Network on Risk Assessment in Plant Health: Toolkit for surveillance of *Xylella fastidiosa* in the EU Member States
Minutes of the 14th meeting

Held on 06-08 March 2019, Parma

(Agreed on 30 April 2019)

Meeting Participants

Country	**Name**
Network Representatives of EU Member States	
Austria	Robert STEFFEK
Belgium	Jan VAN AUTREVE
Bulgaria	Nikolay SPASOV
Cyprus	Anthemis MELIFRONIDOU-PANDELIDOU
Croatia	Dario IVIC
Denmark	Anne Christine HELMS
Estonia	Birger ILAU
Finland	Jari POUTANEN
France	Pauline DE JERPHANION
Germany	Eva FORNEFELD
Greece	Dimitrios PAPACHRISTOS
Hungary	Gabor HOLLO
Ireland	Oliver MCEVOY
Italy	Carlo Francesco CESARONI
Italy	Domenico RIZZO
Latvia	Liga GRISANE
Lithuania	Loreta REZGYTE
Malta	Andrea SCIBERRAS
Netherlands	Jeroen STELLINGWERF
Poland	Anieszka SAHAJDAK
Portugal	Maria Clara SERRA
Slovenia	Erika ORESEK
Spain	Ana JIMENEZ MURO
Spain	Amparo FERRER MATOSES
United Kingdom	Dominic EYRE

Observers	
European and Mediterranean Plant Protection Organization (EPPO)	Françoise PETTER
European Commission (DG SANTE)	Maria MIRAZCHIYSKA
European Commission (DG SANTE)	Pasquale DI RUBBO
Hearing expert	
PLH Panel Member	Stephen PARNELL
PLH Panel Member	Antonio VICENT
Instituto Valenciano de Investigaciones Agrarias	Elena LÁZARO
Università degli Studi di Torino (UNITO) - Department of Agricultural, Forest and Food Sciences	Domenico BOSCO
EFSA	
ALPHA Head of Unit	Nikolaus KRIŽ
ALPHA, PLH Team Leader	Giuseppe STANCANELLI
ALPHA	Sybren VOS (Chair)
ALPHA	Makrina DIAKAKI
ALPHA	Melanie CAMILLERI
ALPHA	Gabriele ZANCANARO
ALPHA	Gritta SCHRADER *Tasking Grant JKI (DE)*
ALPHA	Martijn SCHENK *Tasking Grant NVWA(NL)*
AMU	José CORTIÑAS ABRAHANTES

1. Welcome and apologies for absence

The Chair welcomed the participants.

No apologies were received.

2. Adoption of agenda

The agenda was adopted without changes.

3. Declarations of Interest

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes and the Decision of the Executive Director on Declarations of Interest, EFSA screened the Annual Declaration of Interest filled in by the Network members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

Additionally, all participants were asked to sign a declaration of confidentiality.

4. Agreement of the minutes of the 14th meeting of the Network on Risk Assessment in Plant Health held on 6-8 March 2019

The minutes were adopted by written procedure on the 30 April 2019.

5. Topics for discussion

Following a request from the European Commission to EFSA, a toolkit for surveillance of *Xylella fastidiosa* in the EU Member States is currently being developed in the context of a working group that includes expertise on the pest and on pest surveys. The project involves the joint efforts of the EFSA Animal and Plant Health unit (ALPHA) and the Assessment and Methodological Support unit (AMU), and the collaboration with Member State institutions through tasking grants, namely the Netherlands Food and Consumer Product Safety Authority (NVWA) and the Julius Kuehn Institute (JKI), in Germany.
This meeting of the EFSA Network on risk assessment in Plant Health was aimed at providing tailored support to the Member States in the planning and implementation of the annual surveys for the detection of *X. fastidiosa*. In particular, the main objective of the meeting was to share the tools and approaches with the MSs and to gather their feed-back and proposals to improve the fit for purposeness of the final outputs revising them accordingly.

