Euphresco
Network for phytosanitary research coordination and funding

Final Report

VirusCollect

Fulfilling the need for a common reference collection of plant viruses and viroids

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Contents

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2. Executive Summary

Project Summary

VirusCollect: Fulfilling the need for a common reference collection of plant viruses and viroids

Background
Plant diseases, including many plant viruses, are a continuous threat to the cost effective and safe production of food and cause significant losses in yield and quality of many important crops. It is therefore essential to minimise the impact of plant diseases through an effective and coordinated system of legislation, plant passports and testing laboratories. In such system, reliable and cost-effective diagnosis methods for plant viruses are essential. These only can be developed, validated and implemented when suitable reference materials are available. Historically, reference materials were supplied from collections maintained by European universities and research institutions. However the maintenance of such collections had come under severe pressure due to a decrease in the number of scientists (virologists in particular) and allocated budgets. For plant viruses and viroids, type isolates (if still available) were dispersed between different public and/or private collections (Roenhorst *et al.*, 2013). The Euphresco project VirusCollect aimed to establish a common reference collection of viruses and viroids by linking collections of individual institutions via Q-bank.

Quality standards
To establish international collaboration on virus collections between National Plant Protection Organisations and allied institutions, basic requirements had to be fulfilled. Standard operating procedures (SOP's) were developed and implemented in participating laboratories to guarantee the quality of isolates and data, i.e. on characterisation and inclusion, maintenance, release and production of reference materials (Figure 1).

Q-bank Viruses and Viroids database (http://www.q-bank.eu/Virus/)
The Q-bank Viruses and Viroids database provides information on the nature of viruses and viroids. To ensure better accessibility to the public, the design and contents of the database were improved. Over 1000 virus species were included and relevant information for each species is now provided. EU-regulated species and/or species recommended for regulation were marked as such and linked to the EPPO Global Database whenever possible. The inclusion of additional data and corresponding nucleotide sequences will allow provisional identification of 'unknown' virus isolates using the 'search' and 'BLAST' functions of Q-bank. To further extend the usability of the database specific search options were included and information on the availability of over 500 specific virus isolates in Q-bank can be easily obtained. These options are of interest for both scientists (plant virologists, breeders) and policy makers.

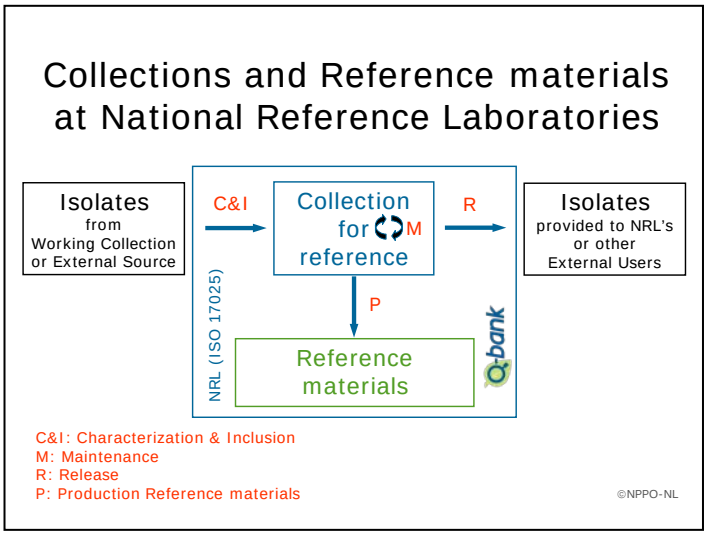

Figure 1 Interrelationships of standard operating procedures for characterisation & inclusion, maintenance, release and production of reference materials.

Characterisation of isolates

Within the VirusCollect project, 135 virus isolates were characterised and/or made accessible and corresponding data included in Q-bank. These include isolates of phytosanitary and/or economically important virus species already present in collections, as well as 'new' and 'emerging' species. Biological and serological properties were determined and sequence data generated. Characterised isolates were adequately stored to maintain their viability and guarantee their availability for future reference and use. VirusCollect enabled the first step in the collaboration and standardisation of virus collections, resulting in common views on quality standards as a prerequisite for the establishment of an international network. These fundamental standards laid the basis for improving the quality of individual collections as well as the layout of Q-bank as a platform to share data and information. The achievements towards a common reference collection were appreciated and resulted in a follow-up Euphresco VirusCollect II project, in which eight countries have expressed an interest to join.

Main conclusions

1. The project VirusCollect improved the quality of both the infrastructure and 'contents' of virus collections at participating laboratories by development and implementation of SOP's and characterisation and inclusion of relevant isolates.
2. Q-bank Viruses and Viroids database plays a key role in sharing data, information and isolates, and as such is an indispensable tool for linking virus collections.
3. The best way to improve the 'contents' of collections is to focus on the 'spin-off' of other projects by securing data and isolates
4. The interpretation of the GMO legislation, the Convention on Biodiversity, in particular the Nagoya protocol, need attention at an international level due to the foreseen impact on the sustainability of existing collections, in particular on the availability and exchange of relevant reference materials.

References

Roenhorst JW, Boonham N, Winter S, Menzel W, Van der Vlugt RAA (2013) The plant viruses and viroids database and collections of Q-bank. EPPO Bulletin 43, 238–243.

3. Report

3.1 Introduction

Plant virus and viroid collections in Europe
During the last few decades, the maintenance of collections of plant pathogens and pests has come under severe pressure due to a decrease of scientists and available budgets. For plant viruses, viroids and phytoplasmas, type isolates, if still available, are dispersed between different scientific and local working collections (Roenhorst et al., 2013). In contrast to collections of arthropods, bacteria, fungi, nematodes and invasive plant species, there is no obligation to deposit type material (type isolates) of (newly) described virus species in an official curated collection. Moreover, in Europe there is only one publicly available collection, Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, that meets quality criteria for depositing plant viruses and viroids. At DSMZ, however, only a small portion of the currently listed quarantine organisms is available and as a consequence reference isolates of those viruses and viroids are not easily accessible for virologists working in research and diagnostic laboratories.

Quality control and accreditation
An increasing need for quality assurance of test procedures in diagnostic laboratories performing official testing in plant health, was the reason for EPPO to start drafting standards in this field, i.e. PM 7/84(1) *Basic guidelines for quality management in plant pest diagnosis laboratories* (EPPO, 2007), PM 7/98(2) *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* (EPPO, 2014a) and PM 7/122(1) *Guidelines for the organization of inter-laboratory comparisons by plant pest diagnostic laboratories* (EPPO, 2014b). These guidelines made clear that the use of quality controls and validation of tests is essential to demonstrate the reliability of a diagnosis, which is particularly important when official action is taken as a follow up of a diagnostic result. During implementation of Standard PM7/98, however, many laboratories experienced significant difficulties in obtaining isolates and adequate reference materials. Laboratories needed positive controls to monitor the validity of tests in daily use. In addition, for validation they needed all known variants of the target organism as well as its relatives and look-alikes. In practice often the majority of the time needed for validation was spent trying to collect relevant specimens, often without success.

Also in plant health policy, the need for reference material is becoming more prominent (EC, 2009). On an EU level, discussions are being held on the establishment of a network of national and community reference laboratories (European Union Reference Laboratories (EURL's)), similar to those in the domains of animal health and food and feed. These reference laboratories in plant health will become responsible for the availability of reference materials (positive controls), validated tests and the organisation of proficiency tests for specific species or groups of organisms. For all these tasks a reference laboratory will need specimens and/or isolates from physical collections.

Q-bank virus collections and reference data

In addition to the role of reference material from a quality perspective, advances in molecular biology also revealed its importance for taxonomy and diagnostics. Nowadays, for taxonomy, sequence data are indispensable for identification of (new) virus and viroid species. In addition, the number of molecular tests used for detection and/or identification is still increasing. Unfortunately, sequences deposited in NCBI GenBank are not always correctly annotated. It is clear that fully exploiting the use of molecular techniques in both taxonomy and diagnostics relies on the availability of isolates whose identity has been confirmed by experts so that their (sequence) data can be used for reference. From this perspective, Q-bank, a comprehensive database on quarantine plants pests and diseases (http://www.q-bank.eu), is providing an excellent platform to share data and information on available isolates and materials. The Q-bank database was the outcome of a Dutch project to strengthen the infrastructure in plant health, and was financed by the Ministry of Economic Affairs. Also data generated within the EU project QBOL on barcoding of quarantine organisms (http://www.qbol.org), were deposited in Q-bank, thereby further strengthening its position. Q-bank thus provides an excellent platform to share data on reference materials and to make them publicly available.

Euphresco VirusCollect project

The Euphresco VirusCollect project, of which the results are described in this report, aimed to establish a common reference collection by linking collections of individual institutes and/or countries via Q-bank to make them publicly available. To guaranty the quality of data and reference material, common standards on characterisation, handling and storage were needed. The implementation of such standards would allow laboratories to share responsibilities and to focus on specific genera or groups of viruses. Basically each participant of this project worked on selected species of their own interest, thereby respecting the agreed requirements for identification and preservation. Finally, data and isolates were made publicly available via Q-bank and the collections linked to this database.

Starting with the objectives and tasks of the project, this report will describe the methods used and results obtained for each work package. The results will be discussed in general, providing the main conclusions on the implications and dissemination, and recommendations for future work.

3.2 Objectives and tasks of the project

The Euphresco project VirusCollect aimed to establish a common reference collection of viruses and viroids* by linking collections of individual institutes or countries via Q-bank to make them publicly available.

To achieve this goal the following activities were described in the proposal:

1. Define the desired contents of Q-bank within this framework (what to be achieved and what not)
2. Draft standards for Q-bank-linked collections: to characterise (identify), handle and store reference material; to deliver data to Q-bank
3. Identify gaps in data and specimens in present (including hidden) collections
4. Draft overview of genera and/or species of interest and divide work over participants (collection of missing species, characterisation, etc)
5. Implement standards and perform work at participating laboratories
6. Deliver data to Q-bank and isolates to Q-bank-linked reference collections

*In this report viroids are included in the term viruses and will not be mentioned separately.

3.3 Methods and results obtained

To address the different activities, the work has been divided over different work packages.

1. Coordination, in particular project and topic descriptions, division of work, planning and reporting.
2. Identification of gaps in Q-bank with regard to regulated and emerging viruses and viroids.
3. Description of quality standards for Q-bank data and maintenance of isolates in collections to be implemented in participating laboratories.
4. AT-AGES: Description of deliverables, i.e. indication of virus species, type of data, type of reference materials, etc., based on needs, expertise and interests of the institute.
5. DE-JKI/DSMZ: Description of deliverables, i.e. indication of virus species, type of data, type of reference materials, etc., based on needs, expertise and interests of the institute.
6. NL-NVWA/PRI: Description of deliverables, i.e. indication of virus species, type of data, type of reference materials, etc., based on needs, expertise and interests of the institute.
7. UK-SASA: Description of deliverables, i.e. indication of virus species, type of data, type of reference materials, etc., based on needs, expertise and interests of the institute.
8. Deliverance of data to Q-bank database and isolates to Q-bank-linked reference collections.

The work packages 1, 2, 3 and 8 are of general concern, the others focus on the specific work done at the different institutes. Details on the methods and results are described for each work package in the following paragraphs.

3.3.1 Work package 1

Coordination of the VirusCollect project

The topic coordinator drafted the topic description and general work plan. The other partners, provided details on the work they planned to do on particular virus species and/or isolates (See WP 4-7). In addition the topic coordinator ensured the adjustment with Q-bank Plant Viruses and Viroids database and the EU project Q-collect.

