TECHNICAL REPORT

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Work-plan and methodology for EFSA to develop plant pest survey guidelines for EU Member States

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Abstract

The European Commission requested EFSA to facilitate the Member States in the planning and execution of their survey activities. In particular, EFSA is asked to provide scientific and technical guidelines in the context of the new plant health regime (Regulation (EU) 2016/2031), in which prevention and risk targeting are given an extra focus, and the European Commission co-financing programme of the annual Member State survey activities for pests of EU relevance (Regulation (EU) No 652/2014). In order to address this mandate EFSA is requested to deliver by the end of 2019: (i) 47 pest survey cards that contain practical information required for preparing survey design; (ii) survey guidelines for 3 different pests that will be case studies to be developed in collaboration with the EU Member States; and, (iii) support to the Member States on the underpinning statistical methods and use of the EFSA WEB-based tools RiBESS+ and SAMPELATOR to inform sampling strategy design, including sample size calculations. This technical report describes the methodological approach and the work-plan EFSA will implement to deliver the requested outputs.

Key words: Plant health, Surveillance, Pest survey, RiBESS+, SAMPELATOR

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1. Introduction

Following a request from the European Commission to EFSA for supporting and assisting the EU Member States (MSs) in the plant pest surveillance activity, EFSA compiled this document to describe its work-plan, the framework and the methodological approaches used to identify, harmonise gathering and integrate the evidences essential to achieve a scientifically based survey design.

1.1. Background as provided by the requestor

Council Directive 2000/29/EC lays down the phytosanitary provisions and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union. In its annexes the list of harmful organisms (pests) whose introduction into or spread within the Union is prohibited, is detailed together with specific requirements for import or internal movement.

An evaluation of the plant health regime led to Regulation (EU) 2016/2031, on protective measures against pests of plants, which was adopted on 26 October 2016 and will be applying from December 2019, repealing Directive 2000/29/EC. An extra focus on prevention and risk targeting has been given.

In addition, Regulation (EU) No 652/2014 is laying down the provisions for the management of expenditure relating to the food chain, animal health and animal welfare, and relating to plant health and plant reproductive material. The Commission co-finances annually the survey activities of Member States for plant harmful organisms ("pests") relevant to the EU's plant health policy. The specifications are included in the Commission's work programme.

In line with the principles of the above-mentioned legislation and within the spirit of preparedness and early prevention for plant health, EFSA is requested to offer technical assistance in surveillance. The aim is to facilitate the Member States in their planning and execution of their survey activities.

1.2. Terms of Reference as provided by the requestor

EFSA is requested, pursuant to Article 31 of Regulation (EC) No 178/2002, to provide scientific and technical assistance in the field of plant health.

TASK A

EFSA is requested to deliver survey data sheets, for the pests included in the list below (list may be further adapted by the Commission) (see table 3). The factsheets are expected to be practical and appropriate for end-users, focusing i.e. on host plants, areas and timing of survey, sampling procedures, and list of available detection methods.

TASK B

EFSA has developed software that allows the calculation of statistically significant sampling for pests during surveillance activities. The RiBESS tool was developed in the context of the animal health and was aiming at supporting the Member States to demonstrate absence of *Echinococcus multilocularis*. In addition, the SAMPELATOR tool enables the pest prevalence estimation in countries/areas that are not free of a particular pest.

EFSA is requested to give access to European Commission and Member States to these tools and present them to the Member States. The aim is to allow Member States to use these tools in the planning and design of their annual survey programmes for plant pests. This support is expected to have an initial pilot phase to allow the adaptation and further development of the IT tools for the plant health purposes. Up to 3 plant pests will be used for this pilot phase which are included in category A or B from the list of pests given below (table 3). The Commission will be consulted on the choice of pests for the pilot phase before the decision is taken. A technical/methodological report describing the surveillance framework, its goals and applications for plant health, including possible grouping of some pests will be provided for the onset of the pilot phase with the Member States.

Subsequently, EFSA is requested to produce pest surveillance guidelines for the initial pilot of 3 plant pests included in category A or B. These guidelines are expected to be concise and in accordance to ISPM 6 guidelines for surveillance on specific surveys. The output is expected to be a practical guidance document, fit to the needs of the end-users.

The outcome of the pilot and the need to develop pest surveillance guidelines for the other pests included in the list below will be considered by the Commission.

Upon decision from the Commission, EFSA will be requested to produce survey guidelines for the other pests included in the list below (list may be further adapted by the Commission).

Where appropriate, the surveillance guidelines may combine more than one pest, e.g. for potato pests, citrus pests, deciduous trees etc.

In the case of guidelines for surveillance for *Xylella fastidiosa*, EFSA is expected to comment and adapt if needed the existing guidelines.

1.3. Interpretation of the Terms of Reference

The new EU plant health regulation (EU 2016/2031[1]) provides an extra focus on the prevention of risks and risk-targeted actions in Plant Health. In particular Article 22 provides further information on the survey of Union quarantine pests, and pests provisionally qualifying as Union quarantine pests, indicating that:

(i) Member States shall carry out risk-based surveys, over specific periods of time, in all areas where the pest of concern is not known to be present

(ii) The design of the surveys shall consist, at least, of visual examinations by the competent authority and, where appropriate, the collection of samples and performance of tests. The surveys should be based on sound scientific and technical principles.

In addition, with regards to Multiannual survey programmes and collection of information, Article 23 indicates that the specific objectives of the survey should be defined and with regards to the priority pests Article 24 states that "surveys shall include a sufficiently high number of visual examinations, sampling and testing, as appropriate for each priority pest, to ensure, as far as it is possible given the respective biology of each priority pest and the eco-climatic conditions, with a high degree of confidence, the timely detection of those pests" .

Moreover, regulation EU 652/2014[2] addresses the Commission co-financing of the annual MS surveys on condition that their scope includes at least one of the two critical categories of pests, namely pests which are not known to occur in the Union and pests which are subject to Union emergency measures. This financial contribution could indirectly enhance the EU MSs survey capacity.

[1] 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

[2] Regulation (EU) No. 652/2014 of the European Parliament and of the Council laying down provisions for the management of expenditure relating to the food chain, animal health and animal welfare, and relating to plant health and plant reproductive material, amending Council Directives 98/56/EC, 2000/29/EC and 2008/90/EC, Regulations (EC) No 178/2002, (EC) No 882/2004 and (EC) No 396/2005 of the European Parliament and of the Council, Directive 2009/128/EC of the European Parliament and of the Council and Regulation (EC) No 1107/2009 of the European Parliament and of the Council and repealing Council Decisions 66/399/EEC, 76/894/EEC and 2009/470/EC, OJ L 189, 27.6.2014, p. 1–32.

The latter regulation also lists the minimum requirements for national programmes that will be the subject of evaluation and approval by the Commission. Survey programmes should contain:

- the pests included in the programme;
- a description and demarcation of the geographical and administrative areas in which the programme is to be applied and a description of the status of those areas as regards the presence of the pests concerned;
- the duration of the programme;
- the number of visual examinations, samples and tests scheduled for the pests and plants, plants products and other objects concerned;
- the estimated budget;
- the targets to be attained by the completion date of the programme and the anticipated benefits thereof; and
- appropriate indicators to measure the achievement of the targets of the programme.

In this context EFSA is requested to support and assist the EU Member States with the development of a tool-kit for the survey of plant pests, in line with the guiding principles described in ISPM 6 (guidelines for surveillance (FAO, 2016)), which could contribute to a harmonised pest survey approach across the EU to inform both risk management and risk assessment.

The aim of this technical report is not to provide guidance on surveys but to describe how EFSA will address the different tasks defined in the mandate in terms of the methodological approaches and in terms of the deliverables of the project. Also this document describes the role of the different partners of the project and in particular the involvement and shared responsibility between EFSA and the Member States to develop practical, concise and fit for purpose tools for performing scientifically based surveys in the EU.

The preparation of a general data collection framework and the provision of a common reporting strategy on the pest surveys are not within the scope of this mandate.

The expected outputs to be delivered by this project and the corresponding end users in the EU MSs are presented in the table 1 below. In summary, EFSA expects to deliver 22 pest survey cards by the end of 2018, 25 by the end of 2019 and survey guidelines for three pilot organisms by the end of 2019. Further details on the milestones and expected timelines of delivering the outputs are described in section 5.

Table 1: Expected outputs to be delivered in the context of the mandate and the corresponding end users in the EU MSs

	End users	Role of the end-user	Expected EFSA deliverable	Objective of the output
Pest identification and detection	Inspectors in the MSs	Persons who will implement the surveys in practice in the field, including visual inspection and sampling	47 concise and practical pest survey cards on the quarantine organisms provided by the Commission (Table 3). The cards will summarise the key biological information relevant for the detection and identification of the pests	Facilitate the detection and identification activity and sampling by the inspector
	Laboratory technicians in the MSs	Persons who will perform the diagnostic tests on the samples delivered by the inspectors		Facilitate the laboratories in the choice of the most appropriate diagnostic method for identifying the pests
Survey design and planning	Survey designer and planners in the MSs	Person who will design the pest surveys and plan their execution in the most rationale manner making best use of the available resources	Guidelines for survey for 3 pilot pests.	Provide the pest-generic and theoretical background of the EFSA sampling tools for survey design for the pilot pests. Provide the support to using EFSA tools for survey design Provide the relevant practical information for the implementation of surveys.
Harmonisation and support to the survey activity	Risk managers in the MSs	Person who will decide if it is appropriate to implement the survey guidelines for the pilot pests and who will provide the feedback on the results of the survey	Discussion and final revision of the guidelines	Interaction with the Member States after implementation of the pilot guidelines for revising and ensuring there are fit for purpose and harmonised across the EU.

2. Statistical tools for survey design

The quality of data used to produce statistics and inferences is essential to ensuring dissemination of reliable and accurate information (EUROSTAT, 2003). Collection of data to support risk assessment regarding plant health is an important task, and therefore needs to ensure that the quality of information is appropriate for such assessments which are crucial to inform policy making.

EFSA recognises that in accordance with the principle laid down in the article VII.2(j) of the IPPC[3], surveys will be conducted to the best of the contracting parties abilities. However, from a technical perspective, the specific objective of a survey has an influence on the most appropriate survey design as well as on sample size calculations. The objective of a survey can in general be divided into two main groups:

- Detection surveys: the surveillance programs (or surveys) that are carried out for pests *that are not known to be present* in a specific area. These focus on (i) the early detection of new incursions (emerging/exotic pests) in the area/region of interest, or (ii) are used to demonstrate freedom from a specific pest in the area/region of interest.

- Monitoring surveys: Surveys that are focused on pests *that are known to be present* in an area/region for which the interest is in describing the prevalence or distribution of that specific pest, or identification of cases which trigger further actions (contingency plans using control measures to contain the spread or establishment of the pest in question).

To assist with this, in this project EFSA will provide separate tools to address these two survey objectives (see sections 2.2 and 2.3). As indicated above (section 1.3) EFSA will address in this work only the compilation of the relevant data for performing scientifically based surveys and the preparation of the guidelines for three pilot pests. The preparation of contingency plans is not within EFSA remit in virtue of the separation between risk assessment and risk management in the EU.

