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Review

International Cooperation to Support the Diagnosis of Forestry Pests: The Role of Eppo and Euphresco

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Abstract: Plant pests are a threat to biodiversity, food security and the economy. The challenges posed by the introduction of plant pests have triggered the establishment of cooperative mechanisms such as the International Plant Protection Convention (IPPC), an international treaty to protect plant health, but also of Regional Plant Protection Organizations (RPPOs) such as the European and Mediterranean plant Protection Organization. RPPOs participate in activities in their regions to promote and achieve the objectives of the IPPC. Because accurate and reliable detection and identification of pests are essential in order to be able to take appropriate measures against a pest and thus avoid or reduce the economic, social, and environmental costs that it can cause, the IPPC and EPPO have established a specific programme on Diagnostics. Diagnostic activities conducted at international level with a focus on the EPPO region are presented.

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1. Introduction

Plant pests are a threat to biodiversity, food security and the economy. Human activities play an important role in favouring the emergence and spread of pests, for example via the increased long-distance trade of plants and plant products, changes in cultivation practices or the cultivation of new plant species and tourism. Forests are also at risk, and losses due to the introduction of pests in forests have been highlighted in many review papers [e.g. 1, 2, 3]. The challenges posed by the introduction of plant pests (which has greatly increased in the last century) have triggered the establishment of cooperative mechanisms to protect agriculture, biodiversity and the economy from pests. These mechanisms include, the International Plant Protection Convention (IPPC), an international treaty to protect plant health adopted in 1951. The purpose of the IPPC is to coordinate joint actions to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control. The Convention includes provision for the establishment of Regional Plant Protection Organizations (RPPOs) which are inter-governmental organizations functioning on a regional level as coordinating bodies for National Plant Protection Organizations (NPPOs), i.e. the official services which are responsible for plant protection in countries. RPPOs coordinate and participate in activities in their regions to promote and achieve the objectives of the IPPC.

The RPPO for European, Mediterranean and Central Asian regions is the European and Mediterranean Plant Protection Organization (EPPO). It was created in 1951 and today, 52 countries are members of the Organization. Some of the key aims of the Organization are to:

- Develop an international strategy against the introduction and spread of pests (including invasive alien plants) that damage cultivated and wild plants, in agricultural and natural ecosystems .

- Encourage harmonization of phytosanitary regulations and all other areas of official plant protection action.
- Provide a documentation and information service on plant protection.

In order to achieve these aims, different activities are conducted. Because EPPO's work is focussed on pests presenting a risk to the Euro-Mediterranean and Central Asian regions, activities are conducted to:

- Identify pests that represent a risk before they reach the region or spread within it.
- Recommend phytosanitary measures to prevent entry or spread of such pests.
- Develop guidance on how pests can be detected, identified and controlled.

Since 1999, EPPO has maintained an Alert List (https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list) and provides information on pests possibly presenting a risk to EPPO member countries and achieve early warning. It is also used by EPPO to select candidates which are then submitted to a Pest Risk Analysis (PRA). The aim of PRA is to decide whether pests should be regulated as quarantine pest and propose risk management options [4]. A number of forestry pests are recommended for regulation by EPPO e.g. *Agrilus planipennis* (Emerald ash borer) and *Anoplophora glabripennis* (Asian long-horned beetle), *Bursaphelenchus xylophilus* (Pine wood nematode) and *Phytophthora ramorum* (the causal agent of sudden oak death).

Once a pest has been identified as presenting a risk for the EPPO region, recommendations are developed to assist NPPOs in conducting their activities including:

- Guidance on how and when to perform inspection at import and export and for surveillance of their territory.
- Guidance on how to detect and identify pests may be developed.
- Guidance on how to eradicate and control pests may also be developed to guide NPPOs in case an outbreak is detected.

Recommendations are approved by EPPO member countries and published as EPPO Standards known in the IPPC context as "regional standards".

Facilitating the cooperation in research on pests is also one of the functions of EPPO. As research does not pertain to NPPOs only, Euphresco, a network now including 75 important actors involved in the phytosanitary research (i.e. research funders, policy makers and research implementing organizations) has been developed and is hosted within EPPO since 2014. The goal of the network is to increase coordination of plant health research programming and support transnational collaboration. The benefits of this coordination are multiple and have been described in [1 & 2]. Many projects commissioned through the Euphresco network relate to diagnostic issues but also facilitate preparedness regarding pests (such as the project 'Tree Borers: risk assessment, risk management and preparedness for emerald ash borer and bronze birch borer (PREPSYS)[1]'. Participation in projects is possible through different mechanisms: in-kind contribution, alignment of existing research activities and ad-hoc funding from national funders that support participation of their national scientists.

