Executive Summary:

Development of accurate identification tools for plant pathogens and pests is vital to support European Plant Health Policies. For the QBOL project Council Directive 2000/29/EC is important, listing some 275 organisms for which protective measures against introduction into and their spread within the Community needs to be taken. Those threats are now greater than ever because of the increases in the volumes, commodity types and origins of trade, the introduction of new crops, the continued expansion of the EU and the impact of climate change.

Currently identifying pathogens (in particular new emerging diseases) requires a staff with specialized skills in all disciplines (mycology, bacteriology, etc.); which is only possible within big centralized laboratory facilities. Taxonomy, phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction. Modern molecular identification/detection techniques may address the decline in skills since they often require much less specialist skills to perform, are more amenable for routine purposes and can be used for a whole range of different target organisms. Recently DNA barcoding has arisen as a robust and standardized approach to species identification. A DNA barcode is a short region of the DNA which can be used to identify a species. QBOL made DNA barcoding available for plant health diagnostics and focused on strengthening the link between traditional and molecular taxonomy as a sustainable diagnostic resource. Within QBOL, collections harboring plant pathogenic Q-organisms were made available. Informative genes from selected species on the EU Directive and EPPO lists have been DNA barcoded from vouchered specimens. The sequences, together with taxonomic features, have been included in a new internet-based database system: Q-bank: www.q-bank.eu. A validation procedure on developed protocols and the database have been undertaken across worldwide partners to ensure robustness of procedures for use in a distributed network of laboratories across Europe.

Project Context and Objectives:

Development of accurate identification tools for plant pathogens and pests is vital to support European Plant Health Policies. For the QBOL project Council Directive 2000/29/EC is important, listing some 275 organisms for which protective measures against introduction into and their spread within the Community needs to be taken. Those threats are now greater than ever because of the increases in the volumes, commodity types and origins of trade, the introduction of new crops, the continued expansion of the EU and the impact of climate change.

Currently identifying pathogens (in particular new emerging diseases) requires a staff with specialized skills in all disciplines (mycology, bacteriology, etc.); which is only possible within big centralized laboratory facilities. Taxonomy, phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction. Modern molecular identification/detection techniques may address the decline in skills since they often require much less specialist skills to perform, are more amenable for routine purposes and can be used for a whole range of different target organisms. Recently DNA barcoding has arisen as a robust and standardized approach to species identification. A DNA barcode is a short region of the DNA which can be used to identify a species. QBOL made DNA barcoding available for plant health diagnostics and focused on strengthening the link between traditional and molecular taxonomy as a sustainable
Four principal project objectives have been formulated within the QBOL project and are shown below:

1. Obtain or produce relevant vouchered sequence data for individual pests or pest groups and position them in a correct taxonomic context. We will determine which and how many genes (barcodes) are informative for correct Q-species identification and what are the species limits for relevant Q-organisms and morphologically and/or taxonomically related organisms, to enable the accurate identification/diagnosis of all taxa on the EU Council Directive and EPPO A1 and A2 lists.

2. Developing generic diagnostic tools based on these barcode sequences. We will investigate bioinformatics tools to enable the correct identification of Q-organisms based on DNA barcode sequences, and develop a database that will enable the storage and searching of related diagnostic metadata, to link vouchered sequence information to published biological information.

3. Develop strategic approaches and methodologies to enable the establishment of DNA banks and access to digital voucher specimens. We will develop methods that enable the storage of DNA/RNA samples (a DNA bank) for the selected set of Q-organisms and their relatives to enable access of material to all national plant protection services for positive and negative controls.

4. We will support better collaboration between EU and third-world country diagnostic laboratories and also the international ‘DNA barcoding’ community.

Project Results:
Main Results
To meet the objectives of QBOL, we performed research on the different aspects of DNA barcoding for the selected quarantine organisms, the database, DNA-bank, validation and dissemination within the different work packages. The results of all these work packages are described below:

Work package 1 – Coordination and Management
QBOL Project activities were continuously coordinated and managed by the project team of the coordinator PRI. The webportal, which was set up for partners for internal communication between partners/Advisory Board and exchange of presentations, reports, minutes etc., was regularly updated. Seven project meetings were organized in Wageningen (May 2009), Montpellier (October 2009), York (May 2010), Bologna (October 2010), Slagelse (March/April 2011) and Gent (September 2011). The final meeting was held in Haarlem on 21 May 2012.