2.1. Key parameters

Regardless of the objective (detection or monitoring) it is essential that surveys are scientifically based. In the context of this project EFSA will define and estimate several parameters to inform the statistical tools (described below in section 2.2 and 2.3) that can be used to inform appropriate survey and sampling intensities, locations, and detection methods. These key parameters are described in the following sections.

2.1.1. Biological parameters

Pest population dynamics involve multiple interacting processes occurring across different biological and spatial scales which must be accounted for in pest detection. A hierarchical approach is applied to ensure the goal of the survey is achieved. Three levels are distinguished and are described below and summarised in figure 1.

Target population: This is 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 and/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

[3] FAO (Food and Agriculture Organization of the United Nations), 2011. International Plant Protection Convention (IPPC) (1997), 18pp. FAO, Rome, Italy. Available online: https://www.ippc.int

Epidemiological unit: It is a homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result into 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, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery).

Inspection unit: The inspection units are the plants, plant parts, commodities, pest vectors that will be scrutinised for identifying and detecting the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place.

When deciding the appropriate choice of epidemiological unit and of inspection units, it is important to consider the following:

- the statistical tools developed within this mandate can be used to identify how many epidemiological units need to be surveyed and how many samples need to be taken from within an epidemiological unit.
- the resulting data will be used to determine pest prevalence; this will be expressed as the number of epidemiological units that are infected or infested with a pest within the target population. For example, the prevalence can be estimated on the number of infected fields or glasshouses where roses are grown in a given country.

As an example, consider a survey for a pest of roses that occurs in glasshouse production. The target population could be all the roses in the MS. The epidemiological unit could be the glass houses producing the roses in the MS. And the inspection unit could be individual rose plants in a glass house (Figure 1).

Pest diagnosis: is defined in ISPM 5 (Glossary of phytosanitary terms, FAO, 2017) as *"the process of detection and identification of a pest"*. Two types of diagnostic methods can be distinguished, visual inspection and testing of samples. On a case by case basis, it is necessary to consider whether it is appropriate to use a single method or a combination of methods for pest detection and identification. The two diagnostic methods are briefly described below:

(i) Visual Inspection: Inspections are defined in the ISPM 5 as: *"Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations"*. Visual examination is defined in ISPM 5 as: *"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*. Visual inspections are mentioned in ISPM 6 as actions to consider when developing a survey procedure. A visual inspection is an action of visiting a specific epidemiological unit looking for symptoms and/or looking for the pest that could fully or partly contribute to a diagnosis. Two cases have to be considered when performing visual inspection:

- **symptoms of the pest are clearly and quickly expressed**, in this case each "observation" of an epidemiological unit can be considered a detection method with an output (e.g. infected / uninfected) which has a given probability of correctly detecting pest presence. In this sense, it is necessary to estimate the sensitivity of the method as the risk of misidentification of the organism and the possibility for it to escape detection (Type II error or false negative, see glossary) need to be considered. In other terms it corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive.
- **asymptomatic infections or cryptic pests** and in particular for diseases with long incubation and latent periods. It should be noted that in this case the sensitivity of the visual inspection method can be considered to be zero and the method will not be useful for early detection. This is because when the symptoms are first detected, the pest might have already spread to other plants and areas where it is in an asymptomatic period. In which case the prevalence of infection will not be detectable until it is already very high, and thus visual examination would not be appropriate for

a detection survey (and sampling and laboratory testing of *asymptomatic* tissue would be required).

(ii) Sampling and laboratory testing: The tests that are defined in ISPM 5 as: *"Official examination, other than visual, to determine if pests are present or to identify pests"*. The methodology for performing the tests might be described in existing diagnostic protocols that contain the essential information to ensure that for a specified pest the methods are appropriate for use in the full range of circumstances. General guidance on diagnostic protocols is provided in ISPM 27 (Diagnostic protocols for regulated pests (FAO, 2006)) and in the EPPO standards on diagnostics: PM7/76 (4) Use of EPPO diagnostic protocols (EPPO, 2017); PM 7/122 (1) Guidelines for the organization of inter-laboratory comparisons by plant pest diagnostic laboratories (EPPO,2014); and PM 7/77 (2) Documentation and reporting on a diagnosis (EPPO, 2016). The methods included in the diagnostic protocols are characterised and selected mainly based on their sensitivity and specificity (see Glossary). It should be noted that in the case of diseases with long asymptomatic periods, for the purposes of early detection, there is an important difference between using a test to confirm visually detected symptoms, and using a test to identify asymptomatic infection.

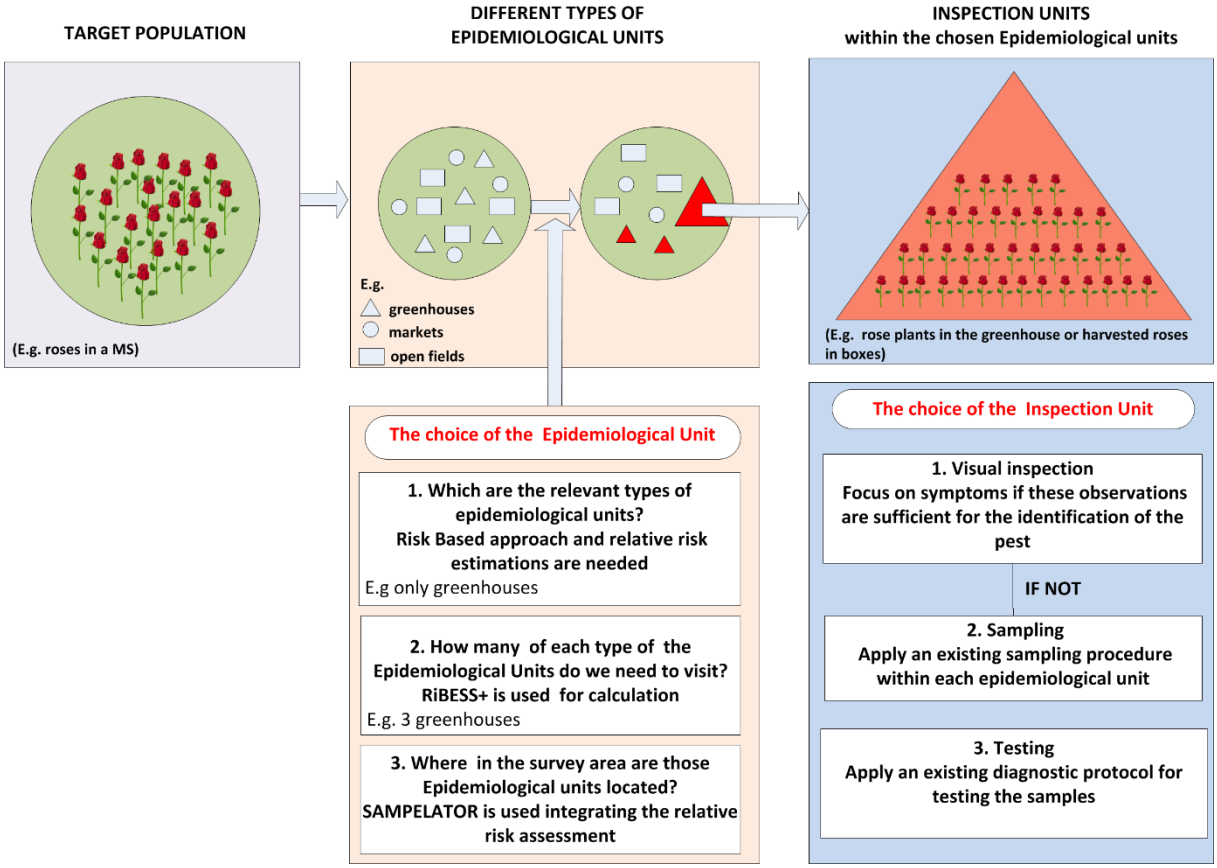


Figure 1: A hierarchical approach in three levels: target population, epidemiological units and inspection units

2.1.2. Confidence level and design prevalence

The parameters listed and described in this section have to be defined with the risk managers as linked to the acceptability of the related risk and the confidence and robustness of the estimates provided by

the surveillance activity. In the context of the pilot organisms, EFSA will provide support to the risk managers for estimating these parameters:

- **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. In other words, 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. Clearly, the more intensive and sensitive the survey, the smaller the design prevalence.
- **Expected prevalence**: in prevalence estimation approaches, it is necessary to have an initial estimation of the proportion of epidemiological units expected to be infected or infested. If an initial estimation is not possible, the expected prevalence can be set at 50% (pure chance), but this will result in the highest sample size possible, keeping the other parameters constant.
- **Desired confidence**: the desired level of confidence of the statement on the design prevalence. It is normally set at 95% or 99%. For example, a confidence of 95% means that if the sampling process was repeated with the same method, the true prevalence would fall between zero and the design prevalence 95% of the time (i.e. 19 times out of 20). A confidence of 99% means that the true prevalence would fall between zero and the design prevalence 99% of the time (i.e. 99 times out of 100).

2.2. Detection surveys: pest freedom and the use of RiBESS+

In situations in which the pest to be considered is not known to be present in an area/region, or to be able to promptly detect a pest when it is entering in an area/region as it was described before (first group of surveillance type), pest freedom survey designs could be used. It is well known and intuitive that demonstrating that a population is truly free of a pest is not possible. For example, Kalaris et al (2014) explain that even testing all units in a target population does not provide sufficient evidence regarding pest status, since tests are in general imperfect and, even in the case in which a perfect test exists, by the time that the whole population is tested, there is no certainty that those that were tested earlier are not infested by the time the last units in the target population are tested. For this reason, this problem has been tackled using a different view point, in which confidence is built up about the pest status, so that the risk of making a misclassification reaches acceptably low levels (i.e. specified by the design prevalence). The methodology for sample size calculations in this setting is well established (e.g. Cannon (2001)). A WEB app following the principles described by Cannon (2001) was developed within an EFSA project (R4EU) that provides a user-friendly interface to perform sample size calculations (Verbeke and Varewyck, 2016). Whereas the general methods available assume a representative sample (i.e. simple random sampling), the EFSA tool allows for improved efficiency of survey resource allocation by accounting for information that is available to target resources based on pest risk i.e. looking for a pest in the locations it is most likely to be present. In order to be able to design a risk-based survey, a number of requirements should be considered:

- a good understanding on the risk factors that determine pest occurrence;
- readily available information on the host population distribution, as well as the geographical distribution of the risk factors under consideration for the design.

In the absence of any information of risk factors for a particular pest, the RIBESS + tool can still be used. The inputs needed are:

- Host population size (which is based on hosts to be sampled, for instance, in the case of a pest affecting citrus plants, estimation of the acreage (or number) of citrus plants in the region to be surveyed)
- Sensitivity of the sampling and test used to detect the pest of interest in each epidemiological unit
- Design prevalence (what could be considered as acceptably low levels)

Appendix C provides details on the illustration of the mathematical concepts in the context of *E. multilocularis* surveillance that were integrated in the RiBESS+ application for the calculation of the sample size both for finite and infinite populations and for simple random sampling and for risk based sampling.

For illustration purposes in the field of plant health we illustrate the use of the RiBESS+ tool using two scenarios: (i) No risk factors are available or can be estimated; (ii) Risk factors are available or can be estimated.

