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DELIVERABLE N° 4.1

Title: Report on stakeholder priorities for tests and general prioritisation framework

Validation of diagnostic tests to support plant health

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Abstract:
Main aim of work-package 4 is a better understanding of the demands for current and future testing options. This is to be achieved via a qualitative assessment of stakeholder requirements using online surveys supplemented by desk-based research, as well as impact assessments. To make the most of work-package 4 and to ensure that Test Performance Study (TPS) targets are determined considering the latest developments in demand, it was decided - prior to the project start - to use the results from the stakeholder survey for prioritising target pests. This also reflects the importance of co-design and the multi-actor approach, which are central to this project. Deliverable 4.1 reports on identified stakeholder priorities for tests as well as the general prioritisation framework, which was created in collaboration with all affected work-packages. After applying the framework to the long list of target pests obtained through the surveys, the selection of pests for TPS round 2 was the following: Arabis mosaic virus, *Cryphonectria parasitica*, *Melampsora medusa*, Plum pox virus, Tobacco ringspot virus, Tomato Brown Rugose Fruit Virus, Tomato ringspot virus, Tomato spotted wilt virus, *Xanthomonas citri pv citri*, *Xylophilus ampelinus*. Of those the following have been selected: *Cryphonectria parasitica* (UNITO), Tomato Brown Rugose Fruit Virus (CREA), Plum pox virus (ANSES), Tomato spotted wilt virus (NIB), *Xanthomonas citri pv citri* (ANSES), *Xylophilus ampelinus* (FERA).

Partners involved: all partners

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1. Introduction

This deliverable serves two main purposes: first, to provide guidance for the test selection in the second round of Test Performance Studies (TPS); second, to provide a basis for determining impact case studies.

VALITEST has integrated a strong stakeholder focus across all work-packages to ensure the delivery of practical and relevant outputs throughout the project's lifetime. Uniquely, this also includes a flexible component for TPS targets, whereby pests and tests for the second round of TPSs are selected based on stakeholder needs in parallel with conducting the initial round, rather than being specified before the start of the project.

2. Methodology

To maximise the relevance of tests selected for the second round of Test Performance Studies, information on current testing priorities was gathered directly from a range of stakeholders and supplemented through online research.

Primary means of data collection were two online surveys hosted by the European and Mediterranean Plant Protection Organization (EPPO) and sent to laboratories and national plant protection organisations. The laboratory survey (**Q1**) was designed in collaboration with work-packages 1, 6 and 7, to ensure consistency and collect up-to-date validation data, as well as with the Euphresco Virfast project, to minimise stakeholder fatigue. It consisted of 5 sections: (1) current testing priorities, (2) requirements for new or improved tests, (3) validation data, (4) the use of on-site testing kits, and (5) the use of High Throughput Sequencing technologies (HTS). For details on the sections see Appendix 1. In addition, EPPO designed a brief survey for national plant protection organisations (**Q2**), which asked representatives to rank their top 10 priority pests. Results from these surveys have been combined and a pest ranking - supplemented with additional information on their national and international status - was presented to the TPS organisers in work-package 1.

For this deliverable we were specifically interested in parts 1 and 3 of Q1, as they directly inform the prioritisation process (see Figure 1). Other parts of the survey will be analysed in more detail later in the project and form part of the wider discussion with risk managers and test providers.

