Final Report Summary - QDETECT (Developing quarantine pest detection methods for use by national plant protection organizations (NPPO) and inspection services)

Executive Summary:

Detection methods are the first tools used by national plant protection organisations (NPPO) and inspection services in order to find incursions of quarantine plant pathogens or pests (Q-pests) across a border, a crucial step to implement Council Directive 2000/29/EC. This is often done visually in the first
instance, with support from a laboratory for confirmatory testing and subsequent monitoring. Reliance on laboratory testing causes significant delays when action is only taken on the return of results from the laboratory to which the samples were sent. Thus, there is a need for rapid, simple and robust detection methods that can be deployed by NPPOs in the field with inspection services to enable early detection of Q-pests. The Q-detect consortium aimed to develop detection methods based on biochemical, acoustic, remote imaging and trapping techniques. The careful selection of traded products (primarily potato and forestry/trees) ensured the methods were developed on high priority targets such as potato brown rot, potato ring rot, Asian longhorn beetle and red palm weevil (along with the project PalmProtect).

Workshops were also completed with NPPO and inspection services from EU and third countries along with EPPO to ensure the methods were relevant to end user needs. Whilst, SME partners ensure access to technology and routes for exploitation after the project ends.

The interdisciplinary Q-detect project worked with a range of technologies seeking to develop solutions to a range of problems. Work was completed investigating volatile detection as an approach for targeting inspection effort. Focusing on bacterial pathogens of fruit trees and potatoes, the work successfully identified some volatiles that may be associated with disease, in addition an instrument based on laser spectroscopy was developed for specific volatile detection and several electronic nose instruments were compared and showed promising results discriminating groups of health and infected plant material.

Trapping plays an important role in monitoring, surveillance and control of invertebrate pests. We have addressed trap design, lure combination, generic lures, trap density and automatic detection for a few of the most important target insects such as wood beetles (Coleoptera) and whiteflies (Aleyrodidae) associated with plant pathogens. Remote imaging has great potential to monitor progression of disease fronts and help target inspections at a landscape level. A desk study concluded that remote imagery collected from unmanned aerial vehicles (UAVs) is probably the most cost effective approach in plant health. Software was developed to enable automated object based analysis of images and studies on the spectral profile of several host/pathogen combinations identified wavelengths at which discrimination of healthy and infected plants may be possible. Both acoustic and vibrometer methods proved useful for the detection of the larvae of wood boring beetles, and the database of sounds generated enables automated identification of detection. However the techniques have different applications, the vibrometer is more expensive, but useful for recording at a distance, for example in the tree canopy. DNA confirmation methods were developed based on LAMP chemistry running on the Genie II platform. The method proved highly popular with inspectors who trialled it during the Q-detect/EPPO workshops run as part of the project. Several NPPOs (UK and Switzerland) are involved in deployment of the techniques with import inspectors as a result of the project. Finally a number of computer models were developed to simulate the deployment of the techniques developed. the aim of these models varied from tools that would enable front line workers to deploy traps at the most effective density to methods that would enable policy makers and inspection services to evaluate investment in new techniques to aid front line inspection.

Project Context and Objectives:
Project context and the main objectives
The aim of the Q-detect consortium was to develop reliable detection methods for quarantine pests and pathogens (throughout the proposal referred to as q-pests) for use by national plant protection organizations (NPPO) and inspection services. The FAO defines a quarantine pest (Q-pests) as follows (IPPC, ISPM No.5: Glossary of phytosanitary terms): A pest of potential economic importance to the area
endangered thereby and not yet present there, or present but not widely distributed and being officially controlled. Invasive alien species that directly or indirectly affect plants or plant products may be quarantine pests. These are organisms that are not present, or with a limited distribution, in the EU and are listed in regulations as being very harmful. Each member state has to prevent introduction and spread of these organisms. In addition, there is a threat from newly discovered organisms that may qualify as Q-pests. The consortium investigated the potential of biochemical (detection of volatile organic compounds (VOC) and nucleic acid), acoustic (including resonance), remote imaging (incorporating spectral and automated data analysis) and trapping (especially Q-insect pests and Q-pathogen vectors) methods. Additionally work was included to optimise monitoring and sampling methods using interdisciplinary work on statistics and modelling. The methods were primarily developed for use on-site and at the point of entry, though they are also suitable for use ‘at-origin’. The project focused on economically and environmentally important quarantine pests where currently we lack adequate methods in the agricultural, horticultural and forestry areas. The target pests were reviewed and revised along with the EU where ever possible to ensure it is aligned with EU priorities and in support of NPPO’s in the implementation of Council Directive 2000/29/EC.

The consortium identified the following four principal project objectives:
1. Investigate and develop a range of detection technologies for important q-pests for use by NPPOs.
2. Validate the methods using real material both inside and outside the EU where the target organism is present.
3. By working with inspection services and NPPOs, model the complex role and relationships between plant health inspections, the use of different detection methods, monitoring and sampling techniques.
4. By working with NPPOs, European Plant Protection Organisation (EPPO) as well as third countries, disseminate the findings of the work widely within the community, both within and outside of Europe.

The Q-Detect consortium were aware of and in some cases were members of other EU funded projects in the area, to put this into context the following projects are relevant and on-going dlialog was maintained with those projects still running:
• FP5 DIAG-CHIP – RTD - Developing microarrays for quarantine potato pathogens and pests.
• FP6 PORTCHECK – STRP - Development of on-site real time PCR methods for quarantine pathogens and pests.
• FP6 PEPEIRA – STRP - Pepino mosaic virus: epidemiology, economic impact and pest risk analysis.
• FP7 PRATIQUE - Collaborative project - Enhancements of pest risk analysis techniques
• FP7 Q-BOL – Collaborative project – Developing DNA barcoding methods for the identification of Q-Pests

The role of detection techniques:
It is important first to define the terms used before the importance of the methods can be defined in an EU plant health policy perspective. Diagnostics, identification and detection are terms often used interchangeably but this belies subtle differences in meaning and therefore differences in the type of methods that may be appropriate to achieve the desired goal. Diagnosis can most readily be defined as ‘identifying the nature or cause of disease’. Identification on the other hand could be defined as ‘accurately naming the causal agent of disease’. By contrast detection could be defined as ‘discovering something that is hidden’.
Another reason for defining terminology precisely is the nature of the techniques to be developed and the way these methods may be used by NPPO’s. In practical terms inspection services use different methods in a cascade to achieve first ‘detection’ followed by ‘confirmation’ of the presence of a regulated pest, finally ‘monitoring’ the spread of a pest. In the simplest sense if an inspector is looking for suspected disease plants he/she is in essence using a detection method – observation of symptoms. Upon finding a suspect sample they may then send it to a laboratory to confirm the presence of a specific regulated pathogen or pest. If confirmed the inspectors may then return to the sites and monitor the spread of the pathogen or pest.