(i) No risk factors: Using the example of a survey for a citrus pest, where the epidemiological unit is a single citrus tree. The number of citrus trees in the area under scrutiny is 10 000, represented by the population of citrus trees in which we would like to establish evidence for pest absence. The sampling procedure and the test used to detect the pest within the epidemiological unit (i.e. in a single tree) has a sensitivity of 90%, and the acceptable level of prevalence of the pest (i.e. the design prevalence) is considered to be 0.1% (0.001). Using the RiBESS+ tool (see Figure 2) the number of trees to be sampled to have a 95% confidence that the prevalence of the pest is less than 0.1% (assuming no pest is detected) is 2876. If fewer than that number of trees are sampled, then the design prevalence will increase. For example, if at the end of the sampling period (normally 1 year) only 250 trees were sampled then the design prevalence would be 1.3% i.e. 130 trees could be infested even though they were not detected in the survey. It is evident, therefore, that the sample size was not adequate to fulfil the pre-set / desired requirements (in this case, maximum 0.1%, i.e. 10 trees infested out of 10 000).

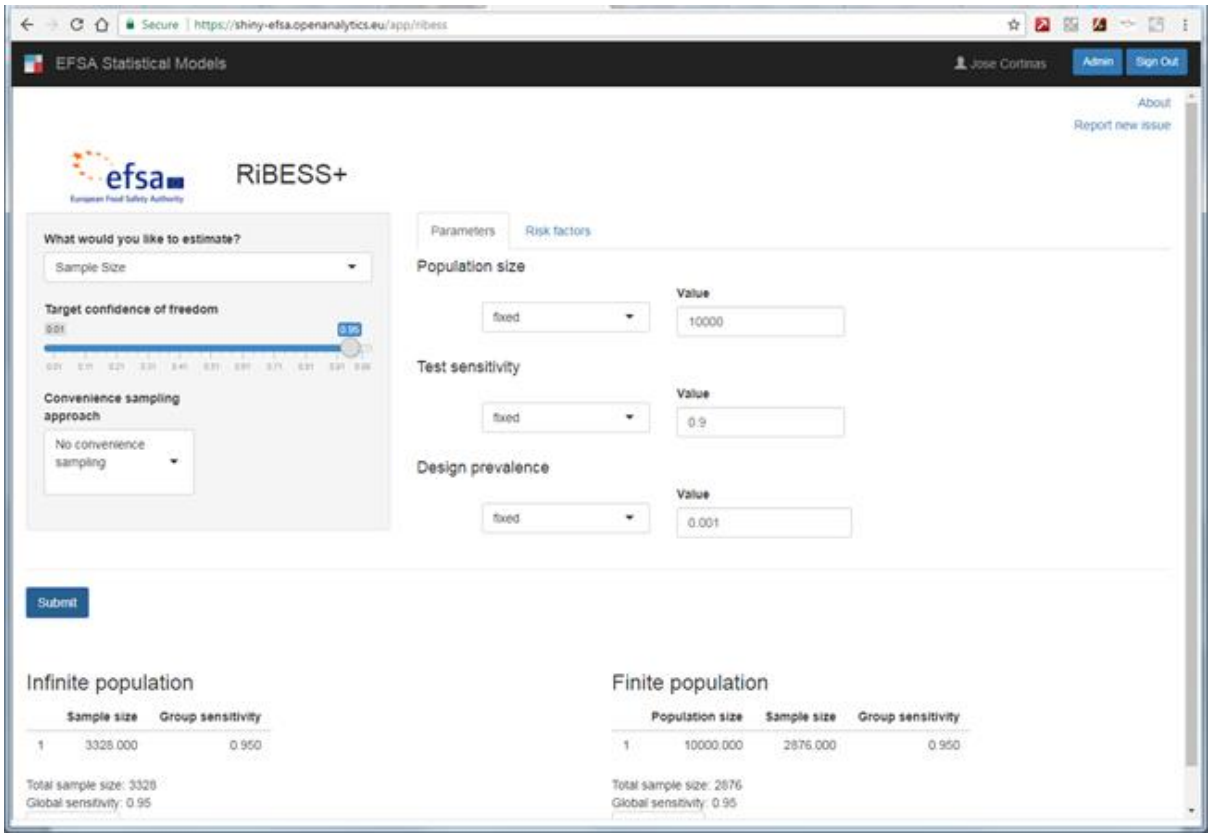


Figure 2: Screenshot of the RiBESS+ WEB app calculating the number of citrus trees to be sampled to demonstrate pest freedom.

(ii) Use of risk-factors: If instead risk factors can be identified for the pest under consideration, considering the same inputs in terms of population size, design prevalence and test sensitivity, additional inputs are needed. The additional inputs required are: number of levels of the risk factor, the relative risk of each of the levels in comparison to a baseline level and the proportion of trees in the population that belong to each of the levels of the risk factor identified. Consider a risk factor with two levels in which the relative risk of Level A is 3 times higher than for Level B, and the proportion of citrus trees having Level A is 0.3, then the sample size based on risk-based surveillance (see Figure 3) would be 1376, which is reduced by more than half with respect to when a representative survey was implemented in scenario (i).

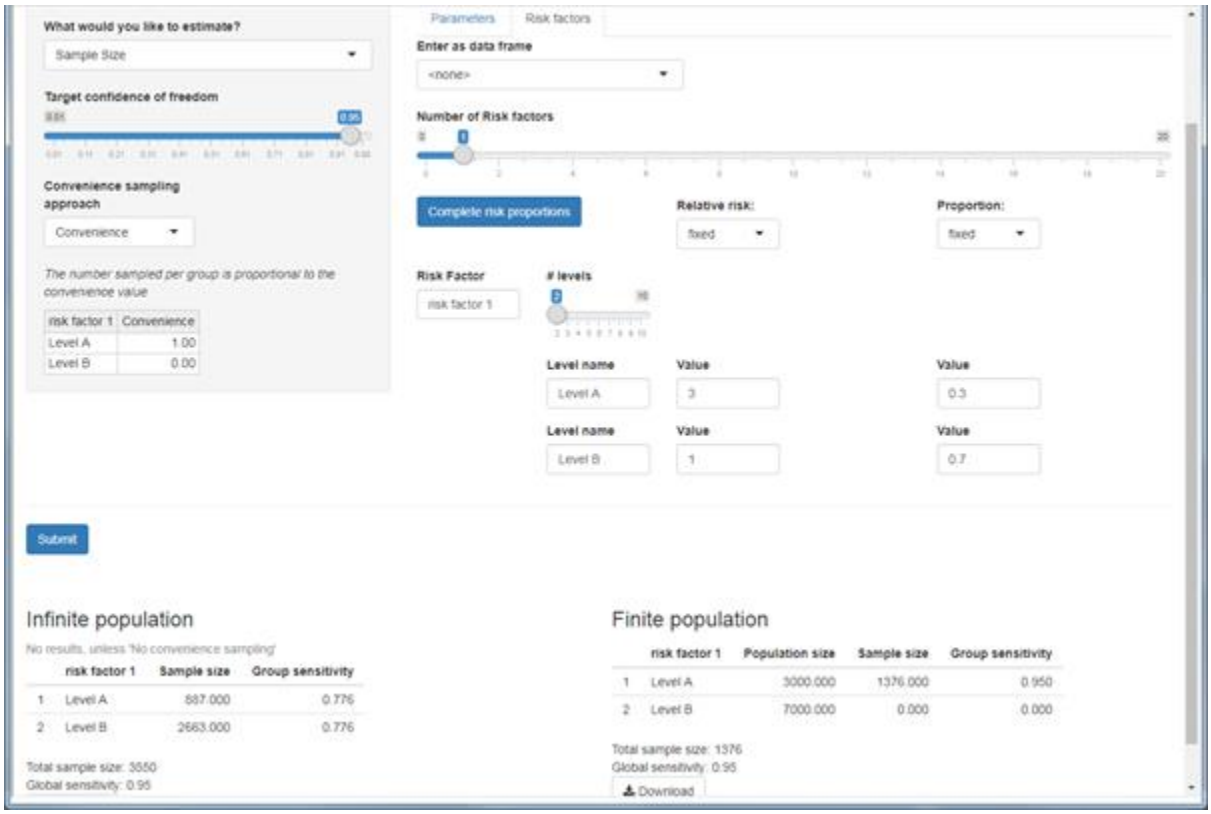


Figure 3: Screenshot of the RIBESS+ WEB app calculating the number of citrus trees with Level A to be sampled to demonstrate pest freedom in the case of risk-based surveillance implementation.

2.3. Monitoring surveys: Pest prevalence estimation using SAMPELATOR

In the case that the purpose of the survey is to estimate the prevalence of a specific pest that is known to be present in an area, ensuring the representativeness of the selection of survey locations and samples is crucial. Data representativeness refers to a dataset obtained from a survey or study (a sample) which accurately resembles/reflects the population under study. This is only possible after clearly knowing the target population from which inference is needed and what is the purpose for collecting the data. Having a large sample does not imply representativeness; the manner in which the sample was collected plays an important role in ensuring representativeness and should be randomised. In general, introducing bias when collecting data should be avoided, for example by employing the principles of sampling design as described in Knottnerus (2003). The use of a well-designed probability sampling strategy minimizes the risk of having selection bias. This relates to both the selection of epidemiological units (e.g. plants, trees, woodlands, fields, orchards) and also to the collection of samples within an epidemiological unit.

Sampling design refers to the whole process and considerations concerned with obtaining descriptive or inferential statistics of a population of interest by studying a portion of the population instead of the whole population (Barnett, 1991; Foreman, 1991; Kalton, 1983). The first stage in designing a survey is a clear definition of the target population and of the objectives. It is important to identify the elements which compose the target population i.e. the units that make up the population from which information is sought.

Logically, the definition of the population should be linked to the objectives of the survey. Objectives can be broadly divided into two groups: estimation and inferential. Estimation objectives mainly involve

production of quantitative and numerical descriptions (estimation) of relevant aspects of a target population, for example the proportion of the population with a characteristic of interest (prevalence of a pest in an area/country of interest). On the other hand, inferential objectives are about testing a particular hypothesis about the population of interest. When a survey is conducted with the aim of estimating a parameter of interest in a population, some level of certainty (usually expressed as a confidence/credible interval) is associated with the estimate. Intervals give a range of values in which it is believed the true parameter value lies, and if the true value is not comprised in this range, a type I error (see glossary), is committed. The probability of committing this error is pre-specified in advance and incorporated in sample size calculation during a surveillance design so as to keep it under control.

