PEST SURVEY CARD

efsa European Food Safety Authority Supporting Publications

APPROVED: 30 July 2020

doi:10.2903/sp.efsa.2020.EN-1915

Pest survey card on *Thekopsora minima*

European Food Safety Authority (EFSA),
Wolfgang Maier, Anne Wilstermann, Alice Delbianco, Sybren Vos

Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137) at the request of the European Commission. Its purpose is to guide the Member States in preparing data and information for *Thekopsora minima* surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. *Thekopsora minima* is not regulated in the EU but is included in the EPPO A2 list of pests recommended for regulation as a quarantine pest. It is the causal agent of blueberry leaf rust on the American highbush blueberry (*Vaccinium corymbosum*). In the EU, the pathogen has been reported in Belgium, Germany, the Netherlands, Portugal and Spain. Host availability seems to be the only limit to the spread of the pest due to its broad climatic tolerance. Surveys should be performed mainly in spring or autumn (in regions with a Mediterranean climate) or late summer and the beginning of autumn (in temperate climates). Detection surveys should mainly target *Vaccinium corymbosum* in nurseries, plantations and naturalised plants, whereas for delimiting surveys *Tsuga* and *Rhododendron* spp. should also be monitored. Surveillance of *Thekopsora minima* in the EU should focus on the visible symptoms on *Vaccinium* plants caused by the uredinial stage of the fungus that can be identified morphologically by experts. However, reliable identification of *T. minima* requires molecular methods and DNA sequence analysis.

Keywords: blueberry leaf rust, hemlock–blueberry rust, plant pest, Pucciniales, risk-based surveillance, survey, *Vaccinium corymbosum*

Requestor: European Commission

Question number: EFSA-Q-2019-00281

Correspondence: ALPHA@efsa.europa.eu

Acknowledgements: EFSA wishes to acknowledge the Julius Kühn-Institut (JKI), in Germany, for the support provided to this scientific output in the context of grant GP/EFSA/ALPHA/2017/02. EFSA also wishes to thank Annemarie Fejer Justesen for reviewing this document as well as the working group members that contributed to its preparation. EFSA also wishes to thank especially Giulia Mattion (trainee in the Animal and Plant Health Unit – Plant Health Team) for her support to the finalisation and publication of this survey card.

Suggested citation: EFSA (European Food Safety Authority), Maier W, Wilstermann A, Delbianco A and Vos S, 2020. Pest survey card on *Thekopsora minima*. EFSA supporting publication 2020:EN-1915. 25 pp. doi:10.2903/sp.efsa.2020.EN-1915

ISSN: 2397-8325

Table of contents

Introduction

The information presented in this pest survey card was summarised from the Pest Risk Analysis for *Thekopsora minima* prepared by the European and Mediterranean Plant Protection Organization (EPPO, 2017a), the EPPO Global Database (online) and other scientific documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *Thekopsora minima* in EU Member States (MSs) following the methodology described in EFSA (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 international standards for phytosanitary measures (ISPM 6: FAO, 2018; ISMP 31: FAO, 2016a) and International Plant Protection Convention Guidelines for surveillance (FAO, 2016b). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on *Thekopsora minima*[1]
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual[2]
   c. The statistical tools RiBESS+ and SAMPELATOR[3].

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Thekopsora minima* (Arthur) Sydow & P. Sydow

Class: Pucciniomycotina **Order**: Pucciniales **Family**: Pucciniastraceae **Genus**: *Thekopsora* **Species:** *Thekopsora minima*

Synonyms[4]: *Uredo minima* Schw., *Pucciniastrum minimum* (Schw.) Arth., *Pucciniastrum vaccinii* Jørst., *Peridermium peckii* Thuem.

EPPO Code: THEKMI

Common names: Blueberry rust; blueberry leaf rust; hemlock–blueberry rust

Like all rust fungi (Pucciniales) *T. minima* is an obligate biotrophic plant pathogen and the causal agent of blueberry leaf rust.

The *Tsuga* Ericaceae rusts had a rather complex systematic history leading to unclear synonymy and species circumscriptions (Arthur, 1934; Gäumann, 1959), and the exact host ranges of individual species in this group of rust fungi have not been finally resolved. In the absence of comprehensive molecular phylogenetic data, the latest systematic classification of this group of rust fungi based on morphological characteristics has been followed (Sato et al., 1993). Three rust species, affecting different *Vaccinium* species were accepted, namely: *T. minima*, *Naohidemyces vaccinia* and *N. fujisanensis.* The three species were defined using morphological differences in the aecial, uredinial and telial stages. *Thekopsora minima* is probably native to eastern North America and Japan and parasitic to *V. corymbosum* among others. Despite the complex systematic of this group, all recent disease reports of leaf rust on cultivated *V. corymbosum* from various countries that have employed DNA-based identification suggested that the causal agents of the rust disease on the American blueberry were identical and were attributed to *T. minima* (EPPO, 2017a). It is also important to note

[1] The Pest Survey Card will be updated in the form of Story Map that will be available in the Plant Pests Story Maps Gallery available online: https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://shiny-efsa.openanalytics.eu/
[4] Synonyms from the EPPO Global Database (online).

that phylogenetically, this species is clearly different from the rust parasitising the European blueberry or bilberry (*V. myrtillus*), which is caused by *N. vaccinii.*

Conclusions on taxonomy

Thekopsora minima is the causal agent of blueberry leaf rust on the American highbush blueberry (*Vaccinium corymbosum*) and it can clearly be distinguished from the other *Tsuga* Ericaceae rust fungi.

1.2. EU pest regulatory status

Thekopsora minima is not a Union quarantine pest but is included in the EPPO A2 list of pests recommended for regulation as a quarantine pest.

With regards to its host plants, Annex VI of Commission Implementing Regulation (EU) 2019/2072[5] prohibits the import of plants for planting of *Tsuga* spp. into the EU from several third countries, including some third countries where *T. minima* is known to occur. In addition, plants and fruit of *Vaccinium* and parts of plants of *Rhododendron*, other than fruit and seeds, are listed in Annex XI of the Regulation, requiring a phytosanitary certificate for them to be imported into the EU.

There are currently no legal obligations in place in the EU for the surveillance of *T. minima*.

Overview of the EU regulatory status

Thekospora minima is not regulated in the EU. There are currently no legal obligations in place in the EU for the surveillance of *T. minima*.

1.3. Pest distribution

Thekopsora minima is present on all continents. In recent years, the pest has been reported for the first time on *Vacciunium corymbosum* in various countries around the world. The impact of the disease varies in different geographic regions. An extensive review of the distribution of the pest in the EU and worldwide is presented in the Pest Risk Analysis for *T. minima* (EPPO, 2017a). The current global distribution of the pest is shown in Figure 1.

The pathogen is thought to be native to eastern North America and Japan, where the alternate hosts – three species of *Tsuga* – are also present (Sato et al., 1993).

In the EU, the pest status is reported as follows in EPPO (online):

- Transient, actionable, under eradication in Belgium (EPPO, online);
- Present, only in some parts of Germany (EPPO, online);
- Present, only in some parts of the Netherlands (EPPO, 2017b);
- Present, under eradication in Portugal (EPPO, 2017c);
- Present with few occurrences in Spain (EPPO, online).

In Asia, besides Japan, the disease was recently reported from China (Zheng et al., 2017).

It is present in eastern parts of Central and North America, (Schilder and Miles 2011), on the west coast of the USA (Wiseman et al., 2016; Shands et al., 2018) and also in Mexico (Rebollar-Alviter et al., 2011).