Thus detection techniques are those methods that guide or lead inspectors and NPPOs to the location of potential quarantine pests, in order to complete the task. Confirmatory and monitoring tools are then necessary to identify the presence of a regulated pathogen – in some instances these may be performed using the same technique, or they are two different tools used in a cascade, both are within the scope of the Q-Detect project.

New detection methods in the context of increasing risks and costs:
The development of effective methods for the detection of plant pests is vital for underpinning European Plant Health Policies. For this project The Council Directive 2000/29/EC of 8 May 2000 is important; it currently lists some 275 organisms (viruses and virus like organisms, bacteria, fungi, insects, mites, nematodes and one parasitic plant) and “concerns protective measures against introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community”. A key aspect of the “protective measures” is the ability to detect and identify accurately and rapidly the introduction of the harmful organism in the plant or plant product being moved. But the threats posed by new plant pests are now greater than ever because of:

- considerable increases in the volumes, commodity types and origins of trade in plant material from third countries
- introduction of new crops
- continued expansion of the EU with new border countries added
- and the impact of climate change affecting the boundaries of pests and their vectors

Although there are no published figures estimating the cost of ‘all’ non-native pests and diseases for the ‘whole’ of Europe, there are some estimates for certain pests and certain countries that give an indication of the size of the problem. For example the introduction of the western corn rootworm (Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae)) has brought with it an estimated cost to Europe in the range of €147 million per annum (Baufeld & Enzian, 2005). For one member state (the British Isles) Pimentel (2002) estimated that invading insect pests and plant pathogens cause $5 billion damage to crops and forests annually. As a result, UK government departments contribute about €18 million per annum to quarantine plant health activity, mostly in the area of risk reduction.

Looking further afield in the USA the cost of non-native pests and diseases and their control is estimated to be $120 billion (Pimentel et al., 2005) and invasive alien species may cause over $314 billion per year worldwide in damage and control costs (Pimentel et al., 2002). In the USA, APHIS increased its annual spending on emergency eradication programs more than twenty-fold during the 1990s to $232 million from $10.4 million. In a recent study, the number of plant pests establishing in Europe has been predicted to increase significantly in the next 10 years based on current trends (Waage et al., 2005; EU project DAISE at www.europe-aliens.org). Such organisms cause considerable economic and societal damage within agriculture, horticulture, forestry, and natural ecosystems. This has been amply demonstrated by the
impacts of a range of different exotic plant pests:
• The introduced bacterial pathogens Clavibacter michiganensis subsp. sepedonicus and Ralstonia solanacearum
• The introduced sudden oak death pathogen, Phytophthora ramorum
• The introduction of the tomato pathogen Pepino mosaic virus
• The introduced pine wood nematode, Bursaphelenchus xylophilus
• The introduced western corn rootworm (Diabrotica virgifera virgifera)
• The introduced Asian and Citrus longhorn beetles (Anoplophora spp.)

New detection methods in the context of EU Plant Health Policy:
Effective detection methods are critical for the efficient functioning of inspection services and NPPO laboratories. Yet in many cases inspection services in particular lack techniques that will enable efficient detection of quarantine pests. Inspection services typically rely on ‘intelligence’ which directs them to the location and type of produce to inspect – typically this is through knowledge of high risk imports and high risk trade routes. After this point visual inspection takes over, with individual inspectors utilising experience to locate potential incursions by quarantine pests. Only a relatively small number of tools are currently available to aid inspectors; for example hand held devices such as Lateral Flow Devices (LFD) and methods such as portable real-time PCR as developed in the FP6 funded project PortCheck (SSPECT-2004-502348 PORTCHECK); these are however primarily confirmatory tools, of use when a potential pest has been found. They also have serious shortcomings; LFDs are not sensitive enough in many situations, whilst portable real-time PCR is too complex and expensive. As might be expected, the availability of tools in NPPO laboratories is much greater, though again these tend to be diagnostic tools for investigational purposes, identification tools to confirm a pest to the species level (for example techniques being developed in the FP7 funded project Q-BOL) or methods most suited to surveying for the presence of a known pest, for example as part of a monitoring scheme post incursion. In this sense the NPPOs are still reliant on the relatively poorly equipped front line inspectors to detect quarantine pests. Timely detection of these organisms is of utmost importance. This is frequently achieved by the deployment of specialist inspection services, who along with NPPOs and typically with laboratory support, are tasked with identifying incursions of these quarantine pests into the EU. At the EPPO Colloquium on "scientific services in support of NPPOs in the EPPO region", which was organized in Madeira (2004-09-23/24), the urgent need to maintain good scientific support for plant health services was stressed. Knowledge erosion was perceived as a very serious threat to the activities of the plant health sector. A state of emergency was declared and the Council Colloquium agreed on an EPPO declaration: plant health endangered. The work of NPPOs relies on scientific expertise, but the services providing this expertise increasingly lack staff, funds and training.

Front line detection of regulated pests (in particular new emerging diseases or new pathotypes) is difficult partly due to the need to sustain a large number of staff with specialised skills. Maintaining specialised staff of this kind is a costly and extremely difficult task. Improved methods that can be deployed to support these specialist staff are critical for the maintenance of these services.

Target pests
The project focused on developing methods for economically and environmentally important quarantine pests where currently we lack adequate methods in the agricultural, horticultural and forestry areas. In terms of importance a preliminary analysis was completed by the consortium of the FP7 funded PRATIQUE project on the most important economically and environmentally damaging quarantine pests in
terms of risk to the EU; this analysis generated a priority list of target pests. In order to assess which quarantine pests currently lack adequate methods several NPPOs around Europe were contacted to give the consortium an opinion on which target pests are currently difficult to detect due to lack of methods. These pests were then short listed to be used as model systems in this project and revised as the project progressed. One notable example of the revision was the inclusion of work on the red palm weevil (Rhynchophorus ferrugineus) which became an ever increasing priority as the project progressed. The work plan was altered to include work on this pest and by working with partners with the FP7 funded project PalmProtect work on acoustic and vibrometric detection of red palm weevil larvae was completed. The Q-DETECT project was an ambitious multidisciplinary one in that the consortium aimed to develop and investigate a range of diverse yet complementary technologies. In order to make this ambitious undertaking possible it was necessary to match up the pests carefully with the most appropriate technology. In addition to this match up a pathology or pest expert within the consortium with a technology expert from a non-plant background. Using the quarantine pests identified from the PRATIQUE project as a model system for development purposes gave two main benefits to the project, firstly it enabled a focus for the technology development and research to be performed using a good model system. Secondly, since the model targets being used have been identified as being high priority the project developed innovative technologies suitable for surveillance-use by inspection services in EU Member States directly at the end of the project (see PUDF).