The WEB app is designed to calculate sample size for various sample designs in accordance with the principles stated above. The sample designs supported are:

- **Simple Random Sampling (SRS)**: Drawing elements from the target population, such that each population member has equal and a non-zero probability of being selected. An example would be like assigning unique number (e.g. 1 to 200) to all citrus trees in an hectares of land and randomly chose any 25 trees (e.g. 5,12,1,…) in a hat for testing.
- **Cluster Sampling**: Drawing existing clusters that form the population, which could include further sampling elements within the cluster sampled if they are considered large, assuming that elements within each cluster are more alike than elements between groups. Thus, each element sampled does not necessarily bring new information about the population. This method could apply in the case of greenhouse crops where the variability within the same greenhouse is very low while it could be much higher between greenhouses. For example, if the objective of the survey is to detect a specific pest in a MS, it might be a very large geographical area to cover and SRS can be difficult. Therefore, if sampling will be limited to only 4 regions out of the total of 20 regions in the MS, this method allows to assign a unique number to each region and use a probabilistic sampling methods to select 4 regions to be included in the survey.
- **Stratification**: When the population of interest falls naturally into strata that compose the population, sampling may be organized within each of these strata, for instance different MSs involved in EU surveillance. In this type of sampling, the characteristic of interest is surveyed and analysed within each stratum, after which the results are combined, to provide an overall sample result. For the detection of a specific pest in a MS on its host plants, a stratification method could be used. Each region in the MS will be a stratum, and a SRS can be used to select a number of samples to be included in the survey from each region.
- **Multi-stage Sampling**: Where a combination of the sampling strategies defined above are used to draw a sample from the population, two-stage and three-stage sampling schemes are implemented in the WEB app. For example, it will be nearly impossible to list all the host plants infested by a specific pest in Europe and sample them. A simple approach is to perform a multi-stage sampling. The first step, we can divide Europe into regions with host plants, then in the next stage we select some MSs from those regions to include in the survey. The next stage, state/province can be selected within the MSs. Then finally fields are selected within the states/provinces.
- **Designs for Measuring Change Over Time**: In general, repeated survey designs are recommended for measuring change over time. These can either be using panel designs or repeated cross-sectional surveys. Panel designs allow measurement of both net and gross change while repeated cross-sectional surveys only allow for gross change. A longitudinal survey is a well-known form of panel designs where the initial selected sample is followed for the whole period of the survey and they produce precise net change estimates (option implemented in the WEB app).

A screenshot of the WEB app is presented in Figure 4.

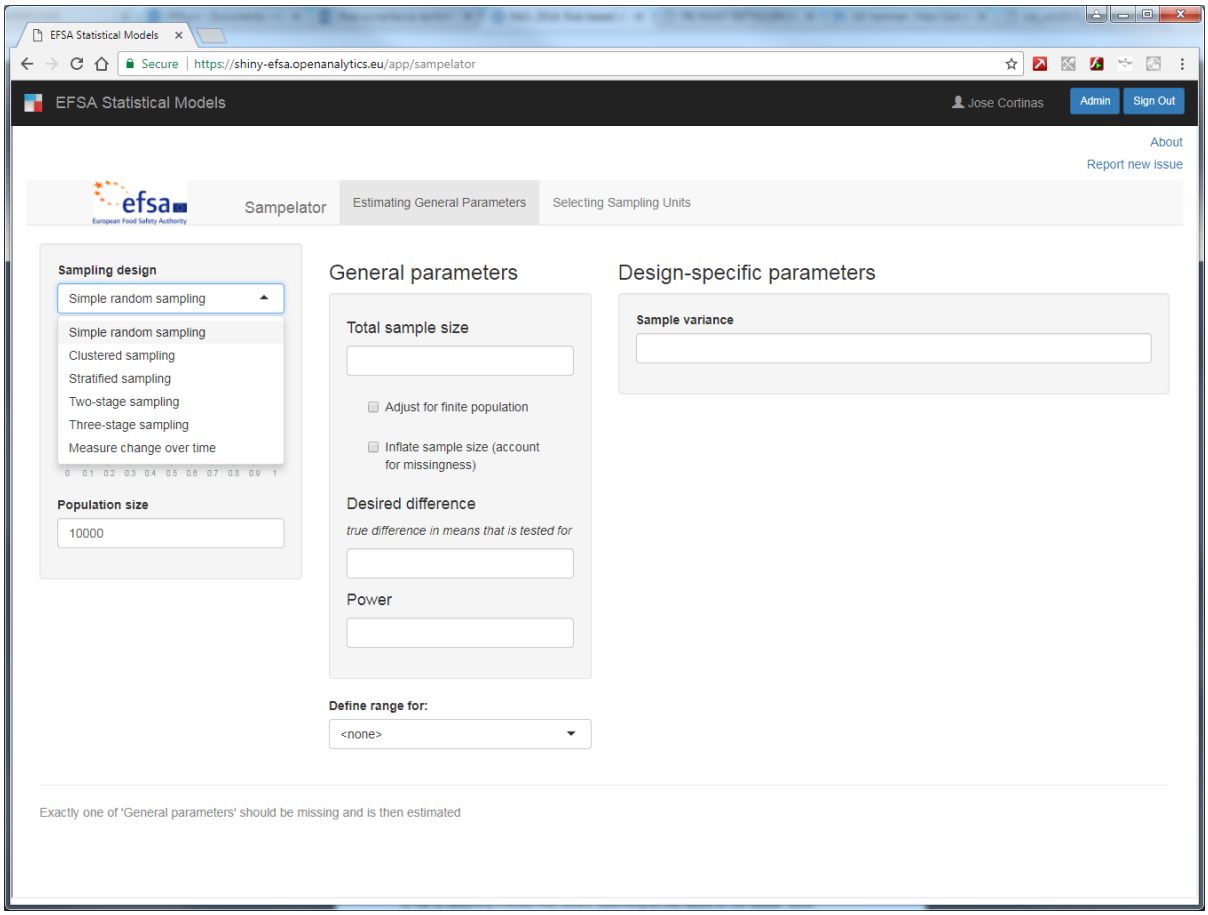


Figure 4: Screenshot of the SAMPELATOR WEB app showing the designs supported.

Further details are available in Milanzi et al (2015) on the mathematical concepts that were integrated in the SAMPELATOR application for the calculation of the sample size in the context of the monitoring and delimiting surveys where the pest prevalence measurement is the ultimate purpose of the surveillance.

2.4. Sampling strategies

It is necessary to optimise sampling with respect to the specific objective of the survey. A decision tree will be developed to support the surveyor in the survey design in tailoring the approach by choosing the most appropriate methodology and statistical technique to address his concern. Different approaches are suggested in the following sections:

2.4.1. Risk based survey design

The design of a survey heavily depends on the goal of the survey itself. In fact, samples can be collected for a variety of purposes, among which the most relevant for this mandate are: (i) demonstrate freedom from a given pest; (ii) estimate the actual prevalence at a given point in time.

The data needed to feed the statistical models underpinning the chosen approach and goal may be the same, but this is not always the case. The description of the existing tools developed and made available by EFSA (RiBESS+ and SAMPELATOR, see sections above 2.2 and 2.3) makes clear what type of data are required.

It is crucial at this point to identify exactly which are the parameters relevant for the goal of this mandate as this information will be part of the Pest Survey cards. Considering the level of detail required by these

methods, it is unlikely that all information is readily available on, e.g., official dedicated websites, and, therefore, a targeted scientific literature search must be performed on each identified parameter.

The sections below aim at listing and defining the parameters of interest for each approach and goal.

2.4.2. Risk factor and relative risk

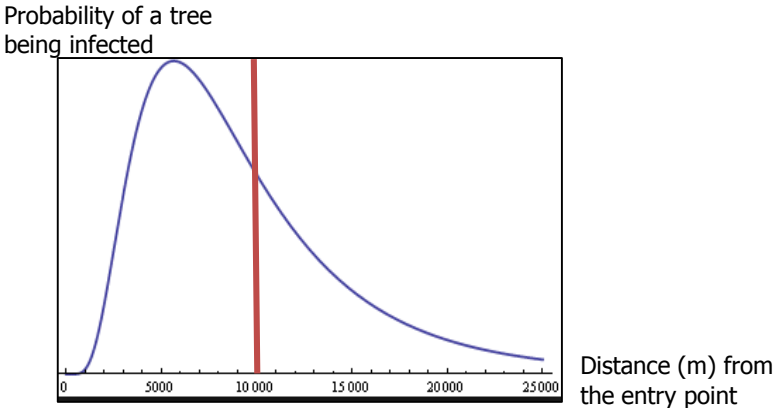
The identification of risk factors entails the identification of subgroups within a target population, each with epidemiological units (e.g. trees) characterised by a different chance of being infected. In Freedom from Pest approaches, once the risk factor(s) has (have) been identified, it is necessary to know (or at least, estimate) the proportion of epidemiological units belonging to each subgroup. As a last step, it is necessary to quantify the relative risk of the different subgroups, compared to a baseline, for each identified risk factor.

The most relevant risk factors need to be identified on an *ad hoc* basis for each pest and, likely, for each specific environmental condition. Below a non-exhaustive list of risk factors that might be relevant for plant pests:

- Vectors (presence/absence OR density)
- Climate conditions (temperature / humidity / wind / etc.)
- Water (presence/absence OR distance form water)
- Biosecurity (presence/absence OR semi-quantitative evaluation, e.g. good/poor)
- Roads (presence/absence OR distance from roads and roads effective as pathway)
- Entry points (presence/absence OR distance from entry points)
- Management options (field / greenhouse)

The identification of the risk factors and related relative risk is essential to implement a risk based sampling strategy. Once this information is identified and gathered, they can be used in the RiBESS+ tool with the advantage of increasing the confidence around the results with less samples. For illustrative purposes only, it is useful to describe the process.

Assuming that the distance from an entry point is relevant for a given pest "PT", the probability of a tree being infected depends on the distance (the greater the distance, the lower the probability of being infected) (see figure 5). In this case, the analyses of the available evidence result in the estimate that the trees within 10,000 meters have double the probability of being infected compared to the others (further than 10,000 meters) that are considered as the baseline. The proportion of trees within 10,000 meters from the entry point is estimated to 15% of the total number of trees. Figure 5 summarises this information on the risk factor and relative risk.

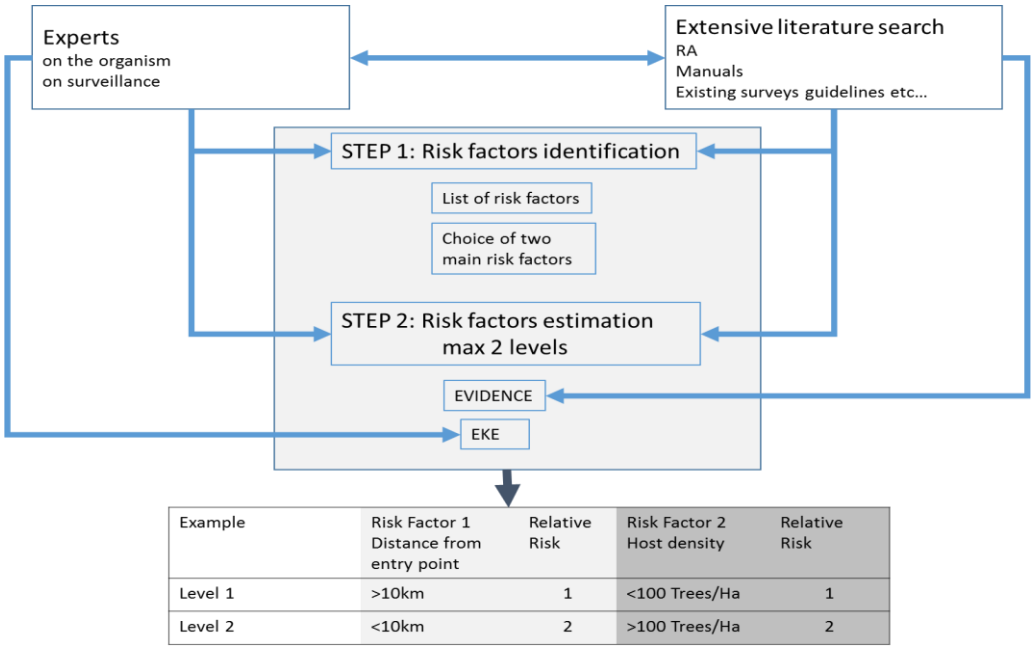


Risk Factor = distance (m) from entry point	Relative Risk	Proportion of trees
< 10,000	2	15 %
> 10,000	1	75%

Figure 5: Example of a risk factor and relative risk: probability of a tree being infected in function of the distance from the entry point

Based on scientific sound evidences, for each relevant risk factor, the relative risks should be estimated for ensuring the survey efforts are increased in the epidemiological units where the probability of infection is higher.