Why is diagnostics important?

Accurate and reliable detection and identification are essential in order to be able to take appropriate measures against a pest and thus avoid or reduce the economic, social, and environmental costs that it can cause. Some diagnostic activities conducted at international level are presented below.

2. EPPO regional programme on diagnostics

2.1. Pest-specific Diagnostic Standards

[1] https://drop.euphresco.net/data/f4164235-1e51-4333-aabd-d89f341c43fd

The first initiatives in developing diagnostic protocols (= diagnostic Standards) were taken by EPPO in 1998 with the establishment of a specific programme on Diagnostics. It was recognized that rapid and accurate identification is key to any action to be taken on an imported consignment or in the case of an incursion in the region, and that harmonization of diagnostic procedures was important to ensure safe movement of plants and plant products. The first diagnostic protocols were published in 2001 [7] and as of May 2023, more than 150 diagnostic protocols have been approved. As the number of protocols to be developed in the different disciplines increased, the EPPO Secretariat suggested to its member countries that specific expertise was needed, and 5 Panels were established on different disciplines including the Panel on Diagnostics in Entomology in 2010. Panels are composed of diagnostic experts nominated by NPPOs of EPPO member countries. The composition of the EPPO Panels can be viewed on the EPPO Website (https://www.eppo.int/ABOUT_EPPO/eppo_bodies).

- Preparation of an EPPO diagnostic protocol

Diagnostic protocols provide the information necessary to detect and positively identify a particular pest. Each first draft is prepared by a team of experts with a lead author according to a common format. Although the lead author should preferably be a member of the Panel, other members of the drafting team do not have to be Panel members and can also be from outside the EPPO region. This is particularly valuable for protocols on pests which are not present in the EPPO region and for which expertise in the region is limited. Protocols on arthropods include detailed descriptions and keys for morphological identification but nowadays molecular tests are also included. Diagnostic protocols are illustrated by figures to support their implementation (e.g. Figure 1) and when needed flow diagrams presenting the diagnostic procedure are also included (e.g. Figure 2). Molecular tests included in EPPO diagnostic protocols are described in detail and the most relevant performance characteristics of the tests are included such as analytical sensitivity, analytical specificity, repeatability, and reproducibility. This information is important as many laboratories in the EPPO region are now required to be accredited for the tests they perform for official diagnostic activities. They need to demonstrate that they can perform the tests in accordance with the performance characteristics described. The number of molecular tests included in diagnostic protocols for arthropods has increased in the last decade and experts are also asked to investigate if 'quick tests' that can be used on-site with portable equipment, such as those based on isothermal amplification, are available and can be included. Horizontal protocols related to a specific method are also developed by EPPO. For example, PM 7/129 *DNA barcoding as an identification tool for a number of regulated pests* [8] describes tests that can be used for the identification of many pests and provides guidance on how to perform sequencing and data analysis.