Work package 2 – Barcoding Fungi
Within WP2 a short list of 19 Q-species were selected for barcoding. For those species several gene regions were screened to identify suitable barcoding loci. Protocols for efficient DNA extraction, generic amplification and sequencing of the selected loci were evaluated.

For some species it was difficult to obtain larger numbers of isolates per quarantine species. This work package succeeded to make available to the Q-bank database: 791 sequences for 193 strains from 25 quarantine species; as well as 6107 sequences from 1145 strains of 612 related species. Of these sequences, 360 sequences for 81 species were extracted from studies in peer-reviewed journals (mainly for the related species of the unculturable obligate biotroph genera Melampsora, Puccinia and Thecaphora). For each species of quarantine importance in the Q-bank database, hyperlinks to EPPO and EU Council Directive documents are provided. A field “Diagnostic locus for identification in Q-bank” is present for each quarantine species to help the end-user to determine which locus is needed for identification in the database and polyphasic identifications are possible per genus group. A molecular decision scheme showing the route to an identification starting with DNA isolation and amplification of the internal transcribed spacers (ITS) of the nrRNA operon as primary barcode is provided on the Q-bank website. A link to MycoBank, a database for the taxonomy of fungal names, is also provided for each species.
Work package 3 – Barcoding Arthropods

Within WP3 198 species of Q-arthropods have been divided into two priority groups. These lists have been erected on the basis of the economic value of the Q-arthropods, their availability and their habitat, trying to cover both agriculture and forest pests. Many contacts with colleagues and many field trips have been made to get the required specimens.

We tested several DNA extraction methods that are commonly used with arthropods and selected three that performed better and/or are easy to use by non-trained people. Also, a non-destructive protocol was developed. Cox-1 region and the ITS-2 region of the rDNA were selected to be barcoded and primers for those regions were developed and tested. The initial objectives were to generate from 5-10 barcode sequences (COI and ITS) for about 100 species of Q-arthropods (priority 1), and about 50 closely related species. For priority group 1, 83 Q-species (79.8%) were sequenced for a total of ca. 2500 sequenced PCR amplicons with an average of 20 COI and 10 ITS sequences per species. For priority group 2, 52 Q-species (54.7%) were sequenced for a total of ca. 1300 sequences with an average of 16 COI and 8 ITS sequences per species.

We also included 20 species that are not yet considered quarantine species, though they represent a threat to Europe. This priority 3 list was established during the project, and we really believe that including these species make our identification tool more adapted to European needs. For these species, a total of ca. 200 sequences with an average of 7 COI and 3 ITS sequences per species were obtained. Instead of the 50 outgroup species initially proposed (i.e. species that are congeneric of or could be confused with the Q-arthropods), we sequenced 128 species, producing about 1300 sequences with an average of 7 COI and 4 ITS sequences per species.

To improve the representativeness of our database (i.e. include Q-arthropod species included on priority 1 and 2 lists but not yet available to us or increase intraspecific variability for better identification), 334 COI sequences mostly produced by USDA and mined from GenBank have been added to our sequence library.

All sequences were carefully validated before inclusion in our database (detection of contamination and pseudogenes). Altogether, about 5300 sequences have been generated during the project and 334 sequences have been mined from Genbank. Our database now includes ca. 5600 COI and ITS barcodes for 153 species of Q-arthropods (ca. 77% of the priority 1 and 2 lists), 20 species of arthropods that are considered a serious threat for Europe and 140 outgroup species, far surpassing the original aim. All specimens have been identified by taxonomists, vouchers in INRA and LNPV Montpellier.

Work package 4 – Barcoding Bacteria

Within WP4 work focussed on the Q-species within the genera Ralstonia, Xylella, Clavibacter and Xanthomonas. Most of the Q-species within Xanthomonas on the EU Directive and EPPO list are on the pathovar level, which makes it difficult to select the barcoding gene as pathovars do not necessarily form a single taxonomical group.

Strains were retrieved from official collections, with a substantial amount being contributed from the BCCM-LMG collection hosted at LM-UGent. Especially for the Xanthomonas, other official and working collections were contacted in regions of the world where the pathogens recently occurred.