To present the work and discuss the achievements on the general topics, two meetings were organized, the first at DSMZ in Braunschweig (24-25 June 2014) and the second and final meeting at the NPPO in Wageningen (29 September – 1 October 2015).

During these meetings, each participant presented the work done at the particular institute. In addition, more general topics like definitions of terms, guidelines for quality management, minimum requirements for collection and reference materials, desired development of Q-bank and the inclusion of data in Q-bank were discussed. Several options for dissemination of the achievements were launched. Furthermore, future possibilities for promoting, extending and ensuring the continuity of the newly established ‘network of virus collections’ were discussed. For reports of these meetings see Appendix 1 and 2.

3.3.2 Work package 2

Identification of gaps in Q-bank with regard to regulated and emerging viruses and viroids

Introduction
To improve Q-bank gaps in its present contents should be identified, so that efforts can be made to trace and collect (missing) species relevant to plant health.

Aim
Extend Q-bank by including virus and viroid species relevant to plant health.

Materials & methods
- Make an inventory of (regulated) species available via public accessible collections.
- Select relevant species that are missing and are suitable for inclusion in the collection
- Trace these isolates and try to purchase them for inclusion in the collection (via other work packages)

Results
During the first year it appeared that the efforts mainly concerned virus species of interest for the participating institutes and countries. The main reason was that time and budget available for this project, only allowed them to work on tasks of current relevance for their institute. Therefore, inventories did not yield the desired overviews of the availability of virus and viroid species of interest. In addition, since such inventory was already planned within the framework of the EU project Q-collect (http://www.q-collect.eu/), it was decided not to take further actions and wait for the results of this project.

Therefore an alternative plan was agreed to improve the Q-bank Plant Viruses and Viroids Database by including all virus and viroid species recognised by the International Committee on Taxonomy of Viruses (ICTV: http://www.ictvonline.org/). For further details see Paragraph 3.8.

As a result the current design and contents of the Q-bank Plant Viruses and Viroids Database, enables searches for regulated species, but also can provide a list of the almost 300 (regulated) virus species that are transmitted by *Bemisia tabaci*. Also a list of potato-infecting virus and viroid species can be retrieved from the database. These data might be of interest for both scientists (plant virologists) and policy makers.

3.3.3 Work package 3

Description of quality standards for Q-bank accessions and their maintenance and handling in contributing collections

Introduction
The characterisation of isolates and production of reference materials is the key activity of collections. Common quality standards are required to ensure the quality and allow comparability across borders. The availability of characterised isolates as standardised reference materials will allow end-users like breeders, diagnosticians and scientists, to obtain reliable and reproducible results at any time.

Aim
The aim of this work package was to generate workflows for the characterisation of virus accessions which later could be made available via Q-bank. These workflows should provide guidance on the characterisation, maintenance and release of virus isolates, and the production of reference materials. The developed standards should support best practice in the production process in collections and include the documentation and traceability. Such defined Q-bank standards would improve the transparency and facilitate a better worldwide acceptance of the virus isolates provided by the contributing collections.

Material and methods
If applicable and reasonable, the description of quality standards for Q-bank virus isolates *should be based on the two relevant international standards ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories* and ISO guide 34:2009 *General requirements for the competence of reference material producers*. Although it was clear that not all requirements could be fully implemented in non-accredited institutions, they provided a valuable guideline to derive common quality standards for all reference collections contributing to Q-bank.

Results

Quality requirements and implementation
Based on the experiences of DSMZ a table was drafted that listed the most important quality related requirements for plant virus reference collections (Appendix 3). DSMZ already had implemented standard operating procedures (SOP's) for their collections and had an accreditation for both standards. The requirements were grouped into the following sections:

- General quality management
- Characterisation and identification
- Reference material production
- Quality control
- Order processing and shipping

This draft table was distributed to and discussed with the other partners during the first year. During the 2nd year, the other partners started to implement the relevant parts in their quality systems. Based on the scheme illustrated in Figure 3.3.3.1, this

resulted in the implementation of the following SOP's for the reference and working collections at the NPPO-NL and WUR:

Characterisation and inclusion of isolates in (reference) collection
- Characterisation by two independent tests, including at least a partial sequence, and preferably additional purity check by Electron microscopy (EM)
- Identification according to ICTV species demarcation criteria (King et al, 2012)

Maintenance of isolates in (reference) collection
- Preparation of a new batch, including verification of identity by at least one test and purity check by EM

Production of reference materials
- Preparation of positive controls from reference isolates, including check(s) as 1st line control in relevant test(s)

Release of isolates from (reference) collection
- Release of aliquots directly from the (reference) collections or after propagation on a host plants, in which case verification is performed on request
- Complying with international legislation

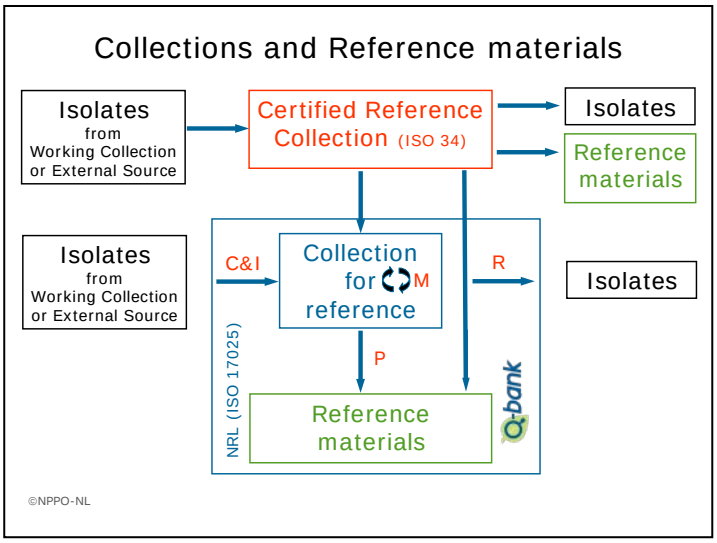

Figure 3.3.3.1 Interrelationships between a certified reference collection and local 'collection for reference'and implemented standard operating procedures for characterisation & inclusion (C&I), maintenance (M), release (R) and production of reference materials (P).

EU project Q-collect

In parallel to the VirusCollect project, the EU project Q-collect (http://www.q-collect.eu/) explored the status of reference collections important to plant health. This project had a broader scope and focused on the coordination and collaboration between reference collections of plant pests and diseases in general. In certain aspects this project overlapped with the VirusCollect project and therefore collaboration and exchange in the field of viruses was ensured by the fact that two partners of VirusCollect also participated in Q-collect.

3.3.4 Work package 4

Detection of viruses in stone and pome fruits, cereals and maize

Introduction

Viruses are very important pathogens. For the diagnostics of viruses with molecular tools it is necessary to have a variety of isolates. With different isolates it is possible to improve the quality of the molecular detection. So a reference collection of various isolates is important for virologists working in research and diagnostic laboratories.

Aim

Collect virus species from cereals, corn, stone and pome fruits crops. Provide isolates of viruses.

Material and methods

Isolation and characterisation of different viruses from maize and cereals plants and stone and pome fruits. The detection was carried out with PCR technique.

Results

During the project different viruses from various crops have been detected by using specific RT-PCR's in cereals and corn, which are important crops in agriculture. (Table 3.3.4.1). Isolated RNA was stored for future use as reference material for diagnostic work. Isolates were not included in collections.

Table 3.3.4.1 Overview of viruses detected in cereals, corn and fruit crops at AGES

Virus species	Acronym	Host plant	Original code
*Apple chlorotic leaf spot virus**	ACLSV	*Malus domestica*	ACLSV_MD
*Apple stem grooving virus**	ASGV	*Malus domestica*	ASGV_MD
*Apple stem pitting virus**	ASPV	*Malus domestica*	ASPV_MD
*Barley yellow dwarf virus**	BYDV	*Avena sativa*	BYDV_AS
*Barley yellow dwarf virus**	BYDV	*Hordeum vulgare*	BYDV_HV
*Barley yellow dwarf virus**	BYDV	*Zea mays*	BYDV_ZM
*Barley yellow mosaic virus**	BYDV	*Hordeum vulgare*	BYDV_HV
Brome mosaic virus	BMV	*Triticum aestivum*	BMV_TA
Cherry necrotic rusty mottle virus	CNRMV	*Prunus avium*	CNRMV_PA
Cherry virus A	CVA	*Prunus avium*	CVA_PA
Citrus dwarfing viroid	CDVd	*Citrus limon*	CDVd_CL
Little cherry virus 1	LChV-1	*Prunus avium*	LChV-1_PA
*Maize dwarf mosaic virus**	MDMV	*Zea mays*	MDMV_ZM
*Plum pox virus**	PPV	*Prunus domestica* subsp. *syriaca*	PPV_PDS
*Wheat dwarf virus**	WDV	*Triticum aestivum*	WDV_TA
*Wheat streak mosaic virus**	WSMV	*Triticum aestivum*	WSMV_TA
*Tomato spotted wilt virus**	TSWV	*Capsicum anuum*	TSWV_CA
*Zucchini yellow mosaic virus**	ZYMV	*Cucurbita pepo* subsp. *pepo* convar. *giromontiina*	ZYMV_CPP

* Nucleic acid extracts are available for others

3.3.5 Work package 5

Characterisation of various whitefly-transmitted viruses of significance for tomatoes and cucurbits in Europe

Introduction

The efforts of DSMZ focused on whitefly-transmitted virus species of significance for tomatoes and cucurbits belonging to the genera *Begomovirus*, *Carlavirus* and *Crinivirus*. Carlaviruses are usually naturally transmitted by aphids, but two species infecting cucurbits and tomato were proved to be transmitted by whiteflies, the recently described species Cucumber vein clearing virus and *Cowpea mild mottle virus* (CPMMV) and its serologically related isolates infecting tomatoes and eggplants. Four of these serologically related isolates of the latter species were available for characterisation. In addition, already available begomovirus and crinivirus isolates were characterised and attempts were made to obtain other relevant isolates for characterisation and inclusion in the reference collection. Further work concerned a number of viroid isolates and other virus species of interest to plant health.

Aim

To collect and further characterise whitefly-transmitted virus species belonging to the genera *Begomovirus*, *Carlavirus* and *Crinivirus*, and include them in the certified reference collection of DSMZ.

Material and methods

Virus isolates were characterised according to the documented management system and SOP's based on ISO/IEC 17025 and ISO Guide 34 standards.

Results

Begomoviruses

For the currently most important emerging begomovirus in Europe, *Tomato leaf curl New Delhi virus* (ToLCNDV), two isolates were obtained from Spain. One isolate originating from a zucchini plant (PV-1109) and another from a tomato plant (PV-1111). In addition, an ELISA-suitable polyclonal antiserum was raised for the specific detection of this virus. For ToLCNDV, nucleic acid extracts, infectious material, ELISA kits and control material can be provided. For a number of other begomoviruses infecting solanaceous crops, isolates or infectious clones were produced and further characterised (Table 3.3.5.1).

Carlaviruses

Regarding the carlaviruses, four serologically related isolates of *Cowpea mild mottle virus* (CPMMV) were characterised. Two could be identified to be almost identical, i.e. an isolate causing pale chlorosis in tomatoes from Israel and an isolate causing mosaic in eggplants from Jordan. These isolates, however, appeared to represent a putative new species within the carlaviruses, rather than being isolates of CPMMV. Two other isolates originating from Sudanese tomato samples also appeared to differ substantially from CPMMV. Furthermore, a groundnut isolate of CPMMV was received from Sudan which was added to the collection (PV-1114).

