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Perspective

What Did We Achieve Through VALITEST, an EU Project on Validation in Plant Pest Diagnostics?

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Abstract

Ensuring the reliability of diagnostic activities is an essential cornerstone of plant health strategies to reduce the risk of entry and spread of plant pests in a region and ultimately their impacts. Diagnostic tests should be validated to ensure that they are fit for purpose. Validation is usually done by diagnostic laboratories, although companies commercializing diagnostic kits also produce validation data for their products. Due to the high number of pest, matrix, and method combinations and given the significant resources required to validate tests, it is essential that validation data are shared with the entire diagnostic community and produced in a harmonized way to facilitate their use by different stakeholders. Indeed, the selection of tests to be used in specific contexts is not the sole responsibility of diagnostic laboratories but also involves national plant protection organizations. The VALITEST EU project (2018 to 2021) was established to tackle all these issues. New validation data for tests targeting important pests for the European and Mediterranean Plant Protection Organization region were produced. Guidelines to improve and harmonize the validation framework were developed. Sharing of validation data and experience was ensured through the development of new or existing databases, the organization of training courses, and the dissemination of the project outputs in scientific publications and standards. Finally, the involvement of researchers, diagnosticians, policy makers, inspectors, and industries and the establishment of the European Plant Diagnostic Industry Association were important actions to strengthen the interactions between plant health stakeholders.

Keywords: high-throughput sequencing, plant pest diagnostics, reference material, test performance study, training, validation

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The Food and Agriculture Organization (FAO) estimates that, annually, between 20 and 40% of global crop production is lost due to pests. Each year, plant diseases cost the global economy around 220 billion USD and invasive insects around 70 billion USD (FAO 2019a). Protecting crops against these losses from farm to fork is critical to ensure global food security, achieve sustainable and competitive agriculture, and protect biodiversity and ecosystems. Efficient surveillance mechanisms are key to the fulfilment of these important goals as they enable effective monitoring and control of the introduction and spread of plant pests (Carvajal-Yepes et al. 2019). Early diagnosis and a rapid response are crucial to reduce the risk of entry and spread of plant pests and ultimately their impacts. Plant pests can be managed most effectively when detected in time and when control measures are implemented at an early stage of infestation (Koch et al. 2020).

National plant protection organizations (NPPOs) routinely conduct inspections, supported by testing for export certification, import, pest surveillance, and eradication programs. Accurate identification of a pest is a prerequisite for taking phytosanitary action. In addition, to enable safe trade, testing must be completed quickly and to a high level of confidence.

In the European Union (EU), the need to validate (including through test performance studies [TPSs]) existing and new tests for the detection and identification of pests using harmonized approaches was recognized for both the animal and plant health fields, and a specific topic, "Validation of diagnostic tools for animal and plant health," was included in the EU's research and innovation funding program for 2014 to 2020 (called Horizon 2020). A contribution from the EU of around 3 million EUR was granted for this plant health topic. One of the requirements of the EU Commission was that projects should involve different stakeholders in plant health and that cooperation with international and standardization bodies should also be ensured. A consortium of 16 partners composed of research institutions, private companies (such as diagnostic kit providers), and NPPOs was formed. The European and Mediterranean Plant Protection Organization (EPPO), an intergovernmental organization responsible for international cooperation in plant protection for the European, Mediterranean, and Central Asian region, was also part of the consortium. In 1998, the EPPO established a work program in pest diagnostics to harmonize procedures across its region. This involves the preparation of pest-specific diagnostic protocols, as well as horizontal standards providing, for example, guidance on the validation of tests or on interlaboratory comparisons.

The VALITEST (validation of diagnostic tests to support plant health) project started on May 1, 2018, and finished on October 31, 2021 (Trontin et al. 2021). The main achievements and lessons learned from the project are presented here. All deliverables and outputs of the project are (or will soon be) available on the VALITEST website (https://www.valitest.eu/index) and on the zenodo repository of the project (https://zenodo.org/communities/valitest/?page=1&size=20).