5.1 Session 1: Introduction

The first session was chaired by Nikolaus Kriz, head of the EFSA Animal and Plant Health Unit (ALPHA). The chair welcomed all participants and shared with the participants the objectives and challenges of the workshop. This was followed by Maria Mirazchiyska (DG-Sante European Commission) presentation on the EU pest survey program and reporting obligations and Pasquale Di Rubbo (DG Sante European Commission) presentation on the legal obligations of surveillance of *Xylella fastidiosa* in the Union territory. Next, Sybren Vos, the coordinator of the EFSA-ALPHA pest survey project presented the EFSA Plant Health Pest survey mandate. Finally, Giuseppe Stancanelli, the ALPHA Plant Health Team Leader presented the past and current activities of EFSA on *Xylella fastidiosa*.

During the discussions, it was noted that there are currently no legal obligations for vector sampling in pest free areas. In addition, the use at local level of the global Xylella distribution maps was discussed and it was suggested to derive from the map, the infected areas with similar climatic conditions as the area of interest. This information will be soon available as the update of the pest risk assessment of *Xylella fastidiosa* currently in preparation will provide EU maps that will show the potential establishment of the different subspecies of the bacterium in the EU. In this way, a country could focus surveillance on those host plant species that are already affected in areas of similar climatic conditions.

5.2 Session 2: *Xylella fastidiosa* in the EU

The session was chaired by Giuseppe Stancanelli, team leader of the ALPHA Plant health team. Stephen Parnell's talk was about the current situation of *Xylella fastidiosa* in the EU with a focus on the ongoing outbreaks in France, Italy, Spain and Portugal. The Italian representative Carlo Francesco Cesaroni provided details on how the detection surveys are conducted in Italy. Furthermore, the surveillance strategy and efforts deployed in Argentaria, Tuscany, Italy, were presented by Domenico Rizzo. The possible origins of the recent reported outbreak were discussed in view of further identifying high risk locations to better concentrate the survey efforts. In this context, the tourism related activities were mentioned in relation to the movement of people and transport means from areas where the pest is known to occur (including boats and coastal areas).
Finally, the Spanish representative Amparo Ferrer presented how the delimiting surveys are currently undertaken in Alicante, Spain, and highlighted the important sampling efforts and difficulties.

5.3 Session 3: Statistically risk-based surveillance

The session was chaired by Antonio Vicent, member of the EFSA PLH Panel and member of EFSA working group on pest surveys. Jose Cortiñas Abrahantes from the EFSA – Assessment and methodological support Unit) delivered a first presentation on the methodological approach for the survey design and the principles behind a statistically based sampling. The basic concepts that are considered when sampling and their application to the detection surveys for plant pests were described. The mathematical basis of the software RiBESS+ for calculating sample sizes for demonstrating pest freedom was presented. Then the data requirements for risk-based survey design were presented in more details by Martijn Schenk from the Netherlands Food and Consumer Product Safety Authority (NVWA). The following presentation by Gabriele Zancanaro from the EFSA ALPHA Unit was showing how these data are integrated in the survey design in a stepwise approach: from the country to the field and to the tree level. For each data requirement a specific presentation was provided: (i) defining the target population of the survey and the derived epidemiological units (by Sybren Vos from ALPHA); (ii) describing the drivers of the spread of *X. fastidiosa* and the corresponding distances (by Stephen Parnell – EFSA ALPHA WG on pest surveys); (iii) relevant information on the vectors of the bacterium, in particular in terms of diversity, variability in abundance and more practically to use vectors in *X. fastidiosa* detection and diagnostics (by Domenico Bosco from Università degli Studi di Torino); (iv) the key information required for the detection and identification of the pest, in particular the EPPO Diagnostic Protocol (by Françoise Petter from EPPO) and the need to estimate the detection methods sensitivity (by Melanie Camilleri from ALPHA); (v) the risk factors identification and an explanation of their use in detection and delimiting surveys (by Makrina Diakaki from ALPHA).

The *Xylella fastidiosa* survey card was presented (by Makrina Diakaki from ALPHA). It brings together the relevant biological regulatory and historical information required for characterising and estimating the above-mentioned data requirements for a risk based and statistically sound survey design.