DNA extraction procedures were evaluated and final protocols were written. Several genes were evaluated for their performance as a barcode region. Finally a decision scheme was published to lead the end-user through the identification process. This scheme clearly shows when to use which barcoding genes. Extensive sequencing also confirmed the taxonomic position of most of the target Q bacteria (within Ralstonia, Xylella, Clavibacter michiganensis and the Xanthomonas species) and also supports recent proposals for the taxonomic division of the Ralstonia solanacearum complex. On the other hand, our research also revealed that some Q-pathogens are represented by heterogeneous strains (e.g. X. axonopodis pvs. dieffenbachiae, p. phaseoli and p. allii). Their classification under the same Q-pathovar name is questionable and needs further investigation by sequencing more genomic domains and performing host range experiments on plants. Within Clavibacter, the three Q-C. michiganensis subspecies are identified by the gyrB-based barcode. Also many non-pathogenic strains of the species (look-a-likes) were included in the study.

In total, the QBOL working collection increased to 1,008 strains and 3,667 sequences have been generated. Based on these sequences and on other strain characteristics (such as host, geographic origin and symptom type) a subset of reference strains has been identified for the end-users. The barcodes have been deposited in the Q-bank database and the strains are
stored and available from the certified public service culture collections BCCM-LMG (BE), NCPPB (UK), and CFBP (FR). Under the initiative of ILVO, these culture collections collaborate on a reference collection of plant health-affecting bacteria.

Work package 5 – Barcoding Nematodes
Within WP5 a base list of 32 nematode species was created for which barcodes needed to be collected. This list contained all quarantine nematodes as well as a number of close relatives. In addition, 43 nematodes species were nominated which would be sequenced if time permitted. These additional species were composed of further relatives of Q-organisms as well as a number of other agronomically relevant nematode species. Material for most of the species on the base list has been acquired as well as material for a large number of additional species.

Five DNA isolation methods were compared, including both commercial kits as well as published methods, and the best two methods were selected for further use.

Primers were developed for the amplification of six potential barcoding regions: the small subunit (SSU) ribosomal RNA gene, the D1-D2 and D2-D3 regions of the large subunit (LSU) ribosomal RNA gene, the second intragenic spacer region (IGS2) of the ribosomal RNA cassette, a fragment of the RNA polymerase II gene and the mitochondrial cytochrome oxidase c subunit 1 (COI) and subunit 2 (COII) genes. A subset of nematode species was assigned to assess both the inter- and intra-species variation of these potential barcode regions and based on these results the SSU, LSU, COI and COII genes were chosen for sequencing in the remaining nematode species.

A total of 1600 sequences for up to 58 species, distributed over the various priority groups, was promised in the project. For each priority group the required amount of sequences were generated and in some cases well surpassed. Of all generated sequences, 1683 were of a high enough quality for inclusion in Q-Bank, originating from a total of 121 species.

Work package 6 – Barcoding Viruses
Since viruses don’t contain a generic barcode gene, we decided within WP6 to sequence the whole genome of viruses using Next Generation Sequence Technology.

The consortium produced an initial list of viral targets for which little sequence was available at the start of the project. Material was obtained for each of these species and the genome sequences were produced using 454 and Solexa technology. With the acquisition of a 454 GS-FLX by one of the partners was possible to optimise the complete sequencing process for virus genome sequencing. Methods have now been developed within the consortium which allow the cheap combining of multiple samples prior to the sequencing processing. These methods, along with an optimum virus RNA specific extraction process have reduced the cost of viral genome sequencing.

Genome sequence data has been produced for Arracacha virus B, oca strain, Potato black ringspot virus, Potato virus T, Potato yellowing virus, Tomato infectious chlorosis virus, Chrysanthemum stem necrosis virus, Iris yellow spot virus, Tomato torrado virus, Tomato marchitez virus, Potato yellow vein virus and Tomato chocolate virus.

A number of different RNA extraction methods have been tested and used to successfully produce virus genome sequence. It has been discovered that to maximise virus sequence recovery and thus minimise sequencing cost total RNA extraction of plants containing virus is not the best approach. Methods have now been developed within the consortium to purify virus RNA away from plant RNA. These methods have been being compared to determine the optimum method for a particular sample type. These methods include double stranded RNA isolation, small interfering RNA isolation, partial virus purification prior to RNA isolation, subtractive hybridisation and the use of capture probes. Results suggest that no one method is optimal for all samples.