Validation terminology varies between different international and national organizations. The terminology used in this article is according to EPPO terminology defined in the EPPO Standard PM 7/76 *Use of EPPO Diagnostic Protocols* (EPPO, 2018).

EVALUATION OF TESTS

By providing information on the performance of the tests used in diagnostics, validation is essential to ensure the reliability of a diagnostic activity. Validation studies can be performed in one laboratory or involve several laboratories. They consist of the evaluation of different performance criteria such as analytical sensitivity, analytical specificity, selectivity, repeatability, and reproducibility (EPPO 2021a). Diagnostic sensitivity and specificity (also called rate of true positives and rate of true negatives) can also be evaluated during validation studies. A test can be considered validated when its performance characteristics meet the level required for a specific intended use. Tests are currently mostly validated on an intralaboratory basis or through limited interlaboratory comparisons (i.e., TPSs, sometimes referred to as ring tests). In addition, sharing validation data in publicly available resources remains limited. Thus, the first goal of VALITEST was to produce new or additional validation data for the detection and identification of plant pests through the organization of two rounds of TPSs. In total, 12 TPSs, targeting 11 pests of interest for various stakeholders in the EPPO region, were organized in the VALITEST framework and produced validation data (e.g., diagnostic sensitivity, diagnostic specificity, and reproducibility) for 83 tests (Table 1). The two rounds of TPSs included laboratories from 31 countries spread over four continents. Between 11 and 34 participants from 8 to 20 different countries were selected for each TPS (Trontin et al. 2021). Prior to each TPS, preliminary studies were carried out by TPS organizers to support the selection of the tests to be included in each TPS. These also contributed to the production of a substantial amount of validation data (e.g., analytical sensitivity, analytical specificity, and repeatability) for a total of 131 tests.

HARMONIZING AND IMPROVING THE VALIDATION FRAMEWORK

In addition to the production of new validation data, one objective of VALITEST was to further harmonize and improve the validation framework and to adapt it to new technologies used in diagnostics. Based on the expertise of the partners and on the experience gained through the organization of several TPSs, different guidelines were produced.

Substantial knowledge gained on the organization of TPSs

TPSs are the ultimate approach to evaluate and compare the performance of tests. However, the organization of a TPS is a complex process that requires time and resources. In addition, TPS organizers need a high level of expertise to ensure a smooth process and reliable results.

Substantial knowledge and experience were gained in the two rounds of VALITEST TPSs. The organization of TPSs was shown to be easier if timelines, rules, and criteria, which need to be followed, are defined and formalized in advance. In the first round of TPSs, a framework and associated documentation for the preparation (including definition of the scope, the selection of tests,

VISION STATEMENT

This concept note presents the outcomes of VALITEST, an EU-funded project on diagnostic test validation. Beyond the evaluation of the performance of specific tests used in plant pest diagnostics, this project improved diagnostic procedure by tackling areas such as the organization of test performance studies, the statistical analysis of data generated during validation studies, and the development of guidelines for the use of reference material and high-throughput sequencing technologies in plant pest diagnostic laboratories. Additionally, it strengthened interactions between stakeholders in plant health, including companies producing diagnostic kits to achieve better diagnostics.

and the selection of participating laboratories) and organization of TPSs were created. For the analysis of TPS results, harmonized documents for the calculation and graphical representation of performance criteria such as diagnostic sensitivity and specificity were also developed. Those documents were used and further improved and finalized in the second round of TPSs.

One of the major outputs of VALITEST is a book written by the TPS organizers, *Critical Points for the Organisation of Test Performance Studies in Microbiology: Plant Pathogens as a Case Study* (Vučurović et al. 2022). This book provides further details on each step of the process and examples of TPS documents and forms. Those general recommendations for the organization of TPSs are applicable to any TPS organization and can help diagnostic laboratories in the field of plant health.