Criniviruses
One isolate each of the tomato infecting criniviruses *Tomato chlorosis virus* (ToCV PV-1023) and *Tomato infectious chlorosis virus* (TICV PV-1108) was characterised. For ToCV an ELISA-suitable antiserum was already available. For TICV the coat protein (CP) gene was cloned in an expression vector in order to raise an ELISA-suitable antiserum. This work is still in progress. In addition, a Greek cucumber isolate of the cucurbit-infecting *Cucurbit yellow stunting disorder virus* (CYSDV, genus Crinivirus) was obtained and characterised (PV-1144).

Viroids
Two viroid isolates, *Chrysanthemum chlorotic mottle viroid* (CChMVd, PV-1009) and *Chrysanthemum stunt viroid* (CSVd, PV-1116) were collected and characterised to make them available as reference material. In addition, five viroid isolates were characterised; of which three infect fruit trees, an *Apple scar skin viroid* isolate (ASSVd, PV-1135) and a *Pear blister cancer viroid* isolate (PBCVd, PV-1136), both belonging to the genus Apscaviroid, and an isolate of the species *Peach latent mosaic viroid* (PLMVd, PV-1134) of the genus *Pelamoviroid*. All were obtained from Italy and are maintained in their original hosts, apple, pear or peach, respectively. Furthermore, the number of physically available viroid isolates of the genus *Pospiviroid* infecting solanaceous crops was increased by characterisation of an isolate of *Tomato apical stunt viroid* (TASVd , PV-1151) found in *Solanum jasminoides* from the Czech Republic and an isolate of *Tomato chlorotic dwarf viroid* (TCDVd, PV-1148) from a German petunia sample. Both isolates were added to the public collection.

Other viruses
Two non-European potato virus isolates from pepino samples from New Zealand (*Potato virus M*, PV-1102; *Potato virus X*, PV-1101), were collected and extensively characterised in order to make them available as reference materials. Furthermore, two *Citrus tristeza virus* isolates (CTV, genus *Closterovirus*, PV-1129 and PV-1130) originating from Malta were added to the reference collection.

All isolates are already or will soon be made available as infectious material and nucleic acid extracts, except those which are derived from infectious full-length clones which will only be provided as nucleic acid extracts. If species specific antisera are available at DSMZ, ELISA positive controls can also be provided.

Table 3.3.5.1 Virus species available as isolates, ELISA controls or nucleic acid extracts from DSMZ

Virus species	Acronym	Host plant	Original code	Q-bank code
Begomoviruses				
Chilli leaf curl Pakistan virus	ChiLCPKV	*Capsicum* sp.	PV-1140	ChiLCPKV_2014_001
Cotton leaf curl Gezira virus	CLCuGeV	*Abelmoschus esculentus*	PV-1122	CLCuGeV_2013_001
Mungbean yellow mosaic virus	MYMV	*Vigna radiate* var. *radiata*	PV-1100	MYMV_2013_001
Pepper yellow leaf curl Vietnam virus	PepYLCVV	*Capsicum* sp.	PV-1150	PepYLCVV_2014_001
Tomato leaf curl New Delhi virus	ToLCNDV	*Cucumis sativus*	PV-1109	ToLCNDV_2013_001
Tomato leaf curl New Delhi virus	ToLCNDV	*Solanum lycopersicum*	PV-1111	ToLCNDV_2013_002
Tomato leaf curl Vietnam virus	ToLCVV	*Solanum lycopersicum*	PV-1080	ToLCVV_2012_001
Tomato leaf curl China virus	ToLCCNV	*Solanum lycopersicum*	PV-1085	ToLCCNV_2012_001
Tomato leaf curl Hainan virus	ToLCLHaV	*Solanum lycopersicum*	PV-1082	ToLCLHaV_2012_001
Tomato leaf curl Hanoi virus	ToLCLHanV	*Solanum lycopersicum*	PV-1081	ToLCLHanV_2010_001
Tomato leaf curl Langson virus	ToLCLsV	*Solanum lycopersicum*	PV-1084	ToLCLsV_2012_001
Carlaviruses				
Cowpea mild mottle virus	CPMMV	*Arachis hypogaea*	PV-1114	CPMMV_2013_002
Tomato mild leaf roll virus	TMLRV	*Solanum lycopersicum*	PV-0914	TMLRV_2005_001*
Tomato pale chlorosis virus	TPCV	*Solanum lycopersicum*	PV-1119	TPCV_2014_001*
Criniviruses				
Cucurbit yellow stunting disorder virus	CYSDV	*Cucumis melo*	PV-1088	CYSDV_2014_002
Cucurbit yellow stunting disorder virus	CYSDV	*Cucumis sativus*	PV-1144	CYSDV_2013_001
Tomato chlorosis virus	ToCV	*Solanum lycopersicum*	PV-1023	ToCV_2011_001
Tomato infectious chlorosis virus	TICV	*Solanum lycopersicum*	PV-1109	TICV_2013_001
Viroids				
Apple scar skin viroid	ASSVd	*Malus* sp.	PV-1135	ASSVd_2014_001
Chrysanthemum chlorotic mottle viroid	CChMVd	*Chrysanthemum* sp.	PV-1009	CChMVd_2010_001
Chrysanthemum stunt viroid	CSVd	*Argyranthemum* sp.	PV-1116	CSVd_2014_005
Peach latent mosaic viroid	PLMVd	*Prunus persica*	PV-1134	PLMVd_2014_001
Pear blister cancer viroid	PBCVd	*Pyrus* sp.	PV-1136	PBCVd_20104_001
Tomato apical stunt viroid	TASVd	*Solanum jasminoides*	PV-1151	TASVd_2014_002
Tomato chlorotic dwarf viroid	TCDVd	*Petunia hybrida*	PV-1148	TCDVd_2014_008
Other viruses				
Citrus tristeza virus	CTV	*Citrus* sp.	PV-1129	CTV_2014_003
Citrus tristeza virus	CTV	*Citrus* sp.	PV-1130	CTV_2014_002
Potato virus M	PVM	*Solanum muricatum*	PV-1102	PVM_2013_001
Potato virus X	PVS	*Solanum muricatum*	PV-1101	PVX_2013_005

*Note that in some cases isolates/sequences will become available after publication

3.3.6 Work package 6

Characterisation of known and unknown viruses from plant virus collections

Introduction

In an earlier project a limited number of isolates of different regulated viruses currently present in the plant virus collection of DSMZ/NVWA/PRI have been subjected to Next Generation Sequencing (NGS). For a number of isolates substantial sequence information has been generated. In addition, sequences of new virus isolates or even new viruses were identified. For further characterisation and to allow a better comparison with already known sequences, the sequences of the isolates under study needed to be completed and analysed in more detail.
In addition, reliable standards were needed for validation of diagnostic tests. A number of (regulated) viruses pose problems in this respect because of lower levels of stability. More research on the development of stable standards was required.

Aim

1. To further complete, characterise and analyse the genomic information of regulated and other agriculturally important viruses, in particular *Andean potato latent virus* and related viruses (APLV, genus *Tymovirus), Andean potato mottle virus* (APMoV, genus *Comovirus*), *Strawberry latent ringspot virus* (SLRSV, unassigned species in the family Secoviridae) and a number of species within the genus *Tospovirus*.
2. To develop more stable standards for validation of diagnostic tests in particular for *Leek yellow stripe virus, Plum pox virus* and *Potato virus Y* (genus *Potyvirus*).

Material and methods

For a selection of viruses partial sequences obtained by Next Generation Sequencing (NGS) were completed to full genome sequences by conventional techniques. If more isolates of a virus were available sequences were compared and further analysed to gain more insight in the variability of the species and consequences for diagnostic tests.

Results

For a number of regulated viruses complete genomes of different isolates were determined using NGS technology and conventional Sanger sequencing (Table 3.3.6.1). Sequence data as well as additional biological and serological data on these isolates were compiled and included in Q-bank. For most of these species physical specimen have been stored in the WUR Plant virus collection under liquid N_2 and are available for future reference.

Table 3.3.6.1 Virus species available as isolates from WUR

Virus species	Acronym	Host plant	Original code	Q-bank code
Nepoviruses				
Arabis mosaic virus	ArMV	*Vinca minor*	PD 22001651	ArMV_2002_001
		Rheum rhabarbarum	PD Naktuinbouw INS 05-05312	ArMV_2005_012
	ArMV	*Tulipa* sp.	PD 5958-1	ArMV_2006_002
	ArMV	*Tulipa* sp.	PD 5790-17	ArMV_2006_003
	ArMV	*Gladiolus* sp.	PD 3059261	ArMV_2006_004
	ArMV	*Grapevine* sp.	DSMZ PV-0045	ArMV_2007_008
Arachaca virus B	AVB	*Arracacia xanthorrhiza*	PRI DSMZ PV-0082	AVB_2011_001
Potato black ringspot virus	PBRSV	*Solanum tuberosum*	PRI PBRSV PV-0056 Peru	PBRSV_1987_002
Potato black ringspot virus	PBRSV	*Solanum tuberosum*	PRI PBRSV-EC	PBRSV_1981_002
Tobacco ringspot virus	TRSV	*Lobelia* sp.	PD 9702383	TRSV_1977_001
	TRSV	*Portulaca* sp.	PD 20004098	TRSV_2000_002
	TRSV	*Portulaca* sp.	PD 3240256	TRSV_2006_003
	TRSV	*Portulaca* sp.	PD 3096239	TRSV_2000_004
	TRSV	*Hemerocallis* sp.	PD 3398191	TRSV_2006_005
	TRSV	*Hemerocallis* sp.	PD 3248618	TRSV_2008_006
	TRSV	*Iris ensata*	PD 3608193	TRSV_2006_007
	TRSV	*Iris ensata*	PD 3281223	TRSV_2007_008
	TRSV	*Iris ensata*	PD 3281311	TRSV_2007_009
	TRSV	*Iris ensata*	PD 3281485	TRSV_2007_010
	TRSV	*Iris ensata*	PD 3281290	TRSV_2007_011
	TRSV	*Pelargonium* sp.	PD TRSV-CSL	TRSV_2007_012
	TRSV	*Tulipa* sp.	PD TRSV-PRI	TRSV_2007_013
	TRSV	*Portulaca* sp.	PD 3337709	TRSV_2007_014
	TRSV	*Mentha* sp.	PD TRSV-Martin	TRSV_2008_015
	TRSV	*Celosia* sp.	PD 3594692	TRSV_2008_016
	TRSV	*Phaseolus vulgaris*	DSMZ PV-0236	TRSV_2008_017
Tomato black ring virus	TBRV	*Solanum tuberosum*	DSMZ PV-0070	TBRV_1987_001
Tomato ringspot virus	ToRSV	*Malus* sp.	'Chikadee'	
Secoviridae				
Strawberry latent ringspot virus	SLRSV	*Lilium* cv Tiber		SLRSV_2012_005
Tospoviruses				
Alstroemeria necrotic streak virus	ANSV	*Alstroemeria* sp.	Colombia	ANSV_2014_001
Alstroemeria yellow spot virus	AYSV	*Alstroemeria* sp.	Alstroemeria2000	AYSV_2015_001
Capsicum chlorosis virus	CaCV	*Capsicum annuum*	Thailand	CaCV_2015_001
Chrysanthemum stem necrosis virus	CSNV	*Chrysanthemum*	HiCh06A, PD 4412741	CSNV_2010_002
Groundnut bud necrosis virus	GBNV	*Arachis hypogaea*	India	GBNV_2015_001
Impatiens necrotic spot virus	INSV	*Petunia hybrida*		INSV_1991_001
Lisianthus necrotic ringspot virus	LNRV	*Lisianthus* sp.	Japan, PD 6269713	LNRV_2015_001
Tomato necrotic ring virus	TNRV	*Solanum lycopersicum*	Thailand	TNRV_2015_001
Watermelon silvery mottle virus	WSMV		Thailand	WSMoV_2015_001