[5] 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.

In South America it has been reported from Colombia (Yepes and Céspedes, 2012), Brazil (Pazdiora et al., 2019) and very recently from Peru (Huarhua et al., 2020).

In Africa the disease has been reported from South Africa (Mostert et al., 2010).

In Oceania it has been reported from Australia (McTaggart et al., 2013) and New Zealand (Padamsee and Mc Kenzie, 2019).

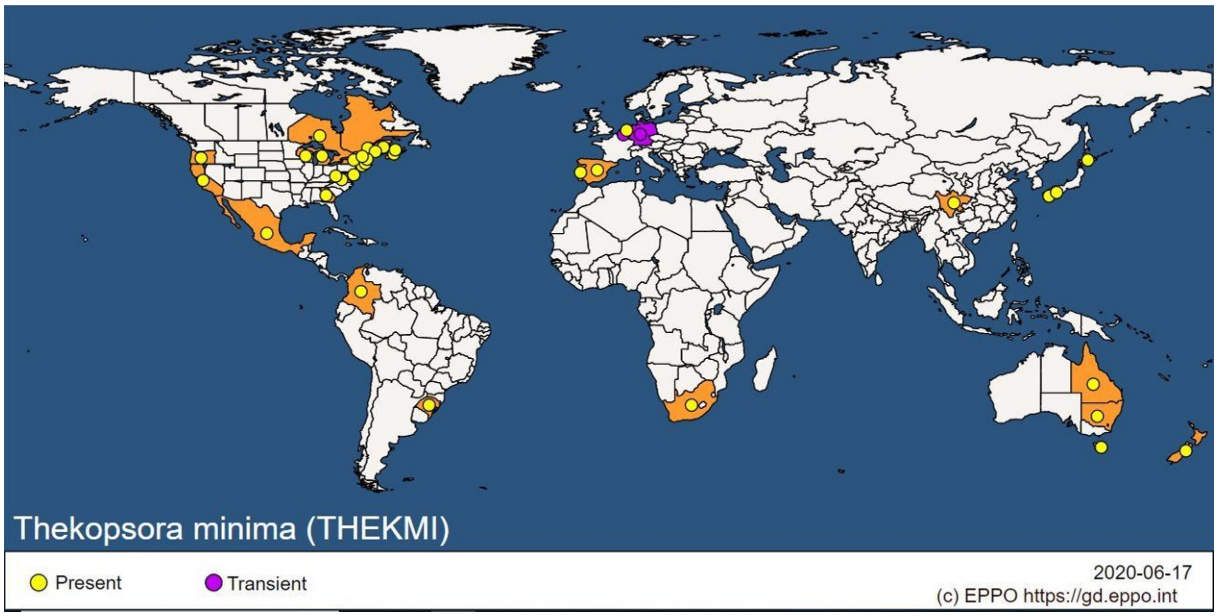

Figure 1: Global distribution of *Thekopsora minima* (Source: EPPO Global Database, online)

Conclusion on pest distribution

In EU the pathogen has been reported on blueberries in Belgium, Germany, the Netherlands, Portugal and Spain.

1.4. Life cycle

Thekopsora minima is a heteroecious macrocyclic rust fungus with reported host alternation between three *Tsuga* and several Ericaceae species needed to complete its life cycle. It is the causal agent of the blueberry leaf rust, which is a significant disease in plantations of high bush blueberries or American blueberries (*Vaccinium corymbosum*).

The life cycle of the fungus (Figure 2) contains several sporulation stages (EPPO, 2017a):

- Aeciospores are produced in the spermogonial and aecial stages on *Tsuga canadensis* in eastern North America and on *T. diversifolia* in Japan, respectively (Sato et al., 1993). The aeciospores cannot reinfect the *Tsuga* host but infect the primary hosts, which are Ericaceae hosts including *V. corymbosum* and *V. angustifolium* (Sato et al., 1993).
- Urediniospores are produced in the uredinial stage on the infected *Vaccinium* species. Uredinia develop from late spring to autumn releasing urediniospores whenever conditions are appropriate. The optimum temperature for their germination ranges between 15 and 25°C with sufficient humidity (Pfister et al., 2004). The released urediniospores reinfect the leaves of the same, close-by or more distant host plants. The rust pustules, which are typical symptoms for these fungi, usually develop on the lower side of the leaves but have also, rarely, been reported on blueberry fruit (EPPO, 2017a).
- Teliospores are produced in the telial stage. In autumn, telia develop and hibernate in the (shed) blueberry leaves. Teliospores germinate in spring, producing basidia.

- Basidiospores are produced in the basidial stage. Basidiospores infect the alternate host, *Tsuga*, if present. If the alternate host is not present, new infections are only possible through the dispersal of urediniospores (i.e. clonal reproduction).

Outside of its native range, *T. minima* has so far mainly been reported from *V. corymbosum* and recently from *V. corymbosum* x *angustifolium* (Wichura et al., 2020). The vast majority of reports regarded urediniospores only, which cause the most prominent symptoms.

Based on current knowledge the best timing for surveys would be in late summer or the beginning of autumn in cool–temperate Member States, while in a Mediterranean climate spring and autumn seem to be the best sampling times, when humidity is sufficient.

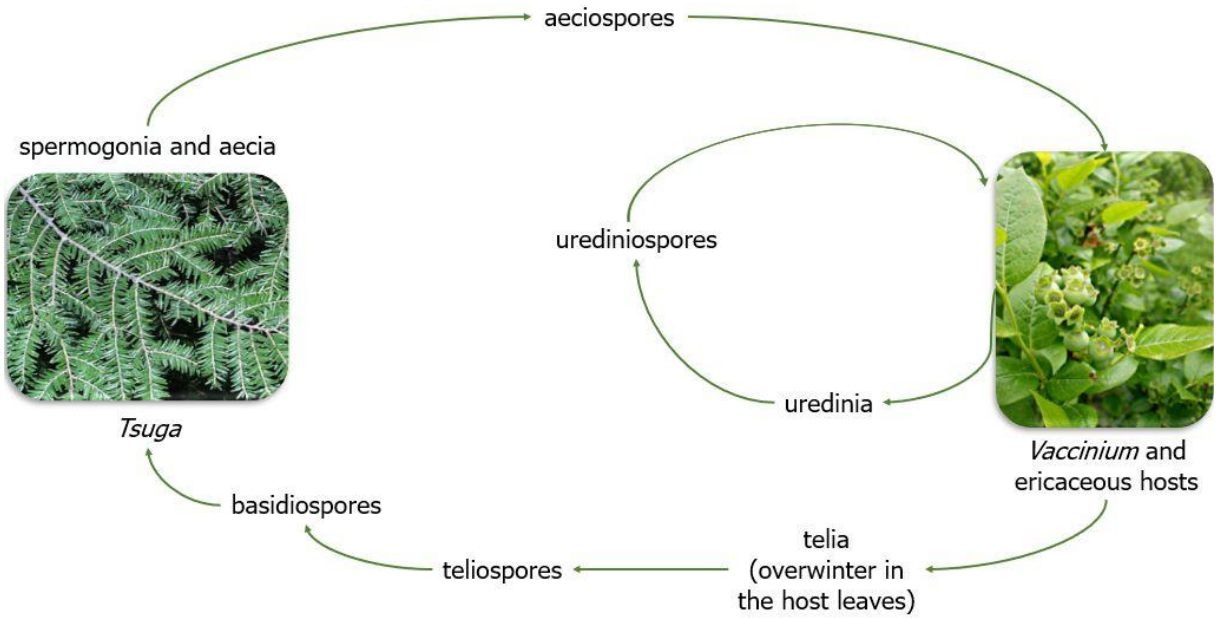

Figure 2: Life cycle of *Thekopsora minima*, adapted from EPPO (2017a) (Source: (left) Paul Wray, Iowa State University, Bugwood.org; (right) Ansel Oommen, Bugwood.org)

Conclusion on life cycle

Thekopsora minima is a heteroecious rust fungus, infecting different hosts to complete its life cycle, which contains several sporulation stages. Surveillance of *T. minima* in the EU should focus on the readily visible symptoms caused by the uredinial stage. Depending on the climatic region, the surveys should be performed mainly in spring or autumn (in regions with a Mediterranean climate) or late summer and the beginning of autumn (in temperate climates).