Better insight into statistical analysis of data

An appropriate and harmonized approach for the statistical analysis of validation data is important to facilitate the interpretation of performance characteristics and the comparison of tests, as well as to increase confidence in the conclusions drawn from the validation data. However, up to now, there has been limited guidance on the use of statistical analysis in the context of plant health diagnostics. During VALITEST, a framework proposing new statistical tools to be used for the analysis of validation data was prepared by a group composed of diagnosticians and statisticians. This framework was evaluated using 10 datasets obtained from the TPSs, which allowed the recommendations to be refined. The choice of the statistical methods for determination of the performance characteristics was based on the applicability of the method in the context of plant health diagnostic laboratories, the minimum number of samples and replicates required for a statistical method to perform correctly, the ease of application, and the interpretation of results. These guidelines also provide information on how to establish the panel of samples, how to deal with inconclusive and missing results, and how to identify and deal with outliers. The proposed statistical tools will facilitate the comparison of the performance characteristics between tests. A paper was published in the EPPO bulletin (Massart et al. 2022a).

Understanding and specifying needs for the routine use of high-throughput sequencing in plant pest diagnostics

High-throughput sequencing (HTS) is one of the most significant advances in molecular diagnostics since the advent of PCR methods in the 1980s. With the potential to detect the nucleic acids of any organism present in a sample, HTS provides new possibilities and opportunities in routine plant health diagnostics (Olmos et al. 2018). However, standardized best-practice guidelines to ensure the harmonized and proper implementation of this new technique were lacking up to now. A recommendation on "Preparing the use of HTS technologies as a diagnostic tool for phytosanitary purposes" was adopted by the Commission on Phytosanitary Measures governing body of the International Plant Protection Convention (IPPC) in 2019 (FAO 2019b). This recommendation encourages the development of best-practice operational guidelines covering analysis results and quality control measures for HTS that "ensure HTS data outputs are robust and

TABLE 1

Summary of the test performance studies organized in the framework of VALITEST (adapted from Trontin et al. 2021)[a]

Pest	TPS organizer	Number of tests evaluated in preliminary studies	Number of tests selected for TPS	Publication of the results (PM 7 = EPPO Standards on Diagnostics)
Bursaphelenchus xylophilus	ANSES	6	5 tests (conventional PCR, real-time PCR, LAMP)	PM 7/004 (under revision)
Citrus tristeza virus (CTV)	ANSES	16	11 tests (ELISA, TPIA, conventional RT-PCR, real-time RT-PCR, RT-LAMP, and LFD)	PM 7/031 (under revision)
Cryphonectria parasitica	UNITO	3	3 tests (conventional and real-time PCR)	PM 7/045 (under revision)
Erwinia amylovora	NIB	9	6 tests (real-time PCR, LFDs, and LAMP)	PM 7/020 (3)
Fusarium circinatum	Fera	7	6 tests (plating, PCR, real-time PCR)	PM 7/091 (revision to be started)
Pantoea stewartii subsp. *stewartii*	NIB	8	6 tests (real-time PCR, conventional PCR)	PM 7/060 (revision to be started)
Plum pox virus (PPV)	NVWA	20	8 tests selected (conventional RT-PCR, real-time RT-PCR, DAS-ELISA)	PM 7/032 (under revision)
Plum pox virus (PPV) onsite tests	ANSES	4	3 tests (LFD RPA, LFD)	PM 7/032 (under revision)
Tomato brown rugose fruit virus (ToBRFV)	CREA	9	5 tests (conventional and real-time RT-PCR)	Luigi et al. 2022, PM 7/146 (2)
Tomato spotted wilt orthotospovirus (TSWV)	NIB	19	8 tests (DAS-ELISA,on-site tests, conventional and real-time RT-PCR)	Vučurović et al. 2022
Xanthomonas citri pv. *citri*	ANSES	20	13 tests (conventional and real-time PCR, LAMP and direct molecular tests performed from LFDs or Whatman FTA cards)	PM 7/044 (under revision)
Xylophilus ampelinus	Fera	10	9 tests (ELISA, IF, conventional and real-time PCR)	PM 7/096 (revision to be started)
Total of 11 pests	Total of 6 institutions	Total of 131 tests	Total of 83 tests	

