Phytosanitary diagnosis and collections
White paper of Q-collect

Introduction

International trade and movement of plants and plant products into and from Europe has increased tremendously in recent years leading to higher numbers of consignment imported. The type of commodities imported and the origins have also diversified. As an illustration; imports of woody plants from Asian origins have become more frequent than in the past where most woody plants were traded between European countries. As a result, the rate of introduction and establishment of new organisms in Europe has increased steadily as well as the phytosanitary risk of introducing such new organisms.

Organisms relevant in plant health include plant pathogens, arthropods and invasive plants. Some may have already been identified as posing an unacceptable risk to agricultural and horticultural crops, forests and the wider environment and currently approximately 250 organisms are included in the Annexes of the Directive 2000/29/EC as pests of the European Union (also called quarantine pest lists). However, it should be noted that some of the pests recently introduced in Europe had not been identified as posing an unacceptable risk before they established in Europe. Consequently the phytosanitary vigilance to detect unknown pests needs to become more and more efficient.

As plants may be latently infected, inspection of consignments and of places of production of plants and plant products often involves laboratory analysis of samples collected during these inspections. In outbreak situations the accurate identification of the causal agent relies on laboratory testing as well. In all these situations the capability of National Plant Protection Organizations (NPPOs) and the affiliated laboratories to quickly and reliably detect and identify organisms is critical for effective phytosanitary measures to be taken. Plant pest diagnostic is also essential to support the phytosanitary certification of consignments of plants and plant products exported from Europe.

In order to better ensure the reliability of diagnostics, laboratories are increasingly working under quality assurance systems (including accreditation, ISO 17025) and this is becoming a requirement of National Plant Protection Organizations for laboratories performing official tests.
Under such circumstances, laboratories need to have access to well characterised biological reference material for morphological identifications and the use for development and validation of tests. Moreover, in tests biological reference material is needed as a base for positive and negative controls.
Validation of tests is carried out to provide data on the performance of a test and in particular regarding its analytical sensitivity and specificity. Reproducibility of a test is also essential and is often determined in test performance studies. In order to be able to establish these performance characteristics, well-defined biological reference material is needed for the organism to be identified with the test but also for those organisms with which the target organism may be confused with (look-alikes). Availability of biological material coming from reliable, curated collections is thus essential for test development and validation. In order for laboratories to confirm its competence to correctly perform tests, participation in proficiency testing schemes is recommended. Although offers to participate in proficiency testing are currently still limited in the plant health sector, they are increasing. Laboratories organizing proficiency testing need to have access to well-defined biological material of the target organism but also material of look-alikes.
Finally biological material is also essential to ensure the quality of analyses at different levels: for each diagnosis as well as for global quality control of the laboratory. Although molecular tests are based on nucleic acid sequences, biological material is essential for the development, validation and quality assurance of these tests. Reliability of the sequences on which the tests are based depends on the correct identification of the original biological material.
The biological material also needs to be kept in collections as a current source of reference DNA. New molecular technologies like Next Generation Sequencing (whole genome sequencing) will increase new taxonomic insights in essence on the short term for bacteria, viruses (viroids) and fungi. Therefore it is essential to be able to re-examine the original biological material. Biological material is also needed for quality control of nucleic acid

isolation. Biological material is kept in collections and these should provide appropriate guarantee regarding this material in term of quality.

New legislations

The Nagoya Protocol

The Nagoya Protocol on 'Access to Genetic Resources and the Fair and Equitable Sharing for Their Utilization' was adopted in 2014 and will have impact on all researchers working with biological resources[1]. It is a legal binding instrument for the implementation of the Convention on Biological Diversity (CBD). The CBD has three main goals i) the conservation of biological diversity; ii) the sustainable use of its components and iii) the fair and equitable sharing of benefits arising from its utilization. Basically the CBD recognises the sovereign rights of countries over their own biological resources and the so-called genetic resources contained therein. Access to genetic resources in a country that is party to the CBD (i.e. a country that has signed and ratified the CBD) requires prior informed content (PIC) from the competent authority in that country and a setting of mutually agreed terms (MAT) between provider and user. Parties are free to decide whether access to genetic resources is subject to such requirements or not. But the Party must assure that, within their territory, genetic resource, originating from other Parties (countries) are handled according the CBD and that benefits arising from the use of the genetic resources or traditional knowledge associated with these resources are shared fairly and equitable.

The Nagoya Protocol aims to provide guidance for the parties to implement the CBD's fair and equitable sharing of benefits arising from the utilization of the resources by adopting their national access and benefit sharing (ABS) legislation. The biological material (including DNA extracts) held in collections is covered by the Nagoya Protocol. This Protocol is now going to be implemented in the EU [2] and guidelines are being prepared and are currently under consideration by different groups. The objective at European level is an implementation of the Nagoya Protocol on the 12th of October 2015. It should be noted that this legislation seems to create conflict with obligations under the IPPC, for example it is not clear if specimens isolated from imported consignments can be stored and used for diagnostic and research purposes. This needs to be clarified.