1.5. Host range and main hosts

The following are reported as hosts of *Thekopsora minima* (Sato et al., 1993):

- Spermogonial and aecial hosts: *Tsuga canadensis*, *T. diversifolia*, *T. sieboldii* (Pinaceae).
- Uredinial and telial hosts: *Vaccinium corymbosum*, *V. angustifolium* var. *laevifolium*, *V. erythrocarpum*, *Gaylussacia baccata*, *Lyonia neziki*, *Rhododendron canadensis* (= *Rhodora canadensis*), *R. canescens*, *R. lutescens*, *R. pericyclemenoides* (= *Azalea nudiflora*), *R. pilosum* (*Menziesia pilosa*), *R. ponticum*, *R. prunifolium*, *R. viscosum* (*A. viscosa*), *R.* x *grandavense*, (all belonging to Ericaceae).

In the EU, *T. minima* has so far only been reported from *V. corymbosum*. The cultivated area of *V. corymbosum* has significantly grown in recent years in many Member States. In addition, in the EU,

V. corymbosum trade as an ornamental plant for private gardens is increasing. American blueberry plants have also been naturalised[6] and are regarded as invasive species in several European countries. Thus, the hosts for *T. minima* occur in urban (i.e. blueberries planted in domestic gardens), wild and semi-wild (i.e. naturalised blueberries), and agricultural areas (i.e. blueberry plantations and nurseries) (EPPO, 2017a).

Conclusion on host range and main hosts

In the EU, detection surveys should mainly target *Vaccinium corymbosum* in nurseries, plantations and naturalised plants.

1.6. Environmental suitability

Infection studies by Pfister et al. (2004) with *Thekopsora minima* on *Rhododendron* showed that epidemiological factors, such as the time needed for uredinia production, the number of uredinia produced per leaf and the urediniospore germination, were near to optimum at temperatures between 15 and 25°C. Moreover, urediniospores did not germinate at 10°C, and infection efficacy was significantly reduced at 30°C. Humidity also plays a role in promoting disease incidence (Schilder and Miles, 2011).

Given the results of the infection studies and the broad geographic region where the pathogen has been in the last 20 years, it can be assumed that it could become established everywhere that blueberries are grown (Figure 3). The countries where it has been reported have temperate to Mediterranean to subtropical climates (Köppen–Geiger Dfa, Dfb, Cfa, Cfb, Csa, Csb[7]) (Figure 4).

[6] For the purposes of this output, 'naturalised plants' are those established in the wild in a non-native area.

[7] Dfa = snow climate, fully humid with hot summer; Dfb = snow climate, fully humid with warm summer; Cfa = warm temperate climate, fully humid with hot summer; Cfb= warm temperate climate, fully humid with warm summer; Csa = warm temperate climate with hot and dry summer; Csb = warm temperate climate with warm and dry summer (Kottek et al., 2006).

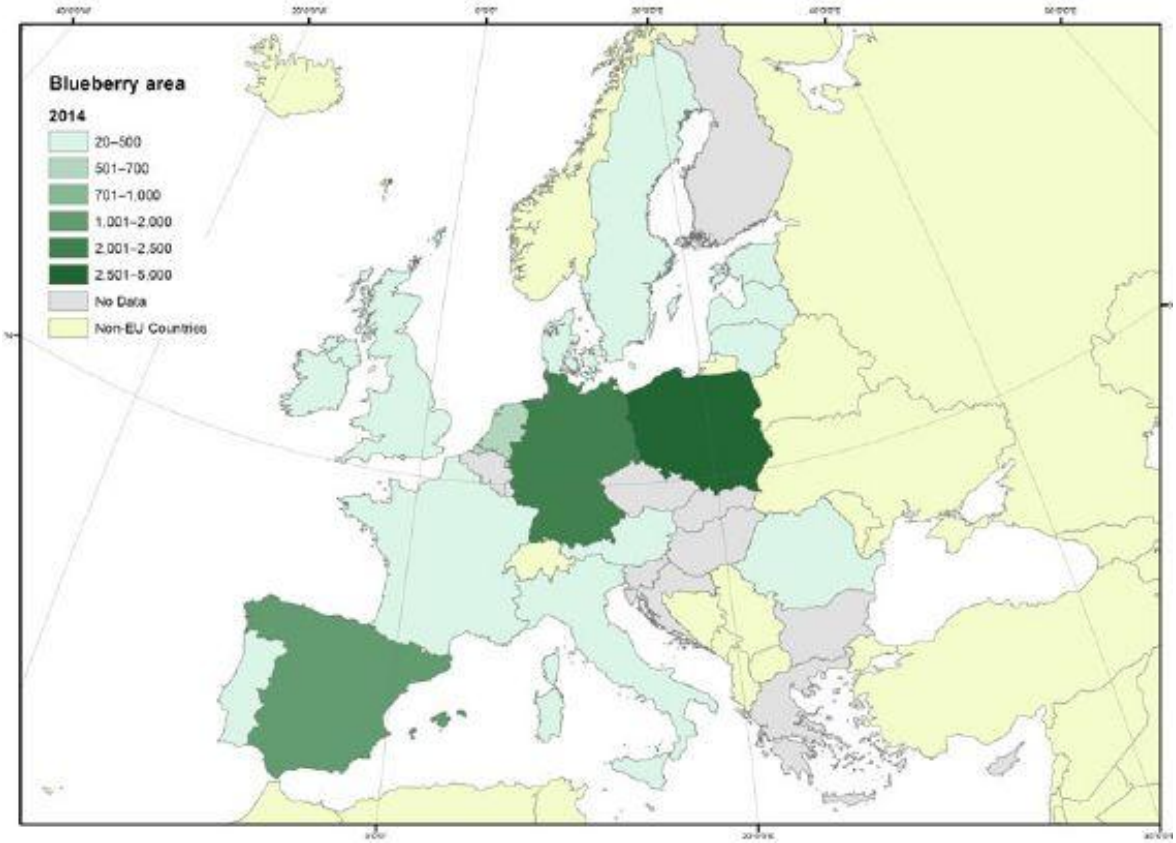

Figure 3: Blueberry cultivated areas in EU countries in 2014. Values are in hectares. (Data source: Brazelton (2016), taken from EFSA PLH Panel (2017))