The technologies:
The project developed detection methods as well as confirmation and monitoring techniques that could be used by NPPO and inspection services for early surveillance of quarantine pests. Since no single detection method would be suitable for the full gamut of Q-pests likely to be encountered by inspectors and NPPOs on a daily basis a range of different methods were investigated. The methods fell into the following type of methods:

- Detection of volatile organic compounds (VOCs) released by pathogens upon infection was used predominantly for bacterial pathogens of fruit trees and potato.
- Detection of pests using acoustic or vibrometry methods targeted the larvae of wood boring beetles in trees, palm trees and wood products.
- Remote visual methods were assessed and developed for a range of different pests and pathogens. Spectroscopy work sought to identify differences between healthy and infected samples that were not visible to the naked eye, and software developed to automatically evaluate image data.
- Novel pest trapping was approaches were developed based on Smart Traps containing cameras, which enabled automated recording of trap catches, whilst for glasshouse pests novel lures were developed to attract damaging pests.
- Generic DNA methods were developed based on isothermal amplification techniques (LAMP) and the Genie II platform for the identification of pests located using other methods.

The techniques developed were not discrete and are likely to be deployed as part of a toolkit of techniques by NPPOs in order to detect pests and then confirm the identity of the pest.

Evaluation of the detection system:
In addition to developing the methods, work was completed on modelling the use of the methods to evaluate how best the methods could be used as well as what the cost benefits of using the different methods may be. In order to achieve this, the Q-DETECT project took two approaches, in both the consortium engaged with NPPOs and inspection services within Europe to gain information on the tasks to
be achieved, the limitations and practicalities and the processes involved. A model of the inspection process was developed; this will enable the use of new methods to be compared and outcomes evaluated. It will also enable the evaluation of different detection parameters from different sampling strategies and regimes, to the effect of deploying methods with varying limits of detection or diagnostic ability. Several other models were also developed to specifically evaluate various aspects of deployment from density of deployment of traps for different invertebrate pests to the evaluation of aerial remote imaging platforms for detecting pathogens in various landscapes.

Project Results:
S&T Results
WP2: Modelling and sampling
At the start of the project there were no models that could be used to compare the cost-effectiveness of detection instruments. For such comparison it is required that instruments are applied in a practical setting, or in other words, the context in which the inspection takes place is important to calculate the cost-effectiveness. ISPM documents and interaction with inspectors provided this information. Inspectors form the NGGO’s of the Netherlands and the UK were engaged in information-gathering for the description of the inspection process and resulting schemes. During some interviews aspects of sampling and sampling rates were discussed. In Deliverable 2.1 the interview reports and available information was used to describe in broad sense the Dutch inspection processes aided by graphs (flowcharts and UML diagrams). Processes are graphically presented in deliverable D2.2. In deliverable D2.3 the inspection processes are generalized and abstracted in generic schemes, this is used as a framework for the Inspection Model.

Developers of the instruments (WP3-7) were questioned - by means of a questionnaire - to gain a better understanding of how potential instruments of the Q-Detect project should work and fit in with the inspection process. The schemes of the inspection processes as well as the information from the instruments guided the development of the models.

The first model to be developed was the Inspection Model. This model is very generic, it can be applied for import, export or field inspections. Sampling adheres to the ISPM31 standards. The Inspection Model has several purposes: gaining insight in the current inspection process, an educational tool for inspectors and students to gain understanding, compare cost-effectiveness of detection instruments (justification to buy), and evaluate the inspection policy. In this model a detection instrument can be applied at different stages: inspection, 1st line confirmation or laboratory. Despite its generic application possibilities, the Inspection Model cannot be used by the work packages on trapping (WP4) and remote sensing (WP5). This problem was recognised one year after the start of the project and led to the development of three additional models: 2 trap models for two different situations/cases and a remote sensing model. One trap model is for Thrips palmi in a glasshouse and one for Thaumetopoea pityocampa (pine processionary moth) in a pine forest. Insects are modelled as agents with their specific characteristics (e.g. gender) and behaviour (e.g. flying, mating). Patches or grid cells have also characteristics (location, scent). The objectives of the trap models are: identify the “best” number and location of traps, the probability to catch insects, cost-effectiveness of traps and lures, and insight in the effect of sustainable production, i.e. usage of traps to reduce insecticides.

In the Remote Sensing Model pest spreads according to biological parameters. The likelihood of detection of a disease is assessed from a high spatial resolution, visible camera mounted on a Low Earth Orbiting
Data supplied by WP4 and WP5 were incorporated in the trap models and remote sensing model. All models are described in great detail in Deliverable 2.4. For deliverable D2.5 (case studies) information was requested from the other WPs. WP3, WP6 and WP7 supplied time, costs and quality data from the instruments (and their applications on organisms). These data are used as input for a comparison model. This model is a simplified version of the Inspection Model and has been developed to compare all instruments with each other when applied for a variety of cases.

Several methods are used in WP2. For the Inspection Model these are Discrete Event Simulation, Waiting Theory, Monte Carlo Simulation, Object-Oriented programming, and the use of several statistical distributions. The modelling environment of the Inspection Model is Visual Basic with Excel as user-interface. Agent Based Modelling techniques are used to develop the trap models and the remote sensing models, the models are exported as Java applets that make it possible to consult on the web. The modelling environment is NetLogo.

To summarize, WP2 has achieved inspection-process descriptions for different inspection situations, five models that can be used by the inspection services to calculate the cost-effectiveness of instruments and to support inspection decisions (e.g. sampling), and an extended document of case studies in deliverable 2.5. In WP2 we have not made the progress as intended. It was agreed that part of the necessary information should be delivered by partner 1. Despite frequent contact we (partner 2) only received limited information. Partner 2 was therefore compelled to compensate the data collection with extra effort by means of direct interaction with several inspectors.

At each biannually Q-Detect meeting WP2 had one or more presentation on the progress of the work and especially with respect to the models (demonstrations). Models were also presented at meetings with inspectors, and especially at the two EPPO/Q-Detect workshops in Italy and Slovenia. There are possibilities to use the inspection models by NPPO or to make adaptations to fit specific circumstances or requirements. Intended end users are policymakers or decision makers from NPPOs (dealing with buying/applying new detection instruments).

WP3: Volatile detection
The work was focused on detection of quarantine bacteria, responsible for the fire blight in pomaceous plants (Erwinia amylovora, Ea), and brown rot and ring rot potato diseases - caused by the bacteria Ralstonia solanacearum (Rs) and Clavibacter michiganensis subsp. sepedonicus (Cms) respectively - by means of (i) identification of volatile markers or specific fingerprints of diseases using GC-MS and PTR-MS analysis and (ii) diagnosis of diseases using electronic nose (e-nose). This approach, although less reliable in comparison with standard molecular methods, has several advantages including lower time consumption, no need for training, and no sampling error.