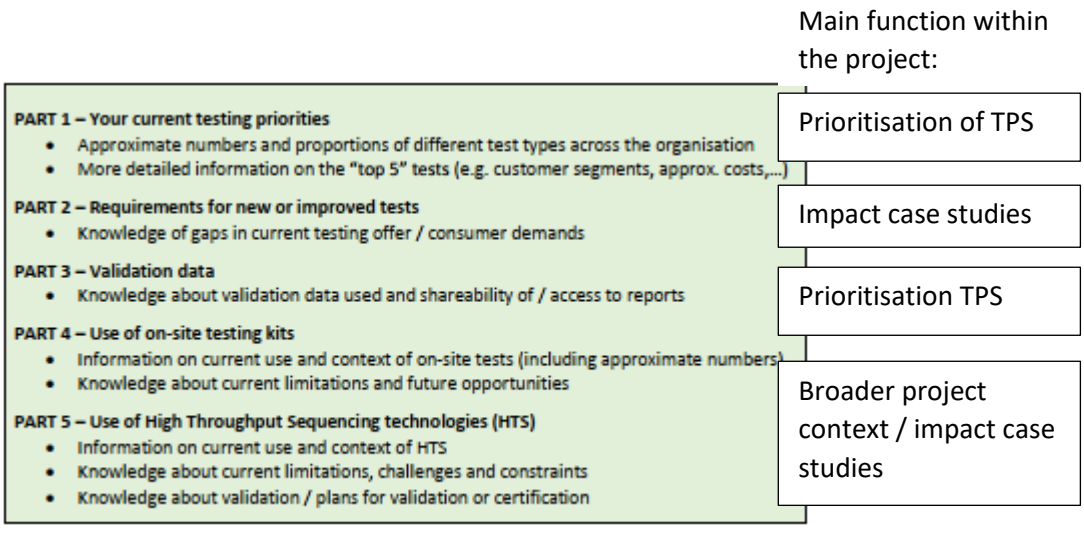

Figure 1. Survey content

3. Prioritisation Framework

In order to select tests for the second round of TPSs, several sessions were organised between all affected work-packages and specifically with WP1 partners working on the actual organisation of Test Performance Studies. During these discussions, it became clear that what work-package 4 should provide is a list of pests, rather than tests, where tests are defined as the combination of pest, matrix and method. This has several reasons: first and foremost, the selection of tests is a complex process which needs to take into account a number of practicalities of organising a TPS, test context, as well as specific competencies of partner organisations. In addition, from a project point of view a certain breadth of organism types and other characteristics was required, as well as considerations whether other projects are already covering certain organisms; for example, a lot of work is currently done on *Xylella fastidiosa*. Therefore, a framework was to be created, which aggregated the ranked results from both surveys (Q1 and Q2) according to the priorities given by respondents. The combined ranking was then to be transformed into an interactive excel table, including supplementary information on each pest's status, like whether it is currently a European priority, and whether respondents are using kits or on-site test. In order to determine whether it currently is a European priority several sources were used, which are summarised in Table 1. This list was then to be passed on to WP1 partners, to exclude pests already covered by other research or sufficiently validated. After adding additional high-priority pests of interest due to their phytosanitary importance, WP1 partners then were to volunteer for those pests their labs could cover. Lastly, selected labs were to identify methods and sample types and combine them into a scope definition; with the help of WP2 sample numbers were defined. This process is depicted in Figure 2 and finalised scope descriptions can be found in Appendix 3: Scope.

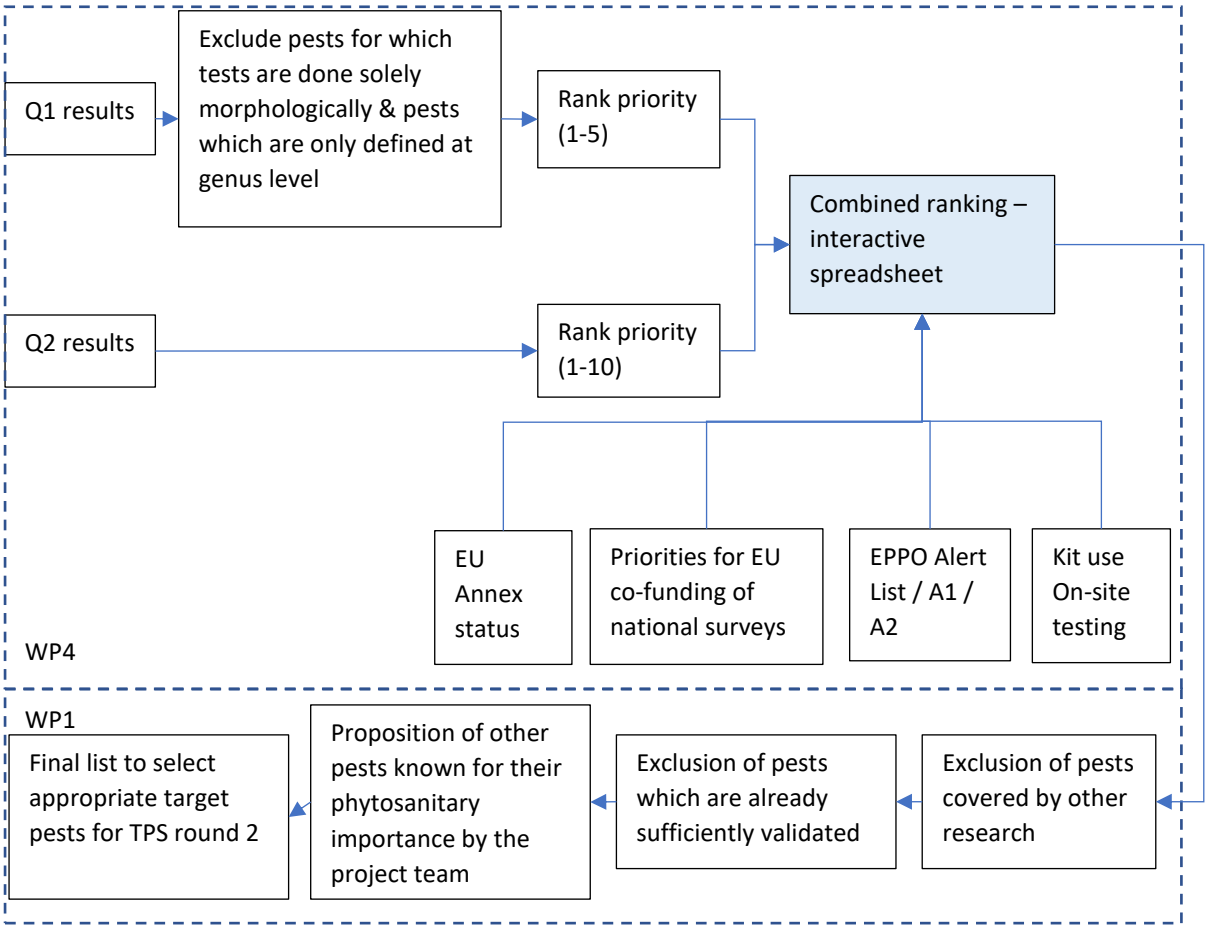

Figure 2. Prioritisation Framework

Due to the large discrepancy between test numbers described in section 4.1 it was decided to base the ranking on listed priorities rather than overall test numbers. In other words, for both surveys a pest would get points according to their rank. For Q1 where we asked for the top 5 pests, this meant giving 5 points to all pests which have been ranked as most important, 4 to the second, etc.; for Q2 weights started at 10 as we asked for the top 10 pests. These were then added across pests, creating the basis for the ranking.