[a] ANSES, French Agency for Food, Environmental and Occupational Health & Safety (FR); CREA, Council for Agricultural Research and Economics (IT); DAS-ELISA, double antibody sandwich ELISA; Fera, Fera Science Limited (U.K.); IF, immunofluorescence; LAMP, loop-mediated isothermal amplification; LFD, lateral flow device; NIB, National Institute of Biology (SI); NVWA, Netherland Food and Consumer Product Safety Authority (NL); PCR, polymerase chain reaction; RPA: recombinase polymerase amplification; RT, reverse transcriptase; TPIA, tissue print immunoassay; TPS, test performance study; UNITO, University of Turin (IT).

accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way, including test validation and quality assurance" (FAO 2019b). In addition, the recommendation highlights the need for validating HTS tests. During VALITEST, guidelines were developed for the use of HTS as a routine test in plant diagnostic laboratories. These were reviewed externally by 42 experts from 18 countries (from 5 continents) and 29 institutes (universities, research centers, diagnostic laboratories, NPPOs, and the EPPO) with expertise in pest diagnostics. The guidelines provide technical recommendations for each step of the test, including laboratory work and bioinformatic analyses. They also include recommendations on test selection, development and optimization, validation and verification, internal and external quality checks (including the use of proper external and internal controls), and interpretation and reporting of test results. The guidelines were developed irrespective of the chemistry, equipment, and software and are applicable to any plant pest in any matrix. They were designed to allow for flexibility within this fast-evolving technology. The guidelines target plant health diagnostic laboratories that intend to routinely use HTS technologies for the detection and identification of pests and are applicable to any organism (e.g., arthropods, bacteria, fungi, nematodes, invasive plants, protozoa, viroids, viruses, or weeds) and any type of matrix (e.g., pure microbial culture, plant tissue, soil, water), regardless of the type of HTS technology (e.g., amplicon sequencing, shotgun sequencing) and its application (e.g., surveillance program, phytosanitary certification, crop protection). In addition, their adoption by research laboratories would improve the overall reliability of generated HTS datasets and their comparison. Two publications (Lebas et al. 2022; Massart et al. 2022b) were published in 2022.

Ensuring the production of high-quality reference material

Reference material is essential to ensure traceability when performing diagnostic activities. In plant health, reference material is usually produced by individual diagnostic laboratories due to the limited commercial offer. To help TPS organizers in that task, quality guidelines were developed for the production of reference materials to be used in interlaboratory studies. First, a list of criteria (i.e., the intended use of the reference material and its identity, traceability, commutability, homogeneity, stability, assigned value, and purity) to consider for the description of reference material was established. Then, a general standard operating procedure for the production of reference material for use in plant health diagnostics was developed. The steps required during the production process (e.g., identification of the material, multiplication, and verification of the homogeneity, stability, commutability, purity, quantity, and identity of the material) depend on the sources of the reference material (e.g., field material, working collection, reference material, or certified reference material) and on the intended use of the material. For each step in the process, critical points should be identified, as well as the criteria that reference material have to meet and their minimum required levels. Further details are available in Chappé et al. (2020) and Chappé et al. (2019). These guidelines were used to develop a new EPPO standard (see below).

Guidelines are important; however, their production alone is not sufficient, and it is essential that access to reference material is enhanced. As recommended in the white paper "Phytosanitary diagnosis and collections" developed in the framework of another EU-funded project (Q-Collect), it is important that appropriate basic funding is secured for reference material collections and that a common policy toward collection management is established to ensure sharing of reference material (see report of the second Q-Collect workshop: https://www.eppo.int/MEETINGS/2015_meetings/wk_q_collect_workshop).

DISSEMINATION OF VALITEST OUTPUTS

EPPO database on diagnostic expertise

The Standard ISO/IEC 17025:2017 (ISO 2017) requires that all the tests for which a laboratory is preparing accreditation should be validated. Validation data should be generated by the laboratory or should be publicly available, in which case, the laboratory should provide objective evidence that it can perform the test according to the established performance characteristics. Therefore, it is important that validation data are made available to the diagnostic community in an easily accessible way.