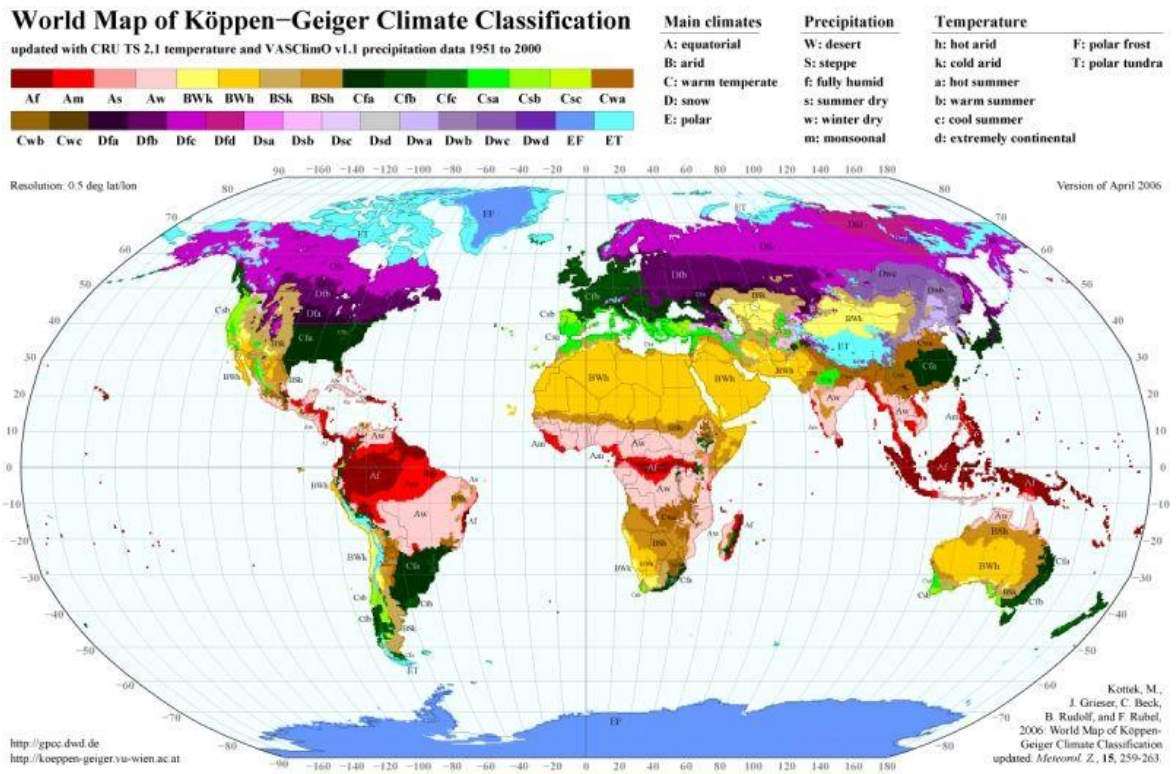

Figure 4: World map of Köppen–Geiger climate classification (Kottek et al., 2006)

Depending on the climate of the region, the best timing for surveys would be in late summer or the beginning of autumn in cool–temperate climates, while in Mediterranean climates spring and autumn seem to be the best sampling times.

The winter survival of the pest in the EU remains uncertain (EPPO, 2017a, Wichura et al., 2020).

Conclusion on environmental suitability

In the EU, host availability seems to be the only limit to the establishment of the pest due to its broad climatic tolerance. Sufficient humidity and temperatures between 15 and 25°C will enhance the disease incidence.

1.7. Spread capacity

Natural spread

Thekopsora minima can spread naturally by wind transport of spores to susceptible plants. This is the usual and very effective dispersal of rust fungi. Wind dispersal of urediniospores happens over short distances up to several hundred metres but may also occur over longer distances. Rain splash dispersal can also occur over short distances (Wilk et al., 2016). Due to the low densities of blueberry plantations, rapid spread by long-distance dispersal is not likely (Brown and Hovmøller, 2002). However, there are no specific data available on the natural spread of *T. minima*.

Human-assisted spread

The disease could also be spread by tools, equipment, packaging, humans (e.g. on clothing, hands), and potentially on fruit (fruit infections have so far only been reported from Australia (EPPO, 2017a)). Import into the EU and movement within the EU of infected plants is the biggest threat.

Conclusion on spread capacity

If suitable host plants are present, rust fungi can spread quickly once established. However, the patchy distribution of blueberry plantations or naturalised plants reduces the likelihood of a rapid spread of the pest over larger areas.

The introduction of *Thekopsora minima* in several countries, on all continents, was probably human assisted by trade of infected plants.

1.8. Risk factor identification

Identification of risk factors and their relative risk estimation is 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 have 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 one example of risk factor for *Thekopsora minima* (Table 1).

To identify the risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of *T. minima*. These activities should then be connected to specific locations. Risk areas can be defined around these locations, bearing in mind that their size depends on the spread capacity of the pest and the availability of host plants around these locations.

Example: Movement through trade of host plants for planting

Large shipments of blueberry plants for planting originating from the USA, from production areas where *T. minima* is present, are a likely source of introduction of the disease to the EU Member States, as the first outbreak in Germany in 2015 shows (Schrader and Maier, 2015).

No specific requirements are currently in place for the import and movement into and within the EU of *Vaccinium* plants for planting or fruit from countries or areas where *T. minima* occurs. Therefore, the movement through trade of infected host plants is the most likely pathway for the introduction into the EU and for further spread of *T. minima* within the EU (EPPO, 2017a).

As a consequence, a risk activity could be defined as the trade of *Vaccinium* plants for planting from areas where the fungus occurs. The corresponding risk locations would be the potential entry points, nurseries and greenhouses in the Member States from where those host plants are traded and dispatched. The risk areas would be the areas around those risk locations that are within the spread distance of the fungus and where host plants from those risk locations are grown.

Table 1: Examples of risk activities and corresponding risk locations that are relevant for surveillance of *Thekopsora minima*

Risk activity	Risk locations	Risk areas
Import into the EU and trade within the EU of *Vaccinium corymbosum* plants for planting from areas where the fungus occurs	Potential entry points, trade hubs, nurseries, greenhouses in the Member States from where those host plants are grown, traded and dispatched	Areas surroundings risk locations where *Vaccinium corymbosum* plants and other potential hosts are grown

2. Detection, sampling and identification

2.1. Detection

2.1.1. Visual examination

The goal of the visual examination is to detect the symptoms caused by *Thekopsora minima* on the leaves of *Vaccinium corymbosum*. Visual examination of the symptoms should be carried out when climate conditions are favourable (i.e. warm weather, rain, dews) (Wilk et al., 2016). However, the pest is difficult to detect at the early stages of infection (Biosecurity Tasmania, online).

Symptoms

Thekopsora minima can infect host plants of all ages. It causes symptoms mainly on the leaves (Figures 5, 6 and 7) but has also been reported on the scar area of the fruit (Barrau et al., 2002). The yellow-orange uredinia are found on the underside of leaves in groups up to 5 mm in diameter, creating yellow-orange powdery pustules, and on necrotic spots with the corresponding upper leaf surface dark brown to blackish, often with a bright red border (Biosecurity Tasmania, online; Wilk et al., 2016). Individual uredinia are subepidermal, erumpent, round and about 100–200 µm in diameter. In the case of severe infection, premature shedding of leaves is common and it could lead to the death of the plant (Wilk et al., 2016). It is helpful to use a hand lens (10–20× magnification) for verification of the rust symptoms – especially the powdery urediniospores.

Telia are formed in the lower epidermis at the end of the growing season, but they have generally not been reported from the EU Member States where *T. minima* occurs. Telia are produced intraepidermally, causing inconspicuous smooth and brown symptoms that are of little to no importance for the monitoring process.