Table 1. European Priorities

EU Annexes	At the time of writing this report the European plant health legislation contains a number of annexes categorising identified harmful organisms: **IA** Harmful organisms whose introduction into, and spread within, all member states shall be banned, where **IAI** Harmful organisms not known to occur in any part of the community and relevant for the entire community and **IAII** Harmful organisms known to occur in the community and relevant for the entire community; **IB** Harmful organisms whose introduction into, and whose spread within, certain protected zones shall be banned; **IIA** Harmful organisms whose introduction into, and spread within, all member states shall be banned if they are present on certain plants or plant products, where **IIAI** Harmful organisms not known to occur in the community and relevant for the entire community and **IIAII** Harmful organisms known to occur in the community and relevant for the entire community; **IIB** Harmful organisms whose introduction into and spread within certain protected zones shall be banned if they are present on certain plants or plant products)
EPPO Alert lists	**A1:** List of pests recommended for regulation as quarantine pest (absent from the EPPO region); **A2:** List of pests recommended for regulation as quarantine pest (locally present in the EPPO region); **Alert List:** In order to keep the Alert List reasonably short, pest are included on a temporary basis
Survey co-funded by EU for 2019-2020	As per directive **EU 2000/29** and **Regulation (EU) No 652/2014** and following **Commission implementing decision 2018/2491** identified as priority for 2019-20 for Union financial support, as regards the orientation of national programmes for pest surveys for the protection of the Union territory. For the Commission Implementing Decision of 30.04.2018 establishing a work programme for the years 2019-2020 for the implementation of survey programmes for pests see: https://ec.europa.eu/food/funding/plant-health/survey-programmes_en [last accessed in November 2019]

4. Stakeholder Priorities

4.1. Survey Descriptives

Q1 was sent to representatives of all 107 laboratories registered in the EPPO diagnostic database; 33 of those laboratories are located in European countries of which 24 are EU countries. 38 individual laboratories and institutions across 25 countries responded to Q1 (35% response rate). Please note that bigger organisations with separate departments for bacteriology, virology, nematology, entomology and mycology have been asked to submit individual responses for each department.

14 national plant protection organisations responded to Q2. The majority of NPPOs were from countries which have also participated in Q1 (Figure 3).

Overall 115 priority pests have been identified by respondents of Q1, of which 98 were specified at species level: 18 bacteria, 15 fungi, 14 insects, 10 nematodes, 5 phytoplasmas, 32 viruses and 4 weeds. 31 of those organisms have also been listed by respondents of Q2, who added an additional 25 pests. Around 50% of all organisms listed can be found on an EPPO list (alert, A1 or A2). Similarly, about half of the pests listed in Q1 were also listed in an EU annex, whereas NPPOs seem to have a larger focus on currently unlisted organisms; this likely reflects the horizon scanning responsibilities of NPPOs.

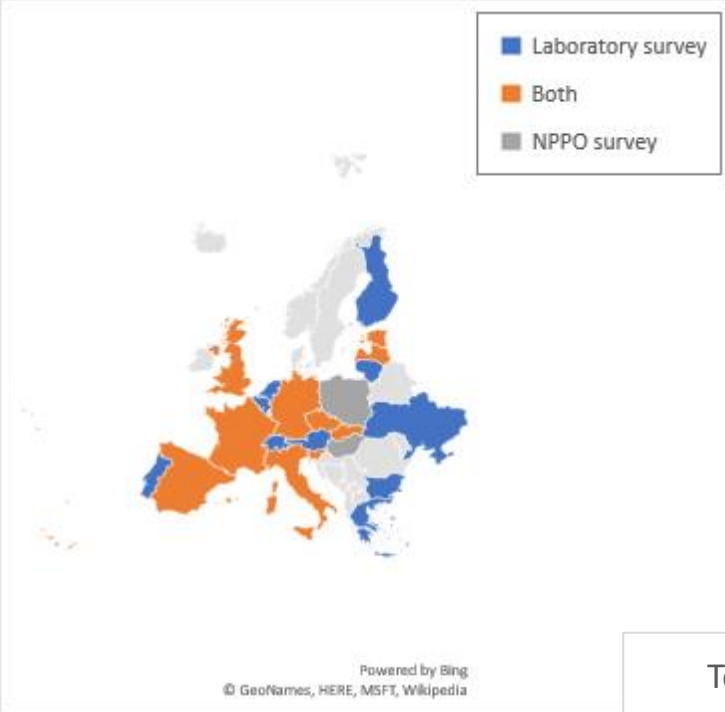

Figure 3. Survey respondents (Europe)

Aggregating the number of reported tests across priority organisms we can see a large discrepancy between countries. For example, Italian institutions have reported a much higher test numbers than any other country (Figure 4). This discrepancy is due to (1) a varying number of labs registered in the EPPO database in each country (ranging from one to twenty-four), (2) large labs submitting responses per department thus increasing response numbers per country, (3) different laboratory / department sizes. While the number of laboratories per country is loosely related to country size, response rates still introduce quite a large bias (see also discussion section). Because of this bias it was decided to rank pests according to a weighted priority rather than total sample numbers.