The EPPO Database on Diagnostic Expertise (https://dc.eppo.int/) was created in 2007 (Roy et al. 2010). Its first aim was to enable identification of experts who can provide diagnosis of regulated pests and who can help in the identification of new or unusual species.

A section, "validation data for diagnostic tests," was created in 2012 at the request of laboratories that were engaging in an accreditation process. It was considered that sharing validation data would save resources and promote collaboration within the EPPO region. The section on validation data includes data for diagnostic tests for regulated pests. Validation data can be deposited by any registered diagnostic laboratory and can be retrieved by the database users in the form of a harmonized validation sheet in PDF format, including the description of the test evaluated (pest × matrix × method) and the associated performance data.

During the VALITEST project, a survey was organized to identify the needs for improvement of the database, which resulted in the following upgrades to the database:

- The database can now be searched using keywords (searchable descriptors are pest, method, plant species, test, matrix, and EPPO-IPPC test).
- Combined and flexible queries (e.g., multiple pest queries) are now possible.
- Sorting of information within different methods has been improved.
- Searches for tests used for detection, identification, or both can now be conducted.
- Searches for kits can now be conducted.

As a result of the project, the format and content of the whole database was further improved and made more user friendly and more searchable, in particular for the section on validation data for diagnostic tests. All validation data generated during the VALITEST project are (or will soon be) available via the database.

Diagnostic kit database

A wide range of kits for serological or molecular diagnostics are available from commercial suppliers worldwide. Each might differ in performance characteristics, intended use, and validation data available.

During the project, a European Plant Diagnostic Industry Association (EPDIA) was formed by the commercial partners of the project. The EPDIA partners created a database that helps potential users to find the diagnostic tool they need. It includes information from different companies on test kits for various pests, suppliers, the purpose of the test, performance criteria, manuals, and more for different techniques such as ELISA

or PCR (https://www.epdia.eu/diagnostic-kits-european-plant-diagnostic-industry-association.php?lang=en).

Training courses

Training activities were organized in the framework of the VALITEST project. Due to the COVID-19 pandemic, the physical workshops planned for diagnostic laboratories could not be organized. All training activities were held online in the format of webinars, practical training sessions, and videos. Three series of activities were organized on the following topics:

- Concept of test validation in plant health.
- TPS organization.
- Use and validation of HTS tests for diagnostics of plant pests.

All webinars were recorded, and videos are available on the VALITEST website to ensure the maximal dissemination of the results of the project (https://www.valitest.eu/training/activities_and_webinars).

In addition to the webinars, several videos were also prepared by the partners to achieve the following:

- Illustrate and describe the whole project.
- Illustrate specific steps in the process of TPS organization and share experience from the TPS organizers via interviews (such as the selection of pests and TPS organizers, tests, and participants).
- Explain specific notions related to the statistical analysis of TPS data.

These videos provide valuable feedback from TPS organizers who explain the difficulties they faced during the organization of the TPS but also provide tips that are useful for laboratories planning to organize TPS.

Videos can be seen on the EPPO YouTube account in a playlist specific to VALITEST.

Dissemination through standards

In addition to being published in international scientific journals and books, most of the VALITEST results and outputs were used to develop EPPO standards to be used by stakeholders or to revise existing ones. Most of the validation data generated in the TPSs and preliminary studies were or will be used to revise EPPO diagnostic standards on specific pests (Table 1). In addition to the experience gained on TPS organization and analysis of validation, data were used to improve the EPPO diagnostic standard on the organization of interlaboratory comparisons (PM 7/122; EPPO 2022a). Finally, two new standards were developed: PM 7/147 *Guidelines for the production of biological reference material* (EPPO 2021b) and another on considerations for the use of HTS in plant health diagnostics (PM 7/151; EPPO 2022b). These standards are published in the EPPO Bulletin with free access and on the EPPO website (https://www.eppo.int/RESOURCES/eppo_standards/pm7_diagnostics) and the EPPO Global Database (https://gd.eppo.int/standards/PM7/).