A range of methods to sequence plant viruses have now been developed and validated within the consortium which allow the cheap combining of multiple samples prior to the sequencing processing. These methods along with advice on accessing this technology have now been published as part of the QBOL project. The methods have been used to sequence in total 46 viruses and have been used in the diagnosis of a number of novel diseases including the discovery of watercress white vein virus and maize lethal necrosis in Kenyan maize.

Work package 7 – Barcoding Phytoplasmas
Within WP7 a prioritized list of all phytopathogenic phytoplasmas relevant for the EU to be barcoded was established and
maintained and expanded using existing collection with relevant phytoplasma isolates. Colleagues and collections have been contacted for specific strains throughout the project, for instance after publication of interesting new strains of phytoplasmas. To obtain new strains we mainly used the COST0807 network.

We have strains from all these Q-phytoplasmas, and have during the last months been able to obtain barcode sequences of the American type of palm lethal yellowing through a scientist in Honduras. As the list of ‘Candidatus Phytoplasma’ species is constantly expanding we are still trying to include these in the collection. We will use our international contacts to get material from these ‘Candidatus’ species. However we have established a list with all 13 Q-phytoplasmas.

Phytoplasmas cannot be cultured in vitro and thus they need to be maintained in planta which requires considerable work. Partner 8 has currently 140 strains in micropropagation which covers the above-mentioned Q-phytoplasmas except palm lethal yellowing which is only available as DNA.

Several DNA extraction methods were evaluated for their effectiveness to extract phytoplasma DNA from infected host material.

Phytoplasma barcode regions 16S, tuf and SecA have been selected to be used as DNA barcodes. Tuf and SecA regions are 400-600 bp whereas the 16S region is app. 1.8 kb.

Barcode data including intra- and interspecies genetic variation on the phytoplasma barcode regions (3 regions: 16S, Tuf and SecA) were collected during the last part of the project. Until now, more than 460 barcodes have been produced. These barcodes enable good separation between phytoplasma groups and are thus ideal for identification of phytoplasmas, including quarantine organisms.

Work package 8 – DNA Banks
Since DNA of quarantine organisms is scarce, we investigated within WP8 different protocols to store, transport and multiply DNA samples of these organisms.

Within WP8 eight different protocols for long term storage and transport of DNA/RNA samples and WGA products were investigated and tested (e.g. filter, beads, other). GenTegra was chosen as storage medium.

Four kits for whole genome amplification (WGA), a method to multiply DNA, were tested on a subset of organisms from each group (fungi, bacteria, arthropods, nematodes, viruses, phytoplasms). The quality of the individual kits was assessed using different methods: TaqMan PCR, conventional PCR, sequence analysis and gel electrophoresis. Based upon results obtained thus far a WGA kit was selected to be used for the rest of the project.

The samples for ring testing in WP10 Validation were prepared and a prototype of DNA bank was established.

Several protocols have been evaluated and final protocols have been written. Using these protocols NPPO’s can better handle DNA samples of rare specimen to be used as positive and negative controls in their molecular identification and detection assays.

Work package 9 – DNA barcode library / Database / Informatics
The database (developed within the Dutch FES project, 2006-2010) has been further developed during the QBOL project within WP9. Together with QBOL WP leaders and associated researchers, we have created six databases: for fungi, arthropods, bacteria, nematodes, viruses and phytoplasmas. All groups have access to their databases via Citrix XenApp. The total database Q-bank is freely accessible via internet (www.q-bank.eu). Contacts with CBOL/BOLD, EDIT WP5, GBIF, StrainInfo, GenBank and EMBL were taken at several occasions during the course of the project in order to import from and export data to the respective projects or databases. A software module to export to and import from Genbank (and therefore EMBL) has been implemented. In discussion with the curators of the database we continuously improved the Internet-based software to comply with the needs of the end-users during pairwise meetings. Additional training was provided during the meetings with WP leaders and associated researchers. Filling of the databases has been made significantly during last stages of the QBOL project but will continue even beyond the end of the project. Publication of the created databases is now complete and Internet visitors are regularly using the system. Websites are therefore not restricted to the users participating in the QBOL project anymore. Usage of the different databases are monitored by Google Analytics.

