2. Target population	Epidemiological units: homogeneous areas that contain both telial and aecial host plants
Where?		Risk areas: areas surrounding risk locations nurseries, garden centres and parks where imported plants (<i>Picea</i> spp. and <i>Arctostaphylos</i> spp.) are stored, traded or planted as ornamental trees
		Inspection units: individual host plants (e.g. both <i>Picea</i> spp. and <i>Arctostaphylos</i> spp.) examined for <i>C. arctostaphyli</i>
How?	3. Detection and identification	Identification of <i>C. arctostaphyli</i> through symptoms confirmed in the laboratory morphologically and or molecularly
When?		Spring-summer for both hosts

Table 3: Preparation of surveys for *Chrysomyxa arctostaphyli* included in Sections 1, 2 and 3



5. Survey framework

Figure 10 shows the next steps after the survey preparation for designing statistically sound and riskbased detection and delimiting surveys. Guidance on the selection of the type of survey, related survey preparation and design is provided in EFSA's general guidelines for pest surveys (EFSA et al., 2020).



Figure 10: 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

Term	Definition*
Buffer zone	An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimise the probability of spread of the target pest into or out of the delimited area, and subject to phytosanitary or other control measures, if appropriate (ISPM 5: FAO, 2021a).
Component (of a survey)	A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruit). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.
Confidence	The sensitivity of the survey is a measure of reliability of the survey procedure (Montgomery and Runger, 2010). The term confidence level is used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2021b).
Delimiting survey	A survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2021a).
Design prevalence	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence.
of detection used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2021b)	In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence (EFSA et al., 2018).
Detection survey	A survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2021a).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2021c).
Epidemiological unit analogous to the term lot used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2021b)	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors would result in a similar epidemiology should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a given geographical area. They are the units of interest for which the sample size is estimated (e.g. a tree, orchard, field, glasshouse, nursery).



Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Expert knowledge elicitation	A systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA, 2014).
Host plant	A host plant is a plant species belonging to the host range on which the pest could find shelter, feed or subsist at least for a period of time.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (ISPM 5: FAO, 2021a).
	This definition is limited to an array of host plant species and does not include commodities other than plants or plant parts.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2021c).
Infected versus infested	Infected is used when a pathogen is referred to in relation to its hosts (e.g. the trees are infected by the bacterium).
	Infested is used when an arthropod pest is referred to in relation to its hosts (e.g. the trees are infested by beetles).
	Infested is used when the pest is mentioned in relation to an area (e.g. an infested zone).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2021a).
Inspection unit analogous to sample unit used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2021b)	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place (EFSA et al., 2018).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2021a).
Method sensitivity analogous to the term efficacy of detection used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2021b)	The conditional probability of testing positive given that the individual is infected (Dohoo et al., 2010). The method sensitivity (MeSe) is defined as the probability that a truly positive host tests positive. It has two components: the sampling effectiveness (i.e. probability of selecting infested plant parts from an infested host plant) and the diagnostic sensitivity (characterised by the visual inspection and/or laboratory test used in the identification process). The diagnostic sensitivity is the probability that a truly positive sample will result positive and is related to the analytical sensitivity. It corresponds to the probability that a truly positive inspection unit or sample will be detected and confirmed as positive.



	The sampling effectiveness depends on the ability of the inspector to successfully choose the infested plant parts in a host plant. It is directly linked to the sampling procedure itself and on the training and expertise of the inspectors to recognise the symptomatology of the pest. Furthermore, symptom expressions are dependent, among other factors, on the weather conditions as well as on the physiological stage of the host plant when the sample is taken.
Pest	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products (ISPM 5: FAO, 2021a).
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2021a).
Pest freedom	Pest freedom can be defined, for a given target population, in a statistical framework, as the confidence of freedom from a certain pest against a pre-set design prevalence (threshold of concern).
Population size	The estimation of the number of plants in the region to be surveyed (EFSA et al., 2018).
Prevalence <i>analogous to the term</i> incidence (of a pest) <i>defined in the 'Glossary of</i> <i>phytosanitary terms' (ISPM 5:</i> <i>FAO 2021a)</i>	Pest prevalence is the fraction of infested units in the total population of host plants. Pest incidence is the proportion or number of units in which a pest is present in a sample, consignment, field or other defined population (ISPM 5: FAO 2021a).
Relative risk	The ratio of the risk of infestation in the exposed group to the risk of infestation in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (FAO, 2014).
RiBESS+	Risk-based surveillance systems. This is an online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of pest freedom. Free access to the software with prior user registration is available at: <u>https://shiny-efsa.openanalytics.eu/</u>
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2021a).
Risk factor	A factor that may be involved in causing the disease (FAO, 2014).
	It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared with a baseline with a level 1.
	efforts to be enforced in those areas where the probability of finding



	the pest is highest.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
SAMPELATOR	Sample size calculator. This is an online application that implements statistical methods to estimate the sample size for pest prevalence estimation surveys. Free access to the software with prior user registration is available at: <u>https://shiny-efsa.openanalytics.eu/</u>
Sample size	The sample size refers to the output of the statistical tools for survey design (RiBESS+ and SAMPELATOR).
	'A well-chosen sample will contain most of the information about a particular population parameter but the relation between the sample and the population must be such as to allow true inferences to be made about a population from that sample.' (BMJ, online).
	The survey sample consists of the required number of 'inspection units' or samples thereof to be examined and/or tested in the survey to retrieve sufficient information on the pest presence or prevalence in the total population. For risk-based surveys, the sample size is calculated on the basis of statistical principles that integrate risk factors.
	If the examination for pest presence is performed by laboratory testing, at least one sample is taken from each inspection unit. These samples will undergo relevant laboratory testing.
Sampling effectiveness	For plants, it is the probability of selecting infested plant parts from an infested plant. For vectors, it is the effectiveness of the method to capture a positive vector when it is present in the survey area. For soil, it is the effectiveness of selecting a soil sample containing the pest when the pest is present in the survey area.
Specified plant	The plant species known to be susceptible to the pest.
	For example, for <i>Xylella fastidiosa</i> , the list of specified plants can be found in Annex II of Commission Implementing Regulation (EU) 2020/1201.
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2021a).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given babitat or area of interact. The
used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2021b)	 different components pertaining to the target population that need to be specified are: definition of the target population: the target population has to be clearly identified; target population size and geographic boundary.
	(EFSA et al., 2018).



Test	Official examination of plants, plant products or other regulated articles, other than visually, to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2021a).
Test specificity	The conditional probability of testing negative given that the individual does not have the pest of interest (Dohoo et al., 2010). The test diagnostic specificity is the probability that a truly negative epidemiological unit will give a negative result and is related to the analytical specificity. In pest freedom it is assumed to be 100%.
Visual examination	The physical examination of plants, plant products or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2021a).

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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 *Chrysomyxa arctostaphyli*: <u>https://efsa.europa.eu/plants/planthealth/monitoring/surveillance/chrysomyxa-arctostaphyli</u>
- Pest categorisation of *Chrysomyxa arctostaphyli*: <u>https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5355</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://www.youtube.com/watch?v=kHAnmRDelx8</u>
- The statistical tool RiBESS+: <u>https://r4eu.efsa.europa.eu/app/ribess</u>

The RiBESS+ manual: https://zenodo.org/record/2541541#.Ys7G5HZByUn

• The RiBESS+ video tutorial: <u>https://youtu.be/qYHqrCiMxDY</u>