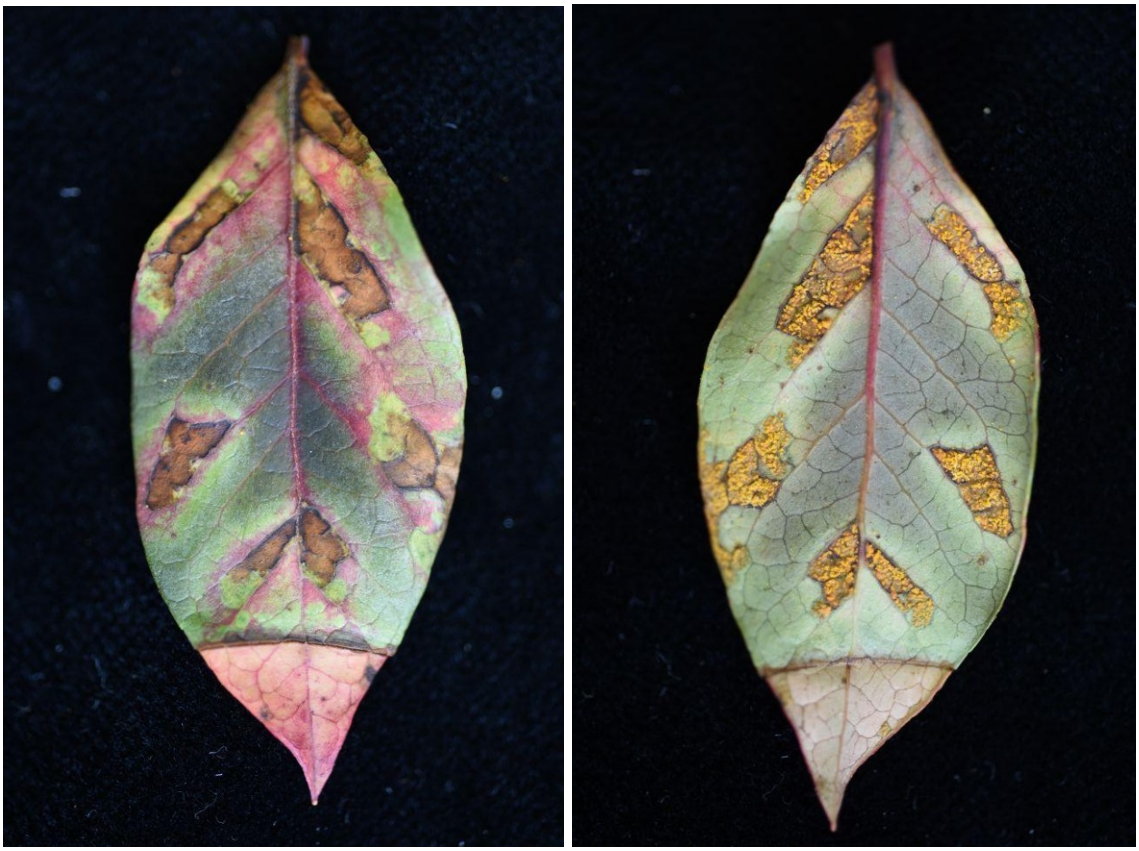

Figure 5: Symptomatic leaf of *Vaccinium corymbosum* collected in a greenhouse in Germany. Left: upper side with discoloration and partially necrotic areas; right: underside of the leaf showing dense growth of uredinia with yellow-orange protruding urediniospores (Source: Wolfgang Maier, JKI (DE))

Figure 6: Symptoms of *Thekopsora minima*: young uredinia with erumpent urediniospores and a little necrotic patch in the middle of the uredinia (Source: EPPO Global Database (online), courtesy of Wolfgang Maier, JKI (DE))

Figure 7: Symptoms of *Thekopsora minima*: necrotic patches on the upper side of a *Vaccinium corymbosum* leaf (Source: EPPO Global Database (online), courtesy of Wolfgang Maier, JKI (DE))

Conclusions for detection methods

Visual examination should be done using a hand lens focussing on the symptoms caused by uredinia. *Thekopsora minima* causes typical rust symptoms on *Vaccinium corymbosum* leaves: orange uredinia on the underside of the leaves, which rupture the plant epidermis and release the powdery urediniospores. On the upper side of the leaf, dark brown to blackish leaf discolouration, often with a bright red diffuse border, can be observed.

2.2. Sampling

If diseased blueberry plants are detected, 5–10 symptomatic leaves per plant should be collected, preferably in paper bags, and sent to the laboratory for identification. Depending on the number of symptomatic bushes in a survey site, 3–5 symptomatic leaves per plant could be sufficient. The leaves showing the strongest symptoms should be selected. Leaves from different plants should not be pooled, but collected separately. Preparations for microscopic or molecular analyses are either done immediately upon return to the laboratory or after the samples have been pressed and dried. Samples must be pressed and dried in a plant press to avoid contamination by moulds and other fungi, which frequently occur when samples are preserved fresh in plastic bags. The paper in the plant press should be changed after ca. 24 hours and, if needed, again after ca. 72 hours. Afterwards, samples should be dry, flat and all structures should be visible. Both morphological and molecular analyses can be easily performed on such recently dried herbarium specimens. These dried samples can also be kept, preferably in a desiccator for documentation or to repeat analyses later, even years later.

Conclusions for sampling

5–10 symptomatic leaves per plant should be collected for identification. It is important not to pool the symptomatic leaves from different plants. Symptomatic leaves should be collected, preferably in paper bags, pressed and dried in a plant press before being sent to laboratories for further identification.

2.3. Identification

Thekopsora minima can be identified during the uredinial stage using light microscopy requiring cross-sections of uredinia and experience in (rust) fungal identification. Morphologically, *T. minima* is similar to other *Vaccinium* rusts (EPPO, 2017a), especially considering the sizes of *Naohidemyces vaccinii*

urediniospores. However, while *N. vaccinii* has a uredinial peridium with conspicuous ostiolar cells (Figure 8), these are lacking in *T. minima* (Figure 9) (Sato et al., 1993). Using this method, a symptomatic leaf with a single uredinial pustule can potentially be analysed and diagnosed. However, the identification requires specific experience with microfungal sample preparation and interpretation of microscopic morphological features of rust fungi. Therefore, the use of molecular methods is recommended.