Tymoviruses				
Andean potato latent virus	APLV	*Solanum tuberosum*	DSMZ PV-0060 Colombia (Col), PD 4062137	APLV_1987_001
Andean potato latent virus	APLV	*Solanum tuberosum*	DSMZ PV-0062 (Col2), PD 4062153	APLV_1979_002
Andean potato mild mosaic virus	APMMV	*Solanum tuberosum*	DSMZ PV-0061 (Hu), PD 4062145	APMMV_1977_001
Andean potato mild mosaic virus	APMMV	*Solanum tuberosum*	PD Q930460	APMMV_1993_002
Eggplant mosaic virus	EMV	*Solanum melongena*	SCRI, PD 4225178	EMV_1966_001
Physalis mottle virus	PhyMV	*Solanum lycopersicum*	NPPO PD 1968347	PhyMV_2004_001
Scrophularia mottle virus	ScrMV	*Scrophularia nodosa*	DSMZ PV-0870, PD 4225557	ScrMV_2002_001
Turnip yellow mosaic virus	TYMV	*Brassica napa*	DSMZ PV-0299	TYMV_1992_001
Other potato viruses				
Andean potato mottle virus (B)	APMoV	*Solanum tuberosum*	PRI DSMZ PV-0057 Strain B	APMoV_2012_001
Andean potato mottle virus (H)	APMoV	*Solanum tuberosum*	PRI DSMZ PV-0059 Strain H	APMoV_2012_002
Potato virus T	PVT	*Solanum tuberosum*		PVT_1980_001
Potato virus Y – C strain	PVY^{C}	*Solanum tuberosum*		PVY_1969_001
Other viruses				
Bean common mosaic virus	BCMV	*Phaseolus vulgaris*	Chile A5	BCMV_2015_001
	BCMV	*Phaseolus vulgaris*	Kaiser Iran	BCMV_1968_002
	BCMV	*Phaseolus vulgaris*	NL1	BCMV_2015_003
	BCMV	*Phaseolus vulgaris*	NL1 B139	BCMV_1977_004
	BCMV	*Phaseolus vulgaris*	NL2 Imuna	BCMV_2015_005
	BCMV	*Phaseolus vulgaris*	NL6 Colana	BCMV_1969_006
	BCMV	*Phaseolus vulgaris*	NL7-B53	BCMV_1974_007
	BCMV	*Phaseolus vulgaris*	NL8 B54	BCMV_1974_008
	BCMV	*Phaseolus vulgaris*	PR1 PR9M	BCMV_1973_009
	BCMV	*Phaseolus vulgaris*	US4 Western strain	BCMV_1973_010
	BCMV	*Phaseolus vulgaris*	US Florida	BCMV_1966_011
	BCMV	*Phaseolus vulgaris*	Vu7 Blackeye	BCMV_1966_012
Bean yellow mosaic virus	BYMV	*Pisum sativum*	E198 pea mosaic strain	BYMV_1966_003
	BYMV	*Pisum sativum*	E199 pea necrosis strain	BYMV_1966_011
Beet mosaic virus	BtMV	*Pisum sativum*	SVP	BtMV_2015_001
	BtMV	*Pisum sativum*	SVP_1	BtMV_2015_002
	BtMV	*Pisum sativum*	SVP_2	BtMV_2015_003
	BtMV	*Pisum sativum*	IPO	BtMV_2015_004
Carnation mottle virus	CarMoV	*Carnation*	Hakkaart	CarMV_2015_001
Cucumber necrosis virus	CuNV	*Cucumber*	Tremaine	CuNV_2015_001
Hydrangea ringspot virus	HRSV	*Hydrangea*	PD109	HdRSV_1998_001
Narcissus mosaic virus	NMV	*Narcissus*	Brunt	NMV_2015_001
Parsnip mottle virus	ParMoV	*Apium graveolens*	Angst246II	PaMoV_1999_001
	ParMoV	*Apium graveolens*	Ag119	PaMoV_1977_002
Parsnip mottle virus	ParMoV	*Heracleum*	Hs 247 I	PaMoV_1983_003
Parsley latent virus	PaLV	*Petroselinum crispum*	Pe6	PaLV_1975_001
Pea early browning virus	PEBV	*Pisum sativum*	E116	PEBV_1959_001
Pelargonium flower break virus	PFBV	*Pelargonium zonale*	Salmon Irene	PEBV_1999_002
Pepino mosaic virus	PepMV	*Solanum lycopersicum*	EU-tomato (PD 9991066)	PepMV_1999_001
	PepMV	*Solanum lycopersicum*	PRI PCH-006/104	PepMV_2009_003

	PepMV	*Solanum lycopersicum*	DSMZ PV-1022 (BBA-137)	PepMV_2011_004
	PepMV	*Solanum lycopersicum*	PRI PepMV-US1	PepMV_2008_002
Pepper veinal mottle virus	PVMoV	*Capsicum annuum*	Paprika	PVMoV_1996_001
Plantago asiatica mosaic virus	PlAMV	*Lilium* sp.	Li 1	PlAMV_2015_002
	PlAMV	*Lilium* sp.	PRI-PlAMV012014	PlAMV_2014_001
Plum pox virus	PPV	*Prunus domestica*	Van Oosten	PPV_1981_001
	PPV	*Prunus domestica*	PRI PPV-Rankovic	PPV_1984_002
Squash mosaic virus	SqMV	*Cucurbita pepo*	1 Ark	SqMV_1997_001
Tulip virus X	TVX	*Tulipa* sp.	TVX-PRI1501	TVX_2015_001
Turnip mosaic virus	TuMV	*Brassica oleracea* sp.	Brass25	TuMV_1976_001

*Note that in some cases isolates/sequences will become available after publication

A new method for the production of coat protein for use as positive control in ELISA tests was successfully tested on *Onion yellow dwarf virus*, a potyvirus closely related to *Leek yellow stripe virus*. This method will be further employed for other viruses, in particular potyviruses, in the near future.

3.3.7 Work package 7

Establishment and maintenance of a potato virus species reference collection for a regulated potato viruses and generation of stable positive controls (non-live reference material) for molecular tests for a range of virus species and genera

Introduction

Currently SASA holds potato-infecting virus collections of indigenous and non-indigenous viruses as live material:

- Virology and Zoology Section holds a collection of indigenous viruses belonging to seven genera see (Table 3.3.7.1) as live material (glasshouse grown potatoes and indicator plants or freeze dried material).
- Plant Biosecurity and Inspections Section holds non-indigenous and some indigenous viruses as live material (in vitro plants and indicator plants or freeze-dried material).

For some viruses nucleic acid and plasmid clones are also available as positive amplification controls (PAC) for PCR-based diagnostic tests.

This work package focused on the following activities:

1. Assessing the infectivity status of the current collections (freeze dried material and microplants) and checking of other material if it is still fit for its intended use. Gaps in characterisation data will be identified and where appropriate isolates will be sequenced. A comprehensive database will be developed of material held (this can be used to populate the project database).
2. Addition of new potato-infecting viruses (particularly to the microplant collection).
3. Inclusion of a range of virus isolates (pathotypes/strains/phylogenic groups from specific virus species) mainly potyviruses.
4. Identification of the most valuable material and deposition where relevant, with other organisations as a backup.
5. Evaluation of the production of stable reference material (DNA, cDNA, PCR fragments) for use as template controls for molecular tests.

Aims

Milestone 1: Establishment of new and maintenance of current ‘live’ virus reference collections: propagation of plants infected with a range of virus isolates, e.g. glasshouse-grown plants, in vitro potato plants, freeze-dried leaves, tubers.
Milestone 2: Validation of virus reference material: molecular and serological characterisation of infected plants by ELISA and PCR-based, partial and/or full-length sequencing.
Milestone 3: Generation and validation of ‘stable positive controls’, i.e. (c)DNA PCR fragments from a range of virus genes from different species and genera cloned into plasmids by:

- PCR amplification and purification of (c)DNA template from reference virus material.
- Validation of generated plasmids.
- Definition of a standard for using DNA template as a positive control in a molecular test.

Material and methods

Virus isolates were characterised according to the SASA quality management system and following EPPO recommendations (EPPO PM 7/98, 2014) using serological tests (DAS-ELISA with monoclonal and polyclonal antibodies) and PCR-based tests (End-point RT-PCR and Real-Time RT-PCR) as previously described (Boonham et al, 2009, Fox et al, 2005, Lacomme et al, 2015, Mumford et al, 2000). Further characterisation of virus pathotypes and phylogenetic groups was achieved by indexing on a suitable range of indicator plants and by partial or full-length genome sequencing (Sanger sequencing). Phylogenetic analysis was performed using MEGA5 software (Neighbour Joining method). PCR products corresponding to specific viral genes were cloned into the pGEM®-TEasy vector and sequenced. Plasmid DNA was purified following QIagen DNEasy protocol and *E.coli* clones stored at -80°C.

Results

3.3.7.1 Indigenous viruses

The SASA Virology laboratory hosts a virus collection of eleven virus species of Scottish origin representing seven genera: *Carlavirus*, *Nepovirus*, *Polerovirus*, *Pomovirus*, *Potexvirus*, *Potyvirus* and *Tobravirus* (Table 3.3.7.1).

Table 3.3.7.1 Indigenous virus species held in the SASA collection

Virus species	Acronym	Host plant	Transmission (vector)
Nepovirus			
Tomato black ring virus	TBRV	*Solanum tuberosum*	Nematodes (*Trichodorus* spp.)
Carlavirus			
Potato virus M	PVM	*Solanum tuberosum*	Aphids, non-persistent
Potato virus S	PVS	*Solanum tuberosum*	Aphids, non-persistent
Polerovirus			
Potato leaf roll virus	PLRV	*Solanum tuberosum*	Aphids, persistent
Potexvirus			
Potato aucuba mosaic virus	PAMV	*Solanum tuberosum*	Mechanically
Potato virus X	PVX	*Solanum tuberosum*	Mechanically
Pomovirus			
Potato mop top virus	PMTV	*Solanum tuberosum*	Fungus (*Spongospora subterranea*)
Tobravirus			
Tobacco rattle virus	TRV	*Solanum tuberosum*	Nematodes (*Longidorus* spp.)
Potyvirus			
Potato virus A	PVA	*Solanum tuberosum*	Aphids, non-persistent
Potato virus V	PVV	*Solanum tuberosum*	Aphids, non-persistent
Potato virus Y	PVY	*Solanum tuberosum*	Aphids, non-persistent

During the course of the project the virus collection has been developed for different purposes:

1. Maintaining a live collection of virus species on growing plants for validation and quality control of serological and PCR-based assays (Table 3.3.7.2)
2. Implementing a collection of virus isolates for research and development (R&D) purpose representing different strains, pathotypes or phylogenetic groups (Table 3.3.7.3).

3. Generating a collection of cloned viral (c)DNA's to be used as 'stable' reference materials, i.e. Positive Amplification Controls (PAC) in PCR-based tests and for trend analysis of assay performance.