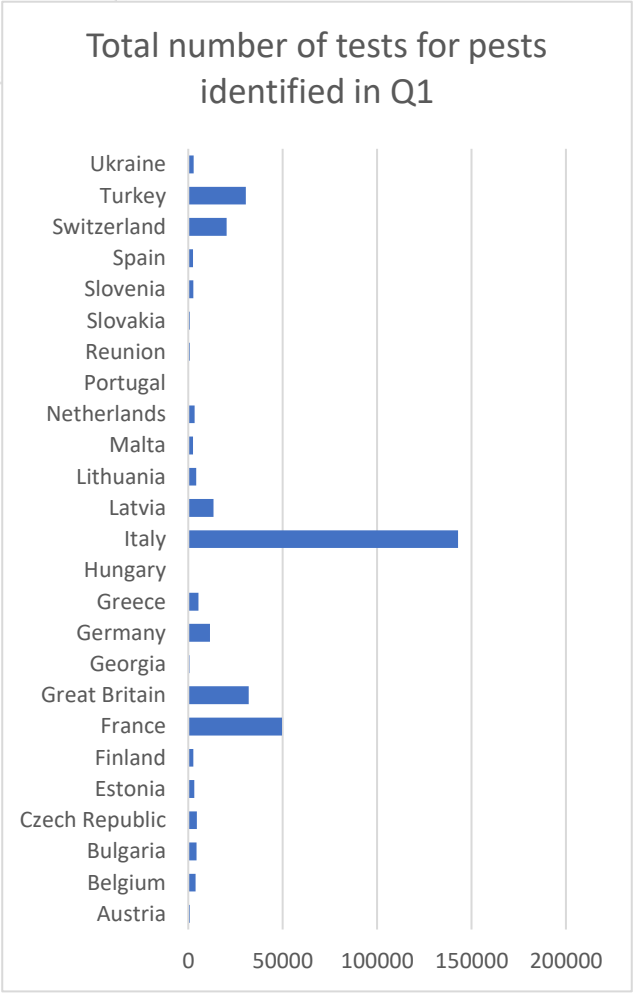

Figure 4. Number of tests per country

4.2. Laboratory Survey

This section reports on current testing priorities collected in Q1. As mentioned above, 98 species were listed in total. Most of the species reported (50%) were primarily tested for using molecular methods, followed by serological methods (30%); morphological, plating and biochemical methods were only used for a limited number of organisms and HTS was only reported in the case of Citrus tristeza virus. For a full list see Appendix 2.

Most tests were performed for plum pox virus, prunus necrotic ringspot virus and grapevine fanleaf virus. This result is primarily influenced by Italy, followed by France, GB and Turkey, as they have reported the highest number of tests for priority pests. To avoid results being skewed by these differences we have further looked at a ranking based on the weighted priority, with the top ranked organisms being *Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia solanacearum* and *Xylella fastidiosa*. Despite the two approaches resulting in different rankings, around three quarters (13) of the top 20 pests remain the same:

Table 2. Stakeholder priorities for testing – laboratory survey

Organism	Method	Matrix	European priority*
Arabis mosaic virus	Biochemical	Leaves, woody cuttings	IIAII
Citrus tristeza virus	Serological, (HTS)	Woody cuttings, leaves	A2, IIAI, survey co-funded by EU for 2019-2020, NPPO priority
Clavibacter michiganensis subsp. *Sepedonicus*	Serological, molecular, (biochemical)	Leaves, tubers	A2, IAII, survey co-funded by EU for 2019-2020, NPPO priority
Erwinia amylovora	Plating, serological, molecular	Leaves, fruits, roots, woody cuttings	A2, IIAII, NPPO priority
Globodera pallida	Morphological, molecular	Soil, tubers, roots	A2, IB, survey co-funded by EU for 2019-2020, NPPO priority
Globodera rostochiensis	Morphological, (molecular)	Soil, tubers	A2, IB, NPPO priority
Grapevine fanleaf virus	Biochemical, serological, (molecular)	Woody cuttings, leaves	---
Pepino mosaic virus	Serological	Leaves, fruit, seed	A2, NPPO priority
Plum pox virus	Serological, (molecular)	Leaves, fruit, woody cuttings	A2, IIAII, NPPO priority
Potato spindle tuber viroid	Molecular	Leaves, fruit, tubers, seeds, herbaceous cuttings	A2, IAI
Ralstonia solanacearum	Serological, plating, biochemical, (molecular)	Tubers, roots, herbaceous cuttings, leaves	A2, IAII, survey co-funded by EU for 2019-2020, NPPO priority
Strawberry latent ringspot virus	Serological	Leaves, seeds	IIAII
Xylella fastidiosa	Molecular	Leaves, herbaceous / woody cuttings	A2, IAII, survey co-funded by EU for 2019-2020, NPPO priority

*European priority: see table 1

4.3. NPPO Survey

Applying a similar ranking approach to Q2 shows that for NPPOs the biggest perceived threat currently by far is *Xylella fastidiosa*, followed by *Bursaphelenchus xylophilus* and *Candidatus phytoplasma vitis*:

Table 3. Stakeholder priorities for testing – NPPO survey

Organism	European priority*
Xylella fastidiosa	A2, IAII, survey co-funded by EU for 2019-2020
Bursaphelenchus xylophilus	A2, IIAI, survey co-funded by EU for 2019-2020
Candidatus phytoplasma vitis	A2, IIAII, survey co-funded by EU for 2019-2020
Clavibacter michiganensis subsp. *sepedonicus*	A2, IAII, survey co-funded by EU for 2019-2020
Phytophthora ramorum	A2
Erwinia amylovora	A2, IIAII
Ralstonia solanacearum	A2, IAII, survey co-funded by EU for 2019-2020
Anoplophora glabripennis	A1, survey co-funded by EU for 2019-2020
Candidatus Liberibacter solanacearum	A1, survey co-funded by EU for 2019-2020
Citrus tristeza virus	A2, IIAI, survey co-funded by EU for 2019-2020
Anoplophora chinensis	A2, IAI, survey co-funded by EU for 2019-2020
Synchytrium endobioticum	A2, IAII, survey co-funded by EU for 2019-2020
Tomato brown rugose fruit virus	AL
Guignardia citricarpa	A1, IIAI, survey co-funded by EU for 2019-2020
Plum pox virus	A2, IIAII

*European priority: see table 1

5. Results and Discussion

As described in section 3 an interactive spreadsheet was produced (Figure 5). After adding two additional pests (*Xylophilus ampelinus* and Tomato Brown Rugose Fruit Virus) the list shown in Table 4 was presented to WP1 partners. This table was completed with information about ongoing research projects and available validation data for the pests listed. Ultimately six pests were selected (Table 4) and test scopes were produced (Appendix 3: Scope).