In the context of annual detection surveys, the discussions were mainly addressing the difficulties of performing visual inspections for *Xylella fastidiosa* in the fields, as infected plants could be asymptomatic at the time of the survey. In particular, the usefulness of performing detection surveys through field inspections only based on the visual examinations was questioned. Visual examination was mentioned to play a key role in the selection of the samples. In addition, the participants commented the low values of sampling effectiveness (50%) used for estimating the sensitivity of methods of detection but these were justified because of the unknown proportion of asymptomatic plants and plant tissues within a plant. For *X. fastidiosa* sampling and testing, both the plant materials and the vectors is required.

The participants also mentioned the limited resources they have for surveillance and that systematic sampling and testing would generate prohibitive costs. In response, it was indicated that this decision is directly linked to the acceptability of the risk assessed by the risk managers and this could be translated in terms of confidence and related pest prevalence levels.

The importance of the timing of the survey activities was discussed. In the case of *X. fastidiosa* surveillance, it aims at effective and rapid removal of infected plants. Since it is assumed that infections occur during summertime, during the flying season of the vector, detection efforts should take place during or right after that period.

The possibility of pooling the insects and plant material was raised in order to reduce the numbers of samples for testing. It was mentioned that it is generally acceptable to pool samples when these are taken from the same species (for both plant and vector material). With regards to the plant sampling, due to the different diagnostic sensitivities for different host plants, pooling samples from different host plant species is also not advised. An output of the XF-actors project is providing guidance on the issue. With regards to the insect vectors, it was indicated that pooling different species wouldn't be advisable. The participants mentioned that the main issue of pooling of samples, insects and or plants, would lead to a loss of information on the species carrying *X. fastidiosa*. However, in reply the

objective of the survey was indicated to help in the decision of pooling samples. The objective of the detection survey being to first early detect the pest but also to substantiate pest freedom statements. Thus, the objective being to prove that the pest is not circulating in an area, pooling of different species would reduce the laboratory testing efforts for achieving the detection of the pest. Instead after a first positive finding, the objective of the survey changes as it aims at delimiting an area. In this last case it is necessary to gather information on the infected host species and infected vectors and thus pooling of samples from different species is not advised.

The difficulty to choose the detection and identification methods among the variety of available methods was discussed. The methods sensitivity and the diagnostic sensitivity of the tests were indicated as being a key parameter to consider in the choice of the methods. This parameter has a direct influence on the sample size. In the case of detection surveys, the molecular methods such as RT PCR are advised. Serological methods could be used in areas where the pest prevalence is high. Currently, an EPPO working group is revising the diagnostic protocol for *Xylella fastidiosa*, in this work information for every host/test combination in terms of diagnostic sensitivity is being scrutinised. However, the comparison of methods is very difficult as the different methods are validated using different units (e.g. bacterium concentration).

5.4 Session 4: Survey design

The session was chaired by Stephen Parnell, member of the EFSA PLH Panel and member of EFSA working group on pest surveys. A brief presentation of the specific guidelines for *Xylella fastidiosa* initiated this session, which was followed by practical exercises. In these, participants were split into groups and were allowed the time to prepare a survey.

The practical exercises started with the preparatory phase of the survey required for characterising the input parameters of the statistical tool for sample size calculation. These include the identification of the survey components (vectors and plants), the choice of the detection methods and related sensitivities, the definition of the host plant population to be targeted by the survey (size and structure), the identification of risk factors (relative risk and proportion of host population).

Guided by Gabriele Zancanaro (ALPHA), in a second phase the groups performed the sample size calculation using RiBESS+. A step-wise approach was applied for estimating: 1) the sampling efforts of plant material and/or vectors within an epidemiological unit 2) the number of epidemiological units in which samples are taken (sampling sites).

During the practical exercises the participants were struggling with the preparatory phase of the survey design, in particular with the definition of the survey scenarios, and the difficulties of the data gathering for characterising the parameters needed for having a risk-based survey and statistically sound sample size calculation.

With regards to the sample size calculations using RiBESS+ software, the importance of including risk factors in the survey strategy was discussed. The participants could assess the reduction in sampling efforts (for achieving the same confidence level with the same design prevalence) when comparing a simple random sampling strategy and a risk-based approach. However, data need to be available to apply the latter approach. It was suggested to benefit from the experience and data acquired in the demarcated areas in the EU following the outbreaks of *Xylella fastidiosa* to better inform the choice of risk factors calculating their relative risks, especially in the case of host species that could be prioritised according to the number of positive detections. It was also suggested to derive from the data of the EU outbreak areas, the relative risks of the different risk areas (in terms of distances from the risk locations).