Work package 10 – Validation / Evaluation
In the first part of the QBOL project a survey was set-up to find out the wishes and expectations of possible end-users (scientists and technicians of NPPO’s) in regard to the data generated by QBOL and stored into the database (Q-bank). Based on the end-users’ expectations, and on the queries submitted, the usability of the QBOL database was improved. Before the start of the test-performance study (TPS) within WP10, the developed tests were harmonised as much as possible and a draft EPPO standard “DNA barcoding as identification tool for EU regulated plant pests” has been made. A selection of specimens to be tested in TPS has been made and treated to be non-infectious and non-viable for sending them without permits. A homogeneity test has been performed with all samples before sending. Obtained sequences in the homogeneity test served as standards for comparison with the TPS outcome.

21 TPS packages (14 TPS partners, 7 training sessions) were prepared providing partners and training session organizers with an instruction booklet including de EPPO standard, all DNA purification kits, primers and samples. All results from 14 TPS partners were analysed and evaluated in terms of 1) Number of samples analysed and % of test correct used, 2) % amplicons obtained. 3) % consensus obtained, 4) % consensus sequence of correct size, 5) % primers trimmed, 6) Diagnostic sensitivity, 7) Diagnostic specificity, 8) Repeatability and 9) Robustness. Pitfalls in the use of the EPPO standard, instruction booklet and the use of Q-bank could be identified and recommendations for future work were made.

Work package 11 – Dissemination
Within the QBOL project dissemination played a major role in order to attract as much interest as possible for the outcome of this EU project. The QBOL project website (www.qbol.org) has been developed, maintained and regularly updated. The aim was to have at least 2000 visitors per year. At the moment we had already more than 10,000 visitors for the website. The website contains also an internal site for project participants and the Advisory Board, which contains minutes of meetings, reports, presentations, discussion forum etc., and an external site for stakeholders and end-users.

A publicity leaflet (1000 copies) and the QBOL poster were made and distributed to stakeholders and can be obtained by the partners from the web-portal. Participants from all WPs presented their work at (inter)national meetings and conferences via oral and poster presentations. Seven training courses were organized: South Africa, Kenya, China, Honduras, Peru, India and the Netherlands. One hundred and thirty participants (mainly people from NPPO’s: national plant protection organisations) from 26 countries attended these seven courses. In those courses the different work packages were presented. Practical work was performed on DNA extraction, barcode amplification and sequencing using the protocols developed within WP2-7 and finally searching in the database developed within WP9 (Q-bank).

A QBOL-EPPO workshop was organized in Haarlem, The Netherlands from 22-25 may 2012. The final QBOL workshop was held together with the EPPO meeting on Diagnostics, which takes place every three-four years. More than 180 people attended one or more days of the meeting. QBOL WP leaders presented the results of their work package. During the project more regular contact with stakeholders and end-users was made. Presentations were given for heads of NPPO’s, CPM (IPPC), Diagnostic panel IPPC, EPPO panel on Quality Assurance and Diagnostics, different EPPO panels, DG-Sanco and EPPO Working Party and Executive Committee.

Within the Netherlands the ministry of Economics, Agriculture and Innovation subsidised the Q-bank database for three years (2011-2013) to set up a long-term plan for Q-bank. A steering committee, a group of curators for the different databases, a coordinator, a program manager and the database managers work now on the quality and continuation of the database. Future incorporation of the database in EPPO activities is under discussion with EPPO and EU.

Conclusions
Within the QBOL project we were able to develop DNA barcodes for many plant pathogenic quarantine organisms present on the EU Directive and EPPO list and closely relatives. Protocols for DNA/RNA extraction, generic amplification of the barcoding region and sequence analysis were written and included in the molecular decision schemes, which were produced by WP2-WP7 and in which end-users can see which protocols to be used for correct identification of the quarantine organisms. Below the total number of sequences obtained is presented:
The developed database, Q-bank, is freely accessible via internet (www.q-bank.eu). Tools have been provided how to search the database and perform BLAST analysis or even multilocus and/or polyphasic identification. Plans for continuation of the Q-bank database have been made and are now being discussed. Many dissemination activities have been performed (website, E-newsletters, flyers, poster, oral and poster presentations at conferences worldwide, training course in 7 countries, publications in refereed journals, etc.). There was much interest from all over the world in the QBOL project and its achievements. The results were presented at the final workshop which was organized together with EPPO in Haarlem, The Netherlands (22-25 May 2012).

Potential Impact:
4.2 Use and dissemination of foreground

Section A

4.2.1 Dissemination and/or exploitation of project results, and management of intellectual property

The joint dissemination, exploitation of project results and management of intellectual property were covered in the project consortium agreement QBOL that was agreed by each partner organisation and the project management committee.