It should be noted that the surveys provide a snapshot of current testing priorities for laboratories and NPPOs across Europe. While most European countries could be reached with either one survey or both, there are differences in the number of responses per country. Ideally, the surveys would therefore be complemented by an impact assessment based on national and / or European risk assessment data, which would add an additional dimension to the ranking. Such an assessment would take standard risk assessment factors such as the host distribution, spread characteristics and impact of the pest into account; furthermore, distributional elements such as the public nature of some hosts or specific industry interests could be considered. While such an extensive approach was not feasible within the timeframe available, it will be discussed within future deliverables and impact assessments. Another interesting dimension is interactions between interests of a number of stakeholders, including laboratories (incl. project partners), kit providers and risk managers. Again, these will be explored in more depth in the second half of the project.

Morphological only	no			
Species level?	yes			
Identified as an EU priority by EPPO and/or the Commission?	yes			
Type	(All)	To be selected by TPS organisers if necessary		
EPPO status	(All)			
EU annex status	(All)			
Co-funded status	(All)			
NPPO priority status	(All)			
NPPO high priority status	(All)			

Row Labels	Weighted priority Q1+Q2 EU ONLY	Weighted priority Q1+Q2 all respondents	Does at least one respondent (Q1)use kits? 0 = no 1 = yes	one respondent (Q1) use on-site tests? 0 = no 1 = yes
Xylella fastidiosa	61	61	0	0
Clavibacter michiganensis subsp. Sepedonicus	59	60	1	1
Ralstonia solanacearum	56	57	1	1
Erwinia amylovora	45	45	1	1
Bursaphelenchus xylophilus	42	43	0	0
Candidatus Phytoplasma vitis	37	37	0	1
Globodera rostochiensis	36	44	1	0
Citrus tristeza virus	36	36	0	1
Phytophthora ramorum	28	28	0	1
Plum pox virus	27	27	0	0
Pepino mosaic virus	25	25	0	1
Candidatus Liberibacter solanacearum	23	23	1	0
Anoplophora glabripennis	22	22	0	0
Xanthomonas citri pv. Citri	18	18	0	0
Globodera pallida	18	22	0	0
Potato spindle tuber viroid	16	16	0	0
Candidatus Liberibacter africanus	15	15	0	0
Meloidogyne chitwoodi	15	15	1	0
Fusarium circinatum	15	15	0	0
Meloidogyne fallax	15	15	1	0
Phyllosticta citricarpa	14	14	0	0
Tomato spotted wilt virus	13	13	0	1
Ralstonia solanacearum race 3	9	9	1	1
Arabis mosaic virus	7	7	1	0
Cryphonectria parasitica	7	7	0	0
Tobacco ringspot virus	6	6	1	0
Tomato ringspot virus	6	6	1	0
Xylella fastidiosa subsp. Pauca	5	5	0	0
Melampsora medusae	5	5	0	0
Strawberry latent ringspot virus	5	5	0	0
Clavibacter michiganensis subsp. Michiganensis	5	5	0	1
Ralstonia solanacearum race 1	5	5	0	0
Ceratitis capitata	5	18	0	0
Potato virus Y	5	5	1	0
Candidatus Phytoplasma mali	4	4	0	0
Prunus necrotic ringspot virus	4	4	1	0
Acidovorax citrulli	4	4	0	0
Ralstonia solanacearum race 2	4	4	1	0
Beet necrotic yellow vein virus	4	4	0	0
Potato leafroll virus	4	4	1	0
Potato stolbur phytoplasma	4	4	0	0
Xanthomonas arboricola pv. Pruni	3	3	0	0
Elsinoe australis	3	3	0	0
Plenodomus tracheiphilus	3	3	0	0
Elsinoe fawcettii	2	2	0	0
Diaporthe vaccinii	2	2	0	0
Cherry rasp leaf virus	2	2	0	0
Potato virus X	2	2	1	0
Tomato leaf curl New Delhi virus	1	1	0	0
Tomato yellow leaf curl virus	1	1	0	0
Diabrotica virgifera virgifera	0	4	0	0
Ralstonia solanacearum sensu lato	0	2	1	0
Alternaria tenuis	0	5	0	0
Tuta absoluta	0	7	0	0
Pantoea stewartii	0	5	1	0
Grand Total	**753**	**804**	**18**	**10**

Figure 5. Ranking process and resulting spreadsheet

Table 4. Selection of pests for TPS round 2

Arabis mosaic virus
Cryphonectria parasitica
Melampsora medusa
Plum pox virus
Tobacco ringspot virus
Tomato Brown Rugose Fruit Virus
Tomato ringspot virus
Tomato spotted wilt virus
Xanthomonas citri pv citri
Xylophilus ampelinus

Table 5. TPS round 2 – pests and TPS organizers selected

Pest	TPS Organiser
Cryphonectria parasitica	UNITO
Tomato Brown Rugose Fruit Virus	CREA
Plum pox virus	ANSES
Tomato spotted wilt virus	NIB
Xanthomonas citri pv citri	ANSES
Xylophilus ampelinus	Fera

Appendix 1: Survey Information Requirements

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Information needs – Summary

For detailed questions please see pp 2-7

PART 1 – Your current testing priorities

- Approximate numbers and proportions of different test types across the organisation
- More detailed information on the "top 5" tests (e.g. customer segments, approx. costs,...)