5.5 Session 5: Conclusion

The session was chaired by Martijn Schenk, expert on surveillance in the Netherlands Food and Consumer Product Safety Authority (NVWA) and member of EFSA working group on pest surveys. During the concluding session, the different groups were invited to present the outcomes of their

discussion as well as bring the difficulties that they encountered forward and reflect on them along with the rest of the participants.

The participants clearly stated that more time was needed to better understand the underpinning principles and concepts of the risk-based surveillance approach and the statistically sound sample size calculations.

The participants shared their views on the use of the RiBESS+ tools and agreed that it would be an added value to tailor it to the plant health surveys. In particular, to align the terminology used with the terms used in the international standards and the EU regulations and to make the stepwise approach more intuitive in the tool, with this the RiBESS+ tool would be even more user friendly, making it more accessible to a broader community of risk managers involved in the planning and implementation of pest surveillance.

The participants found the workshop very useful and expressed the need for further support on the topic. EFSA indicated that the discussions and comments provided during the 3 days meeting were really useful and will be integrated in the different documents that compose the toolkit. EFSA invited the participants to provide their comments on the toolkit in writing and suggested to circulate a short online questionnaire to assess the next steps for better using the toolkit. Results of this questionnaire are presented in the appendix of these minutes.

6. Closure of the meeting

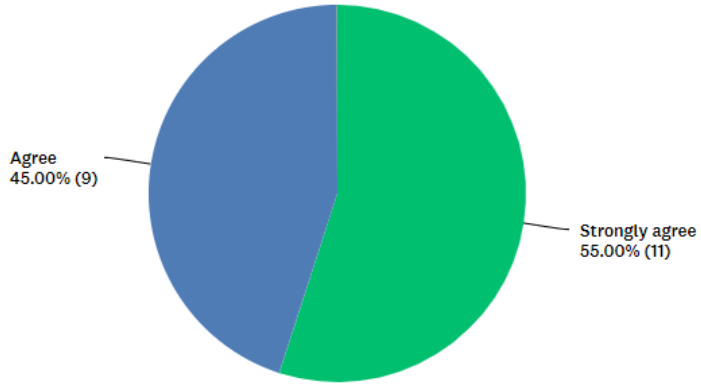
The chair closed the meeting.

Appendix: Questionnaire on the follow-up of the 14th meeting of the Scientific Network for Risk Assessment in Plant Health: Tool kit for surveillance of *Xylella fastidiosa* in the EU Member States

QUESTION 1: First impressions

As a result of this meeting, I have a clearer understanding of the concept of a statistically sound, risk-based surveillance program, in terms of the steps involved in its preparation and implementation. (Strongly agree; Agree; Disagree; Strongly disagree)

Answered: 20 Skipped: 0



Participant Comments:

It will be very important to bear in mind several situations in reality which can differ very much from the cases prepared by EFSA. It was clear in which direction the preparation of the card would continue but I still do not know what this approach will mean for our resources (human as well as financial) which are limited.

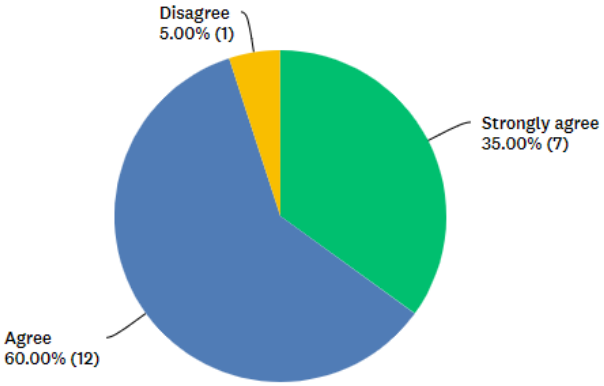
EFSA response:

The use of the approach is independent from the resources available. Following the approach will allow to conclude on the confidence you will achieve for the pest freedom statement based on the detection survey done (inspections, sampling and testing). Nonetheless, the approach of risk-based sampling can be applied in specific situations to align the survey efforts with the available resources.