A special work package (WP11) was committed to dissemination of the project results.

Database

• The results of the project is an Internet-based freely accessible and functional database, called Q-bank, containing barcode sequences of relevant plant pathogens and pests with additional taxonomic data. Q-bank can be searched by internet: www.q-bank.eu.
• Generated DNA barcode sequences were also added to nucleic acid sequence databases (GenBank, EMBL).

Contact with stakeholders

• Regular reports were made to National EU Plant Protection Organisation (NPPO) representatives and via them, and via the EC Desk Officer, to the EU Standing Committee on Plant Health.
• Collaboration with EPPO for the integration of the protocols developed in the EPPO Diagnostic program has been made.
• QBOL collaborated with BOLD, NCBI and EMBL in exchanging data from QBOL.
• Protocols developed within the QBOL project were transferred into a draft EPPO Diagnostic protocol on DNA barcoding. In a Euphresco project (topic description submitted) this draft protocol will be further developed and a final EPPO protocol will be written.
• QBOL and the database Q-bank was presented two times at the IPPC-CPM meeting in Rome, Italy.

Communication

• A website (www.qbol.org) has been developed containing all relevant information of the project for all stakeholders. More than 10,000 persons visited the website during the project time.
• Wider dissemination of the findings of the project has been made to relevant agencies, stakeholders and civil society in all EU member states through the E-Newsletters.
• A project publicity leaflet has been produced at the start of the project outlining its aims and objectives.
• An introductory video for Q-bank has been made and can be accessed via the homepage of Q-bank (www.q-bank.eu)

Collaboration

• Many colleagues throughout the world contributed to QBOL in providing specimens, DNA samples or other and these parties are acknowledged on the QBOL and Q-bank websites.
• Collaboration with EPPO for the integration of the protocols developed in the EPPO Diagnostic program has been made.
CABI has been contacted to investigate future possibilities for collaboration.

DG Sanco has been contacted to discuss future use of the database in their policy.

NPPOs have been contacted to discuss Q-bank as a tool for correct identification in their activities.

International Workshop

An international workshop (combined QBOL-EPPO workshop) to present the project findings has been held at the end of the project and joined to an international symposium on Diagnostics on plant pathogenic Quarantine organisms (22-25 May 2012, Haarlem, The Netherlands).

Conferences and papers

Presentations were held at relevant scientific meetings worldwide with publications in conference proceedings.

High quality papers have been and will be offered for publication in peer-reviewed international scientific journals.

Training

Training of people within Europe but also outside Europe in working with the developed protocols and determining the barcode sequence data and surveying has been given. Seven training courses have been held in South and Middle America, Asia, Africa and Europe attended by 123 participants (mainly from NPPOs) from 34 countries.

Impact and Use

In Cooperation. Theme 2. Food, Agriculture and Fisheries, and Biotechnology, Policy context is stated: “Research......making important contributions to the implementation of existing, and the formulation of future, policies and regulations in the area of public, animal and plant health and consumers protection.” The QBOL project provided research in support of EU’s policy of plant health by developing phytosanitary science expertise and capacity to be offered to the plant health authorities and other users in the EU member states. The project addressed the problem of eroding expertise with users as a consequence of an eroding scientific basis in the phytosanitary field, where many classical taxonomists are retiring and are not replaced, by offering a tool linking the classical taxonomic knowledge with DNA-based technology, which every well-equipped national plant inspection laboratory can handle. Furthermore this project addressed research in order to make important contributions for policy and regulations in the area of plant health.

This problem of eroding phytosanitary expertise has been recognised by the EC through the Chief Officers of Plant Health Services and the European Plant Protection Organisation's (EPPO) common declaration in 2004:

The project also fits very well in EU's focusing on plant health translated into establishing the phytosanitary ERA-network EUPHRESCO with the main purpose to coordinate the national funding of phytosanitary research.

The following stakeholder groups benefit and will benefit from the outcomes of the QBOL project:

- National plant protection and inspection services both within the EU and in third countries
- National and EU policy makers for compliance of legislation
- Growers and breeders of plant material
- Trade
- Society

All of these stakeholders will benefit from the outcome of this project since a centralised, uniform system of identification of plant pathogenic Q-organisms based upon scientific knowledge has been developed, the Q-bank database. Therefore the expected impact for each of these stakeholder groups will be high.