During the first year of work, a GC-MS characterization of VOCs emission from cultured bacteria was obtained. This allowed to identify some key compounds to be used as specific markers, such as dimethyldisulphide for Rs, 2-propanol and 3-methylbutanal for Cms, and 2,3-butanediol for Ea.
Furthermore, VOCs emissions from infected plant samples in controlled conditions (experimentally inoculated potato tubers, enclosed in jars, or in vitro apple explants) were consistent with those of cultured bacteria. This prompted a time-course analysis of emissions, obtained by GC-MS and PTR-MS. The latter, although providing only a temptative recognition of VOCs, is suited for low-molecular weight compounds and real-time monitoring. As a result, 30 hours from infection were shown to be sufficient for Ea detection in apple plantlets; instead, since Rs and Cms take longer time from inoculation to complete rotting, VOCs emission from infected potatoes depend from the disease progression stage. Ethylene emissions were also found in Ea-infected apple plantlets, and may contribute to diagnosis as a generic marker of stress.

With the demonstration of differences in VOCs profiles according to infection, in the second year an experiment was carried out to prove whether the e-nose may be effective in recognizing infected material before the appearance of specific visual symptoms. E-nose was selected as the most promising technology for diagnosis in real conditions (such as customs or nurseries) because of its immediacy in use and data reading. Therefore, two commercial e-nose equipments were tested in the recognition of diseased plant material in laboratory conditions by direct headspace sampling. The recognition power correlated with the severity of disease in potato tubers. A data base of VOCs profiles was built according to severity. Concerning apple plantlets, only one e-nose equipment was able to discriminate Ea-infected from healthy plants, or plants inoculated with a different pathogen (Pseudomonas syringae pv. syringae, Pss).

The third year was dedicated to the scale-up of e-nose recognition protocols to real conditions. To this aim, a VOCs sampling method was set up, consisting in the exposure (7 days) of a VOCs trap (Radiello®, Supelco, USA). The length of the exposure was chosen to be comparable to the time needed for the shipment inside containers. The trap is subsequently heat-desorbed, and the gas sample is collected in an odorless VOCs-sampling bag until e-nose analysis. The plant material consisted in (i) 25-kg potato tuber sacks, in PVC or net bags, and at 4 °C or room temperature; (ii) one-year dormant apple plants, singularly enclosed in PVC bags and stored at 3 °C. The experiment on potatoes was validated after destructive symptom assessment and bacterial reisolation, and fitted well in the data base of VOCs profiles in relation to disease severity (figure 1). With regard to apple plants, the discrimination of Ea-infected samples was successful on pairwise comparisons with Pss- and mock-infected ones. One Ea-infected plant out of 10 enclosed in the same bag could still be detected.

Figure 1: Score plot of healthy and Rs and Cms diseased samples; Radiello® cartridge was used as adsorbent material. The blue dotted line separates samples with low disease severity from the ones with high disease severity.

As a side experiment, GC-MS characterization and e-nose recognition were attempted on in vitro kiwifruit plants, infected with Pseudomonas syringae pv. actinidiae. E-nose diagnosis proved less efficient than in the Ea-apple model, due to the slow progression of disease, since a discrimination may take over 30 days from inoculation. Nonetheless, some compounds were found to characterize VOCs emissions of infected plants, including hexene and oxalic acid.

In spite of the good potential for practical diagnosis supported by our data, the method is not ready for a straightforward application, because of several problems such as low sensitivity and drift effects. In fact, characteristic VOCs profiles are supposedly based on quantitatively small differences in composition. The extension of data bases with real condition samples, a better standardization of samples and procedures
and an improved statistical analysis may help the application of e-nose-based pathogen recognition methods. Presently, e-nose should be considered as a supplementary warning tool which can be used to steer standard molecular analysis.

WP4 - Trapping Quarantine Pests
Quarantine pest trapping is one of the methods used for early detection of pests by National Plant Protection Organizations. In Q-DETECT we aim at improving the design of trapping systems in order to make them more efficient, cheaper, and more widely applied to provide a consistent and reliable surveillance network. An efficient trapping system could thus increase the detection power that is currently limited by the low amount of the commodities that are inspected with traditional methods.

The key challenges that limit widespread deployment include the development of on-site identification, the availability of lure and trap designs suitable for use under special conditions (e.g. at the docks, within cargoes and shipments), an automatic system for recording and communicating captures, and the need to link the catch and the abundance of the organism.

We have addressed trap design, lure combination, generic lures, trap density and automatic detection for a few of the most important target insects such as wood beetles (Coleoptera) and whiteflies (Aleyrodidae) associated with plant pathogens. Tomato plants with a different susceptibility to a whitefly vector of plant viruses (Bemisia tabaci) were screened to detect volatiles that can be used to enhance trapping methods of the whitefly.

We have developed procedures for early detection at ports of entry, such as maritime ports, and tested them over a wide latitudinal range to keep into account climatic variation. Traps were deployed at ports of entry but also in natural conditions (e.g. forests) to validate the techniques with native species taxonomically related to the target exotic ones. Our results demonstrated that the number of alien species was positively correlated to the amount of imported commodities at the port scale, and it was influenced by the composition of forest sites close to the ports. Specifically, alien species richness was higher in broadleaf than in coniferous forests. By contrast, forest cover in the surrounding landscape was positively related with the occurrence of native but not alien species. Finally, the number of both alien and native species was higher in the forest sites close to the ports than in ports.

Two prototypes of an automatic trap that registers catches of insects with a video camera and sends them to a remote computer through mobile phone technology have been developed in collaboration with Biosecurity of New Zealand and tested with various target pests. The first prototype with 1 MP camera has been tested for checking trap-captures of longhorn and bark beetles at ports of entry. Pictures were automatically taken by a camera and sent, by a user-defined frequency, to a safe repository accessible through the web. The picture quality allowed the identification of insects at family or, in some cases, at genus level. The system is logistically effective as well, with a rechargeable battery pack with a three to four week life span. A second prototype with a 3 MP camera and new casing offers greater sensitivity, allowing better species discrimination, and a better integration of the camera with a number of traps. This second prototype is currently being tested with various species of quarantine insects.