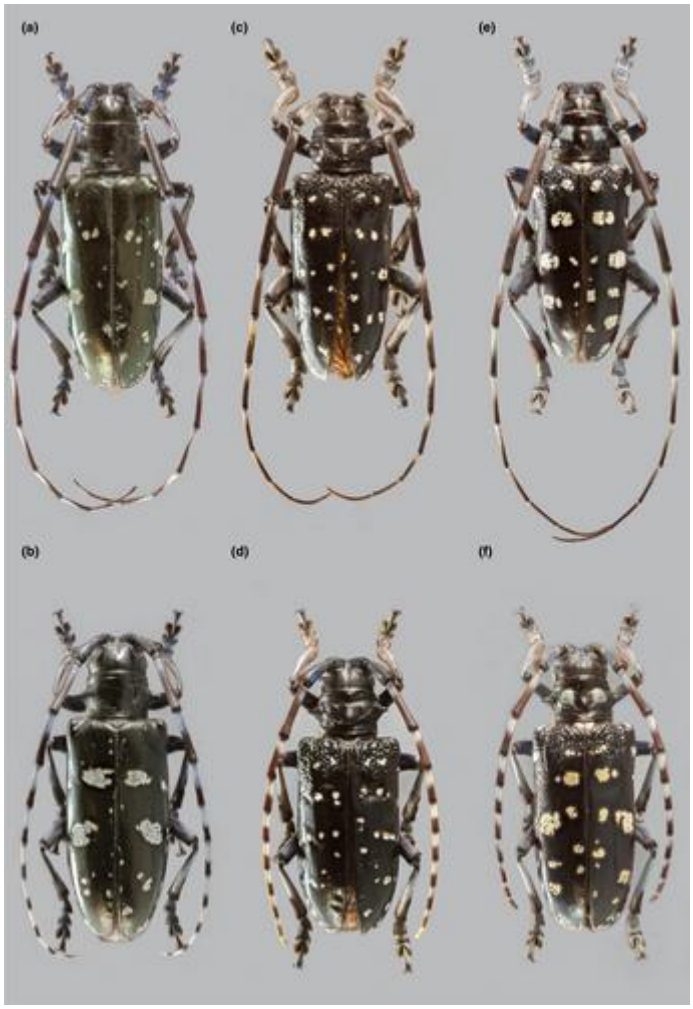


Figure 1. Habitus of adults: (a) ♂, (b) ♀ *Anoplophora glabripennis*, (c) ♂, (d) ♀ *A. chinensis*, (e) ♂, and (f) ♀ *A. chinensis* form *malasiaca*. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).).

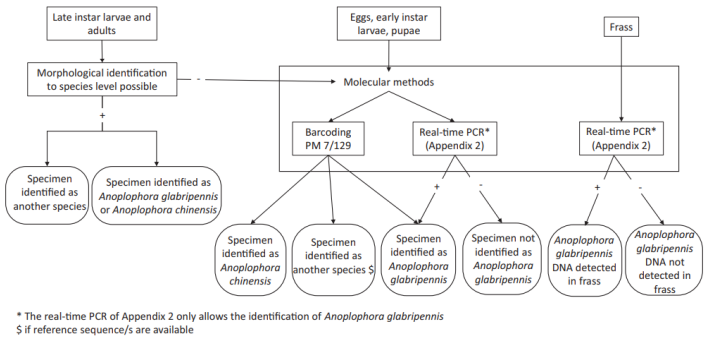


Figure 2. Diagnostic procedure included in the Diagnostic Protocol for *Anoplophora glabripennis* and *A. chinensis*.

- Review of the draft by a Panel of experts

When a first draft of a diagnostic protocol is considered ready by the drafting team it is presented during a meeting of the relevant Diagnostic Panel where it is critically reviewed. The drafting team will then revise the diagnostic protocol taking into account the comments made by the Panel. A revised version of the protocol is then circulated again to the Panel which decides if the protocol is ready to be sent for a formal consultation during which all EPPO member countries have the opportunity to express their views.

- Consultation of EPPO member countries

The approval procedure of EPPO diagnostic protocols is presented in Figure 3. Pest specific diagnostic protocols are approved following a variation from the normal approval procedure. More details are available in the 'Procedure for preparation and approval of EPPO Standards' [9]. Comments received from EPPO member countries are reviewed by the drafting team and the Panel and a revision prepared. This revision is then sent for a last consultation where the 52 EPPO member countries are given the possibility to present an objection if the modifications made after the first consultation are not acceptable.

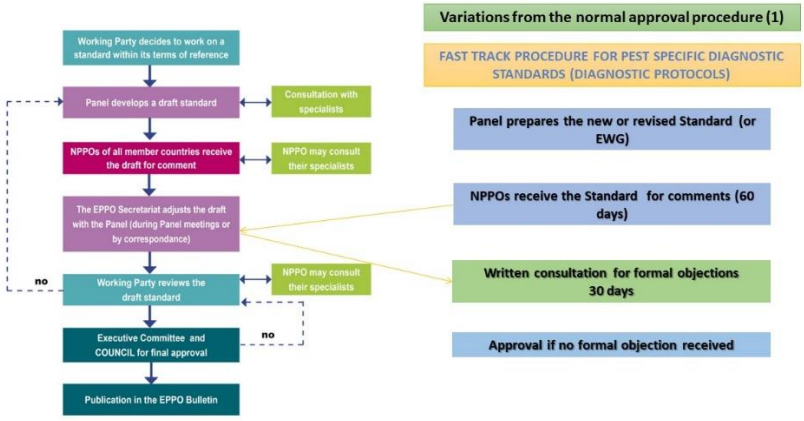


Figure 3. EPPO approval procedure including the variation regarding Pest specific diagnostic protocols.