New EU control directive

For EU member states, a major change in the organization of plant pest diagnostic laboratories performing official diagnostic will happen with the revision of the EU Regulation No. 882/2004 on official controls. The new Regulation proposal of the European Commission will extend the scope of the above Regulation to plant health. One of the changes foreseen will be the establishment in the plant health sector of a network of official laboratories, National Reference Laboratories (NRL) and EU Reference Laboratories (EU-RL). For these laboratories performing tests the requirements will include accreditation and the use of standardised methods. The EU-RL will (among other tasks) play a dominant role in elaboration and harmonization of tests, analyses and diagnosis and providing reference methods, providing reference material, organisation of test performance studies and proficiency testing. The EU-RL therefore needs (the access to) a reference collection. Therefore the need of well-established reference collection will increase even more in the coming years.

Current situation

Results of Q-collect project

The survey organized during the Q-collect EU funded project confirmed that although there is a significant number of plant pathogens and pest collections still present within Europe, they are dispersed, widespread and of very variable quality.

The main findings of the project are as follows:

- Most collections are working or research collections and only few of these collections are likely to be organized to provide services to outside users.
- There is a limited number of information on collection's holdings available online.
- Many collections have no quality system in place, and accreditation of collections is scarce.
- Sharing of material between collections to ensure resilience is not common and can be considered as a high risk for loss of important biological material in case of incidents with buildings or equipment.

[1] For more information see https://www.cbd.int/abs/about/default.shtml/

[2] For more information see regulation (EU) No 511/2014.(http://eur-lex.eruopa.eu/legal-content/EN/EN/TXT/?uri=celex:32014R0511)

- Appropriate basic funding is not secured enough and there is a need for a common policy towards collection management throughout the region.

Achievements of the project

- Guidelines have been drafted on Quality assurance for reference collections and will be further developed with Panels for each group of organisms in order to prepare an EPPO Standard.
- The Project proposed criteria for the establishment of a sustainable network. This network will bring together previously dispersed information on biological material and stimulate collaboration. The criteria will be reviewed in the EPPO framework. The Project recommended that an online platform should be established and maintained to stimulate and facilitate networking and data sharing. This platform will improve accessibility and visibility of biological material (and related information) available in collections. A pilot platform will be established to include a deposit form for information on biological material, a search engine and links to additional relevant information available online.

Other initiatives

Online access to collections can facilitate the availability of collection. Since 2010 a database called Q-bank has been elaborated. This database links information, sequences and collections of the most important organisms (Q-organisms, look-alikes and other important organisms). The database can be used for diagnosis, but gives also the possibilities of finding organisms in collections. This initiative originally started as a Dutch project. The EU funded project QBOL has been using Q-bank to serve as a repository of DNA sequences. Nowadays this database is maintained by a group of international scientific curators responsible for their discipline. In the past 4 years the Dutch government has supported this group and the database was further elaborated. During the Q-collect project more work on this database has been done especially on quality criteria.

Future

Goal for the future: for every organism group/discipline there are at least at 2 locations official reference collections and through online catalogue(s) these reference collections can be accessed by interested users to search and order material.

All the initiatives of the past years (Q-bank, QBOL, Q-collect, etc.) have given a boost to the phytosanitary collections. Much energy, knowledge and hard work has been given to the collections. The challenge is to keep up the good work and follow this road. In the coming years it is unlikely collections will be supported with big additional budgets or project funding. However, a database like Q-bank has proved that it is possible to connect specialists and their collections on an online platform. Other projects have proved that the network of phytosanitary specialists is strong and people are willing to collaborate. In the framework of Euphresco there are many international co-operations especially between official NPPO related laboratories. The laboratories in the EPPO region do not need a reference collection in every country or at every laboratory, but they do need access to these collections. By connecting collections online and clustering collections an international coverage for reference materials can be achieved. With quality systems in place the collections can offer reliable, well defined reference material. The new EU regulation with extra demands on reference material and the forming of EU-RL's can help to speed up this movement. Official collections as part of or related to an EU-RL and partly financed by the EU provide possibilities. Through co-operations like EUPHRESCO an even distribution of the reference collections should be possible.

Recommendations

To reach the above mentioned, first steps should be taken in the near future. The need to improve the infrastructure supporting phytosanitary collections and use them more efficiently and improve collaboration will be the main ingredients.

A:Priority to making/maintaining inventories

It is essential to be able to know which and where biological material is available. Collections should make an inventory of their holdings and provide this information in a format that could allow sharing on a platform.

B: *Establish a long term sustainable online platform*
Encourage and support the creation of a long term single platform to access the information. Choose the online platform to be used and assure a long time hosting.

C: Improvement of Quality systems for collections
The implementation of quality systems in collections is essential to ensure that proper reference material is used in the development and performance of tests. The upgrade of working collections should be a short term objective.

D: Establish networks for collections
Networks of collections should be established in order to share the responsibility of maintaining reference material and also for duplication purposes whenever necessary. When quarantine organisms are involved the network should have a link with Euphresco..

E: Establish a common policy for reference material
A common policy should be established for developing and maintaining reference material among member states as one of the basic requirements for a sustainable network of National Reference Laboratories and EU Reference Laboratories in the new EU control directive on official controls. Exchange of material and concentration of material in yet well-established collections will be a first step.
In case of outbreaks it is essential that not only information on the location of collections is accessible but also prompt and organised/regulated exchange of material is possible. Therefore a common policy for issuing of material and shipment in compliance with legislation should be developed.

F: Nagoya Protocol
Consequences for plant health related collections should be clarified and in case of conflict with plant health legislation, derogations should be sought.

The basis for the above steps originate from the different collection projects. Now there is a strong need for further implementation from within our field and our organisations.