A further improvement consists of an automatic processing of the images according to a set of predefined target species and whenever a positive matching is found, a message is sent to the inspector asking for on-site visit and identification. For this reason we have adopted, in collaboration with WP7, the LAMP method, a molecular biology technique that doesn’t require complex laboratory tools or reagents with special needs of conservation. LAMP is used in association with Genie® II, a thermal cycler that combines high portability and simple use. The LAMP technology is quite insensitive to possible inhibitory substances
and therefore does not need time consuming DNA extractions. If target DNA is present, an increase in fluorescence is detected that is proportional to amplification product. Finally a further step of melting/annealing is added to verify the specificity of the amplification reaction. Detection has been developed for Bemisia tabaci and a number of quarantine viruses that this insect can contain and spread, for the Monochamus beetle and the plant pathogenic Pine Wood Nematode that this beetle can spread, Bursaphelenchus xylophilus, for blue stain fungi associated with bark beetles, for parasitoids of the chestnut gall wasp Dryocosmus kuriphilus, and for the invasive longhorn beetles of the genus Anoplophora.

WP5: Imaging
The remote sensing work package (WP5) of the Q-Detect project has accomplished three distinct objectives that focused on investigating methodologies that enable the detection of disease in trees and plants using standoff techniques. These objectives were based around the three outlined tasks, WP 5.1: A comprehensive review of the potential for different remote sensing platforms in combination with current instrument techniques to be used to detect the spread of disease. WP5.2. The analysis of the reflectance spectra of diseased and healthy leaf samples in the mid-infrared 2 µm (4500 cm-1) to 20 µm (500 cm-1). WP5.3 T

WP5.1. The horizon scanning study reviewed the current state of the art in terms of technologies that could be applied to the area of plant disease detection. This technique, developed at Exeter University and applied to this problem, iteratively uses “relevance feedback” to enhance the quality web-based information retrievals of the search results (Marco et al 2013). The review looked into current and future aerial platform technologies and instrumentation options for detecting and monitoring diseases in vegetation and the mapping of pests through the use of aerial platforms. The aim was to assess the efficacy of using remote sensing techniques (such as direct imaging and spectrally resolving reflected light) from different aerial platforms (from small unmanned aircraft to low altitude satellites), to evaluate and monitor the health of plant life over long periods of time with little human inspection. This was done by looking at the combination of platform and instrumentation technologies to understand how low, medium and high-altitude platforms integrated with high spectral and spatial resolution instrumentation could be used to come up with different performance metrics within the specific user requirement framework. The framework that was adopted for this assessment included cost, endurance, spatial resolution and the frequency of measurements. The report was not meant to target specific diseases, but to provide an overview various, if not all, potential diseases whilst providing a thorough examination of the state-of-the-art in remote sensing instrumentation and platform technology.

WP5.2. The analysis of the high resolution reflection spectra from the leaves of healthy and diseased plants was performed using a laboratory Fourier Transform Spectrometer (FTS) in the mid infrared spectral region 2 µm (4500 cm-1) to 20 µm (500 cm-1). The aim of this work was to determine whether mid-IR spectroscopy could be used to detect a variety of diseases in plants, and so three spectroscopic techniques where used to analyse leaves from healthy, senesced and diseased plants. These methods of optical probing included reflectometry, attenuated total reflection (ATR) and an specifically adapted integrating sphere technique. The healthy, senescent and diseased samples included japanese larch (larix kaempferi) inflected with (phytophthora ramorum) in needles, pine needles (Pinus spp.) infected with pinewood nematode (bursaphelenchus xylophilusis, wheat (triticum aestivum, winter wheat-cv gladiator) infected with rhizoctonia cerealis, tomato (solanum lycopersicum, ‘moneymaker’) infected with potato virus
Y and citrus leaves infected with liberibacter asiaticus and liberibacter africanus. From this study, the differences between the health, diseased and stressed samples have been noted and reported. Typical example of the differences measured can be found in figure 1(a) which shows the healthy sample of citrus leaves that are healthy and samples liberibacter asiaticus and liberibacter africanus.

Figure 2: (a) The differences in the mid-infrared spectra of healthy sample of citrus leaves that are healthy and samples liberibacter asiaticus and liberibacter africanus as measured for WP5.2 (b) the classification of areas in high resolution satellite imagery using the MORPHIS pattern recognition software.

WP5.3 A morphological image processing technique was used to develop an automatic pattern recognition tool for high spatial resolution satellite imagery analysis. The Model for Object Retrievals From Imaging Satellites, MORFIS, was developed as a standalone package to be used to classify regions in an image based on calibration images. The software uses these calibration images as a training set that it uses to classify pixels based on the dominant colours, patterns, geometric shapes and features. These are then used to search all of the pixels in the test image to highlight the areas of interest as well as classifying regions based on the calibration data. Imagery from the World view-2, IKONOS, GeoEye-1 and QuickBird data has been used to look at areas in the UK in 2010 phytophthora ramorum in larch was known to be present. Using 3 and 4 channel visible data from spatial resolutions from 0.8m to 2.3m per pixel, and shown in figure 1(b) the automatic classification was been used to identify regions on larch within imagery and to associate areas where disease such as phytophthora ramorum could be present, enabling a more targeted approach to inspector based sampling.


Work Package 6: ACOUSTICS
Introduction
Target pests: the wood-boring beetles
a) Monochamus galloprovincialis (vector of nematode Bursaphelenchus xylophilus; b) and others of genus Monochamus sp.),
c) Anoplophora glabripennis (the ASIAN LONGHORNED BEETLE (ALB)),
d) Rhynchophorus ferrugineous (the RED PALM WEEVIL (RPW))

The wood-boring beetles have always presented a special challenge for pest managers, since their larvae, which are responsible for most of the damage, are hidden from view inside trees (e.g. shipments of bonsai trees), cut wood and wood packaging material. It is of paramount importance to detect these pests at the larval stage, before the adults emerge and start spreading the infestation to new areas.

Today, the surveys to detect and delimit infestations of ALB are carried out mostly by visual inspection from ground, using bucket trucks and tree climbers looking for oviposition sites and emergence holes, as well as sap and frass. Our WP tested different bioacoustic methods for detecting the wood-boring beetle larvae. These methods exploit the sounds and vibrations that are generated as a by-product of their eating and locomotion. Microphones were generally found to be more useful as airborne sound sensors, while vibration sensors such as laser vibrometers usually interface better with signals produced in solid substrates like soil, grain, or fibrous plant structures like wood.

In the last two decades, laser vibrometry was used in entomology mostly to study insect behavior. A laser
Doppler vibrometer operates on the basis of optical interference and Doppler Effect and makes non-contact vibration measurements using a laser beam (without mass-loading the target). None of the bioacoustic methods developed so far, however, has proven reliable or cost-effective enough to be used regularly, so the research efforts continue.

Methodology
BFW focused on the airborne component of the larval sounds and used the microphone, which was sealed to the measuring surface using modeling clay to ensure maximum contact. The Slovenian partner, NIB, used the laser vibrometer PDV-100 (Waldbronn, Germany). Trips to Italy (ALB) and Israel (RPW) were made for gathering infested wood samples and recording the larval sounds and vibrations from infested palm trees from Oct. 2010 to Feb. 2013.