Live collection of indigenous viruses

The live collection of indigenous virus isolates is maintained in potato plants by tuber-propagation. Each propagated plant is given a QC reference number for each generation/batch of growing plant and virus species diagnosed. Propagated plants are tested by DAS-ELISA using SASA monoclonal antibodies for eleven serotypes of the virus species listed: SASA- MAb-PLRV, MAb-PAMV, MAb-PMTV, MAb-PVA, MAb-PVM, MAb-PVS, MAb-PVV, MAb-PVX, MAb-PVY^{N}, MAb-PVY$^{O/C}$, MAb-TBRV. Additional validation is effectuated by real-time RT-PCR for the following seven virus species: PLRV, PMTV, PVA, PVV, PVX, PVY and TRV. The current list of virus isolates propagated and validated is presented in Table 3.3.7.2.

Table 3.3.7.2 Live collection of indigenous viruses in potato plants at SASA

Virus species	Acronym	Method of validation	Original code	Q-bank code
Potato aucuba mosaic virus	PAMV	ELISA, Real-time RT-PCR	PAMV	PAMV_2016_003
Potato leafroll virus	PLRV	ELISA, Real-time RT-PCR	QC105	PLRV_2002_001
Potato virus A	PVA	ELISA, Real-time RT-PCR	QC108	PVA_2002_007
Potao virus M	PVM	ELISA	QC118	-
Potato virus S (Ordinary)	PVS^{0}	ELISA	QC122	PVS_2006_001
Potato virus V	PVV	ELISA, Real-time RT-PCR	QC110	PVV_2002_004
Potato virus X	PVX	ELISA, Real-time RT-PCR	QC124	PVX_2002_006
Potato virus Y^{C}	PVY^{C}	ELISA, Real-time RT-PCR	QC116	PVY_2006_010
Potato virus Y^{NTN}	PVY^{NTN}	ELISA, Real-time RT-PCR	QC114	PVY_2002_012
Potato virus Y^{O}	PVY^{O}	ELISA, Real-time RT-PCR	QC112	PVY_2002_011
Tomato black ring virus	TBRV	ELISA	QC120	-

*Note that in some cases isolates/sequences will become available after publication

Characterisation of virus strains, pathotypes, and phylogenetic groups

During the course of the project, several virus isolates identified during the annual survey of virus populations in growing potato crops were further characterised. Selected PVA and PVY isolates were typed in order to define potential pathotypes and phylogenetic groups. Partial genome sequences of virus genes, such as Coat protein, Helper-Component and Proteinase, or full-length genome sequences were determined.

The characterisation of these isolates was undertaken by analysing their serology (ability to cross-react with a range of monoclonal antibodies in DAS-ELISA), their pathotype (based on their ability to trigger specific symptoms and disease development on a range of indicator plants) and their association to phylogenetic groups (sequence analysis of their genome and phylogenetic studies with reference virus isolates present in public databases). This study allowed definition of different phylogenetic variants and pathotypes of PVA and PVY (Table 3.3.7.3). These isolates are not yet made available in Q-bank and are only propagated for R&D purposes (stored as either frozen or lyophilised leaf material). Isolates and their nucleotidic sequence data will be made available once published.

Table 3.3.7.3 Identified pathotypes and strains of PVA and PVY available at SASA

Virus species (strain, pathotype, phylogenetic group)[1]	Serotype	Symptoms on indicator plants[2]	Original code
Potato virus A			
Mild mosaic pathotype	PVA	Potato cv Desiree: MM	PVA-285 PVA-331
Severe mosaic pathotype	PVA	Potato cv Desiree: SM	PVA-28 PVA-364
Potato virus Y			
PVY^{E}, PVY^{EU-NTN} European	PVY^{N}	Severe mosaic, PTNRD ++	PVY^{E}-10088
$PVY^{NA-Wilga}$	$PVY^{O/C}$	Vein necrosis	$PVY^{NA-Wilga}$-5467
$PVY^{N-Wilga}$	$PVY^{O/C}$	Vein necrosis	$PVY^{N-Wilga}$-4388
PVY^{NTN}, PVY^{EU-NTN} European	PVY^{N}	Vein necrosis, PTNRD ++	PVY^{EU-NTN}-DV76
PVY^{EU-}	-	-	PVY^{EU-NTN}-10766
PVY^{NTN}, PVY^{NA-NTN} N. American	PVY^{N}	Vein necrosis, PTNRD ++	PVY^{NA-NTN}-DV69
PVY^{NA-NTN}	-	-	PVY^{NA-NTN}-9561
PVY^{O}	$PVY^{O/C}$	Mosaic, PTNRD +/-	PVY^{O}-DV71

[1]Infected material and full length genome sequence available after publication.
[2]For PVY species biotyping is performed on *Nicotiana tabacum* cv White Burley and potato cv Nadine. *PTNRD: potato tuber necrotic ring disease.

Collection of plasmid-borne viral cDNAs

The availability of a 'stable' collection of virus genes as cloned material is a cost-effective alternative to live control material to monitor the performance of PCR-based tests. The obvious advantage is the availability of a template that is more suitable as PAC in PCR-based tests, and is more suitable for trend analysis of test performance over a given period of time. Using plasmid-borne viral cDNA should avoid the inherent shortcomings of using freshly extracted RNA from infected plants as PAC, where variation in virus titre in propagated plants is frequently observed and therefore does not reflect test performance.

During the course of the project a range of viral and plant cDNAs used for the diagnosis of viruses and quality control of real-time RT-PCR assays were cloned and sequenced. PCR fragments of defined viral genes were cloned and individual clones sequenced. This collection of plasmid-borne viral cDNA (stored in *E. coli* glycerol stocks) had been established for the virus genes listed in Table 3.3.7.4. The nucleotide sequences of previous batches of isolate were made available via Q-Bank. Nucleotide sequences of virus genes subject to further R&D will be made available after publication. Cloning of coat-protein cDNA fragments of PVM and TBRV is on-going and will be made available once completed. Similarly, endogenous plant (c)DNAs from Cytochrome C oxidase (COX) and NADH5 (NAD5) were cloned in order to generate stable PAC material for trend analysis of nucleic acid extraction of real-time PCR assays.

Table 3.3.7.4 Cloned and sequenced viral cDNAs available at SASA

Virus species	Acronym	Cloned/sequenced gene	Original code	Q-bank code
Potato aucuba mosaic virus	PAMV	Coat protein	pSASA-PAMV	-
Potato leaf roll virus	PLRV	Coat protein	pSASA-PLRV-QC55	-
Potato mop top virus	PMTV	Part seq Coat protein read-through RNA2	pSASA-PMTV-TD141112	-
Potato virus A	PVA	Coat protein	pSASA-PVA-QC58	PVA_2002_007
Potato virus S	PVS	Coat protein	pSASA-PVS-QC72	PVS_2006_001
Potato virus V	PVV	Coat protein	pSASA-PVV-QC60	PVV_2002_004
Potato virus X	PVX	Coat protein	pSASA-PVX-QC74	PVX_2002_006
Potato virus Y^{C}	PVY^{C}	Coat protein	pSASA-PVY^{C}-QC91	-
Potato virus Y^{E}	PVY^{E}	Coat protein	pSASA-PVY^{E}-10088	*
Potato virus Y^{NTN}	PVY^{NTN}	Coat protein	pSASA-PVY^{NTN}-QC64	PVY_2002_012
Potato virus Y^{NTN}	PVY^{EU-NTN}	Coat protein	pSASA-PVY^{EUNTN}-10766	*
Potato virus Y^{NA-NTN}	PVY^{NA-NTN}	Coat protein	pSASA-PVY^{NA-NTN}-DV69	*
Potato virus Y$^{N-Wilga}$	PVY$^{N-Wilga}$	Coat protein	pSASA-PVY$^{N-Wilga}$-4388	*
Potato virus Y^{O}	PVY^{O}	Coat protein	pSASA-PVY^{O}-QC62	PVY_2002_011
Tobacco rattle virus	TRV	Part seq 16K RNA1	pSASA-TRV-6398	-

*Note that in some cases isolates/sequences will become available after publication

An example of a typical amplification profile of a plasmid dilution series (pSASA-PVY^{NTN}-QC64) to monitor the performance of a real-time PCR assay is given in Figure 3.3.7.1.

Dilution factor of plasmid pSASA-PVY^{NTN}-QC64	Ct value
10E-3	18
10E-5	25
10E-7	31

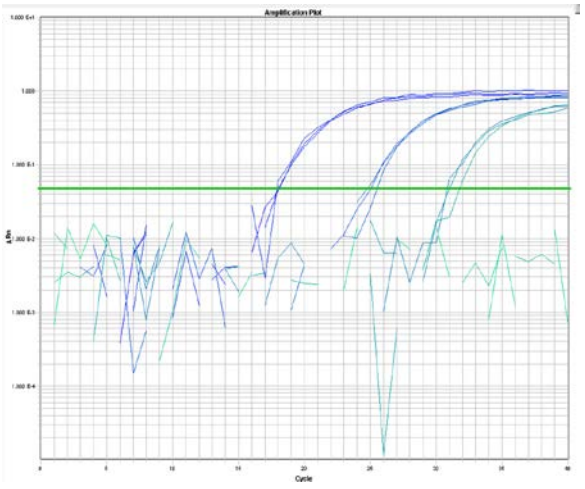

Figure 3.3.7.1 Real-Time RT-PCR amplification profile using PVY primers and probe combinations for testing a plasmid dilution series of pSASA-PVY^{NTN}-QC64 (Boonham et al, 2009; Lacomme et al, 2015). The Ct value is reported for each dilution.

3.3.7.2 Non-indigenous viruses

The UK Potato Quarantine Unit at SASA maintains a collection of virus isolates, from around the world, some of which have been intercepted during quarantine testing (Table 3.3.7.4).

Table 3.3.7.4 Non-indigenous virus species held in the SASA collection

Virus species	Acronym	Host plant	Transmission (vector)
Begomovirus			
Tomato yellow vein streak virus	ToYVSV	*Solanum tuberosum*	Whiteflies (*Trialeurodes vaporariorum*)
Carlavirus			
Potato latent virus	Pot LV	*Solanum tuberosum*	Aphids
Potato virus P	PVP	*Solanum tuberosum*	Aphids
*Potato virus P**	PVP (PRDV)	*Solanum tuberosum*	Aphids
Potato virus S (Andean)	PVS[a]	*Solanum tuberosum*	Aphids
Comovirus			
Andean potato mottle virus	APMoV	*Solanum tuberosum*	Contact
Crinivirus			
Tomato chlorosis virus	ToCV	*Solanum tuberosum*	Whiteflies (*Trialeurodes vaporariorum*)
Potato yellow vein virus	PYVV	*Solanum tuberosum*	Whiteflies (*Trialeurodes vaporariorum*)
Tepovirus			
Potato virus T	PVT	*Solanum tuberosum*	Contact
Tymovirus			
Andean potato latent virus	APLV	*Solanum tuberosum*	Flea beetles; contact

*Potato rough dwarf virus

During this project a range of virus isolates have been maintained / developed for different purposes:

1. Live collection of in vitro plants to be used for quality control of serological and PCR-based tests, validation of new assays and R&D.
2. Cloned viral (c)DNA's to be used as 'stable' positive controls to assist with trend analysis of test performance as part of ISO 17025:2005 accreditation.