STRENGTHENING LINKS WITH STAKEHOLDERS

The last objective of VALITEST was to better understand the need of different stakeholders (e.g., researchers, diagnosticians, policy makers, inspection services, industries, seed companies, growers' associations, etc.) at national and EU levels and to further strengthen their collaboration for better diagnostics.

Identification of the needs of different stakeholders

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. One example is the organization of two online surveys targeting laboratories and NPPOs to identify testing needs.

The survey for laboratories covered different topics: (i) current testing priorities, (ii) requirements for new or improved tests, (iii) validation data available, (iv) the use of on-site testing kits, and (v) the use of HTS. A survey for NPPOs was also conducted and asked representatives to rank their top 10 priority pests. Results from these surveys were combined, and a pest ranking (supplemented with additional information on their national and international status) was the basis for the selection of priorities for the organization of the second round of TPSs. To support this selection, a framework was created to aggregate the ranked results from the two surveys according to the priorities given by respondents.

A mathematical framework has also been developed to support, inter alia, resource allocation for and design of sampling and test programs in different plant health contexts (see Harrison et al. 2023).

Establishment of links with accreditation bodies regarding proficiency testing

Ensuring that laboratories are proficient is essential for a reliable diagnostic service. However, laboratories in plant health cannot undertake proficiency testing (PT) for all the tests they use. The VALITEST partners aimed to develop a horizontal approach that would ensure the proficiency of laboratories through their participation in a limited but specific number of PT.

The needs and expectations of the laboratories were identified, and possible solutions were discussed with representatives of an accreditation body. The most appropriate approach identified to limit the PT participation plan was that a laboratory should identify sets of tests (grouped by methods) for which the outcome of a PT using one test can be directly correlated to the proficiency of the laboratory in the use of other tests. Such an approach is described by the European Cooperation for Accreditation (EA) in the EA-4/18 guidance document on the level and frequency of proficiency testing participation (EA 2021). A case study was developed and will be discussed with EA in the coming months.

Establishment of the EPDIA

At the start of the project, the diagnostic industry was not structured as an entity that can be solicited by other stakeholders. The project provided the opportunity to establish the foundations for a structure to improve communication concerning offers and demands for plant health diagnostic tests in a sustainable manner. The EPDIA (www.epdia.eu) was created during the VALITEST project.

The EPDIA's mission is to engage, on behalf of its members, with all relevant European decision makers to represent their interests and to contribute to the following:

- The promotion of a Quality Charter for the production and development of tools by the plant diagnostics industry.
- The promotion and disclosure of information to the market on phytodiagnostic technologies and their validation.
- The representation of the plant diagnostics industry within European and international institutions.

CONCLUSION, RECOMMENDATIONS, AND BEYOND VALITEST

The Strategic Framework for the IPPC 2020-2030 adopted in 2021 recognizes the importance of diagnostics. It highlights the need for internationally accepted standards for accurate diagnostics but also for networks to help countries identify pests in a more reliable and timely manner. It also underlines the fact that developments in molecular biology and genetic sequences will deliver not only new tools but also new challenges for plant health diagnostics (FAO 2021). Activities conducted during the VALITEST project have contributed to this strategic objective. The guidelines developed to improve the validation framework and the validation data generated throughout the organization of TPSs are the result of international collaborations not only within the EPPO region but also with diagnosticians, researchers, and companies from other parts of the world and are being used to revise major EPPO standards on diagnostics. The preparation of guidelines for HTS is a nice example of successful international collaboration and is an important step toward the development of standardized HTS tests for pest detection and identification. Lessons learned from VALITEST include the following:

- The need to find compromises between what is ideal and what is practical (e.g., when designing panel of samples for optimal statistical analysis or when producing reference material).
- The need for anticipation and the importance of logistics for the good progression of TPSs.
- The need for thorough knowledge of the biological constraints associated with the pest and the plant material for the production of reference material (seasonal availability, survival/stability, delay to produce samples).
- The importance of information on performance of commercial kits being easily retrievable and of companies having a platform for exchange, which is one of the reasons for the creation of the EPDIA.
- The importance of sharing experiences and tips among different stakeholders, which is why a book on TPS organization has been prepared (Vučurović et al. 2022).