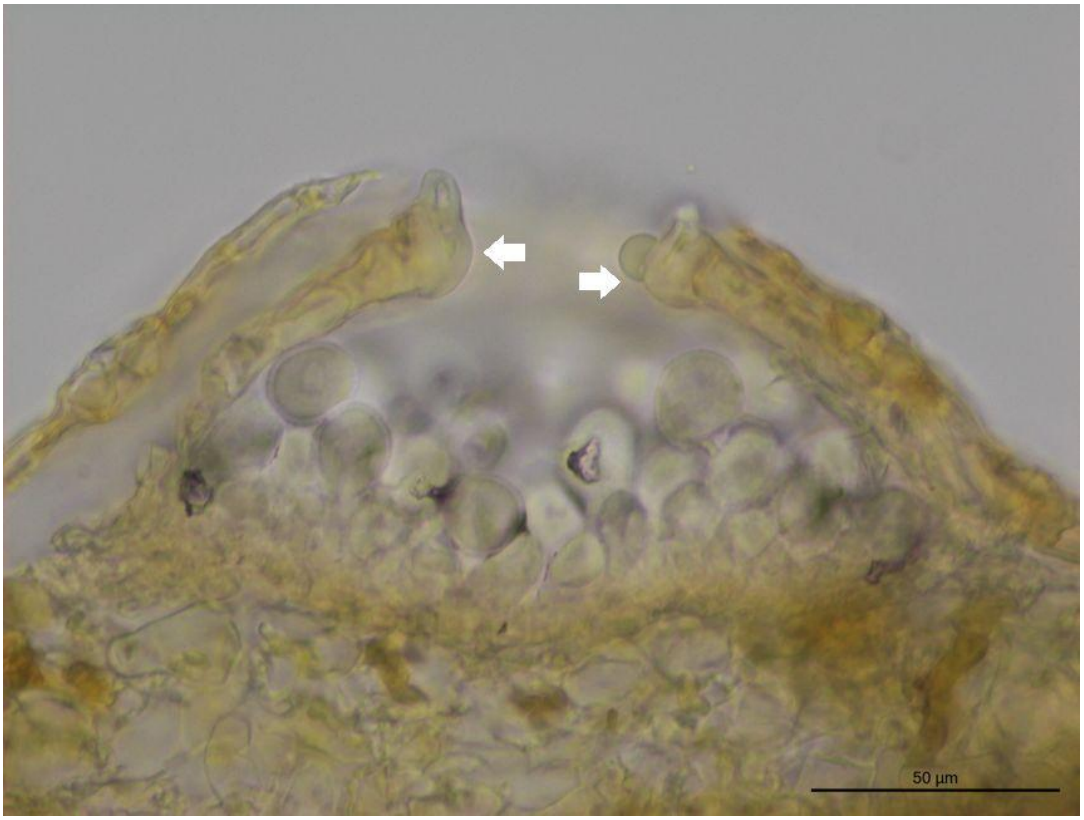

Figure 8: Cross-section of *Naohidemyces vaccinii* uredinia with uredinial peridia and conspicuous ostiolar cells (indicated with white arrows) (Source: Wolfgang Maier, JKI (DE))

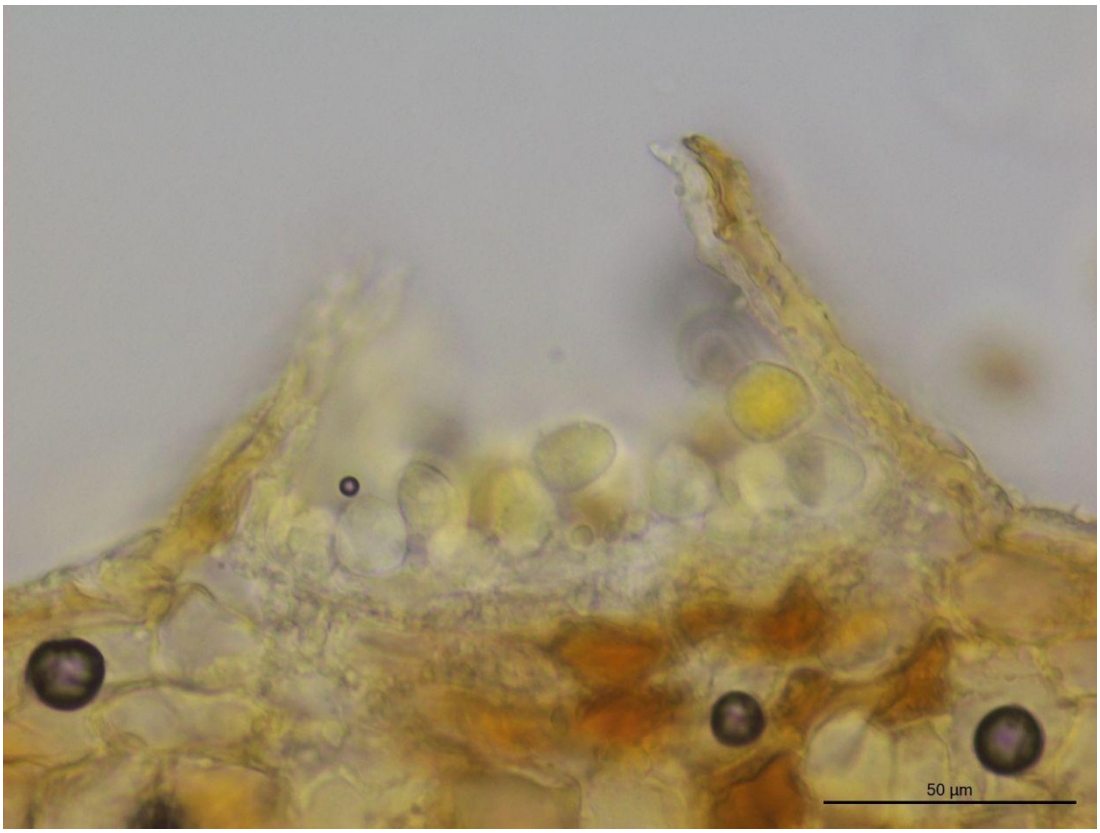

Figure 9: Cross-section of *Thekopsora minima* uredinia with uredinial peridia without ostiolar cells (Source: Wolfgang Maier, JKI (DE))

2.3.1. Laboratory testing

Reliable identification of *Thekopsora minima* requires the use of molecular methods (e.g. DNA sequencing of ITS or LSU rDNA) (EPPO, 2017a). DNA extraction can be performed on a few uredinia cut from leaves and, after DNA amplification through PCR, the PCR products should be sequenced and compared with the ITS or LSU rDNA sequences of *T. minima* available in Genbank. Wichura et al. (2020), in their study on the occurrence of *T. minima* in Lower Saxony in Germany (2015–2016), amplified part of the LSU region by nested PCR, with Rust2inv and LR6 (Aime, 2006) as the first primer set and LR3 (Vilgalys and Hester, 1990) and LR0R (Moncalvo et al., 1995) as the second, which was also used for sequencing the PCR product. Various combinations of primers and protocols for PCR analysis that can be applied for the identification of *T. minima* are reported in Shands et al. (2018), Zheng et al. (2017), Pazdiora et al. (2018) and Schilder and Miles (2011).

In monitoring studies, it is reasonable to only apply sequence-based identification to a few representatives of a homogenous survey site exhibiting the typical symptoms.

Conclusion for pest identification

The uredinial stage of *Thekopsora minima* can be identified morphologically by experts based on the structure of the uredinial peridium. Reliable identification of *T. minima* requires molecular methods and genomic sequence analysis.

3. Key elements for survey design

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation in each Member State.

The size of the defined target population and its structure in terms of the number of epidemiological units need to be known.

When several pests have to be surveyed in the same crop, it is recommended that the same epidemiological and inspection units are used for each pest in order to optimise the survey programme as much as possible. This would optimise field inspections since they are organised per crop visit and not by pest. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for *Thekopsora minima*

	Definition
Target population	All *Vaccinium corymbosum* plants in a Member State
Epidemiological unit	A homogenous area in a Member State in which *V. corymbosum* plants are grown (e.g. nurseries, plantations, natural areas)
Inspection unit	A single host plant

To design a plant pest survey on *Thekopsora minima* the general guidelines provide further details on the following steps that will generally be necessary:

1/ Determine the type of survey based on its objectives. For *T. minima*, the type of survey will depend on the pest status (according to ISPM No. 8 (FAO, 2017)) in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infection or to determine the pest prevalence. The next steps deal with the example of substantiating pest freedom.

The overall confidence level and design prevalence of the survey have to be decided by the risk managers before designing the surveys as they reflect the acceptable level of the risk of infection of the host plants by *T. minima*. The general guidelines for pest surveillance provide further details on the choice of these values and the related consequences in terms of pest surveys.

2/ Define the target population and its size. When determining the target population for surveillance of *T. minima*, the host plants that are relevant for the survey area have to be selected. The size of the target population should be determined. For example, the target population could be all host plants in a Member State.

3/ Define the epidemiological units. The epidemiological units should be single homogeneous areas that each contain at least one individual host plant.