Live collection of non-indigenous viruses maintained in vitro

The live collection of non-indigenous virus isolates is maintained in vitro. Plants are maintained in M&S growth medium and propagated at various intervals. Each virus isolate is given a QV reference number which remains with the isolate through all generations to ensure traceability back to the original isolate. Plants receive various levels of testing to ensure that they are free of other pathogens. Plants may have been tested by DAS-ELISA, RT-PCR (with or without sequencing) and bioassay and this information will be supplied for any isolate. The virus isolates listed in Table 3.3.7.5 have been identified/characterised by the indicated methods.

Table 3.3.7.5 Live collection of non-indigenous viruses in microplants at SASA

Virus species	Acronym	Method of validation	Original code	Q-bank code
Andean potato latent virus	APLV	ELISA (Prime Diagnostics); No sequence data	QV170	-
Andean potato mottle virus	APMoV	ELISA (Prime Diagnostics); No sequence data	QV235	APMoV_2016_004
Potato latent virus	PotLV	Bioassay; ELISA (SASA); RT-PCR, Sequencing	QV133	PotLV_2014_002
Potato virus P	PVP	Bioassay; ELISA (SASA); RT-PCR; Sequencing	QV113/1	PVP_2016_001
Potato virus P	PRDV	Bioassay; ELISA (SASA); RT-PCR; Sequencing	QV108	PVP_2014_002
Potato virus S	PVS	Bioassay; ELISA (SASA); RT-PCR; Sequencing	QV257	PVS_2016_002
Potato virus T	PVT	ELISA (SASA); No sequence data	QV1	PVT_2016_003
Potato yellow vein virus	PYVV	RT-PCR; Sequencing	QV202/3	PYVV_2014_001
Tomato yellow vein streak virus	TYVSV	RT-PCR; Sequencing	QV263	TYVSV_2014_001

*Note that in some cases isolates/sequences will become available after publication

Collection of plasmid-borne viral cDNAs

Under a quality system such as ISO 17025:2005 the use of stable controls is essential for monitoring the performance of a test over time. Live material can be difficult to maintain and be variable in performance, and prepared controls (eg freeze dried /nucleic acid samples) may deteriorate over time. Previous experience using plasmid viral (c)DNA clones of *Beet curly top virus* (BCTV) and PYVV had shown such controls to be stable and suitable for trend analysis. PCR fragments of defined viral genes were cloned and individual clones sequenced. Plasmid viral cDNA's (stored in *E. coli* glycerol stocks) have been established for the virus genes listed in Table 3.3.7.6.

Table 3.3.7.6 Cloned and sequenced viral cDNAs available at SASA

Virus species	Acronym	Cloned/sequenced gene	Original code	Q-bank code
Potato latent virus	PotLV	Part seq Coat protein – 3'end	QV133	PotLV_2014_002
Potato virus P	PVP	Part seq Coat protein – 3'end	QV113/1	PVP_2014_001
Potato yellow vein virus	PYVV	Part seq Coat protein	QV202/3	PYVV_2014_001
Tobacco necrosis virus-D	TNV-D	Part seq Coat protein	QV319	-
Tomato yellow leaf curl virus	TYLCV	Part seq Coat protein	QV166	-
Tomato yellow vein streak virus	ToYVSV	Part seq Coat protein	QV263	TYVSV_2014_001

Plasmid viral cDNA clones do not control the reverse transcription step of the assay. To overcome this, the addition of an internal control such as *nad*5 can be used to control the reverse-transcription reaction in the samples, the healthy control and the plasmids wells (if diluted in healthy RNA instead of water). All tests have been validated with infected RNA to ensure the specific reaction of the reverse transcriptase with the virus specific antisense primer and RNA template, before using plasmid controls.

3.3.8 Work package 8

Data and isolates in Q-bank

At the first VirusCollect meeting in Braunschweig it was decided to improve the Q-bank Plant Viruses and Viroids Database by including all virus and viroid species recognised by the International Committee on Taxonomy of Viruses (ICTV). Therefore the ICTV database was taken as a starting point (2013, V2; http://talk.ictvonline.org/files/ictv_documents/m/msl/4911.aspx). This excel-based Master Species list contained a total of 2827 recognised virus names. From this list all plant-associated viruses were selected, which resulted in a list of 1190 different plant virus and viroid species. During the second year, all ICTV-recognised species were included in the database and basic information was provided on taxonomy, quarantine status, and availability of isolates. If available a link was provided to the EPPO Global Database. In addition, for all species regulated in the EU or recommended for regulation by EPPO, reference sequences from NCBI GenBank were included, so that the database could be used for provisional identification of these species by BLAST searches. Also for a limited number of other (agronomical) important viruses, additional data and information were included. Bibliography data were provided where available.

The next step was the inclusion of the virus and viroid isolates characterised and/or made available during this project, as indicated in the tables in Paragraphs 3.4 to 3.7. To enable efficient loading of the data, templates were designed for providing metadata on a specific isolate as well as to provide data on its biological, serological and molecular characteristics, including sequence data.

With regard to the availability of isolates, the database easily and clearly shows which species are available from public collections and which not. For available species, information is provided on the characteristics of a specific isolate and the location from which it can be purchased. However, as a result of the alternative approach of further development of Q-bank (Paragraph 3.2) information and data of species and isolates might be incomplete. On the other hand, the inclusion of non-available species offers insight in concrete needs and targets for further development of the database and collections.

By the end of the project, Q-bank contained basic information on almost 1200 virus and viroid species. For 329 species, comprising most regulated species, reference sequences from NCBI GenBank have been added to facilitate BLAST searches. Furthermore, Q-bank currently provides data on 269 isolates that are physically available from collections for reference, including those resulting from this project.

4. Discussion of results and their reliability

General
The achievements described in Chapter 3 show that establishing collections of well-characterised virus and viroid isolates gained increasing interest over the last few years. The fact that many diagnostic laboratories prepared accreditation under ISO 17025, made clear that reference collections are indispensable for their work. On the other hand, this Euphresco VirusCollect project showed that for most laboratories it was difficult to acquire budgets for collection and characterisation of viruses that are not causing actual problems. This was the main reason for changing the focus from identifying and filling gaps in collections to establishing the infrastructure for making characterised and available isolates publicly accessible (Paragraph 3.2).

Standards
At the same time, it was clear that quality control was one of the key issues in sharing data and connecting collections. Therefore, minimum requirements on characterisation, maintenance and release were addressed and included in standard operating procedures at the participating laboratories (Paragraph 3.3). In the same period, the EU project Q-collect also formulated minimum requirements for reference collections of quarantine pests and diseases. The outcome of both the VirusCollect and Q-collect project form a good starting point for further development of standards. At this stage it is important to continue by implementing the draft standards to experience if and how they work in practice. Final standards should not only guarantee the quality of the collection and data, but should be feasible in practice as well.

Collection and characterisation
With regard to the collection and/or characterisation of isolates, DSMZ was able to obtain interesting isolates of various whitefly-transmitted viruses, and some other viruses and viroids (Paragraph 3.5). After characterisation, either isolates or nucleic acid extracts derived from infectious clones, and reference materials (positive controls) were made available via Q-bank. Three of the characterised carlavirus isolates will be made available following publication.
WUR characterised many isolates of their collection by application of NGS technology, which appeared very suitable for this task (Boonham et al, 2014; Dullemans et al, 2015; Paragraph 3.6). In general, this technique yields almost full genome sequences that were completed by RACE analysis (Sambrook and Russell, 2001). This technique was successfully used to obtain sequence data for development of a molecular test for SLRSV. This virus showed so much sequence variation that none of the available tests was able to detect all isolates. As a result of this project (almost) complete sequences of over 20 isolates became available. These sequence data allowed a much better understanding of the sequence variability within this group of SLRSV isolates and provide a solid base for development of more reliable diagnostic tests. Unfortunately, not all isolates were physically available for inclusion in the collection. In addition, by performing analysis on NGS data by using a combination of reference and de novo assembly, additional information on the purity of the isolates could be obtained. Finally the WUR collection was further improved by storing isolates under liquid N_2, which hds been shown to

increase long-term viability significantly. To set expiry dates for individual species viability of stored isolates will be followed over a longer period.
Also SASA further characterised many of the indigenous and non-indigenous potato viruses of their collection (Paragraph 3.7). In addition, by cloning partial sequences standard reference materials (positive controls) for molecular tests were produced. All relevant data, isolates and reference materials were included in Q-bank, or will be made available after publication.
It was not possible to include any of the virus species detected by AGES, since isolates were not further characterised and propagated and only RNA had been left (Paragraph 3.4).

Q-bank
The inclusion of all ICTV-recognised virus and viroid species in Q-bank resulted in a comprehensive database for public use (Paragraphs 3.2 and 3.8). This database provides access to general information at the species level and specific data at the isolate level, and links to relevant websites. In addition, it easily generates overviews of 'regulated' species, species transmitted by *B. tabaci*, species infecting potato, as well as the location from which specific (characterised) isolates can be purchased. The inclusion in Q-bank of reference sequences from NCBI GenBank of 'all regulated' species, allows BLAST searches and provisional identification of these species on the basis of (partial) sequences generated by diagnostic laboratories. However, with regard to the inclusion of sequence data from GenBank, it should be noted that these data often have not been checked and validated. Especially for the viruses transmitted by *B. tabaci,* it appeared that for the vast majority of these viruses very little information is available. Although sequences of these viruses have been deposited in GenBank, and for a number also a literature reference is available, virtually no information is available on their physical availability. Validation of the (Genbank) deposited data is therefore not (yet) possible. Therefore, end-users using the BLAST function offered on the Q-bank website, should be aware that results often might be based on the publicly available GenBank data. As a consequence, the verification and interpretation of these results is their own responsibility.

Collections and legislation
The acquisition and release of virus isolates as well as isolates or reference materials derived from cloned viral sequences is subjected to international directives and protocols. In this paragraph, a few remarks are made on the most relevant ones.

Within the EU, the movement of regulated species is described in Commission Directive 2008/61/EC (http://faolex.fao.org/docs/pdf/eur80072.pdf). In practice this implies that a Letter of Authority (LoA) has to be provided by the Member State's phytosanitary authority which allows the material to enter its territory or to be moved within the country. This document has to accompany the commodity during transport. For exchange of material with countries outside the EU, an Import Permit is used in stead of a LoA.
In addition the Convention on Biological Diversity, in particular the Nagoya Protocol might have implications for the exchange of isolates from (plant virus) collections (Annex 2; Convention on Biodiversity; about the Nagoya protocol; https://www.cbd.int/abs/about/ (09-11-2015)). However, different interpretations of the scope of this protocol might hamper a free exchange and limit the availability of

collection materials. Therefore, a global discussion and agreement on the interpretation and consequences of the Nagoya Protocol would be desirable. Finally, it is not clear how 'isolates' derived from infectious full-length clones should be handled. Which legislation does apply on this type of material? GMO regulation (http://ec.europa.eu/food/plant/gmo/legislation/index_en.htm; (http://www.unece.org/fileadmin/DAM/trans/danger/publi/adr/adr2015/ADR2015e_WEB.pdf), Directive 2008/61/EC, or other? This question not only concerns the release and exchange (including transport) of this type of material but also the requirements for handling and storage. Also this question qualifies for a broader discussion at the international level.

Concluding remarks and future developments

The project VirusCollect laid the foundation for further collaboration between laboratories keeping virus collections. The formulation of requirements to guarantee the quality of collection materials and the development of (draft) SOP's, provided basic conditions for exchange between laboratories. Together with the minimum quality standards described in the report of the Q-collect project, these standards form a good starting point for improving the infrastructure and contents of virus collections. In addition to the development and implementation of standards, VirusCollect also improved the 'contents' of the participating virus collections. Isolates that had been stored over many years were revived and characterised according to the new standard. In addition, unknown and emerging viruses were identified and added to the collections.