QUESTION 2: First impressions

The contents of the meetings' presentations were clear and useful (As a result of this meeting, I have a clearer understanding of the concept of a statistically sound, risk-based surveillance program, in terms of the steps involved in its preparation and implementation Strongly agree; Agree; Disagree; Strongly disagree).

Answered: 20 Skipped: 0



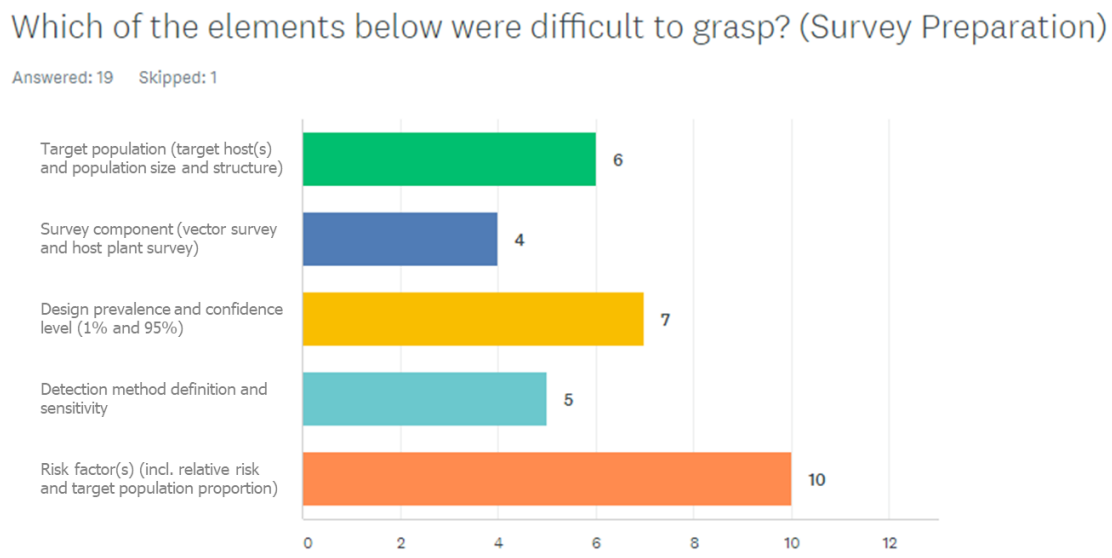
Participant Comments:

1. *It was a lot of information in a few period of time, that's why it could be unclear some time*
2. *More time on the concrete application of the methodology would have been useful!*
3. *It would be really helpful if the presentations could be made available during the meeting in order to look up previous slides or use the information from the presentations during the group work (sometimes it was just too fast to follow and remember or note all the information)*
4. *There was some overlap in the presentations and I think a lot of people had some familiarity with the pathogen. It might have been better to have more discussion time and less presentation.*

EFSA response:

The *Xylella fastidiosa* pilot case is a very complex case study because of (i) its polyphagy, (ii) the fact that it is a vector born bacterium, (iii) the severity of some epidemics, and (iv) the knowledge gaps. This complexity was reflected in the application of the methodology. We recognise that more time is needed for such complex cases. However, the preparatory phase of the risk-based and statistically sound survey is key for defining the survey strategy and for ensuring that the calculated sample sizes correspond to the minimum efforts to deploy for achieving the confidence levels for pest freedom statements. A specific session on the use of the statistical tool RiBESS+ is in discussion after the consolidation of the information in the toolkit.