- Approval and publication

If no objection is received during the last stage of consultation, the diagnostic protocol is approved and published in the EPPO Bulletin. All approved diagnostic standards can also be retrieved from the EPPO Website (https://www.eppo.int/RESOURCES/eppo_standards/pm7_diagnostics) and via the EPPO Global database (https://gd.eppo.int/standards/PM7/). As of May 2023; there are more than 150 diagnostic standards approved.

2.2. Standards on Accreditation and quality management for plant health laboratories

In addition to pest specific diagnostic protocols, EPPO is also developing Standards on horizontal issues such as accreditation and quality assurance.

Since 1999, there have been several discussions on the possibility that EPPO should assist national diagnostic laboratories in obtaining accreditation. It was concluded that EPPO could develop a quality assurance standard for diagnostic laboratories, but that accreditation can only be provided by an official (national) outside body. In order to support its members, EPPO has developed two diagnostic Standards regarding quality assurance and accreditation. The first Standard on quality assurance (PM 7/84 *Basic requirements for quality management in plant pest diagnosis laboratories*) was approved in 2007 and last revised in 2021. It includes the general quality management requirements needed to perform diagnostic tests for plant pests in a laboratory. The second Standard, PM 7/98 (*Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity)*, includes specific quality management requirements for laboratories preparing for accreditation according to the ISO/IEC Standard 17025 General requirements for the competence of testing and calibration laboratories (references to relevant parts of ISO/IEC Standard 17025 are included). Such a Standard was considered particularly useful to promote a harmonized interpretation across the region (it has subsequently been revised 4 times as more experience was gained and due to revision of the ISO/IEC Standard 17025). In 2009, a joint communiqué was signed by EPPO and The European Co-operation for Accreditation (EA) stating that *'EA will recommend that assessors from Accreditation Bodies take note of EPPO*

documents when evaluating plant pest diagnostic laboratories'. A revised version of this communiqué was signed in October 2018. EA is the European network of nationally recognised accreditation bodies located in the European geographical area.

EPPO has also developed Standards on the organization of interlaboratory comparisons (PM 7/122,) and on the production of biological reference material (PM 7/147).

2.3. Other EPPO initiatives in the field of diagnostics

- EPPO databases on diagnostics
- EPPO database on diagnostic expertise

In 2004, EPPO Council stressed that the implementation of phytosanitary regulations for quarantine pests was jeopardized by decreasing knowledge in plant protection and made an official declaration (Plant Health Endangered, also known as the 'Madeira declaration'). The Panel on Diagnostics proposed that an inventory should be made of the available expertise on diagnostics in the region. A database on Diagnostic Expertise (https://dc.eppo.int/) was then created to allow identification of experts who could provide diagnosis of e.g. regulated species or pests possibly presenting a risk to EPPO member countries (EPPO Alert List) or help in the identification of new species or those that are difficult to identify. Approximately 700 experts from more than 100 diagnostic laboratories in the EPPO region have provided details about the pests they can diagnose and the methods they use. The database also provides an inventory of validation data (as referred to in PM 7/98) for tests targeting the above-mentioned pests.

- EPPO-Q-bank

EPPO-Q-bank (https://qbank.eppo.int/) is composed of 7 curated databases covering different disciplines (e.g. entomology, mycology). Each database comprises genetic sequences (e.g. barcodes) of quarantine pests and their look-alikes. Initiated as a Dutch project, it has been hosted by EPPO since 2019. EPPO-Q-bank. The cornerstone of these databases is their curation by a team of scientists with taxonomic, phytosanitary, and diagnostic expertise from National Plant Protection Organizations and institutes with connections to relevant phytosanitary collections. Most strains, isolates or specimens, from which sequences are included in EPPO-Q-bank were obtained from physical collections. EPPO-Q-bank databases also provides valuable information about these specimens, strains or isolates, information about barcoding and sequencing methodologies and tools to perform single and multi-locus blast searches. As of May 2023, the EPPO-Q-bank databases include information on more than 2 000 species, 9 500 specimens, strains or isolates and 25 000 sequences.