More effective diagnostic services by national plant protection and inspection laboratories

The QBOL project provided common tools and methods, a common database of DNA barcodes and a reference collection of
DNA of quarantine organisms to be made available for both the member states ‘plant inspection service’ laboratories and research institutes as institutions outside Europe, since Europe has many trade relationships with countries all over the world. The impact of this will be to empower all countries equally to undertake effective diagnostic services for their own use, and a significant facility will be made available to each member country for relatively little individual input (research and development, collections etc); this would not be easy to achieve as independent national initiatives and a ‘whole-of-Europe’ will be far more effective. Therefore, in comparison to the status quo, where there are only a few ‘centralised’ facilities that are well enough resourced to tackle the gambit of diagnostics activity, and to whom a lot of the samples are out of necessity sent from neighbouring countries, this ‘universal’ method will devolve capability to all member countries whilst maintaining the assurance of accurate identifications. In turn this will expedite the use of diagnostic information for local quarantine operations. Transparency of methods will also better enable inter-laboratory and inter-country trouble-shooting at both the technological and operational levels for more effective actions against invasive pathogens and pests. It will also support the move towards national reference laboratories by providing central approaches and a standardized and vouchered resource for using DNA/RNA sequence data in diagnostics for quarantine plants pathogens and pests.

Looking further outside of Europe (by working with third countries), common and freely available methodologies empower counties trading within the EU. The availability of standardized methods enables third countries to effectively perform ‘at origin’ inspections and testing before export, using the same technology as the importing countries. As already discussed in the proposal, two of the important drivers in the Plant Health arena are globalization of trade (bringing more pests and diseases) and climate change (enabling establishment of plant pathogens and pests); though these two drivers also interact. As more produce is traded globally it puts increased pressure on the producer, trade and consumer network regarding the extent of the combined carbon footprint. Often produce prevented entry in to the EU is disposed of, or returned to origin at the expense of exporting country, in the current climate of increased costs associated with transport and a drive to reduce carbon usage, ‘at origin inspections’ and testing using methods ‘approved’ by the importing country will become increasingly important.

QBOL delivered objective DNA Barcodes of quarantine species mentioned in EU Council Directive 2000/29 and on EPPO A1/A2 lists to be used as species definition for legal purposes.

For the future, a significant strategic impact of establishing DNA barcoding in particular as a universal approach is that the Q-bank database can be continuously ‘added to’ in terms of taxonomic breadth without changing the technological protocols. This bodes well as a predictive measure to deal with the potential and as yet unknown changing needs of quarantine stakeholders that may occur, for example as a result of changing climate or trade agreements. In addition, because of the stringency around producing DNA barcodes this can be reliably achieved by capitalising on data generated elsewhere from very disparate projects and sources, i.e. not necessarily generated for local or quarantine purposes (more “bang for the buck”). Further, as molecular technologies improve and bioinformatic understanding grows, to the point where portable devices become available, the dataset established now will remain worthwhile as fundamental raw data underpinning those technologies.

Impact on producers, trade and society

The products of the QBOL project will significantly help to tackle increasing risks to EU plant health from exotic pests linked to increased globalization of trade in plants/products and in that way will also have an impact on the EU trade and competitiveness. By giving access to quicker and safer diagnosis of quarantine organisms it improves the possibility of preventing introduction and establishment of new plant pests and diseases in the EC. The project will also facilitate quicker tests of plant material to be exported to third countries and support better cooperation between EU diagnostic laboratories. QBOL will contribute to a reduction of incidents with quarantine organisms / exotic organisms and therefore contribute to a reduction of economic losses for the sector.

The 34 countries, which participated in the seven QBOL training courses countries, include many important nations making up
a large proportion of the total export trade of flowers, fruit and vegetables to the European Union. Third country training will both serve to promote EU support of international trade but critically help to prevent accidental introductions of quarantine organisms into the EU by helping the phytosanitary organizations of exporting countries to identify these pests and pathogens prior to export."

The availability of a database system harboring all relevant DNA barcode data and taxonomic features across multiple disciplines, freely accessible over the Internet (www.q-bank.eu) provide a quicker tool for correct identification of unwanted plant pathogens/pests for end users. Now such an advanced integrated database is available it can easily be updated regularly to reflect changes in taxonomy, new pests, etc.