QUESTION 3: Clarity on the basic concepts for Survey Preparation

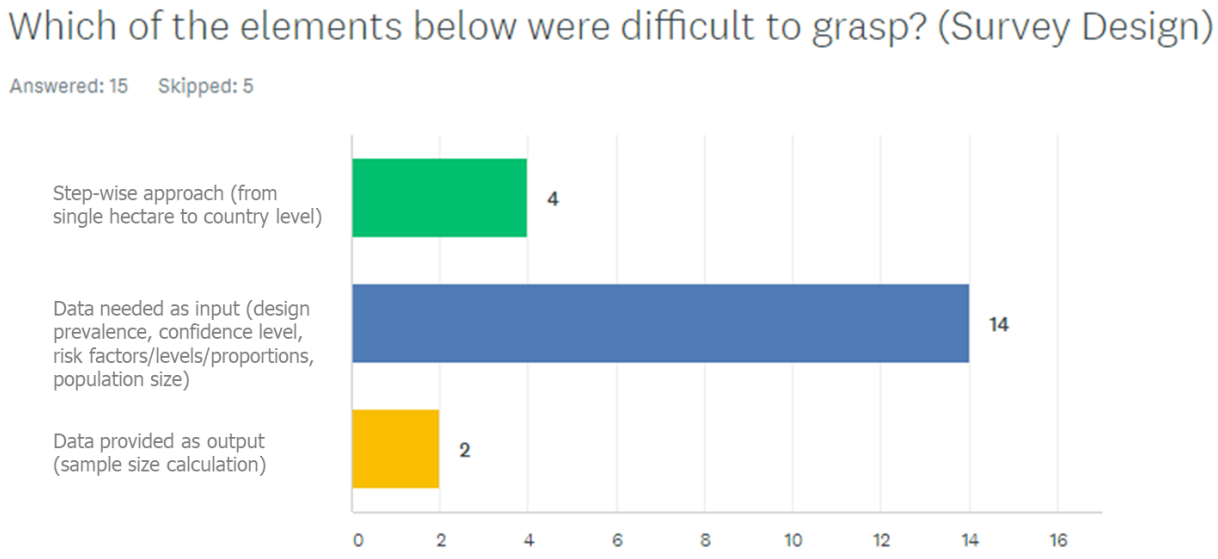


Participants did not provide comments

EFSA response:

The risk factors identification and the estimation of their relative risk and to which part of the target population their correspond are the main challenge when designing a risk-based survey. However, data are required to fully characterise the risk factors and use then in a statistically sound sample size calculation. More clear guidance is needed to assist the MSs through the process and this will be revised in the toolkit and the future workshops.

QUESTION 4: Clarity on the basic concepts for Survey design

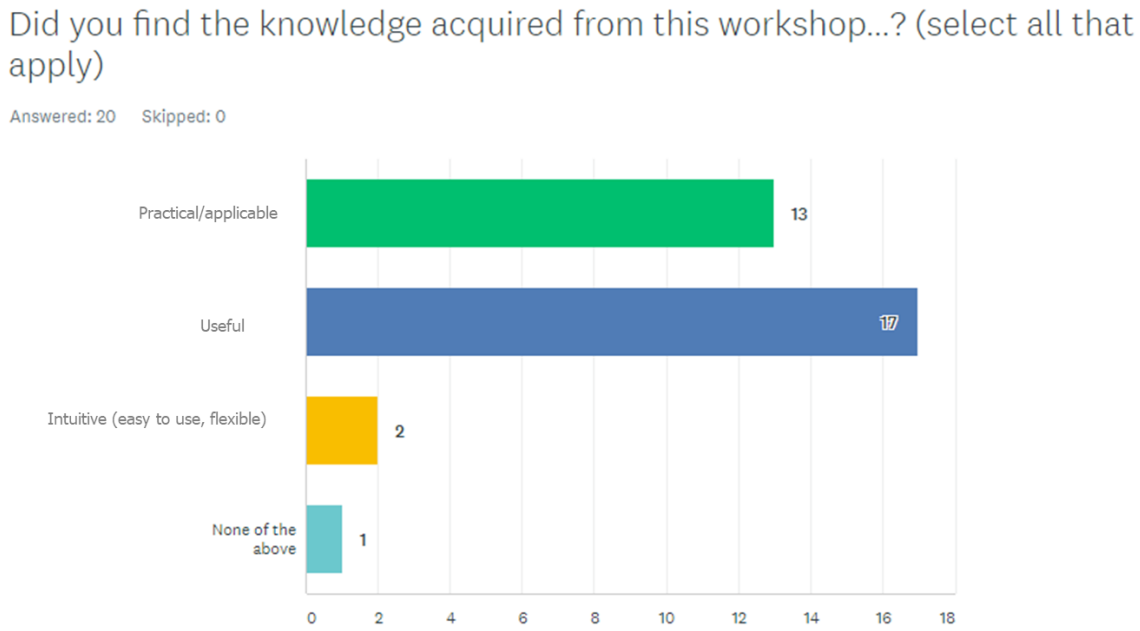


Participants did not provide comments

EFSA response:

More clear guidance is needed to share with the MSs the data requirements that are the key stone of the risk based and statistically sound surveillance. It is necessary to use the "jargon" and the specific terminology in Plant health surveillance to describe these concepts, the related definitions and descriptions, and we recognise that an effort should be done in this aspect in the survey guidelines.