Development of the automated detection method
Clustering experiments were carried out to allow for species-specific classification on recordings made with the microphone (this was carried out as a subcontract by S. Hübner, Sejona R&D, www.sejona.com).

Results
Laser vibrometry was employed successfully for the first time as a novel bioacoustic technique for detecting the wood-boring beetle larvae (Figure 3). Typical vibratory signals emitted by the ALB and RPW larvae are well characterized by their spectral and temporal characteristics. Complete databases of both microphone and laser recorded sounds and vibrations of all three target species are available on the internet; for information and links see www.qdetect.org.

Figure 3:. (A) - Oscillogram and sonagram of a laser vibrometer recording of RPW larvae from a canary palm. Upper trace – several 'clicks'; lower trace – a single 'click'; lower image – a sonagram of the above 'click'. (B) - Oscillogram and sonagram of a laser vibrometer recording of RPW larvae from a date palm. Upper trace – several 'rasps'; lower trace – a single 'rasp'; lower image – a sonagram of the above 'rasp'.

The clustering experiments have shown that it is possible to form species-specific clusters in certain contexts. To what extent this approach is suitable for fully automatic species identification has to be clarified during further experiments. This requires appropriate corpora with more standardized recordings. An automatic differentiation of the three species based on their acoustic emissions is possible in principle.

Table 1: Recording the activity of the RPW inside the palm trees (P. canariensis and P. dactylifera)

<table>
<thead>
<tr>
<th></th>
<th>Laser</th>
<th>Microphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.88</td>
<td>0.84</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.88</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Discussion
Notes on the uncertainties
1. Larvae are not continuously active. Their bioacoustic activity mainly depends on:
   a) the time of year,
   b) the time of day,
c) temperature (best >18°C) and
d) the larval stadium. Also, the knowledge of insect
2. life cycles is crucial because during the stages of eggs and pupa the wood borers cannot be detected
using bioacoustic methods (with rare exceptions). Further studies are needed for each species.
3. Level of sound emissions. Very small larvae are difficult to record because of low emissions.
4. Size of sound database. The accuracy of bioacoustic species identification has to be verified on a large
number of sound samples.

Laser vibrometry as a non-contact bioacoustic detection method

The laser vibrometer (LV) is portable and robust; its great advantage is that it allows a working distance of
several meters, which expands recording possibilities in relation to other acoustic methods. It is a non-
contact method and as such not affected by the test surface. Contrary to the microphone, the LV records
only the substrate-borne vibrations; microphones, for example, lack a complete coupling with the substrate
and therefore detect also the airborne component of the emitted structure-borne signals, hence they
usually demand amplification. The one disadvantage of the LV is its relatively high purchase price
compared to other methods; however, this is outweighed by its high sensitivity, zero loading of the
measuring surface and the option of a high working distance. The maintenance costs are ca. € 1,000
(yearly calibration). No other costs are involved as there are no consumables. One should also always
keep in mind the commodities for which a certain method will most likely be employed. In case of detecting
pests in high cost commodities such as bonsai or palm trees, the benefits are more than likely to outweigh
the costs.

*Collaboration with EU project – Palm Protect

WP7: On-Site confirmation and monitoring

The outputs from WP7 can be summarised in three main areas 1) simplex detection using the Genie II
platform, 2) multiplex detection using universal LAMP and 3) development of a novel, field portable
platform.

1. Simplex detection in the field using the Genie II platform.

Two main outputs were delivered in this aspect of the Q-detect project. Firstly, platforms for field
deployment were investigated and the Genie II platform was selected as being the most suited to use on
the front line by Inspection services. Genie® II is an extremely powerful analytical instrument that was
specifically developed to support all available methods of isothermal amplification of DNA and RNA that
employ fluorescence read-out. The instrument is a highly-flexible device and can also be applied to many
other chemistries requiring temperature control up to 100°C along with highly sensitive fluorescence or
luminescence optical detection. The instrument is simple to operate and is normally used without a
computer. The attractive enclosure is rugged and compact, and there is an internal battery to allow use
without mains power. Genie® II is a state-of-the-art diagnostic tool that is equally at home deployed for
detection at point-of-application as in the laboratory.

The second output from the Q-detect project is the assays (Table 2) that have been developed and
validated for use on the Genie II platform. The exploitation of these assays to make them available to
NPPOs and inspection services for use is described in detail in the PUDF.

Table 2: Details of simplex LAMP assays developed and validated within the Q-detect project.
Target Developed by
Citrus black spot (Guignardia citrocarpa) P1 (Fera)
Ralstonia solanacearum P3 (NIB)
Potato spindle tuber viroid (PSTVd) P3 (NIB)
Clavibacter michiganensis subsp. sepedonicus P3 (NIB)
Bursaphelenchus xylophilus P9 (UNIPAD) and P1 (FERA)
Monochamus galloprovincialis P9 (UNIPAD) and P1 (FERA)
Bacterial spot of stone fruits (Xanthomonas arboricola pv. pruni) P4 (ACW)
Fire blight (Erwinia amylovora) P4 (ACW)
Thrips palmi P4 (ACW)
Pseudomonas syringae pv. actinidae (kiwi) P4 (ACW)
Pantoea ananatis (Eucalyptus spp., onion, pineapple, clinical, etc.) P4 (ACW)
Clavibacter michiganensis subsp. sepedonicus P1 (Fera)
Liriomyza huidobrensis P1 (Fera)
Tomato chlorotic dwarf viroid (TCDVd) P1 (Fera)
Chrysanthemum sten necrosis virus (CSNV) P1 (Fera)
Potato yellow vein virus (PYVV) P1 (Fera)
Curtobacterium flaccumfaciens pv. falccumfaciens P1 (Fera)
Tomato chlorosis virus, (TOCV) Bemisia tabaci P2 (DLO)
Tomato infectious chlorosis virus, (TICV) Bemisia tabaci P2 (DLO)
Cucurbit yellow stunting disorder virus, (CYSDV) Bemisia tabaci P2 (DLO)
Tomato yellow leaf curl virus, (TYLCV) Bemisia tabaci P2 (DLO)
Cotton leaf curl virus (CLCuV) Bemisia tabaci P2 (DLO)
Ophiostoma brunneo-ciliatum P9 (UNIPAD) and P1 (FERA)
Ips acuminatus P9 (UNIPAD) and P1 (FERA)

2. Multiplex detection in the field using Universal LAMP.
Several methods were developed as a proof of concept for the detection of multiple pathogens using a single test (multiplex detection) to make the procedure more efficient for screening for multiple targets in the field situation.
   a) Annealing temperature
When monitored in real-time, LAMP produces amplification plots that are similar in appearance to those generated by real-time PCR (Fig. 4a).
(A)
(B)
Figure 4. Amplification plot (A) and melting curve (B) of two LAMP reactions followed in a Genie II. The red and blue line represent a sample with and without target respectively.