PART 2 – Requirements for new or improved tests

- Knowledge of gaps in current testing offer / consumer demands

PART 3 – Validation data

- Knowledge about validation data used and shareability of / access to reports

PART 4 – Use of on-site testing kits

- Information on current use and context of on-site tests (including approximate numbers)
- Knowledge about current limitations and future opportunities

PART 5 – Use of High Throughput Sequencing technologies (HTS)

- Information on current use and context of HTS
- Knowledge about current limitations, challenges and constraints
- Knowledge about validation / plans for validation or certification

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 1

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Part 1 – Your current testing priorities

1.1. Thinking of the past 5 years, how many **individual tests** do you perform **on average annually** in your lab / organisation? (Please provide a rounded estimate)

1.1.1. Based on the number of individual tests performed annually in your lab / organisation (5-year average), please indicate the proportion represented by different methods *(molecular, morphological, serological, biochemical, planting)*

1.1.2. Based on the number of individual tests performed annually in your lab / organisation (5-year average), please indicate the proportion represented by each pest type *(bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods, invasive plants)*

1.2. Please indicate the importance (in %) of the following categories:

- Testing for **quarantine pests** on samples sent in by the **national plant health agency**
- Testing for **regulated non-quarantine pests** (certification pests) on samples sent in by the **national plant health agency or delegated official bodies**
- Testing for **quarantine pests** on samples sent in by **private entities** (organisations or individuals)
- Testing for **regulated non-quarantine pests** (certification pests) on samples sent in by **private entities** (organisations or individuals)
- Testing for **non-regulated pests**

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 2

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1.3. Please list your "top 5" tests in terms of overall quantity (annual):

	Pest	Method	Matrix	Main customer segment(s)	Do you use commercial kits?	Number of individual tests per year (rounded estimate)	Main advantages of this test over others	Approximate costs for running one test (for one sample or one batch if samples are processed in batches) for hand-on-time in minutes (*1)
1								
2								
3								
4								
5								

Drop-down fields and checkboxes

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 3

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Part 2 – Requirements for new or improved tests

2.1. New tests (i.e. no tests are currently available) are required for:

Pest	Matrix	Method (if preference exists)	Key requirements the test will need to meet
1			
2	*Drop-down fields and checkboxes*		
3			
4			
5			

2.2. Existing tests that need to be improved (this includes relatively small changes like the design of a new primer):

Pest	Method	Matrix	Reason for improvement need:
1			
2	*Drop-down fields and checkboxes*		
3			
4			
5			

Part 3 – Validation data

3.1. Do you have validation data and reports that you are willing to share on tests for plant pests that you have not already submitted? (either in the first VALITEST survey or provided to the EPPO Database on Diagnostic Expertise). The validation report can be in your own languages.

Yes / No

If yes, please complete the following and upload the report
Pest (name) (scroll down list)
Method (scroll down list)
Matrix (scroll down list)

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 4

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Part 4 – Use of on-site testing kits

4.1. Are you currently using on-site testing kits?

4.1.1. Which tests are you currently using? *(LFD, portable real-time PCR, LAMP or RPA, Immunoprinting, other)*

4.1.2. In which context are you using these tests? *(Certification, field-inspection, import inspection, post-entry quarantine, other)*

4.1.3. On average, for the past 5 years, what is the number of annual on-site tests carried out for:

- 4.1.3.1. Certification
- 4.1.3.2. Field inspection (general surveillance)
- 4.1.3.3. Field inspection (pest specific survey)
- 4.1.3.4. Import inspection
- 4.1.3.5. Post-entry quarantine
- 4.1.3.6. Other

4.1.4. What are the 5 most targeted pests for on-site testing?

4.1.5. Ranking advantages of on-site testing (which foster their use)

	Essential	Important	Nice to have	Not important
Orientation testing				
Immediate decision				
Save time				
Save Cost				
Easy to use				

4.2. What are the current limitations for using on-site testing? *(Cost, absence of tests for the targeted pest, limited sensitivity, false negative results, cross-contamination issues, limited validation data, methods are not validated, difficulties in technological transfer to field partners, decision scheme depending on output, lack of trust in results (e.g. by the industry, plant health inspectors, diagnosticians), not useful for my activities, other)*

4.3. In which context do you see a primary opportunity for using on-site testing in the near future? *(Certification, field-inspection, import inspection, post-entry quarantine, other)*

4.4. What are the 5 pests for which an on-site testing kit is needed the most?

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 5

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Part 5 – Use of HTS technologies

5.1. Do you currently use HTS technologies?
Yes /No

If yes:

5.1.1. In which context do you currently use HTS technologies? *(Certification, resolving unknown etiology of symptoms (**after** classical testing), resolving unknown etiology of symptoms (**instead** of classical testing), field inspection (general surveillance), import inspection, post-entry quarantine, other)*

5.1.2. Which technology/technologies do you currently use? *(Illumina, Ion Torrent, Pacific Biosciences, Oxford Nanopore Technologies, other)*

5.1.3. Which are the two most challenging steps in the HTS-based diagnostic procedure? *(Wet lab processing, sequencing, data analysis, interpretation of results, other)*

5.1.4. What are the two main difficulties in interpreting HTS data for diagnostic purposes? *(Distinguishing between real and false positive hits in bioinformation results, detecting contaminants, determining the priority for the confirmation studies on detected taxa, distinguishing between integrated or activated viral sequences, other)*

5.1.5. Do you use any quality controls in your HTS-based diagnostic procedures?

5.1.5.1. Please describe your quality controls.

5.1.6. What is your decision procedure to determine the presence of a pest in HTS data? (Option to upload a flow-chart)

5.1.7. Are you planning to validate HTS for diagnostics?

5.1.7.1. What do you think the bottlenecks will be?

5.1.8. Are you planning to request ISO accreditation of HTS protocols? *(In the next two years, in the next five years, no)*

5.1.9. Do you encounter challenges in communicating the results (with NPPOs or other customers)?

5.1.9.1. What are these challenges?

5.1.10. Please let us know the reason for not using HTS technologies. *(Cost, complexity, lack of expertise in application, lack of expertise in interpretation, risk of novel findings, not relevant for my organisation, other)*

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 6

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5.2. What is needed for your laboratory / organisation to adopt HTS technologies in diagnostics?