With regard to the collaboration, it should be emphasized that the viruses and viroids database of Q-bank plays a key role in sharing data, information and isolates (Figure 4.1). This platform provides public access to available data and isolates of an increasing number of virus and viroid species. The VirusCollect partners made relevant data generated during this project available via this database, so that they became accessible for other the partners as well as others.

From the results obtained so far, it is clear that this first step to a further collaboration between virus collections is a success which is appreciated by other laboratories. It is promising that the VirusCollect project will be continued with a new Euphresco project, VirusCollect II, in which at least four additional partners will join the network that links virologists and virus collections together.

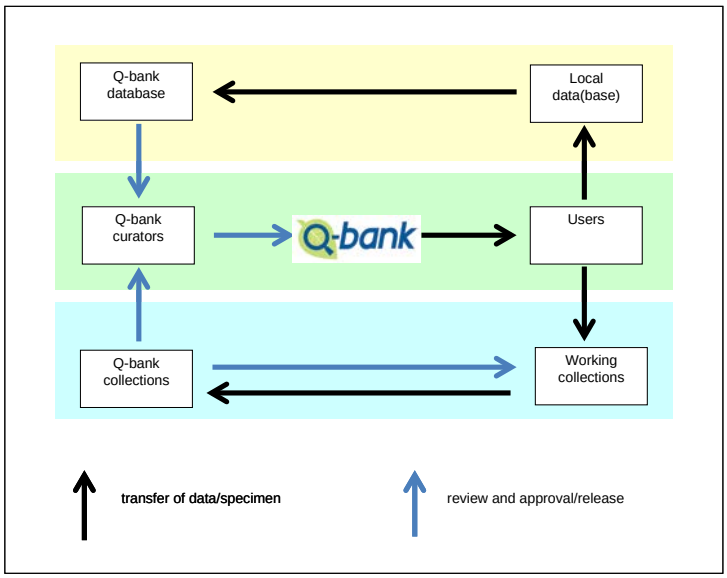

Figure 4.1 Central role of Q-bank for sharing data and information (database) on plant viruses and their availability (collection). From Roenhorst et al, 2013.

5. Main conclusions

1. The project VirusCollect improved the quality of both the infrastructure and ‘contents’ of virus collections at participating laboratories by development and implementation of (draft) standard operating procedures and characterisation and inclusion of relevant isolates.

2. Q-bank viruses and viroids database plays a key role in sharing data, information and isolates, and as such is an indispensable tool for linking virus collections.

3. The best way to improve the ‘contents’ of collections is to focus on the ‘spin-off’ of other projects by securing data and isolates. Spending time on gaps in collections is difficult, since priorities are dictated by actual problems.

4. The interpretation of the GMO legislation, the Convention on Biodiversity, in particular the Nagoya protocol, need attention at an international level due to the foreseen impact on the sustainability of existing collections, in particular on the availability and exchange of relevant reference materials.

6. Dissemination activities

Presentations, posters, other

Author(s)	Title (type)	Occasion	Location	Date
Menzel W	Q-bank – Eine umfassendes Informationssystem für regulierte Pflanzenviren und ihre Verfügbarkeit in Sammlungen (presentation)	59. Deutsche Pflanzenschutztagung	Freiburg (Germany)	24.09.2014
Van der Vlugt RAA	VirusCollect (presentation)	Q-Collect project meeting	Kleinmachnow (Germany)	26/11 – 28/11-2014
Van der Vlugt, RAA and Roenhorst JW	Viruscollecties (presentation)	Begeleidingscommissie topsector Teelt en Uitgangsmateriaal, Fytosanitair fundament,	Wageningen (Netherlands)	17-02-2015
Roenhorst JW, de Krom C and Flint L	How to build collections and more (presentation)	EUPHRESCO Fellowship meeting	York (United Kingdom)	25-02-2015
Menzel W	Die Pflanzenvirus-sammlung des Leibniz Institut DSMZ (presentation)	Sitzung der Abteilung Zierpflanzen des Bundesverband Deutscher Pflanzenzüchter (BDP)	Hannover (Germany)	27.02.2015
Roenhorst JW	Rol Q-bank database en (virus) collecties in Plantgezondheid (presentation)	1. Symposium Fytosignalering NVWA; 2. Lunch meeting NVWA	Wageningen (Netherlands)	15-04-2015 20-04-2015
Van der Vlugt, RAA and Roenhorst JW	Viruscollecties en Q-bank (presentation)	Werkgroep Plantenvirologie	Lisse (Netherlands)	17-09-2015
Roenhorst JW	National Reference Centre & Role of Q-bank database and virus collections in Quality control (presentation)	Technical mission, CIQ	Kunming (China)	12-10-2015
Roenhorst JW, Flint L, Menzel W, Winter S & van der Vlugt RAA.	Q-bank for sharing data and information on plant virus and viroid isolates in collections (poster)	1. Fifth Conference International Working Group on Legume and Vegetable Viruses 2. COST Action DIVAS	Haarlem (Netherlands) Ljubljana (Slovenia)	30/08-03/09-2015 16/11-18/11-2015
Roenhorst JW, Lacomme C, Leichtfried T, Menzel W, Nisbit C, Winter S & van der Vlugt RAA.	VirusCollect: Building an international network of reference collections for regulated and other important viruses (poster)	1. Fifth Conference International Working Group on Legume and Vegetable Viruses 2. COST Action DIVAS	Haarlem (Netherlands) Ljubljana (Slovenia)	30/08-03/09-2015 16/11-18/11-2015
Van der Vlugt RAA	VirusCollect (presentation)	Q-Collect project meeting	Rome (Italy)	07/09 – 09/09-2015

Publications

Authors	Title	Reference
Roenhorst JW, Boonham N, Winter, S, Menzel RAA, Van der Vlugt RAA	The plant viruses and viroids database and collections of Q-bank.	EPPO Bulletin 43, 2013, 238–243

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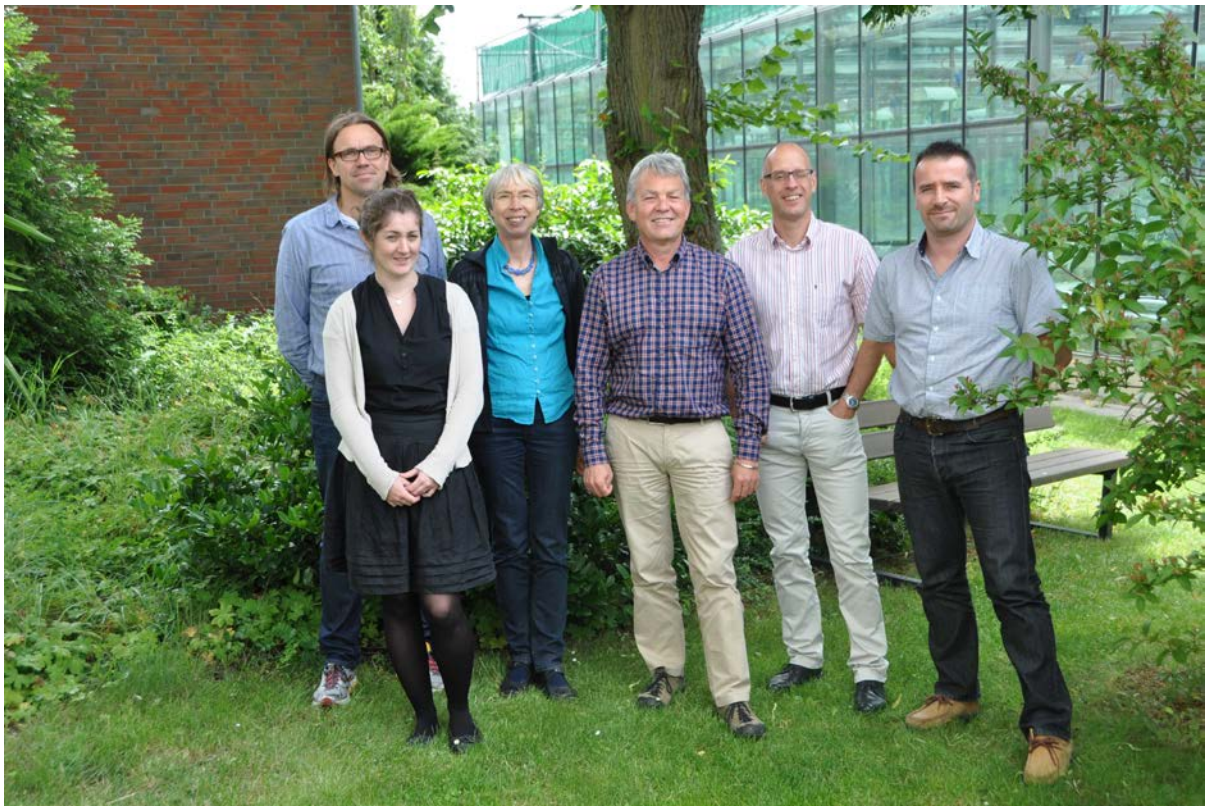
Annex 1

Summary 1st year meeting at DSMZ, Braunschweig, Germany, 24-25 June 2014

Particpants
Christophe Lacomme (SASA, UK), Wulf Menzel (DSMZ, DE), Annelien Roenhorst (NPPO, NL), René van der Vlugt (PRI, NL) and Stephan Winter (DSMZ, DE). Unfortunately Thomas Leichtfried (AGES, AT) was not able to join the meeting. Laura Flint (FERA, UK) participated because of her involvement in Q-bank.

General introduction
The 1st year meeting of the Euphresco project VirusCollect was combined with a meeting of the curators of the Q-bank Plant Viruses and Viroids Database. This offered the opportunity to discuss the results and future plans on physical collections in relation to their accessibility. All Euphresco partners* presented the results of their work performed over the last year (WP's 4 to 7). Agreements were made on the choice of isolates to be included in Q-bank. Practical issues on data transfer were addressed, especially for those partners not involved in Q-bank (WP 8). Furthermore, fundamental issues related to the quality and maintenance of a virus collection were discussed (WP's 2 and 3). Finally, DSMZ offered a tour around their facilities and presented on the ISO 17025 and ISO 34 accreditation of the DSMZ Virus collection. The following paragraphs provide the highlights of the discussions and agreements of the meeting.



First year meeting Euphresco project VirusCollect, DSMZ Braunschweig Germany, 24-25 June 2014

Collection versus reference material
Before starting the discussion on quality criteria, the term collection should be defined more explicitly. Two terms are relevant, i.e. collection material and reference material. A collection includes virus isolates that fulfil the criteria of authenticity (isolates true to type), purity, viability and preservation. Reference material is a product derived from a collection and related to its use in a diagnostic test. As such reference materials do not have to fulfil the criteria of a collection and may consist of different derivatives of the collection material, e.g. non-infectious virus for ELISA, nucleic acid extract or plasmid for PCR.

Purpose of a virus collection
The main purposes of a virus collection are: 1) preservation of particular virus isolates and 2) supply of reference materials for diagnostic purposes. The VirusCollect consortium focuses on preservation of isolates fulfilling the criteria, but at the same time produce and offer reference materials for diagnostic purposes. Test laboratories as end users might often be only interested in reference material for diagnostics.