- Workshops and conferences

EPPO regularly (co-) organize(s) workshops and conferences on diagnostics to enhance collaboration between laboratories and networking, to share good practices and tackle specific diagnostic issues. For example, workshops for heads of plant pest diagnostic laboratories are regularly organised to discuss, at the management level, horizontal diagnostic topics such as quality assurance, accreditation under ISO 17025, and proficiency of laboratories. Specific technical workshops have also been organized on various topics such as the maintenance of collections and the use of high throughput sequencing for plant pest diagnostics. Finally, training courses are also organised, sometimes in the framework of specific projects (e.g. PRACTIBAR a project on barcoding, VALITEST an EU funded project on the validation of plant pest diagnostic tests). In 2022, The EPPO Secretariat and the IPPC Secretariat co-organized a dedicated session entitled 'Plant pests diagnostic: its importance and its relation to food security' during the first International Plant Health Conference (London 2022-09-21/23).

3. Diagnostics at Global level: IPPC programme

3.1. Pest specific diagnostic protocols

The need for harmonization of diagnostic procedures is not only valid at regional level but also at global level. In reviewing global needs for plant protection in 2003, the Commission on Phytosanitary Measures (governing body of the IPPC) recognized that countries had a strong interest in international diagnostic protocols [10]. It considered in particular that international diagnostic protocols would provide harmonized detection and identification procedures worldwide and contribute to greater transparency and comparability of diagnostics of regulated pests. The IPPC has consequently established a diagnostic programme. To date 31 diagnostic protocols have been approved at global level (See ISPM 27 and its annexes; https://www.ippc.int/en/core-activities/standards-setting/ispms/). IPPC diagnostic protocols are developed in a comparable way to EPPO regional protocol. It is often the case that regional diagnostic protocols are the basis for the development of IPPC diagnostic protocols (e.g. the EPPO Diagnostic Protocol on *Phytophthora ramorum* was used to develop the IPPC one). EPPO is not the only RPPO developing diagnostic protocols (e.g. the North American Plant Protection Organization and the Comité de Sanidad Vegetal (COSAVE) are also developing diagnostic protocols for their members which are used as a basis for IPPC diagnostic protocols.

3.2. Development of a network of diagnostic laboratories

The development of diagnostic protocols requires input of experts on the pests, and the subsequent implementation of these protocols also requires skilled diagnostic staff. The IPPC Strategy 2020-2030 [11] recognizes diagnostic expertise as one of the major capabilities for the proper functioning of any NPPO and proposes that the Commission on Phytosanitary Measures (governing body of the IPPC) helps to address the lack of access to diagnostic capacity in many countries by establishing a voluntary network of diagnostic laboratories. The development of this network is in progress.

4. Euphresco activities

Harmonization in standard setting is complicated by the diversity of tests used in different countries, which often have comparable performances and have been used for many years. This diversity explains why consensus on international Standards is often only found on a minimum common basis. In order to enhance harmonization and adoption of common practice early on in the laboratory activities, international collaboration and coordination of research that underly the official diagnostic activities has been promoted in recent years. Since 2006, Euphresco has supported the coordination of plant health research programming activities that support policy-making in the EU, facilitated exchange of knowledge and capacity building on research matters, and allowed international collaboration between countries that share the same interests.

Starting as a network of several western European countries, Euphresco has now developed into a network of 75 organizations from more than 50 countries worldwide.

Diagnostics is an important activity in Euphresco research projects. More than 80 research projects (out of more than 120 projects commissioned through Euphresco so far) have a diagnostic component. Euphresco projects have allowed test development and the organization of test performance studies and proficiency tests. The projects are used to support capacity building, exchange of reference material, and are also an opportunity to create better links between researchers and policy makers and to engage with the end-users of research (e.g. farmers, nursery workers). As an example, the project 'Developing and assessing surveillance methodologies for *Agrilus* beetles[2]allowed information to be gathered on existing surveillance protocols and for novel methods to be evaluated.

The success of Euphresco as a primarily European network for phytosanitary research coordination has set the ground for discussions on the development of initiative(s) to address the needs of other regions of the world and global phytosanitary research coordination. In 2019, during the Meeting of the Agricultural Chief Scientists of the G20

[2] https://drop.euphresco.net/data/68aca0f8-1b49-436a-8d7d-e0c5445119e3

(MACS), the need '*to strengthen research collaboration to develop effective measures against transboundary pests and to participate in joint funding networks such as Euphresco*'[12] was noted. In 2020, the article 'Science diplomacy for plant health'[6], co-authored by experts from the IPPC, several RPPOs, NPPOs, research coordination networks and research organizations, highlighted the need for '*a global network for phytosanitary research coordination that can shape research agendas across countries and accelerate the development of science to support regulatory phytosanitary decision makers*'. In 2021, the IPPC Strategic Framework 2020-2030 [11] was adopted at the 15th session of the Commission on Phytosanitary Measures (CPM-15). Global phytosanitary research coordination is one of the development programmes identified, and it is expected that by 2030 '*possibilities for establishing an international research collaborative structure have been explored and, if appropriate, the structure has been established*'.