The figure 6 suggests a two steps procedure for the identification of the relevant risk factors and the estimation of their relative risks.



Example	Risk Factor 1 Distance from entry point	Relative Risk	Risk Factor 2 Host density	Relative Risk
Level 1	>10km	1	<100 Trees/Ha	1
Level 2	<10km	2	>100 Trees/Ha	2

Figure 6: Simplified procedure for the identification of the relevant risk factors and the estimation of their relative risks.

2.4.3. Spread models

In addition to static risk-factors that are based on biotic and abiotic variables (e.g. climate, host availability), a significant factor determining whether a location will be occupied by a pest is its connectivity within the host landscape. That is, a pest requires both suitable environmental conditions and available hosts to spread, and its direction and speed of spread will thus be partly determined by the 'stepping stones' and 'corridors' of host fields (and other discrete patches of host) within the landscape.

For example, combining simple spatially-explicit and stochastic spread models with data on the spatial distribution of hosts, e.g. using Geographic Information Systems, allows spread to be simulated on real landscapes. Using available data on the distribution of (a) host(s) species in a landscape and knowledge of the dispersal, transmission and survival characteristics of a pest, pest-generic spread models can thus be utilised to determine the probability of spread to any location in a landscape. This can be assessed as the risk at a particular point in time or the risk up to a particular pre-defined prevalence of a pest in a region.

Simulation based on predictive spread models starting from potential entry points, allows the relative risk of a pest spreading to a particular location (where each location may be specified at the epidemiological unit scale or a cluster of multiple epidemiological units) to be estimated. This can be combined with other risk factors in determining the overall relative risk of a particular sub-group. For pest surveillance, it will be imperative to use simple models with few parameters to estimate spread risk, given the time and resource requirements to accurately parameterise more complex spread models. Model selection is supported by recent pest spread model reviews (EFSA, 2015) and the availability of generic and simple pest spread models online (e.g. Savage and Renton, 2014).

3. Pest survey cards

3.1. Template

For each one of the 47 pests listed in the table 3 to the request from the European Commission a practical and concise pest survey card will be prepared. These documents will contain the key information that are necessary to inform the development of the survey guidelines of the pests providing:

- essential biological information on the pest, its distribution in the EU and its epidemiology to better target the surveillance;
- data and information that define the key parameters for survey design using the EFSA statistical tools for sample size calculation as described in section 2.1.1. In particular, for defining the target population (e.g. host range, host distribution, vectors and their distribution) the epidemiological unit (e.g. field, farm, glasshouse, region), the visual inspections, laboratory testing;
- if available, relevant information on the risks posed by the organism under scrutiny (e.g. information extracted from pest risk assessments) for improving the sampling strategies applying a risk based approach.

In the first phase of the project, a template of the pest survey cards will be developed and agreed with the European Commission and fine-tuned with some Member States based on the examples of the survey cards for the 3 pilot organisms before preparing all the other pest survey cards.

3.2. Data collection process

A process for systematic data gathering has been developed (Figure 7) to ensure that recent and most relevant available information on the key parameters are collected. The methodology on data collection

regards the identification of statistical key parameters such as target population, risk factors, visual inspection and sample testing methods for the calculation of sample sizes in the surveillance process using the statistical tools (RIBESS+ and SAMPELATOR). Three different steps are distinguished in the data collection process:

Step 1: Pest Risk Assessments and Diagnostic Protocols, identification of existing pest risk assessments and pest identification methods and diagnostic protocols from different information sources (e.g. Internet searches, EFSA opinions, IPPC, EPPO, USDA, CABI Crop Protection Compendium, CABI Forestry Compendium, EFSA pest categorisations). All the documents found will be retrieved and screened in order to extract data on the key parameters of interest. In case the collected data is not sufficient, the search will be extended to Step 2.

Step 2: Scientific and grey literature including technical documents and reports. In case the collected data is still not sufficient, the search will be extended to Step 3.

Step 3: Experts, the remaining knowledge gaps on the key parameters will be addressed through consultation of scientific experts. If needed, expert knowledge elicitation techniques can be applied for estimating the missing parameters (EFSA, 2014).

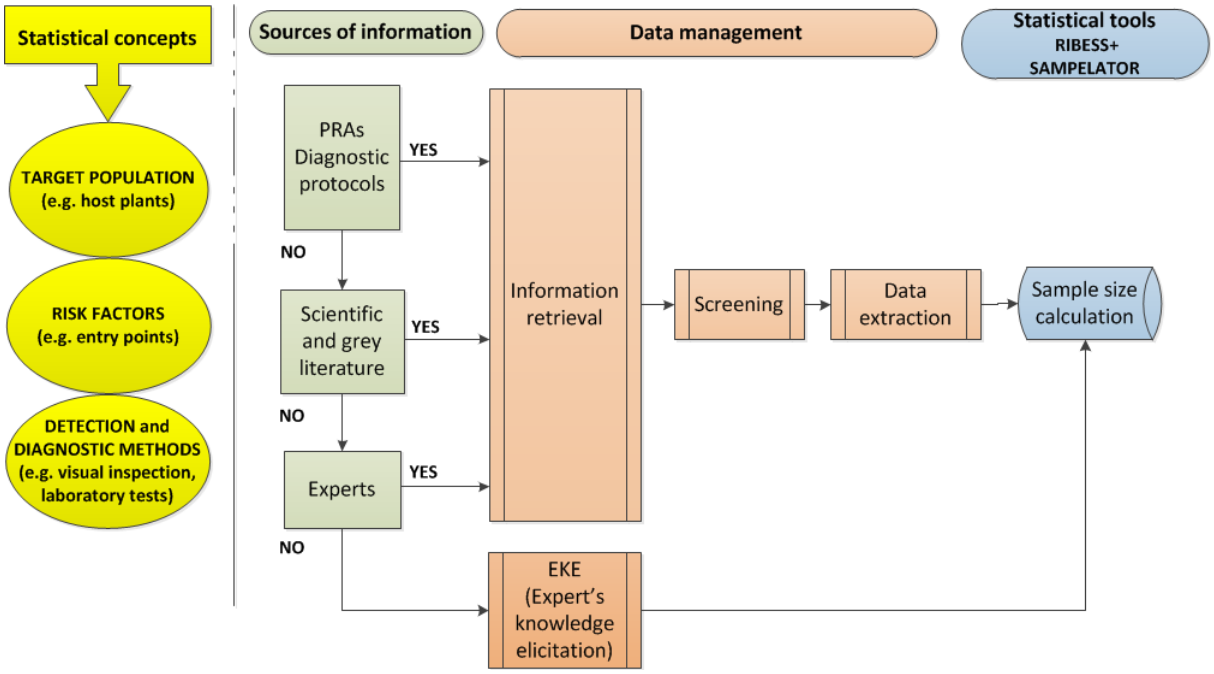


Figure 7: Data collection process for pest survey cards

3.2.1. Extensive literature searches

As shown in Figure 7 and explained above, when the pest risk assessments and the diagnostic protocols do not have sufficient data, extensive literature searches (ELS) will be performed and search strategies will be developed and applied to the databases described in Table 2. In this activity the guiding principles for an ELS will be followed as described in the EFSA guidance on systematic review methodologies (EFSA, 2010).

First the resulting papers will be screened for relevance and data will be retrieved from the relevant papers for use in the tools for sample size calculation.

If the data is still not sufficient, as indicated in figure 7, it might be necessary to estimate the missing parameters using expert elicitation techniques. The entire process will be documented for ensuring transparency and reproducibility as recommended by EFSA (2015; EFSA Prometheus project).

3.2.1.1. Data retrieval

The citations found by the literature search will be retrieved. Searches will be carried out in order to identify scientific literature on the key parameters of interest (See section 1.4) for each pest.

Table 2 shows the databases that will be searched.

Table 2: Example of databases that could be consulted in the extensive literature searches for the key parameters for pest surveillance

Database	Platform
Web of Science Core Collection: Science Citation Index Expanded (1975-present) Conference Proceedings Citation Index- Science (1990-present) Emerging Sources Citation Index (2015-present)	Web of Science
BIOSIS Citation Index (1926-present)	
CABI: CAB Abstracts (1973-present)	
Chinese Science Citation Database (1989-present)	
Current Content Connects (inception-present)	
KCI-Korean Journal Database (1980-present)	
MEDLINE (1950-present)	
SciELO Citation Index (1997-present)	
Russian Science Citation Index (2005-present)	
Crop Protection Compendium (inception-present)	CABI

In house EFSA expertise will be used to develop the search strings and to test them in order to identify as many relevant studies as possible. The search strings will focus on the terms describing the pests. Additional elements to describe the key parameters/outcomes will be used and combined with the pest string. Searches in databases will be first carried out for the last ten years, and extended in case information is not sufficient. Language limits might be applied depending on the number of hits. The search strategies will be adapted according to the configuration and features of each source of information.

Examples of draft search strategies for *Phyllosticta citricarpa* and *Agrilus planipennis* are shown in Appendix A.

The output from the searched sources of information, including all indexed fields per hit (e.g. title, authors, abstract), will be exported into Endnote bibliographic management software file, and duplicate records will be removed.

3.2.1.2. Screening

The screening process will be conducted using specific software, such as DistillerSR (Evidence Partners, Ottawa, Canada). A two-step selection procedure is foreseen:

- Initially, title and abstracts will be screened, to identify studies that contain information on the key parameters of interest. Studies not containing this information will be excluded. In case of doubts or unclear studies, the records will be moved to the full-text screening.

- Screening of full-text documents, to identify studies with relevant information.

3.2.1.3. Data extraction

Data will be extracted from each relevant study using pre-defined forms to guide the reviewers in the process. The forms include a section for evaluating the reliability of the study and a section for the data extraction itself on the key parameters of interest. The approach implicitly considers all uncertainty with regard to data found and data handling. The data extraction forms might be created using specific software, such as DistillerSR (Evidence Partners, Ottawa, Canada) or alternatively, Excel files might be used.

3.2.1.4. Pest survey cards delivery

EFSA will deliver the pest survey cards using a template (as described in section 3.1) in two batches over a period of two years. Upon agreement between EFSA and the European Commission, the list of the pest survey cards to be delivered in 2019 could be reprioritised and modified by October 2018 in consideration of the outcomes of other relevant currently ongoing projects.

An indicative delivery plan of pest surveys cards is presented in the table 3 below.