4/ Determine the inspection unit. For *T. minima*, for example, the inspection unit is a single blueberry plant.

5/ Determine the number of inspection units per epidemiological unit. For *T. minima*, this is the number of blueberry plants per epidemiological unit.

6/ Implement the inspections and, if appropriate, the sampling, following the procedures suggested by the competent authorities, within the epidemiological units and estimate the method effectiveness in order to determine the overall method sensitivity (sampling effectiveness × diagnostic sensitivity). A representative number of plants should be examined and if there are suspicious symptoms they should be sampled. RiBESS+ can be used to calculate how many inspection units need to be examined or sampled when using a predefined prevalence level (e.g. 1%) to obtain a particular confidence level. This confidence level is in turn needed to calculate the number of sites to be inspected (Step 8). Note that the more units are inspected the higher the confidence will be. The competent authorities need to align the survey efforts with the resources available.

7/ Define the risk factors. A risk factor affects the probability that a pest will be present or detected in a specific portion of the target population. It may not always be possible to identify or include a risk

factor in the survey design. Risk factors can only be included when both the relative risk and the proportion of the overall plant population to which they apply are known or can be reliably estimated.

8/ Determine the number of epidemiological units to survey. RiBESS+ can be used to determine the number of epidemiological units to survey in order to achieve the objectives of the survey set at Step 1 in terms of confidence level (e.g. 95%) and design prevalence (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. As a result, considering, for example, fields where host plants are present, the number of fields that need to be surveyed are estimated for a Member State in order to state with 95% confidence that the prevalence of *T. minima* will be at 1% or below.

9/ Summarise and evaluate the survey design. At this stage, it is necessary to evaluate whether the above steps have resulted in a survey design that matches the available resources, meaning that a feasible number of inspections can be performed within an acceptable time frame per inspection, and resulting in a feasible number of samples. If not, available resources should be adjusted. This adjustment would result in a modified survey design using different input parameters of the statistical tool RiBESS+ (e.g. varying the number of components, method sensitivity, etc.).

10/ Integrate the pest-based survey into a crop-based survey (optional).

11/ Allocate the calculated survey effort. In the survey area, the output of RiBESS+ should be allocated proportionally to the host plant population or to the number of epidemiological units. In addition, the survey size should be selected from the list of available locations.

12/ Data collection and survey reporting. Consider which data are needed and how these data will be reported together with the related assumptions.

13/ Plan, develop or update the specific instructions for the inspectors. These activities are not addressed by EFSA and fall within the remit of the competent national authorities.

References

Aime CM, 2006. Toward resolving family-level relationships in rust fungi (Uredinales). Mycoscience, 47,112–122. doi:10.1007/s1026 7-006-0281-0

Arthur JC, 1934. Manual of the rusts in United States and Canada. Lafayette, Indiana, USA: Purdue Research Foundation.

Barrau C, de los Santos B and Romero F, 2002. First report of leaf rust of southern high-bush blueberry caused by *Pucciniastrum vaccinii* in Southwestern Spain. Plant Disease, 86, 1178.

Biosecurity Tasmania, online. Plant biosecutiry: Pests and Diseases – Blueberry Rust. Available online: https://dpipwe.tas.gov.au/biosecurity-tasmania/plant-biosecurity/pests-and-diseases/blueberry-rust [Accessed: 29 June 2020]

Brazelton C, 2016. World Blueberry Acreage & Production. World Blueberry Acreage & Production Report, US Highbush Blueberry Council, 77 pp.

Brown JK and Hovmøller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science. 2002, 297(5581), 537–541. doi:10.1126/science.1072678

EFSA (European Food Safety Authority), Ciubotaru RM, Cortiñas Abrahantes J, Oyedele J, Parnell S, Schrader G, Zancanaro G and Vos S, 2018. Technical report of the methodology and work-plan for developing plant pest survey guidelines. EFSA supporting publication 2018: EN-1399. 36 pp. doi:10.2903/sp.efsa.2018.EN-1399

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger M, Bragard C, Caffier D, Candresse T, Chatzivassiliou E, Dehnen-Schmutz K, Gilioli G, Gregoire J-C, Jaques Miret JA, MacLeod A, Navarro MN, Niere B, Parnell S, Potting R, Rafoss T, Rossi V, Urek G, Van Der Werf W, West J, Winter S, Gardi C, Mosbach-Schulz O, Koufakis I and Van Bruggen A, 2017. Scientific Opinion on the pest risk assessment of *Diaporthe vaccinii* for the EU territory. EFSA Journal 2017;15(9):4924, 185 pp. doi:10.2903/j.efsa.2017.4924

EPPO (European and Mediterranean Plant Protection Organisation), online. EPPO Global Database. Available online: https://gd.eppo.int/ [Accessed: 29 July 2020]

EPPO (European and Mediterranean Plant Protection Organisation), 2017a. Pest risk analysis for *Thekopsora minima*. EPPO, Paris. Available online: https://gd.eppo.int/taxon/THEKMI/documents [Accessed: 29 July 2020]

EPPO (European and Mediterranean Plant Protection Organisation), 2017b. First report of *Thekopsora minima* in the Netherlands. EPPO Reporting Service no. 03 – 2017. Num. article: 2017/059. Available online: https://gd.eppo.int/reporting/article-6027 [Accessed: 29 July 2020]

EPPO (European and Mediterranean Plant Protection Organisation), 2017c. First report of *Thekopsora minima* in Portugal. EPPO Reporting Service no. 03 – 2017. Num. article: 2017/060. Available online: https://gd.eppo.int/reporting/article-6028 [Accessed: 17 June 2020]

FAO (Food and Agriculture Organization of the United Nations), 2016a. ISPM (International Standards for Phytosanitary Measures) 31. Methodologies for sampling of consignments. FAO, Rome, 31 pp. Available online: https://www.ippc.int/en/publications/588/

FAO (Food and Agriculture Organization of the United Nations), 2016b. Plant Pest Surveillance: A guide to understand the principal requirements of surveillance programmes for national plant protection organizations. Version 1.1. FAO, Rome.

FAO (Food and Agriculture Organization of the United Nations), 2017. ISPM (International Standards for Phytosanitary Measures) 8. Determination of pest status in an area. FAO, Rome, 16 pp. Available online: https://www.ippc.int/en/publications/612/

FAO (Food and Agriculture Organization of the United Nations), 2018. ISPM (International Standards for Phytosanitary Measures) 6. Surveillance. FAO, Rome, 18 pp. Available online: https://www.ippc.int/en/publications/615/

Gäumann E, 1959. Die Rostpilze Mitteleuropas. Buechler, Bern.

Huarhua M, Acuña R, Aragón L, Soto J, Landeo S, Martínez de la Parte E and Apaza W, 2020. First report of blueberry leaf rust caused by *Thekopsora minima* on *Vaccinium corymbosum* L. in Perú. Plant Disease. doi:10.1094/PDIS-03-20-0585-PDN

Kottek MJ, Grieser C, Beck B, Rudolf and Rubel F, 2006. World Map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift, 15, 259–263. doi:10.1127/0941-2948/2006/0130

McTaggart AR, Geering ADW, Shivas RG, 2013. *Thekopsora minima* causes blueberry rust in south-eastern Queensland and northern New South Wales. Australasian Plant Disease Notes, 8, 81–83.