Quality of a virus collection
Ideally a virus collection meets the criteria of authenticity (isolates true to type), purity, viability and preservation. At the same time, the collection has to be kept up to date. This puts high demands on the collection and curation of the isolates. How to select isolates? How to preserve a virus isolate true to type? How to define a particular strain? Moreover, how to keep virus species that cannot be transmitted mechanically or preserved outside their original host. Often only limited amounts of these isolates can be preserved as reference material, but not as collection material.

Conduct codes
Depositors of isolates agree that their isolates will become public available in due time. Purchasers of collection material are fully responsible for its use, either for beneficial or misuse. Curators of public collections should be able to exchange material on the basis of free interchange.

Future collection and accessibility of regulated viruses
In Europe, virus collections are fragmented, often their contents is unknown and especially regulated and emerging species are hardly available. The VirusCollect consortium aims to characterise relevant isolates and makes them accessible via Q-bank. Since progress in this way is only limited, an alternative strategy has been agreed. Following this alternative approach Q-bank will serve as a central database that provides actual information on especially regulated viruses. On one hand it should list all names of regulated species, on the other hand it should indicate if and where isolates of these species are available. In this way Q-bank offers additional features over other virus databases, i.e. it provides a complete overview of all regulated virus species and at the same time shows if and where they are available. Moreover, it will clearly show which species are missing, and efforts can be initiated to fill these gaps. As a result the Q-bank will no longer show ‘complete’ data sets for a limited number of species, but limited data for all virus species. However, the key feature remains, i.e. that all data included are checked by the curators.

Q-bank as central database

At the short term, the Q-bank curators will include the names of all plant virus species as well as their quarantine status in the database. Curators will continue on filling the gaps, both on the availability and characterisation of virus isolates. In the mean time, they will include the data generated during the VirusCollect project.

Annex 2

Summary of the 2nd and final meeting at NPPO, Wageningen, the Netherlands, 29 September till 1 October 2015

Particpants
Christel de Krom (NPPO, NL), Christophe Lacomme (SASA, UK), Wulf Menzel (DSMZ, DE), Carolyn Nisbet (SASA, UK), Annelien Roenhorst (NPPO, NL) and René van der Vlugt (PRI, NL). Unfortunately Thomas Leichtfried (AGES, AT) was not able to join the meeting.

General introduction
The 2nd and final meeting of the Euphresco project VirusCollect aimed the presentation of work done by all partners, in particular over the last year (WP's 4 to 7). In addition, the developments of Q-bank database on plant viruses and viroids was presented by the curators in relation to the identification of gaps in current collections (WP 2). The discussion focused on the drafting and implementation of quality standards on collection and reference materials (WP 3), the provision of data for inclusion in Q-bank (WP 8), the consequences of the outcome of the EU project Q-collect and the successor Euphresco project VirusCollect II. Furthermore, there were tours around the facilities of Wageningen University and Research Centre and the National Reference Centre of the National Plant Protection Organization of the Netherlands. The following paragraphs provide the highlights of the discussions and agreements of the meeting.

Final meeting Euphresco project VirusCollect, NPPO, Wageningen, the Netherlands, 30 September – 1 October 2015

Definitions of collections
The discussion on the definitions of collection and reference materials during the first year meeting was continued by a discussion on the definition of different types of collections. The following terms and definitions were used in this project:

- Certified reference collection: isolates are included in, maintained and isolates and/or reference materials provided from the collection, following the requirements of an ISO 34 accreditation;
- Reference collection (collection for reference): inclusion, maintenance and provision of isolates and/or reference materials in or from the collection according to SOP's that are part of the quality management system under ISO 17025 accreditation;
- Working collection: isolates from field samples or local collections that do not fulfil requirements to be included and maintained in a (certified) reference collections.

The mutual relationship between the different types of collections is illustrated in figure 1. It should be noted that other terms have been used or are in use in the Q-collect project. The terms used in the VirusCollect project will be changed accordingly if any other terms become the standard.

Developments Q-bank Plant Viruses and Viroids database
As agreed during the first year meeting all plant virus and viroid species, recognised by the International Committee on the Taxonomy of Viruses (ICTV), were included in the Q-bank Plant Viruses and Viroids database. In addition, for all species that are regulated in the European Union or recommended for regulation by the European and Mediterranean Plant Protection Organization, reference sequences from NCBI GenBank were included, so that the database can be used for provisional identification of these species by BLAST searches.

EU project Q-collect and Nagoya protocol
René van der Vlugt highlighted the outcome of the EU project Q-collect, which aims, where it concerns viruses and viroids, at least partially overlap with those of the Euphresco VirusCollect project. The most important conclusions concerned the fact that there are a significant number of virus collections, mainly consisting of small collections of different quality for local use, which are not disclosed. The need for creating sustainable networks was addressed, including the agreement on a minimum quality level. It was ascertained, however, that only a very few collections are currently complying with relevant ISO norms. Another point of concern was the implication of the Nagoya Protocol, a supplementary agreement to the Convention on Biological Diversity that provides a transparent legal framework for the fair and equitable sharing of benefits arsing out of the utilization of genetic resources (Convention on Biodiversity; about the Nagoya protocol; https://www.cbd.int/abs/about/ (09-11-2015)). This protocol entered into force on 12 October 2014, and so far it is not clear how it would affect the inclusion of isolates in collections and the exchange of collection materials in the field of plant health. This subject, however, exceeds the scope of this project.

Dissemination and future plans

To inform others about the ‘network of virus collections’ established as a result of the Euphresco project VirusCollect, ideas have been launched for dissemination and promoting the achievements and its potential for future collaboration. Several partners had presented the project during scientific meetings. To promote its use and increase the scientific value of Q-bank Plant Viruses and Viroids database and collections, virologists should be approached to provide their data and isolates. The inclusion of data from Next Generation Sequencing projects, like COST Action DIVAS and the Euphresco project ‘The application of Next-Generation Sequencing technology for the detection and diagnosis of Non-Culturable Organism: Viruses and Viroids’, would be interesting. In addition, plant quarantine and diagnostic laboratories should become aware of the benefits of the Q-bank collections for quality management. Furthermore, after finalising the project, it was proposed to draft a ‘press release’ for EPPO and IPPC on the state of the art and future developments of Q-bank Plant Viruses and Viroids database and collections.

Annex 3

Quality standards for Q-bank collections of viruses and viroids

General quality management
the whole quality management system and procedures should be periodically reviewed to keep all up to date (internal audits)
onsite audits/inspections of the production facility should be possible/allowed (transparency)
business continuity should be guaranteed
equipment (e.g. pipets, balance, pH meter) should be periodically calibrated to traceable measurement standards
maintenance and calibration reports should be kept
only trained personnel should be allowed to handle reference material (whole process from characterisation to order processing)
facility access control is a prerequisite
appropriate cleaning and decontamination procedures should be installed to avoid contaminations
data (characterisation, production and ordering process) should be stored (including a back up copy), traceability is important
ensure the protection of confidential information
backup storage of the collection should be available (e.g. DSMZ has N_2 storage [seed stock] in one building, freeze dried material [distribution stock] in another)
in order to guarantee sustainability, adequate equipment and reliable funding is needed for long-term stability of the physical Q-bank collections
ensure that personnel is free from undue internal or external pressure that adversely affect the quality
in order to guarantee that the required expertise is maintained, the head of laboratories/curators of the collections should have a long-term employment

Characterisation and identification
isolate origin should be recorded as good as possible (isolation host, country of origin, submitted by, original isolate number/name given by the collector)
procedures for receipt and handling of biological material should be documented
unique numbers have to be assigned which are never reassigned if the material is later discarded
material has to be characterised to the level of its intended use [identity for isolates and specific reaction for controls]
identification with appropriate methods (species level according to demarcation criteria in King et al. 2012-Virus Taxonomy)
at least a partial genome sequence, ideally covering a gene used for species demarcation, should be available (e.g. coat protein gene)
property values have to be assigned
characterisation has to include assessment of viability and purity

characterisation may include a broad range of techniques, especially to assess purity:
- electron microscopy
- dsRNA extraction
- RCA (rolling circle amplification)
- typical symptoms on assay hosts
- group specific tests
- transmission trials (e.g. mechanical, insect vector)

(of course not all have to be applied for each isolate during characterisation, but the selection/combination should give confidence)
suitability of selected/specific conservation method has to be assessed
identity and purity also has to be verified for material received from other sources (collections/scientists), irrespective if it is already characterised or not
raw data should be stored (e.g. sequencing, ELISA)
traceable documentation of the whole characterisation process is required

Reference material production

procedures should be documented in SOPs to guarantee uniform production and consistent batch quality
SOPs should also contain all required data for relevant buffers, media, PCR-reaction mixture, etc.
changes of the production process should be verified by quality
appropriate labelling (collection name, scientific/species name or accepted acronym, no synonyms/colloquial names)
unique collection (accession) number if available (especially important if more than one isolate of a species exists in the collection)
long-term stability: suitability of selected conservation method should be assessed exemplarily for old batches (e.g. 5 years old)
short-term stability: suitability of conservation method should be assessed exemplarily under extreme conditions to simulate transport (e.g. 10 days 37°C)
between vial homogeneity should be assessed exemplarily (in particular for positive controls)
if virus is only available in mixed infection (PC, NA extract and inoculum), this information should be provided to the customer
(not required for only seed transmitted, non-pathogenic viruses of the families Partitiviridae, Endornaviridae and only genome integrated viruses)
traceable documentation of the whole production process is required

Quality control

the processes used in QC should be defined
exemplarily validation (e.g. for DAS-ELISA) and/or participation in interlaboratory comparisons for proof of competence is recommended
each new batch of positive controls should be tested to fulfil QC criteria (should be defined, e.g. PC has at least 5-times higher OD compare to NC/buffer)
each new batch of inoculum or the plant used to produce the batch should be tested to ensure identity of species (e.g. EM, ELISA, PCR)

storage conditions of produced batches should be clearly defined
storage conditions should be controlled to ensure quality (e.g. temperature)
batches should be traceable to the production process and date of production
quality of nucleic acid extracts have to be checked (e.g. gel electrophoresis, nanodrop)
non-conforming work should be controlled and corrective actions taken
traceable documentation of the quality control process is required

Order processing and shipping

it has to be guaranteed that handling always complies with relevant national law and regulations
international safety regulations and Q regulations (letter of authority [EU Directive 2008/61], dual use [EU Council regulation 1232/2011]) have to be considered
appropriate packing and shipping (based on experience "fit for purpose", e.g. dry ice or room temperature, courier service or regular mail)
provide instructions for end-users including storage and handling conditions (eg. certificate, information sheet, or provide information via website)
information what and how much customer gets has to be specified (e.g. dried, freeze dried, fresh leaves; 1 cutting, 2 ml PC etc.)
an expiry date has to be defined for the material provided
a free replacement should be provided within that timeframe if material is not appropriate
free access to related data/information should be granted
customer support after purchase of material should be guaranteed
a permanent contact address should be provided/published (mail, email and telephone)
traceability of orders should be possible for e.g. at least a few years or until the expiry date
customer complaints should be tracked
confidentiality for all orders of past and present customers has to be guaranteed
customers have to be informed if nonconformities are realized (e.g. contaminations, misidentification)
information on order processing time (lead-time) should be provided (regular process, not for exceptional cases that might happen)
distributors have to be authorized by the reference material producer (appropriate storage and handling has to be guaranteed)
if resoled by unauthorized third parties/distributors, the material will loose its reference status (no control/influence on handling, storage etc.)
material will loose Q-bank reference status if reproduced by third parties (no influence on production process, QC, storage and handling)
traceable documentation of the order processing is required