The following steps bring about these impacts:
• a global collaboration of collections harboring plant pathogenic organisms
• standardized approaches for DNA barcoding of relevant genes to define species borders for the different taxa
• an internationally accepted and open access internet accessible database system
• internationally acceptance of the new internet accessible QBOL database system and developed protocols for European plant protections services and national reference laboratories, phytosanitary regulators and stakeholders
• continued testing, training and dissemination among above mentioned labs, exploiting new scientific and technological developments

Within QBOL we implemented an European approach. This is needed because:
• pests do not respect national borders
• many EU member states may be threatened by the same pest species and concerted action must be undertaken to be effective in Plant Health Diagnostics
• Standardisation of identification methods are required for the whole of the EU since changes to the EC Plant Health Directive are made by the EC Plant Health Standing Committee and, in order to agree measures and harmonise EC legislation, this Committee needs to take into account the risks posed to the whole Community
• European and ultimately global agreement on the DNA barcoding methods for identification of quarantine organisms is needed to enhance up-take
• Expertise for identification of pests and establishment of a useful database is readily available within the EU
• Critical mass is essential to tackle species identification for the different taxa
• Coordination of regional, national and international research will help standardization of methods developed within QBOL

QBOL took other national and international activities into account through links with:
• National activities in the EU on DNA barcoding:
  o Most, if not all QBOL participants have experience in identification of plant pathogenic quarantine organisms. Several of them have developed specific diagnostic tools that are used at a national level. For example, the Netherlands and the United Kingdom utilise a shortened PRA scheme in emergencies and in order to indicate whether a detailed PRA is required. Project partners from these countries will ensure that the advantages of shorter schemes will be taken into account when enhancing the EPPO PRA scheme.
  o Connections were made to national programs on barcoding of plant pathogenic organisms, e.g. FES (The Netherlands). The Q-bank database is further build upon the existing FES database.
• International activities in the EU on Development of Diagnostics:
  o A global inventory and network of culture collections with their taxonomic expertise will enhance international collaboration in taxonomy. A new EU project proposal on phytosanitary important collections is being written and will be submitted before 5th Feb 2013. Collections used within the QBOL project will be partners in this proposal.
  o The presence of several of the end-users within the QBOL consortium enhanced the acceptance of delivered products within
EU Plant Health. The outcome of the QBOL project is also discussed with many other end-users through the EPPO panel on Diagnostic Methods & Quality Assurance.

- The presence of EPPO in the Advisory Board enhanced the use of deliverables of QBOL within EPPO and European Diagnostic protocols.
- The presence of Andre Levesque and Paul Hebert in the Advisory Board supported optimal exchange of database and taxonomy knowledge. They were present in one or more meetings and gave their expert opinion on progress made by the consortium.
- Connections were be made to international programs on diagnostics for plant pathogenic organisms, e.g. QUAD, USDA-APHIS-PQR.
- Linking with other running or new FP6 and FP7 projects were made (EMBARC, Q-detect).
- National Reference laboratories are being set up (coordinated by DG-SANCO) in the EU. The QBOL project significantly helped to tackle increasing risks to EU plant health from exotic pests linked to increased globalization of trade in plants/products. It supported better cooperation between EU diagnostic laboratories and potential moves towards reference laboratories by providing central approaches and a standardized and vouchered resource for using DNA/RNA sequence data in diagnostics for quarantine plants pests and pathogens. The steering committee of Q-bank is now in discussion with DG-SANCO to use the database for delivering standards for identification of quarantine plant pathogens.
- The PFC (permanent phytosanitary committee) in Brussels and IPPC CPM members have been informed on QBOL progress for important Q-organisms and the Q-bank database.
- Connections to the USDA (USDA APHIS PPQ Beltsville) and to the QUAD group (USA, Canada, Australia and New Zealand) have been be made.
- Connections have been made with Genome Canada (Andre Levesque and Karen Kennedy).
- Through its Asian, South Africa, South America and New Zealand partners, QBOL has now strong links with research programmes in these leading countries in phytosanitary measures.
- Stakeholders have been informed about the results obtained within the project through e.g. Electronic Newsletters. The outcome of the QBOL project, an internet-accessible database (Q-bank) and attached protocols and procedures for correct identification of plant pathogens, has been discussed with multiple stakeholders.

List of Websites:

www.qbol.org

**Related information**

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