Table 3: Indicative delivery plan of pest survey cards in two batches

2018 batch of pest survey cards	2019 batch of pest survey cards
Xylella fastidiosa	Hop stunt viroid and Citrus bark cracking viroid
Agrilus planipennis	*Polygraphus proximus*
Phyllosticta citricarpa	*Thaumatotibia leucotreta*
Anoplophora glabripennis	*Thrips setosus*
Popillia japonica	Tomato leaf curl New Delhi virus (ToLCNDV)
Scrobipalpopsis (Tecia) solanivora	*Xylosandrus crassiusculus*
Candidatus Liberibacter spp. and its vectors (*Diaphorina citri* and *Trioza erytreae*)	*Anoplophora chinensis*
Synchytrium endobioticum	*Pomacea*
Toxoptera citrida	*Agrilus anxius*
Bursaphelenchus xylophilus	*Anthonomus eugenii*
Monochamus spp. (non-European)	*Dendrolimus sibiricus*
Erwinia stewartii	Grapevine flavescence dorée phytoplasma
Xanthomonas campestris (all strains pathogenic to Citrus)	*Radopholus similis*
Geosmithia morbida and its vector *Pityophthorus juglandis*	*Scaphoideus titanus*
Citrus tristeza virus (non-European strains)	*Aleurocantus* spp.
Epitrix spp.	*Dacus dorsalis*
Gibberella circinata	*Pterandrus rosa*
Pseudomonas syringae pv. *actinidiae*	*Rhagoletis fausta*
Clavibacter michiganensis ssp. *sepedonicus*	*Agrilus auroguttatus*
Ralstonia solanacearum	*Aromia bungii*
Globodera pallida and *G. rostochiensis*	*Scirtothrips* sp
	Atropellis spp.
	Eotetranychus lewisi
	Diaporthe vaccinii
	Pissodes spp. (non-European)
	Candidatus Liberibacter solanacearum

4. Survey guidelines

4.1. Pilot organisms

In the context of the twelfth meeting of the EFSA Network on risk assessment in plant health, held in Parma on 06 and 07 December 2017, the participants were consulted on the criteria for selecting relevant organisms from the list annexed to the request. The following criteria were discussed as relevant:

- The aims of the surveillance (early detection/pest freedom and monitoring/pest prevalence)
- Pest characteristics (hosts, mono/polyphagous, vector role)
- Knowledge (risk assessment, identification and detection methods, diagnostic tests)
- Generalisability and reproducibility of the procedure for other pests
- Importance of the pest: Pest pressure (trade), main crops (production)
- Difficulties for the assessment: Uncertainties, complex, diverse, no existing guidance
- Potential plant health crisis: Unexpected, fast, many hosts, big impact
- Diversity of the organisms: Pests, hosts.

Based on network discussions *Agrilus planipennis and Phyllosticta citricarpa* were considered as relevant for many EU Member States.

In addition, *Xylella fastidiosa* was also included as a pilot organism for developing guidelines on surveillance, in consideration of the recent and current outbreaks in the EU MSs of *X. fastidiosa*, and the need for reviewing in 2018 the current guidelines in the context of this mandate. The *X. fastidiosa* survey card will be prepared during 2018 and the preparation of the surveillance guidelines (focussing on the update of the current guidance) will initiate during 2018 providing the final guidelines during 2019.

In summary, the survey guidelines will be developed in the frame of this project for the 3 following pilot organisms:

- ***Agrilus planipennis***
- ***Phyllosticta citricarpa***
- ***Xylella fastidiosa***

4.2. Expertise required

The involvement of experts of the different Member States in the development of the different pilot guidelines depends on:

- The expertise needed for the activity. The following field of expertise are required for each one of the pilot pests:
  - the identification and detection methods,
  - the epidemiology,
  - the survey design and statistics;
  - the implementation of the surveys;
- The interest in the organisms and the possibility to test and implement the survey design in the MS.
- The availability of MS experts to contribute to the activity.

The development of the guidelines for the pilot organisms will be performed for each one of the 3 organisms involving MSs experts following the EFSA rules for selection of experts[4].

[4] Decision No.: REF. EFSA/HUCAP/DEC/2017/17115037. Effective Date: 22 May 2017; Decision of the Executive Director concerning the selection of members of the Scientific Committee the Scientific Panels, and the selection of external experts to assist EFSA with its scientific work. Available online at: http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/expertselection.pdf

4.3. Survey guidelines for pilot organisms

The survey guidelines that will be developed in the context of this project will follow the guiding principles described in section 2. At the same time, as indicated in the ToR, they should be practical tools that assist the MSs in the implementation of the surveys. Therefore, the involvement of the MSs experts in this activity is essential for combining the theoretical concepts with a more pragmatic approach. The guidelines will be developed for the 3 pilot organisms described in section 4.1 (i.e. *Xylella fastidiosa; Agrilus planipennis; Phyllosticta citricarpa*) and will include the relevant information on the organisms for informing the surveillance plan in terms of the host range, the detection methods and diagnostic protocols for detecting and identifying the pest, the timing of the year for the sampling activity, the plant parts to sample and the other parameters needed for the sample size calculation and applying the risk based approach. In the course of the preparation of the guidelines, EFSA will provide support to the MSs involved in the activity for performing the survey design.

The survey guidelines will be prepared during 2018 for their practical implementation in 2019. After implementation and analyses of the survey results by the Member States, the survey guidelines may be revised for ensuring they are fit for purpose. The sequence of activities for this phase is presented in section 6. It is important to stress that for the pilot phase of the project it is crucial to put in place a data collection activity in order to ensure that all previous steps have been properly implemented. For this purpose, EFSA will temporarily set up an ad hoc data collection process. Once the pilot phase has been concluded, the data collection activity can be dismissed and the Member States will still be able to use the supporting tools for the estimation and the management of the sample.

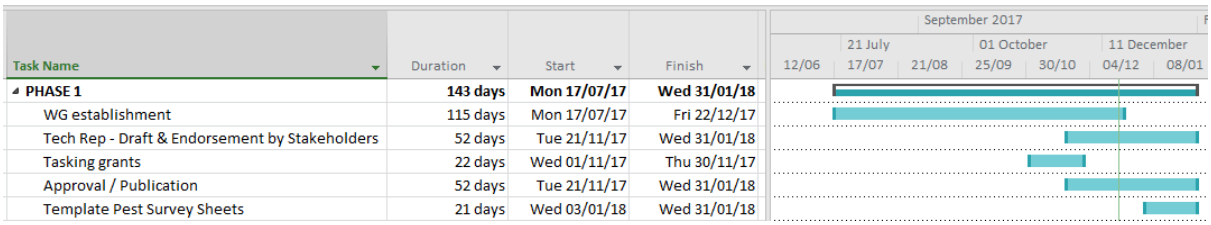
5. Project management and Gantt Chart

A dedicated EFSA working group will involve different experts depending on the topics addressed and will have monthly two day meeting preferably in Parma. In case it is needed ½ day meetings could also be organised and attended using web-conferencing facilities. During the meetings the discussions and contributions will be focussed on different tasks therefore the composition of the WG will vary according to the specific expertise required. The tasks to address comprise:

- Pest survey cards preparation
- Pilot 1 Guidelines for *Xylella fastidiosa*
- Pilot 2 Guidelines for *Agrilus planipennis*
- Pilot 3 Guidelines for *Phyllosticta citricarpa*
- Dissemination and support to MSs

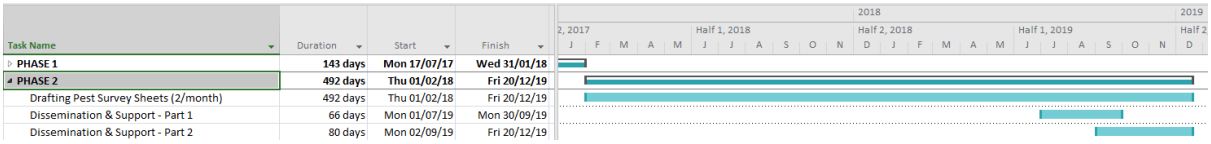
The project has been divided in 4 different phases as indicated in the Gantt charts below:

Phase 1: Planning and methodological approaches



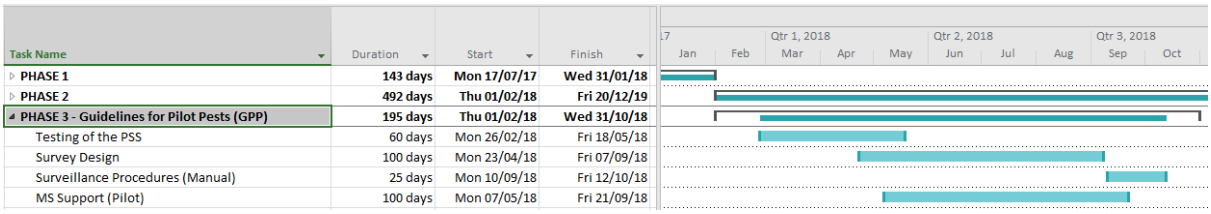
Task Name	Duration	Start	Finish
PHASE 1	143 days	Mon 17/07/17	Wed 31/01/18
WG establishment	115 days	Mon 17/07/17	Fri 22/12/17
Tech Rep - Draft & Endorsement by Stakeholders	52 days	Tue 21/11/17	Wed 31/01/18
Tasking grants	22 days	Wed 01/11/17	Thu 30/11/17
Approval / Publication	52 days	Tue 21/11/17	Wed 31/01/18
Template Pest Survey Sheets	21 days	Wed 03/01/18	Wed 31/01/18

Phase 2: Preparation of the pest survey cards



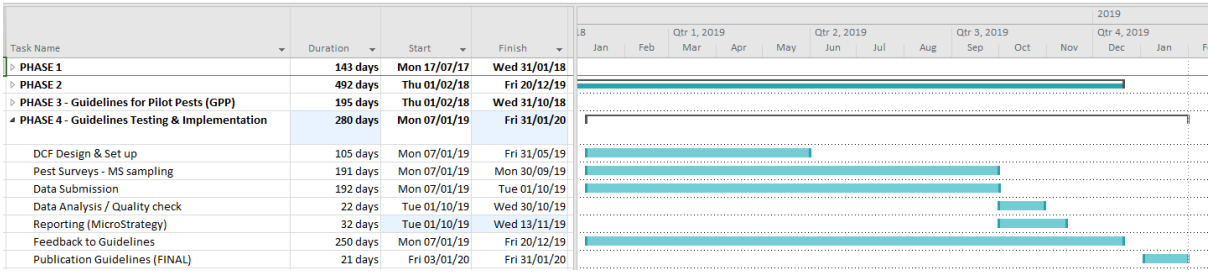
Task Name	Duration	Start	Finish
PHASE 1	143 days	Mon 17/07/17	Wed 31/01/18
PHASE 2	492 days	Thu 01/02/18	Fri 20/12/19
Drafting Pest Survey Sheets (2/month)	492 days	Thu 01/02/18	Fri 20/12/19
Dissemination & Support - Part 1	66 days	Mon 01/07/19	Mon 30/09/19
Dissemination & Support - Part 2	80 days	Mon 02/09/19	Fri 20/12/19

Phase 3: Preparation of the surveillance guidelines for 3 pilot organisms



Task Name	Duration	Start	Finish
PHASE 1	143 days	Mon 17/07/17	Wed 31/01/18
PHASE 2	492 days	Thu 01/02/18	Fri 20/12/19
PHASE 3 - Guidelines for Pilot Pests (GPP)	195 days	Thu 01/02/18	Wed 31/10/18
Testing of the PSS	60 days	Mon 26/02/18	Fri 18/05/18
Survey Design	100 days	Mon 23/04/18	Fri 07/09/18
Surveillance Procedures (Manual)	25 days	Mon 10/09/18	Fri 12/10/18
MS Support (Pilot)	100 days	Mon 07/05/18	Fri 21/09/18

Phase 4: Guidelines testing, implementation and final revision



Task Name	Duration	Start	Finish
PHASE 1	143 days	Mon 17/07/17	Wed 31/01/18
PHASE 2	492 days	Thu 01/02/18	Fri 20/12/19
PHASE 3 - Guidelines for Pilot Pests (GPP)	195 days	Thu 01/02/18	Wed 31/10/18
PHASE 4 - Guidelines Testing & Implementation	280 days	Mon 07/01/19	Fri 31/01/20
DCF Design & Set up	105 days	Mon 07/01/19	Fri 31/05/19
Pest Surveys - MS sampling	191 days	Mon 07/01/19	Mon 30/09/19
Data Submission	192 days	Mon 07/01/19	Tue 01/10/19
Data Analysis / Quality check	22 days	Tue 01/10/19	Wed 30/10/19
Reporting (MicroStrategy)	32 days	Tue 01/10/19	Wed 13/11/19
Feedback to Guidelines	250 days	Mon 07/01/19	Fri 20/12/19
Publication Guidelines (FINAL)	21 days	Fri 03/01/20	Fri 31/01/20

The overall project chart is presented in Appendix B.