	Essential	Important	Nice to have	Not important	Already achieved	Don't know
Lower price per analysis						
Better information technology (IT) structure						
Training of laboratory personal						
Support with / decreased complexity of data analysis solutions						
Faster turnaround time						
Guidelines for appropriate reporting of results						
Framework to validate the results						
Guidelines on validation requirements						
Guidelines on quality controls needed						

Please note: The term pest is used to cover bacteria, viruses, viroids, phytoplasmas, fungi, nematodes, arthropods and invasive plants. 7

Appendix 2: Full Pest List and Preferred Methods

Organism	Molecular methods	HTS	Morphological methods	Serological methods	Plating	Biochem. methods
Acidovorax citrulli	100					
Alternaria tenuis	100					
Ambrosia artemisiifolia			100			
Anoplophora glabripennis	100					
Apple chlorotic leaf spot virus				100		
Apple mosaic virus				100		
Arabis mosaic virus				5		95
Armillaria mellea	100					
Beet necrotic yellow vein virus	100					
Bursaphelenchus xylophilus	56		44			
Candidatus Liberibacter africanus	100					
Candidatus Liberibacter solanacearum	100					
Candidatus Phytoplasma mali	100					
Candidatus Phytoplasma prunorum	100					
Candidatus Phytoplasma vitis	100					
Ceratitis capitata	8		92			
Cherry rasp leaf virus	100					
Citrus tristeza virus		1		99		
Clavibacter michiganensis subsp. Michiganensis	100					
Clavibacter michiganensis subsp. Sepedonicus	16			79		5
Cryphonectria parasitica			23		77	
Cyperus esculentus	100					
Diabrotica virgifera virgifera						
Diaporthe vaccinii					100	
Dothistroma septosporum	100					
Elsinoe australis	100					
Elsinoe fawcettii	100					
Erwinia amylovora	21			34	45	
Fusarium circinatum	9		91			
Globodera pallida	20		80			
Globodera rostochiensis	7		93			
Grapevine bois noir phytoplasma	100					
Grapevine fanleaf virus	1			24		75
Grapevine leafroll-associated virus 1				100		
Grapevine leafroll-associated virus 2				71		29
Grapevine leafroll-associated virus 3						100
Melampsora medusae	100					
Meloidogyne chitwoodi	100					

Meloidogyne fallax	100					
Pantoea stewartii				100		
Pea necrotic yellow dwarf virus	100					
Pennisetum setaceum	100					
Pepino mosaic virus				100		
Pepper mild mottle tobamovirus				100		
Phocine morbillivirus				100		
Phyllosticta citricarpa	100					
Phytophthora ramorum	100					
Pineapple mealybug wilt-associated virus 1	100					
Plenodomus tracheiphilus	100					
Plum pox virus	1			99		
Potato leafroll virus	100					
Potato spindle tuber viroid	100					
Potato stolbur phytoplasma	100					
Potato virus X				100		
Potato virus Y	100					
Prune dwarf virus				100		
Prunus necrotic ringspot virus				100		
Pseudomonas syringae pv. Tomato	100					
Ralstonia solanacearum	9			74	2	15
Ralstonia solanacearum race 1	100					
Ralstonia solanacearum race 2				100		
Ralstonia solanacearum race 3				25	75	
Ralstonia solanacearum sensu lato	100					
Strawberry latent ringspot virus				100		
Tobacco rattle virus	100					
Tobacco ringspot virus	78			22		
Tomato leaf curl New Delhi virus	100					
Tomato leaf curl virus				100		
Tomato mosaic virus				100		
Tomato ringspot virus	86			14		
Tomato spotted wilt virus				100		
Tomato yellow leaf curl virus						
Turnip yellows virus				100		
Tuta absoluta						
Xanthomonas arboricola pv. Pruni					100	
Xanthomonas campestris pv. Campestris						
Xanthomonas citri pv. Citri						
Xiphinema index						
Xylella fastidiosa	100					
Xylella fastidiosa subsp. Pauca	100					

Appendix 3: Scope of Test Performance Studies – round 2

Tomato Spotted Wilt Virus				
	Methods			
	ELISA	**RT-PCR**	**real time RT-PCR**	**other methods applicable for on-site: LFD**
sample type (DNA, plant material with deactiv. pests, etc.)	Infected/ non-infected plant material	Infected/ non-infected plant material	Infected/ non-infected plant material	Infected/ non-infected plant material
matrix (type of plant material: seed, leaves, etc.)	Leaves of tomato	Leaves of tomato	Leaves of tomato	Leaves of tomato
suitable for: symptomatic / asymptomatic sample	symptomatic	symptomatic	symptomatic	symptomatic
purpose: detection / identification	detection and identification	detection and identification	detection and identification	detection and identification
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC NC	PAC PIC NAC NIC	PAC PIC NAC NIC IC	PC NC
no. of samples	22	22	22	22
max no. of participants	20	20	20	20

Number of tests that will be included in TPS: 5-8.