After amplification on the Genie II, products are typically analysed by a slow annealing step with concurrent fluorescence monitoring, because the temperature at which the amplification product anneals/melts is consistent and characteristic for each assay (Fig. 2b). This approach allows two or three amplification products to be resolved in the same reaction if the products have sufficiently different annealing/melting temperatures. The ability to resolve multiple products allow multiple pathogens to be detected in a single reaction; alternatively it has been shown that the pathogen-specific test can be combined with an internal control assay to assist in the interpretation of negative results.
b) Biotin LAMP
Different LAMP assays can also be combined if biotinylated deoxy-CTP is added to the LAMP master mix. Products can directly be monitored with specific hybridisation probes after the LAMP amplification and identification is not disturbed by any aspecific amplification artefact. For demonstration Tomato chlorosis virus (TOCV), Tomato infectious chlorosis virus (TICV) and Tomato yellow leaf curl virus (TYLCV) were added to the LAMP test in different ratio’s (Figure 5).

Figure 5: Biotin-LAMP; LAMP target (RNA/DNA) amplification with biotinylated nucleotides allows on-site screening for multiplex targets (first line screening). Positive samples can be brought to the lab for validation and identification with Luminex or ClonDiag (second line screening).

This approach reduces the amount of on-site field testing substantially because only positive samples have to brought to the lab for direct validation/identification with Luminex, ClonDiag array analysis or via retesting with single LAMP assays, making first and second line screening very efficient.

c) Universal ligation LAMP
To simplify the design and detection of one or more targets per sample, another strategy for multiplex LAMP has also been developed. We tested the ligation based Universal-LAMP procedure to see if different DNA targets could be independently detected in one LAMP reaction. With this procedure two target-specific probes are hybridised to each individual target DNA, followed by a specific 5′-3′ ligation of the probe arms. The ligated probes are amplified with universal LAMP primers in master mix under standard conditions. Amplification products are analysed with Luminex, ClonDiag array systems or melting curve analysis (Fig. 6).

Figure 6: Ligation based Universal-LAMP; A. Ligation of target specific probes to different targets. All LAMP probes have universal primer sites. B. Universal LAMP amplification of the ligated probes in the presence of biotinylated nucleotides, allowing multiplex screening of the targets. C. LAMP amplicon decoding by hybridization to Luminex or ClonDiag array systems.

Probes have been designed according to this strategy for detection of Tomato infectious chlorosis and Tomato yellow leaf curl virus. A ligation detection approach has also been tested for Potato spindle tuber viroid (PSTVd) combined with the detection/discrimination of relevant Phytophthora species. P. kernoviae-specific regions were designed based on an alignment of ITS sequences for P. kernoviae and the closely related species P. morinidae and P. boehmeriae. Probes were designed to exploit sequence differences between target and non-target species, with a mismatch between P. kernoviae and the most closely related species positioned at the 3′-end of the left hand probe.

Ligation based Universal-LAMP results indicate that different DNA targets are independently detectable in one LAMP reaction (Fig. 5). The limit of detection is around 100fg for each target, however to date, point mutation discrimination has not yet been realised. Future work will be carried out on the ligation probe design to improve specificity and developing a protocol for rapid cDNA synthesis aiming to find a procedure that is complete in less than five minutes.

Figure 7: Ligation based Universal-LAMP on TYLCV and TOC viruses; the different DNA targets can be independently detected in one LAMP reaction.
3. A novel multiplex platform for pathogen detection.

In addition to LAMP based isothermal amplification approaches developed an innovative approach based on the detection of products of rolling circle amplification was also developed. In this approach isothermal amplification is based on the interaction of circularisable probes (padlock probes) which following ligation can be amplified using rolling circle amplification. The output from the work was a platform for the detection of these products.

The system (for schematic see figure 8, for a video of the prototype see [www.qdetect.org](http://www.qdetect.org)) is an automated system for analysis of rolling circle products (RCP). RCPs will be small rounded fluorescent spheroids approximately 1 μm in diameter. Samples containing RCP are placed in a sample holder. The samples can either be in single PCR tubes, or in sets of three. The samples are sequentially moved to a sample aspiration position, where a defined portion of the sample(S) is aspirated. This sample portion is then analyzed by the instrument. This readout is performed by pumping the sample through a flow cell where the RCPs are illuminated by laser light of one, two, or three different wavelengths, depending on the configuration of the instrument. Emitted fluorescent light is collected by fast line detectors and transferred to an image processing computer for analysis. Computer image analysis is used to count the number of RCPs in the sampled volume. Image analysis uses two thresholds to process the image data. The first threshold is performed to discriminate RCPs over background. After this object thresholding the number of RCPs is determined and compared to a sample threshold to decide if the sample is positive or negative.

The instrument can be functionally divided into a fluid handling module, a detection disk handling module, an optics module including cameras, an infrastructure module, and a control and analysis module.

Figure 8: The reader, with front hatches removed to show fluid containers and sample loading area(left), and detection channel CD (right).

Potential Impact:
Impacts from the work
The outcomes from the project will significantly help to tackle increasing risks to EU plant health from regulated or emerging exotic pests linked to increased globalisation of trade in plants and plant products. By the provision of sampling and detection methods in particular, the results provide scientific support and also modelling tools to the EU plant health policy who can use them to evaluate new techniques before deployment. The methods developed will help EU countries to enforce monitoring and on-going surveillance of regulated pests and may ensure that imported plants and plant products comply with EC plant health import requirements. The quarantine pests targeted are those where detection methods are lacking, where there is a high priority due to EU policy and where no existing methods are available. The methods developed will also be useful in exclusion, eradication and containment campaigns, and should help to minimise economic and environmental damage.