Documentation provided to EFSA

Request to provide scientific and technical assistance on survey guidelines relevant for plant health for the EU territory, European Commission, Ref. Ares(2017)3377627 - 05/07/2017. Submitted by SANTE.G1 /PMY/MM/ag (2017) 3464185. This request includes the list of pests

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Glossary of terms

TERM	DEFINITION
Actual prevalence	It is the true proportion of infested units in a population infested by one or more pests (McMaugh, 2005)
Confidence	Sensitivity of the survey
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.
Desired confidence	The level of uncertainty in point estimates and indicate the expected range of values that a parameter might have (Dohoo et al., 2010) The desired level of confidence of the statement on the design prevalence
Detection survey	Survey conducted in an area to determine if pests are present (ISPM 5)
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27)
Early detection	The process of searching a population to determine whether or not an invasive pathogen is present The aim is to discover the invader before it has reached high prevalence so that a programme of control or containment can be instigated with as little as possible cost. (Parnell et al., 2015)
Epidemiological unit	A group of individuals with a defined epidemiological relationship that share approximately the same likelihood of exposure to a pathogen of infested units (Dohoo et al., 2010)
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested
Hierarchy of population	The multiple spatial scales and levels where the population of interest occurs
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27)
Inspector	Person authorized by a national plant protection organisation to discharge its functions (ISPM 5)
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (ISPM5)
Monitoring survey	Ongoing survey to verify the characteristics of a pest population (ISPM 5)
Pest diagnosis	the process of detection and identification of a pest (ISPM 5)
Pest freedom	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriated, this condition is being officially maintained (ISPM 5)
Population size	The estimation of the number of the plants in the region to be surveyed
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 (Cameron et al., 2014)
Risk factor	The factor that may be involved in causing the disease (Cameron et al., 2014)
Sample population	A group or individuals within a population that end up in a study (Dohoo et al., 2010)
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005)

Sampling design	The whole process and considerations concerned with obtaining descriptive or inferential statistics of a population of interest by studying a portion of the population instead of the whole population (Barnett, 1991; Foreman, 1991; Kalton, 1983)
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)
Target population	The population to which it might be possible to extrapolate results from a study (Dohoo et al., 2010)
Test	Official examinations, other than visual, to determine if pests are present or to identify pests (ISPM 5)
Test sensitivity	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010) The test diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will test positive and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive.
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 (DSp) is the probability that a truly negative epidemiological unit will test negative and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.
Type I error	A false positive where it is concluded that the outcomes compared in a group are different when in fact they are not (Dohoo et al., 2010)
Type II error	A false negative where it is concluded that the outcomes compared in a group are not different when in fact they are (Dohoo et al., 2010)
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)

Acronyms and abbreviations

ASe	Area Sensitivity
CABI	Centre for Agriculture and Biosciences International
D	Proportion of infected/infested plants
DCF	Data Collection Framework
DP	Design prevalence
EKE	Expert knowledge elicitation
ELS	Extensive literature searches
EPPO	European and Mediterranean Plant Protection Organization
EPI	Effective Probability of Infection
GSe	Group Sensitivity

IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
MS	Member State
N	Population size
n	Sample size
DP	Design prevalence
Pfree	Probability of Freedom
PM	Phytosanitary procedures
PRA	Pest Risk Assessment
r	The number of groups/sub-areas included in the survey
RiBESS+	Risk Based Estimation of the Sample Size and System Sensitivity
RSe	Round of Tests Sensitivity
Se:	Sensitivity
Sp	Specificity
SSD	Standard Sample Description
SSe	System Sensitivity
Test DSe	Test Diagnostic Sensitivity
TSe	Tests Sensitivity
USDA	U.S Department of Agriculture
WR	Weighted risk

Appendix A – Examples of pest specific search strategies

The search strategies are part of a more general data collection process described in section 4. The search of the literature both for scientific papers and for the technical documentations and reports will be initiated only when the available risk assessments and diagnostic protocols specific for the pest do not include the required information. The search strategies presented below have been developed only for the purpose of scoping the literature for two organisms and two different key parameters. These search algorithms still need to be fine-tuned to the requirements of the project.

A.1. Example 1 for *Phyllosticta citricarpa* in the Web of Science platform

1- The first search string is developed to ensure the papers relevant to the pest is found:

TOPIC: ("Phyllosticta citricarpa" OR ("black spot" NEAR/3 (citr* OR orange*)) OR "Guignardia citricarpa" OR "G citricarpa" OR "Phoma citricarpa" OR "Phyllostictina citricarpa" OR "P citricarpa")

2- A second search strings might be developed to ensure specific papers relevant to the key parameters are found. In this example the key parameter of interest is diagnostic protocols.

TOPIC: (diagnos* OR detect* OR sensitivity OR assay OR test*)

3- Combination of the search strings: Pest string AND Key parameter string

TOPIC: (("Phyllosticta citricarpa" OR ("black spot" NEAR/3 (citr* OR orange*)) OR "Guignardia citricarpa" OR "G citricarpa" OR "Phoma citricarpa" OR "Citrus black spot" OR "Phyllostictina citricarpa" OR "P citricarpa") AND (diagnos* OR detect* OR sensitivity OR assay OR test*))

Results for 2012-2018 in Web of Science

Phyllosticta citricarpa (1)	Diagnostic protocols (2)	(1) AND (2)
107	6,281,593	78

A.2. Example 2 for *Agrilus planipennis* in the Web of Science platform

1- The first part of the search string is developed to ensure the papers relevant to the pest are found:

TOPIC: ("Agrilus planipennis" OR "A planipennis" OR "Agrilus feretrius" OR "A feretrius" OR "Agrilus marcopoli" OR "A marcopoli" OR "Emerald ash borer*")

2- A second search string might be developed to ensure specific papers relevant to the key parameters are found. In this example the key parameter of interest is related to the host plants.

TOPIC: (Fraxinus OR elm OR elms OR ulmus OR ulma* OR juglans OR (ash AND tree*) OR host* OR specie*)

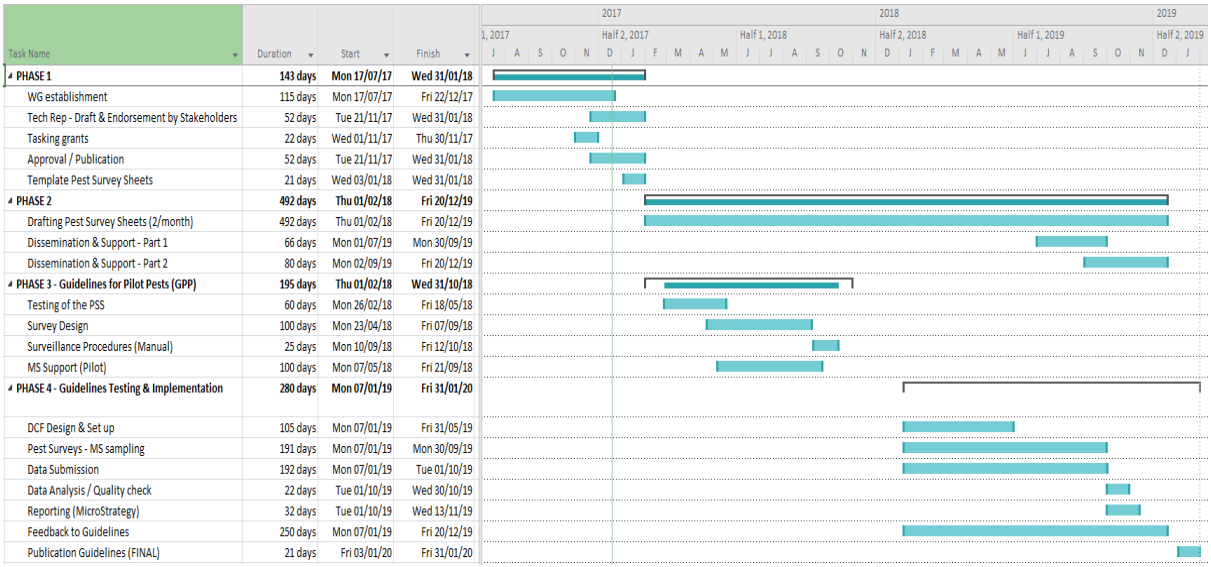
3- Combination of the search strings: Pest string AND Key parameter string

TOPIC: (("Agrilus planipennis" OR "A planipennis" OR "Agrilus feretrius" OR "A feretrius" OR "Agrilus marcopoli" OR "A marcopoli" OR "Emerald ash borer*") AND (Fraxinus OR elm OR elms OR ulmus OR ulma* OR juglans OR (ash AND tree*) OR host* OR specie*))

Results for 2012-2017 in Web of Science

Agrilus planipennis (1)	Host plants (2)	(1) AND (2)
609	1,833,930	567

Appendix B – Pest survey project GANTT Chart



Task Name	Duration	Start	Finish
PHASE 1	**143 days**	**Mon 17/07/17**	**Wed 31/01/18**
WG establishment	115 days	Mon 17/07/17	Fri 22/12/17
Tech Rep - Draft & Endorsement by Stakeholders	52 days	Tue 21/11/17	Wed 31/01/18
Tasking grants	22 days	Wed 01/11/17	Thu 30/11/17
Approval / Publication	52 days	Tue 21/11/17	Wed 31/01/18
Template Pest Survey Sheets	21 days	Wed 03/01/18	Wed 31/01/18
PHASE 2	**492 days**	**Thu 01/02/18**	**Fri 20/12/19**
Drafting Pest Survey Sheets (2/month)	492 days	Thu 01/02/18	Fri 20/12/19
Dissemination & Support - Part 1	66 days	Mon 01/07/19	Mon 30/09/19
Dissemination & Support - Part 2	80 days	Mon 02/09/19	Fri 20/12/19
PHASE 3 - Guidelines for Pilot Pests (GPP)	**195 days**	**Thu 01/02/18**	**Wed 31/10/18**
Testing of the PSS	60 days	Mon 26/02/18	Fri 18/05/18
Survey Design	100 days	Mon 23/04/18	Fri 07/09/18
Surveillance Procedures (Manual)	25 days	Mon 10/09/18	Fri 12/10/18
MS Support (Pilot)	100 days	Mon 07/05/18	Fri 21/09/18
PHASE 4 - Guidelines Testing & Implementation	**280 days**	**Mon 07/01/19**	**Fri 31/01/20**
DCF Design & Set up	105 days	Mon 07/01/19	Fri 31/05/19
Pest Surveys - MS sampling	191 days	Mon 07/01/19	Mon 30/09/19
Data Submission	192 days	Mon 07/01/19	Tue 01/10/19
Data Analysis / Quality check	22 days	Tue 01/10/19	Wed 30/10/19
Reporting (MicroStrategy)	32 days	Tue 01/10/19	Wed 13/11/19
Feedback to Guidelines	250 days	Mon 07/01/19	Fri 20/12/19
Publication Guidelines (FINAL)	21 days	Fri 03/01/20	Fri 31/01/20

Appendix C – RiBESS+ tool and Pest freedom (Detection Surveys)

The same mathematical concepts can be applied to sample size calculation in the fields of plant health for surveys on plant pests and of animal health for surveys of animal diseases. However, this appendix describes the approach that was applied in the field of Animal Health for demonstrating freedom from *Ecchinococcus multilocularis*.