Methods selected for TPSs are those described EPPO standards, from literature search and available commercial kits. The most important criteria for test selection are specificity (exclusivity and inclusivity) and sensitivity.

Xylophilus ampelinus				
	Methods			
	IF	**DAS-ELISA**	**PCR**	**Real-time PCR**
sample type (DNA, plant material with pests, etc.)	Plant material spiked with culture	Plant material spiked with culture	Plant material spiked with culture and DNA extracts	Plant material spiked with culture and DNA extracts
matrix (type of plant material: seed, leaves, etc.)	Stem material	Stem material	Stem material	Stem material
suitable for: symptomatic / asymptomatic sample	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic
purpose: detection / identification	detection	detection	detection	detection
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC; NC (buffer)	PC; NC (plant); NC (buffer)	NIC; NAC; PIC; PAC	NIC; NAC; PIC; PAC
no. of samples	24	24	24	24
max no. of participants	20	20	20	20

Methods select for TPS are those described in EPPO standards, from literature search and available commercial kits.

Analytical sensitivity and specificity will be evaluated on DNA extracted from pure cultures or pure cultures of *Xylophilus ampelinus* from different geographical regions and other relevant species. Repeatability and reproducibility will also be evaluated.

Plum pox virus			
PPV on-site testing	**Methods**		
	On-site LFD serologic	**On-site LFD PCR**	**On-site LAMP**
sample type (DNA, plant material with deactiv. pests, etc.)	Infected/ non-infected plant material	Infected/ non-infected plant material	Infected/ non-infected plant material
matrix (type of plant material: seed, leaves, etc.)	Freeze-dried leaves	Freeze-dried leaves	Freeze-dried leaves
suitable for: symptomatic / asymptomatic sample	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic
purpose: detection / identification	detection	detection	detection
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC, NC	PC, NC	PC, NC
no. of samples	22	22	22
max no. of participants	20	20	20

Tests selected for TPS are available commercial kits and described on internet sites or pending.

Tests that can be performed on site or only few step in a room or lab (tests needing a nucleic acid extraction step or laboratory tests excluded).

The most important criteria for test selection are inclusivity and diagnostic specificity.

Cryphonectria parasitica		
	Methods	
	Conventional PCR	**Real time PCR**
sample type (DNA, plant material with deactiv. pests, etc.)	• Plant material with deactivated pathogen • DNA extracts	• Plant material with deactivated pathogen • DNA extracts
matrix (type of plant material: seed, leaves, etc.)	Spiked wood material	Spiked wood material
suitable for: symptomatic / asymptomatic sample	Symptomatic / asymptomatic sample	Symptomatic / asymptomatic sample
purpose: detection / identification	Detection	Detection
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	**NAC** **NIC** **PAC**	**NAC** **NIC** **PAC**

	PIC	PIC
no. of samples	• 8 Spiked wood material samples • 8 DNA sample extracts	• 8 Spiked wood material samples • 8 DNA sample extracts
max no. of participants	15	15

Methods selected for TPS are those found in literature and only molecular methods were selected. Morphological methods were ignored, including EPPO standard PM 7/45(1). No commercial kits are available.

Xanthomonas citri* pv *citri							
	Methods						
	plating	**IF**	**ELISA**	**PCR**	**real-time PCR**	**LAMP**	**other methods applicable for on-site**
sample type (DNA, plant material with deactiv. pests, etc.)	-	-	-	DNA	DNA	DNA	-
matrix (type of plant material: seed, leaves, etc.)	-	-	-	Leaves/ fruits	Leaves/ fruits	Leaves/ fruits	-
suitable for: symptomatic / asymptomatic sample	-	-	-	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic	-
purpose: detection / identification	-	-	-	detection	detection	detection	-
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	-	-	-	PAC NAC	PAC NAC	PAC NAC	-
no. of samples	-	-	-	20-25	20-25	20-25	-
max no. of participants	-	-	-	20	20	20	-

The TPS aims to compare molecular methods. Molecular methods selected for TPS are those described in IPPC and EPPO standards, from literature search and available commercial kits. The most important criteria for test selection are specificity (exclusivity and inclusivity) and sensitivity.

Tomato Brown Rugose Fruit Virus				
	Methods			
	DAS-ELISA	**RT-PCR**	**Real-time PCR**	**Other methods applicable for on-site: Immunostrip for tobamovirus**
sample type (DNA, plant material with deactiv. pests, etc.)	Freeze dried leaves and fruits extract	Freeze dried leaves and fruits extract	Freeze dried leaves and fruits extract	Freeze dried leaves extract

matrix (type of plant material: seed, leaves, etc.)	Leaves/ fruits of tomato and pepper	Leaves/ fruits of tomato and pepper	Leaves/ fruits of tomato and pepper	Leaves of tomato and pepper
suitable for: symptomatic / asymptomatic sample	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic
purpose: detection / identification	detection	detection	detection	Early warning
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC; NC (plant); NC (buffer)	NIC; NAC; PIC; PAC	NIC; NAC; PIC; PAC	PC; NC (plant);
no. of samples	22	22	22	22
max no. of participants	25	25	25	25

The purpose of the TPS is to compare the performance of different 5-8 tests.

Methods selected for TPS are those described from literature search, ISF protocol and available commercial kits. None EPPO standard is available. The most important criteria for test selection are specificity (exclusivity and inclusivity) and sensitivity.

Lateral flow will be included to have an on-site method for an early ToBRFV warning.