Moncalvo J, Wang H and Hseu R, 1995. Phylogenetic relationships in Ganoderma inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87, 23–238. doi:10.2307/3760908

Mostert L, Bester W, Jensen T, Coertze S, van Hoorn A, Le Roux J, Retief E, Wood A and Aime MC, 2010. First report of leaf rust of blueberry caused by *Thekopsora minima* on *Vaccinium corymbosum* in the Western Cape, South Africa. Plant Disease, 94, 478.

Padamsee M and McKenzie EHC, 2019. *Pucciniastrum minimum* is the causal agent of rust on blueberries in New Zealand. Australasian Plant Disease Notes, 14, 27.

Pazdiora PC, Dorneles KR, Araújo Filho JV, Rossetto EA, Guatimosim E and Dallagnol LJ, 2018. First Report of Blueberry Leaf Rust Caused by *Thekopsora minima* on Blueberry (*Vaccinium corymbosum*) in South America. Plant Disease, 103, 1026–1026. doi:10.1094/PDIS-11-18-1981-PDN

Pazdiora PC, Dorneles KR, Araújo Filho JV, Rossetto EA, Guatimosim E and Dallagnol LJ, 2019. *Thekopsora minima* on Blueberry (*Vaccinium corymbosum*) in South America. Plant Disease, 103. doi:10.1094/PDIS-11-18-1981-PDN

Pfister SE, Halik S, Bergdahl DR, 2004. Effect of temperature on *Thekopsora minima* urediniospores and uredinia. Plant Disease, 88, 359–362.

Rebollar-Alviter A, Minnis AM, Dixon LJ, Castlebury LA, Ramirez-Mendoza MR, Silva-Rojas HV, Valdovinos-Ponce G, 2011. First report of leaf rust of blueberry caused by *Thekopsora minima* in Mexico. Plant Disease, 95, 772.

Sato S, Katsuya K and Hiratsuka Y, 1993. Morphology, taxonomy and nomenclature of *Tsuga-Ericaceae* rusts. Transactions of the Mycological Society of Japan, 34, 47–62.

Schilder AMC and Miles TD, 2011. First report of blueberry leaf rust caused by *Thekopsora minima* on *Vaccinium corymbosum* in Michigan. Plant Disease, 95, 768–768.

Schrader G and Maier W, 2015. Express – PRA zu *Thekopsora minima*. Journal für Kulturpflanzen 67, 348–352.

Shands AC, Crandal SG, Ho T and Miles TD, 2018. First report of leaf rust on southern highbush blueberry caused by *Thekopsora minima* in California. Plant Disease. doi:10.1094/PDIS-09-17-1439-PDN

Vilgalys R and Hester M, 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology, 172, 4238–4246. doi:10.1128/jb.172.8.4238-4246.1990

Wichura A, Brand T and Böhm J, 2020. Occurrence of *Thekopsora minima* on highbush blueberries in Lower Saxony 2015-2016: prevalence, susceptibility of varieties and some aspects of infection biology. Journal of Plant Diseases and Protection, 127, 359–366.

Wilk P, Simpson M and Browne B, 2016. Berry plant protection guide 2016–17. NSW DPI Management Guide. New South Wales Department of Primary Industries. Available online: https://cleangrow.com.au/wp-content/uploads/2017/04/Berry-plant-protection-guide-2016-17.pdf [Accessed: 29 June 2020]

Wiseman MS, Gordon MI and Putnam ML, 2016. First report of leaf rust caused by *Thekopsora minima* on northern highbush blueberry in Oregon. Plant Disease. doi:10.1094/PDIS-11-15-1344-PDN

Yepes M and Céspedes P, 2012. New rusts (Pucciniales) records on crops and ornamental plants in Colombia. Revista facultad nacional de agronomía, Medellín, 65, 6691-6696.

Zheng X, Tang G, Tian Y, Huang X, Chang X, Chen H, Yang H, Zhang S and Gong G, 2017. First report of leaf rust of blueberry caused by *Thekopsora minima* in China. Plant Disease, 101. doi:10.1094/PDIS-09-16-1379-PDN

General glossary for pest survey

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, 2019).
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, 2016b).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2019).
Design prevalence *analogous to the term **level of detection** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	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. 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, 2018).
Detection survey	Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2019).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016a).
Epidemiological unit *analogous to the term **lot** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same 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 geographical area. They are the units of interest to which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
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, 2019). This definition is limited to an array of host plants 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, 2016a).
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 insect 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, 2019).
Inspection unit *analogous to* ***sample unit*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b*)	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, 2018).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2019).
Method sensitivity *analogous to the term* ***efficacy of detection*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	The conditional probability of testing positive given that the individual is diseased (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 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 epidemiological unit 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 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 diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2019).
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 the plants in the region to be surveyed (EFSA, 2018).
Prevalence *analogous to the term* **incidence (of a pest)** *defined in the 'Glossary of phytosanitary terms' (ISPM 5: FAO 2019)*	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 2019)
Relative risk	The ratio of the risk of disease in the exposed group to the risk of disease 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 freedom from disease. Free access to the software with prior user registration is available at https://shiny-efsa.openanalytics.eu/
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, 2019).
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. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas, where the highest probabilities exist to find the pest.
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 https://shiny-efsa.openanalytics.eu/
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 *Xylella fastidiosa*, the list of specified plants, which includes host plants and all plants for planting, other than seeds, belonging to the genera or species, can be found in Annex I of Decision (EU) 2015/789.
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, 2019).
Target population *analogous to* ***consignment*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b*)	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 habitat or area of interest. The 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, 2018)
Test	Official examination of plants, plant products or other regulated

	articles, other than visual, to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2019).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease 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 freedom from disease 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, 2019).

*References

BMJ (British Medical Journal), online. Populations and samples. Available online: https://www.bmj.com/about-bmj/resources-readers/publications/statistics-square-one/3-populations-and-samples [Accessed: 13 March 2020]

Dohoo I, Martin W and Stryhn H, 2010. Veterinary epidemiologic research. 2nd Edition. VER Inc., Canada.

EFSA (European Food Safety Authority), 2014. Guidance on Expert Knowledge Elicitation in Food and Feed Safety Risk Assessment. EFSA Journal 2014;12(6):3734. 278 pp. doi: 10.2903/j.efsa.2014.3734 Available online: www.efsa.europa.eu/efsajournal

EFSA (European Food Safety Authority), 2018. Technical report of the methodology and work-plan for developing plant pest survey guidelines. EFSA supporting publication 2018: EN-1399. 36 pp. doi:10.2903/sp.efsa.2018.EN-1399

FAO (Food and Agriculture Organization of the United Nations), 2014. Risk-based disease surveillance – A manual for veterinarians on the design and analysis for demonstration of freedom from disease. FAO Animal Production and Health Manual No. 17. Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations), 2016a. ISPM (International Standards for Phytosanitary Measures) 27. Diagnostic protocols for regulated pests. FAO, Rome, Italy. Available online: https://www.ippc.int/en/publications/593/

FAO (Food and Agriculture Organization of the United Nations), 2016b. ISPM (International Standards for Phytosanitary Measures) 31. Methodologies for sampling of consignments. FAO, Rome, Italy. Available online: https://www.ippc.int/en/publications/588/

FAO (Food and Agriculture Organisation of the United States), 2019. ISPM (International Standard for Phytosanitary Measures) 5. Glossary of phytosanitary terms. FAO, Rome, Italy. Available online: https://www.ippc.int/en/publications/622/

McMaugh T, 2005. Guidelines for surveillance for plant pests in Asia and the Pacific. ACIAR Monograph No.119, 192 pp.

Montgomery DC and Runger GC, 2010. Applied statistics and probability for engineers. Fifth Edition, John Wiley & Sons. 792 pp.