The Q-DETECT project provided outputs in support of EU’s policy of plant health by developing phytosanitary science expertise and capacity (for numbers of people trained and recruited to work on the project see section 4.3C) which can now be offered to the plant health authorities in the EU member states. The impact of this engagement with actors in various sectors will be maintained since in the development process the project was constructed by teaming NPPOs and other pathology laboratories with specialist technology developers and engineers. the aim of this structure was to bring together the best expertise in a multi-disciplinary project but to also retain the expertise by working with knowledge
custodians within NPPOs and deploying an effective dissemination plan with NPPOs, inspectors and other first responders. In this way the project addressed the problem of an eroding scientific basis in the phytosanitary field, which has previously been recognised by the European Plant Protection Organisation (EPPO) in the common declaration in 2004:

https://www.eppo.int/STANDARDS/position_papers/madeira.htm

The following stakeholder groups have benefited already or will benefit in the longer term from the outcomes of the Q-DETECT project, specifically:

- National plant protection and inspection services both within the EU and in third countries have benefited from almost all outputs from the project, by engaging in the workshops of the project.
- National and EU policy makers for compliance of legislation, have benefited by being involved in a dissemination activity in Brussels and from the use of the tools with the national NPPOs.
- Trade (both importers and exporters) will benefit most immediately by the deployment in 2013/14 of the outputs of WP7 (Genie ii instrument and LAMP assays) at Heathrow and Zurich airports in the first instance, where the storage costs for holding consignments for testing will be reduced considerably.
- Society due to prevention of diseases that damage crops and the environment, and in cost saving from importers being passed on to consumers.

In addition collectively all of these stakeholders will benefit from the outcome of this project since a uniform system of identification of plant pathogenic Q-organisms based upon scientific knowledge will be developed.

EUPHRESCO – The phytosanitary ERA-network:

This proposal fits very well into the research coordinated by the Phytosanitary ERA-network. EUPHRESCO aims to coordinate the national funding of phytosanitary research, such that larger more strategic research can be tackled by consortia working through the framework programs. The Q-DETECT project fitted well with this strategic planning; EUPHRESCO has not funded work on the development of detection methods for use by NPPOs and Inspection Services during the period of the project, but has received proposals for funding to take forward outcomes from the Q-detect project after the project finished. A number of consortium members are working in EUPHRESCO projects and a member of the EUPHRESCO management team is present on the projects Management Steering Committee. As a result both national funding and EU funding for research has been spent efficiently.

More effective detection methods for national plant protection and inspection laboratories:

The Q-detect project has provided tools and methods; from some parts of the project protocols, methods and techniques are available for use by end users. From other parts of the project (by working with SME’s) either prototype detection devices are available or in some cases commercially available equipment is now available for both the member states ‘plant inspection service’ laboratories and research institutes as institutions outside Europe. The impact of this will be to empower all countries equally to undertake effective detection of pests for their own use, 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 full gamut of detection activity, to whom a lot of the samples are out of necessity sent from neighbouring countries, the
Detection methods developed are suitable for use by all NPPOs. In turn this will expedite the use of detection methods by 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 pests. It will also support the move towards national reference laboratories by providing central and standardised approaches for the detection of quarantine plants pests.

A good example of this is the LAMP assays developed in WP7 for quarantine pest detection and identification, that are becoming available through exploitation of one of the SME partners. In addition the validation data for these assays which has been completed to the EPPO standard will become available using the EPPO database of validation, as a result the data will be available to all NPPOs.

Looking further a field outside of Europe (by working with third countries), the availability of common and freely available methodologies empower counties trading within the EU, several of which participated in the project. 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 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 may become increasingly important and may become feasible if common methodologies are used.

Developing portable detection techniques:
Detection of pests is done on the front line primarily by inspection services with support from laboratory-based methods where necessary. In certain situations such as inspection of perishable products imported by air freight, the delays in the decision making cause a number of consequences to both the airports and the importers. In these situations it would be ideal if the people performing the inspections (i.e. the decision makers) would be able to do so without having to break the decision making process by sending samples to a laboratory and waiting for the results. The benefits of this approach are several fold:

• Importers carry the costs of storage and demurrage charges as well as disposal if the product is subsequently found to be infected/infested with a quarantine pest. If the consignment is cleared losses often occur through depreciation of quality of the product during storage which can be significant in high value perishables (e.g. anecdotally mango reduces in value by half each additional unplanned day in storage). Faster decision making would reduce the cost to the importers in each case.
• Airports and ports only have limited storage space and chilled storage for perishable product is even more limited and therefore even more expensive. Long delays in clearance of imported produce causes logistical problems at airports and ports which potentially have costly consequences to other importers, directly or indirectly affected.
• Finally inspection services are on the front line and individual inspectors work in a highly pressurised environment, where both airports and importers both want rapid decisions on movement of imported produce through the airport. This can create stress for individuals that can be alleviated by the use of tools that prevent delays.
For the future:
A significant strategic impact of establishing a panel of interlinked detection methods is the added value that was gained from other projects. For example this project developed pest-trapping approaches, furthermore to use the CAM methods to identify pest species and any viruses they may vector. This single example draws on background information and techniques from the EU funded projects PRATIQUE, PortCheck and Q-BOL, as a result the Q-detect project provided legacy benefits of these other projects. For example, the Q-BOL project developed a comprehensive database of DNA barcodes for quarantine pests that enabled the development of effective CAM methods, subsequent exploitation and validation was provided by the DNA bank of material also developed in Q-BOL.

Impact on producers, trade and society:
The products of the Q-DETECT project will significantly help to tackle increasing risks to EU plant health from exotic pests linked to increased globalisation 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, risk and evidence-based detection of quarantine organisms it improves the possibility of preventing introduction and establishment of new plant pests and diseases in the EC. The methods and products developed will facilitate quicker testing of plant material to be exported to third countries and support better cooperation between EU diagnostic laboratories. Q-DETECT will contribute to a reduction of incidents with quarantine organisms / exotic organisms and therefore contribute to a reduction of economic losses for the sector.

Main dissemination activities
The main dissemination activities were training courses where the aim was to train directly the main stakeholders for the project which are phytosanitary inspectors in Europe and as a secondary objective phytosanitary inspectors from third countries. The European training courses were held jointly with EPPO which enabled the Q-detect project to reach a more comprehensive audience of NPPOs from across Europe. A comprehensive list of all dissemination activities is found in template A2, the most significant Dissemination activities organised were as follows:

Event: EPPO / Q-DETECT Workshop for Phytosanitary Inspectors
Date: 16-18/11/2011
Location: Padova (Italy)
Attendees: approximately 80 Inspectors, NPPOs and Students

Event: Q-DETECT, SASHA & Q-BOL combined training
Date: 16-20/01/2012
Location: ILRI, Nairobi, Kenya
Attendees: Inspectors, NPPOs, diagnostic companies and students

Event: Q-detect training
Date: 16-18/1/2013
Location: Tibet Building, Beijing, China.
Attendees: Approximately 80 - Inspectors, NPPOs, diagnostic companies and students
Event: EPPO / Q-DETECT Workshop for Phytosanitary Inspectors
Date: 19-21/2/2013
Location: Ljubljana, Slovenia
Attendees: Approximately 70 - Inspectors, NPPOs, Students

List of Websites:

www.qdetect.org

Last update: 16 January 2015
Record number: 153184

Permalink: https://cordis.europa.eu/project/id/245047/reporting

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