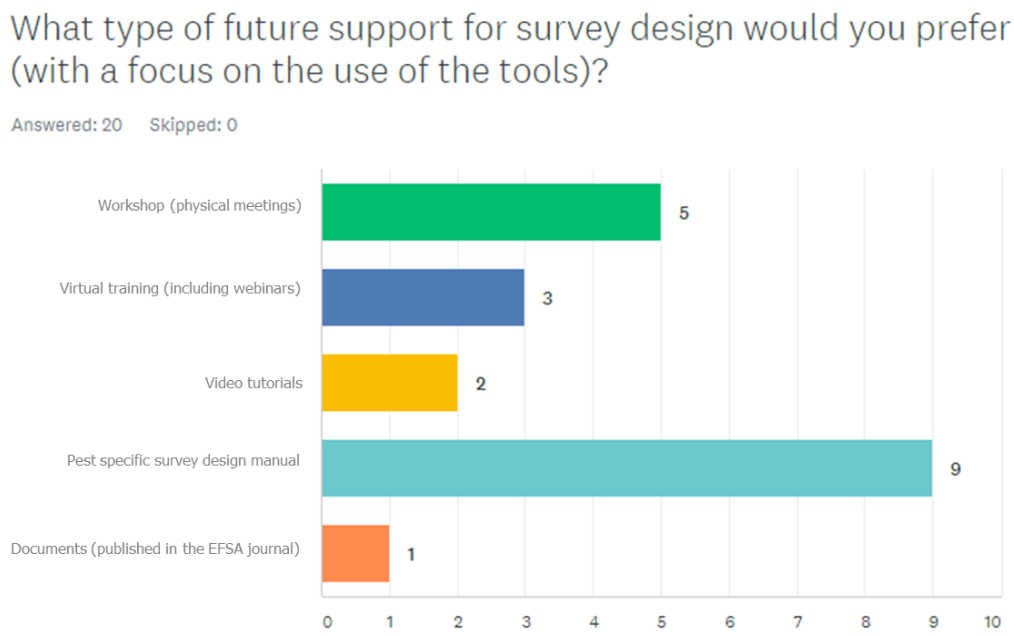
QUESTION 5: Survey implementation



EFSA response:

From the responses of the participants an additional effort should be made to provide a more user friendly and intuitive tool. This is the purpose of the pest specific guidelines that are currently under preparation. In addition, reflexions are currently ongoing in EFSA on the possibility to tailoring of the statistical tool to the field of plant health.

QUESTION 6: Future steps for providing support



Participant Comments:

1. *Preference for Option 1 and 4 (3) Training: more interaction and responding between participants and Trainers (the time for practical work was limited to late afternoon of day 2 and morning of day 3). Many issues of day 1 and 2 could have been summarized and shorter (legal aspects and biology could have been taken for granted) Training Manual and Training may be better aligned.*
2. *A way to ask questions appearing when applying the tools would be great (maybe also a collection of FAQs)*
3. *Also virtual training or workshops will be great.*
4. *Translating the theoretical to the practical is a difficult step.*
5. *A second workshop/training would be appreciated. To be followed by pest specific survey manual and video tutorials in different languages of the EU to spread the concept and facilitate the update at national and regional level.*

EFSA response:

It is clear from the answers that the preferred support EFSA should provide to the MSs following this workshop is possibly through the organisation of a follow up workshop focussing on the use of the statistical tools and in the preparation of a simple and concise manual for guiding the MSs through the process of survey design. However, EFSA trusts that user-friendly online tutorials might be very useful and cost-effective for guiding the users in the survey design process.