However, validation is a continuously evolving story; new tests will be developed and will need to be validated, as will new on-site diagnostic technologies that are coming to the market. In this context, important players to ensure the production of validation data for tests in the EPPO region are presented below.

The laboratories

Plant pest diagnostic laboratories (including national reference laboratories) remain the main source of validation data, and most data included in the EPPO database on diagnostic expertise were generated by individual laboratories. The EPPO will continue to encourage laboratories to share the data produced and to support the validation process by updating the EPPO standards on validation whenever necessary. In 2017, a new EU regulation (EU 2017/625) on official controls entered into force, and European reference laboratories (EURLs) whose activities enhance diagnostic capability and strengthen diagnostic activities in the EU were established. Five EURLs have been designated in the different disciplines (i.e., bacteriology, fungi and oomycetes, insect and mites, plant parasitic nematodes, and virology). One of the EURL activities is the validation of tests to make recommendations to the national reference laboratories. EURLs participate in the six EPPO panels on diagnostics, and validation data generated by these laboratories populate the EPPO database on diagnostic expertise.

Euphresco

To increase the collaboration among those organizations involved in plant health research activities at national and regional levels, Euphresco (European Phytosanitary Research Coordination, www.euphresco.net) was established in 2006 as an ERA-NET project funded by the European Commission. Euphresco has subsequently evolved into a self-sustaining international network hosted by the EPPO. The benefits of such coordination are multiple (Giovani et al. 2015). By fostering collaboration at the research level, Euphresco allows researchers to work on common problems. Euphresco goes far beyond Europe as members of the network come from five different continents.

Every year, Euphresco members identify research priorities to be tackled through transnational collaboration. Many research projects have been commissioned with the aim of developing new tests for the detection and identification of pests, validating diagnostic tests, or evaluating the proficiency of laboratories (examples of pests for which TPSs or PT have been organized include '*Candidatus* Liberibacter solanacearum', *Acidovorax citrulli*, *Xylella fastidiosa*, potato virus Y, Andean potato latent virus, *Ralstonia solanacearum*, and *Clavibacter sepedonicus* '*Candidatus* Liberibacter' spp. causing the Huanglongbing disease on *Citrus* spp.) (Giovani et al. 2019).

The coordination of national activities improves the use of resources allocated to plant health by avoiding duplication and favoring synergies. Synergies have also been pursued with other international initiatives and projects. Recently, the outbreaks of tomato brown rugose fruit virus in several countries pushed countries to validate the use of diagnostic tests. The VALITEST project organized a TPS to validate several tests on plant material, and a Euphresco project was initiated to validate several tests on seed of tomato and pepper.

International collaboration contributes to knowledge exchange, capacity building, and harmonization of best practices (including those with diagnostic aims). Projects have been conducted on DNA barcoding (including training sessions available online: https://youtube.com/playlist?list=PLoVf4Pt04Db53pUVTI8qwcWkWgUgg46gm) as well as on HTS.

The outputs of research projects have an impact beyond research activities, as they also support national policymaking and international standard setting and practices (Giovani et al. 2017).

RECOMMENDATIONS

- Research institutes, companies, and diagnostics laboratories developing tests are encouraged to use the VALITEST outcomes when performing validation studies.
- Resources needed to produce validation data in terms of both expertise and funds should not be underestimated as producing and sharing useful and reliable validation data can be complex.
- Communication between laboratories and other stakeholders is important. For example, as much relevant information as possible should be provided to the risk managers of an NPPO to help them make an informed decision when selecting tests to be used in, for example, surveillance and import inspection.
- Communication between laboratories performing validations and test providers is important to ensure reliable results.
- Reference material is essential for the evaluation of tests, and collections should be sufficiently funded and maintained to provide sufficient diversity regarding the target pests and also the "look-alikes" (species with which they could be confused).

- Research institutes, companies, and diagnostics laboratories developing tests are encouraged to provide validation data and make them publicly available.

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