While Euphresco has been effective in Europe and has attracted non-European members, its current structure and operations limit a full engagement; moreover, there are research areas that are under-represented. Euhresco members are currently reflecting on how to clarify the role of Euphresco in the global plant health research context and how to adapt its structure and operations to better serve a wider and more diverse membership. It will hopefully result in a stronger network with a broader audience and greater impact in terms of plant health research.

European Reference Laboratories and their contribution to the EPPO Diagnostic programme

The 52 Countries members of EPPO include the 27 countries of the European Union (EU). In order to support a harmonized implementation of several EU regulations including plant health rules, a regulation (Regulation EU 2017/625 [13]) was approved in 2017 regarding official controls and official activities. In this context, Plant Health European Union Reference Laboratories (EURLs) were established in 2019. They are key players in the surveillance for and prevention of the introduction of pests, guaranteeing high-quality and uniform testing e.g. for early detection and diagnosis of emerging pests, and supporting the EU Commission activities on risk management and risk assessment. Collaboration between EPPO and EURLs is considered essential to create synergies between the EPPO Diagnostic Programme and activities conducted by the EURLs. Experts from the EURLs are members of the EPPO Diagnostic Panels participate actively in the preparation and revision of diagnostic protocols. For example, they provide validation data from TPS they organise, but also useful feedback based on the outcomes of Proficiency Tests they organize with EU National Reference Laboratories.

Conclusion

Activities on diagnostics have been and continue to be of prime importance for EPPO members and have been one of the main areas in which the Organization has made its mark in recent years, in its publications and on its website. A very valuable international resource has been created, and a basis has been laid for active cooperation between EPPO countries in the provision of diagnostic services. Euphresco research activities provide scientific evidence to support EPPO Standard setting.

Looking to the future of diagnostics

High-throughput sequencing (HTS), also known as next generation sequencing (NGS) or deep sequencing, is one of the most significant advances in molecular diagnostics since the advent of the PCR methods in the early 1980s. It is a good example of a new technology which is now being taken up by diagnostic laboratories. Compared to more traditional methods, HTS can potentially detect the nucleic acids of any organism present in a sample without any a priori knowledge of the sample's phytosanitary status [14, 15] and so, opens new possibilities and opportunities in routine diagnostics [16]. A recommendation on 'Preparing the use of high throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes' was adopted by the Commission on Phytosanitary Measures in 2019 [17]. This recommendation encourages the development of

best-practice operational guidelines covering result and quality control measures for HTS that 'ensure HTS data outputs are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way, test validation and quality assurance' [17]. In addition, it highlights the need to validate HTS tests. In line with this recommendation, an EPPO Standard was developed based on an outcome of the EU funded VALITEST project [18] and describes specific elements to take into consideration for the use of HTS tests in laboratories [19].

Remote Sensing is another technology that could radically change the way NPPOs work. Currently, inspections are mainly targeted, for example the inspection of commodities arriving at ports or airports or of places of production. General surveillance of forests and natural areas is also performed but requires a lot of resources both from the NPPOs and from other bodies and stakeholders involved such activities. Remote sensing, by satellites, manned aircraft or drones, facilitates the work of image acquisition, which is carried out in real time, and the processing of information on both a small and large scale. Remote sensing has the potential to guide surveillance activities on the ground without having to visit a site in person, and analysis of specific spectral bands can in some cases detect stress potentially associated with the presence of a disease before plant symptoms are visible to the naked eye [20]. This allows inspections to be better targeted. Although remote sensing has already demonstrated its potential to strengthen the surveillance for important pests such as *Bursaphelenchus xylophilus* [21], there are however few operational applications to date in the field of plant health. Barriers to implementation have been identified [22] and transdisciplinary collaboration will be needed in the next years to foster the operational use of remote sensing to support plant health surveillance activities.

Although new technologies offer new possibilities, it is essential that classic expertise (including taxonomy) is maintained and new expertise developed, that bridges between disciplines are built or strengthened and that data and information is shared and made available for use and re-use. International organizations will have an essential role to play to ensure collaboration and harmonisation, and that resources are used in the most efficient way possible to protect forest health worldwide.

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