In the EFSA technical report (EFSA, 2012) the mathematical and statistical concepts that are behind the sample size calculation using the RiBESS+ tool is presented and have been extracted for illustration purpose only.

European Food Safety Authority (EFSA), 2012. A framework to substantiate absence of disease: the risk based estimate of system sensitivity tool (RiBESS) using data collated according to the EFSA Standard Sample Description - An example on *Echinococcus multilocularis*. Supporting Publications 2012:EN-366. 44 pp.

C.1. Sample size calculation

The formulae used to calculate the sample size needed to detect an infection when its prevalence is at or above the Design Prevalence (0.01 in the case of *Ecchinococcus multilocularis*) are based on the principles developed by Cannon (2002) and are based either on the binomial or the hypergeometric probability distributions, according to the size of the population under investigation.

If the population can be considered *infinite*[5] (i.e. the individual probability of being positive does not change along the sampling exercise; also referred to as "sampling with replacement"), the Binomial distribution can be used:

$$RSe = 1 - (1 - DP \,.TSe)^n \quad (1)$$

where *RSe* is the sensitivity of a round of tests (e.g. a set of tests performed in the framework of a survey), *DP* is the Design Prevalence, *TSe* is the sensitivity of the test and *n* is the sample size.

From which *n* can be derived as follows:

$$n = \frac{\log(1-RSe)}{\log(1-DP\ .\ TSe)} \quad (2)$$

While, if the population is *finite*, the Hypergeometric adjustment is needed. In this case, the Round of tests Sensitivity is given by:

$$RSe \ \cong 1 - \left(1 - \frac{n\ .\ TSe}{N-0.5\ .\ (N\ .\ DP\ .\ TSe-1)}\right)^{N.DP} \quad (3)$$

Where N is the total population size, D is the proportion of infected/infested plants ($D = DP \times N$), DP is the Design prevalence.

From which we can derive the sample size which is given by:

[5] Though a universal definition of "infinite" and "finite" population does not exist (as it depends on the prevalence, the test sensitivity and the desired level of confidence) the rule of thumb is that a population can be considered "infinite" when n/N < 0.1 (Evans et al. (2000))

$$n \cong \frac{(1-(1-RSe)^{\frac{1}{(N.DP)}})\times(N-0.5\ .\ (N\ .\ DP\ .\ TSe-1)}{TSe} \quad (4)$$

It is important to notice that in formula (4) $N\ .\ DP$ equals to the number of diseased individuals. It may happen that, if the prevalence is very low and the population very small, the result is not an integer (<1). E.g. if N is 30 and the DP is 0.01, the amount of disease animals should be 0.3 which is, obviously, not realistic. In the tool that was developed this problem has been avoided by setting a constraint such as the minimum number of diseased individual could not be less than 1. This will also have an impact on the original design prevalence which, in the example, will not be 0.01 anymore, but 0.033. Despite it could be argued that the corrected design prevalence is higher than the required, it must be considered that: i) the difference may not be so relevant (< 5 fold); ii) most importantly, this is a realistic DP, where the sampling is designed to detect the disease when at least one animal is diseased.

It is also essential to highlight that the formulae used for the sample size calculation assume a diagnostic test with 100% specificity (Sp=1). About this assumption Cannon states that in general, the design of any survey to demonstrate freedom from/absence of infection should specify a sequence of further testing that would be done to clarify the true status when a positive reaction is detected and questioned (Cannon, 2002). Such a sequence would effectively result in a 100%-specific test. In addition, the assumption of perfect specificity has an important consequence: if a positive test result is returned by a system having 100% specificity, freedom from infection can no longer be claimed, as all positive results are true. Each surveillance system should be seen to encompass all necessary follow-up testing to resolve potential false positive results (Cannon, 2001; Dufour et al, 2001; Martin et al., 2007).

C.2. Representative survey (simple random sampling)

An important underpinning assumption that needs to be taken into consideration is that a simple random sampling is adequate when no risk indicator[6] plays a role in the distribution of the pest of concern. In practical terms, this approach assumes that the target population is homogeneously distributed in the study area (e.g. a Member State) and that the infected/infested units are homogeneously distributed across the target population.

In addition, as the simple random sampling assumes that every unit in the target population has an equal probability of being included, a complete list of the source population is required and a formal selection process must be used (e.g., computer-generated random numbers). A violation of this assumption invalidates the results of the formulae (Cameron, 1998).

However, as the two underpinning assumptions on homogeneity do not seem to be applicable, the simple random sampling approach is not recommended for the purpose of detecting a pest both in terms of efforts and reliability. Still, it represents an opportunity when no knowledge is available on possible risk indicators and on the characteristics of the target population (Blickenstorfer et al., 2011). As mentioned above, other options for achieving a representative sample might be used but these are only approximations of a simple random sampling. Nonetheless, a conservative approach should be adopted in this case to account for the potential bias given by the violation of the underpinning assumptions, e.g. by using lower design prevalence and/or a higher confidence level.

C.3. Risk-based sampling (scenario-tree modelling)

Scenario-tree modelling techniques were introduced by Martin et al. (2007) to explicitly account for non-representative sampling approaches. These techniques captured the effect of differential sampling

[6] The terminology "Risk Indicator" is preferred to "Risk Factor" as in a freedom from disease framework the focus is not on the causality. See also Willeberg, 2012.

from population strata with different risks of infection, allowing quantification of the benefits of risk based sampling. The risk based sampling, indeed, refers to the consideration of infection risk indicators when determining the sampling pressure applied in different strata of a population under surveillance (Cameron, 2012).

The principle is that the design prevalence (DP), as a single value, implies that all units within the target population have the same average probability of being infected. Scenario-tree modelling effectively divides the population into multiple risk groups, using the relative risk of infection in each group to adjust the DP to reflect the group-level probability of infection (Cameron, 2012).

The following points put in clear the process step wise.

Once the risk indicators are identified and the associated risk parameter estimated, it is possible to combine the different levels in order to obtain the risk groups. As an example, if 2 risk indicators are identified with 3 levels (categories) each, then 9 different risk groups can be obtained. For each of them, the weighted risk is calculated as follows:

$$WRi = \frac{CombRPi}{\sum_{i=1}^{r}(PopPropi\,.CombRPi)} \quad (5)$$

where ***CombRPi*** is the Risk parameter for a specific risk group "i" (combination of the 2 risk parameters); ***PopPropi*** is the fraction of the total population allocated in that specific risk group "i"; and r is the total amount of risk groups.

Using the WR for each risk group, it is then possible to calculate the Effective Probability of Infection (*EPI*) as follows:

$$EPIi = DP\,.WRi \quad (6)$$

Where DP is the overall design prevalence and WR is the Weighted Risk.

The sample size is then calculated user either the binomial formula or the hypergeometric one, according to the needs (see formulae (2) and (4)).

Once the sample size is calculated, there are two possible ways of implementing a survey:

- To select the group where the adjusted EPI is higher and collect the amount of samples needed to detect the infection when this is present at or above the EPI in that group. This option is based on the concept that it is more likely to find something where this is more likely to be: if all test results are negative in the highest risk group, this means that in the lower risk groups the infection (if present) would affect a smaller proportion of the population if compared to the high risk (i.e. infection is absent or below the overall DP);

- A second option is to collect sample from more than one group (for convenience matters, for instance). In this case, it is possible to calculate the GSe (Group Sensitivity; see formulae (1) and (3)). The overall sensitivity of the surveillance is then calculated by formula (7)

$$SSe = 1 - \prod_{i=1}^{r}(1 - GSe_i) \quad (7)$$

where ***SSe*** is the System (overall) Sensitivity, ***GSe*** is the Group (or sub-area) Sensitivity and ***r*** is the number of groups/sub-areas included in the survey. The *SSe* represents the confidence of 95% required by the Regulation on *E. multilocularis*.

A prerequisite for the risk based approach is the definition of the Risk Groups, which requires knowledge of the main risk indicators, and the Risk Parameter (RP) associated with each of them. This information, along with knowledge on the amount of definitive hosts located in each Risk Group (i.e. the population fraction), allows calculating the Weighted Risk (WR) and, in turn, the Effective Probability of Infection (EPI) in each Risk Group. For each Risk Group either formula (2) or (4) can be used to estimate the sample size.

It is not necessary to have precise estimates for these parameters (i.e. RP, Population Fractions, etc…). There are at least two ways to overcome the problem of a lack of knowledge: the first relies on probabilistic distributions assigned to any parameter; the second investigates different scenarios, using minimum, maximum and most likely values for each parameter. Also in this case, a standard approach does not exist as both methodologies have pros and cons: The stochastic approach (based on probabilistic distributions) has the advantage of including a degree of uncertainty around the best guess of the parameter that needs to be estimated. At the end of the process, the outcome (e.g. the sample size needed) will be expressed, in turn, with a probabilistic distribution. The disadvantage is that, most of the times, there are no data on the type and the shape of the probabilistic distribution that better describes, say, a RP. In such situations usually a PERT distribution is used (Vose, 2008) as only 3 parameters are required: minimum, most likely and maximum guesses of the parameter that needs to be estimated. Recent publications adopted this approach to implement a stochastic model for a risk based sample calculation (Murphy et al, 2012). However, any choice on the type and the shape of the probabilistic distribution to be used is completely arbitrary and arguable if no evidence supports this choice. This consideration is not theoretical as the shape of the distribution chosen for an input parameter will obviously influence the shape of the distribution describing the output. If the premises do not hold, the results will be biased; What-if scenarios do not require assuming any probabilistic distributions for the parameter of interest avoiding unnecessary bias: the outcome is calculated using minimum, most likely and maximum guesses. However, it is not possible to know to what extent the minimum and the maximum values are less probable to be observed when compared to the most likely.

EFSA (2012) also suggests a stepwise analysis for estimating the probability of freedom from disease taking into account the historical data and provides a manual for the use of the RiBESS+ software.