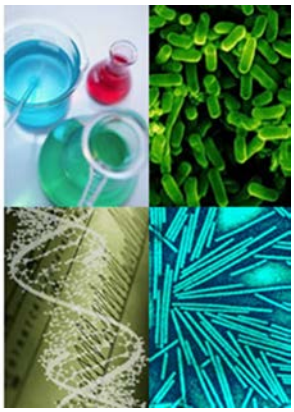
Testa - EPPO Conference on diagnostics for plant pests

(and associated workshops)



Programme, summaries of presentations and posters

2015-11-30 to 2015-12-04
Angers (FR)

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Day 1: Monday 30th November 2015

Testa project - Seed health: development of seed treatment methods, evidence for seed transmission and assessment of seed health

Time	Item	Presenters
12:30[1]-14:00	**Registration for the Testa –EPPO Conference on diagnostics for plant pests** and lunch	
14:00	**Session A: Introduction and sampling**	**Chair: Christine Henry (FERA)**
14:00-14:15	Welcome and introduction to the TESTA project	Christine Henry (FERA)
14:15-14:40	Seed sampling: one plan or many?	Roy Macarthur (FERA)
14:40-15:00	Practical sampling of seed for phytosanitary and quality testing	Valerie Cockerell (SASA)
	Session B: Seed transmission	**Chair: Peter Bonants (DLO)**
15:00-15:20	Efficiencies of bacterial transmission from seeds to plantlets	Marie-Agnès Jacques (INRA)
15:20-15:40	Colonization routes of *Xanthomonas campestris pv. campestris* in Brassica plants that can result in seed infection	Jan van der Wolf (DLO)
15:40-16:10	*Coffee break*	
16:10-16:30	Diversity of seed-borne bacteria and consequences for detection strategies	Marie-Agnès Jacques (INRA)
	Session C: Diagnostic methods	**Chair: Françoise Petter (EPPO)**
16:30-16:50	Detection of viable spores of *Tilletia caries* in a seed lot and a PCR protocol to study their transmission to soil and plants	Geoffrey Orgeur (GEVES)
16:50-17:10	Novel molecular diagnostic methods for seed transmitted pathogens of tomato	Peter Bonants (DLO)
17:10-17:25	Comparison of three DNA extraction kits suitable for PCR-based detection of *Acidovorax citrulli* in watermelon and melon seeds	Emilio Stefani (UNIMORE)
17:30	**Close of Day One**	

[1] A closed meeting of the Testa consortium will be held from 9-12 am for the Testa partners only. This is separate from the conference. Conference participants are invited to arrive at 12.30.

Tuesday 1st December 2015 (morning)

Time	Item	Presenters
09:00	**Session C (cont.): Diagnostic methods**	**Chair: Françoise Petter (EPPO)**
09:00-09:30	"Dead or alive" that is the question, with examples of CGMMV and Xcc	Theo van der Lee & René van der Vlugt (DLO)
09:30-09:50	Comparison of next generation sequencing and VideometerLab for pathogen detection on cereal grain	Mogens Nicolaisen (AU)
09:50-10:10	Implementation of the detection protocol for *Xanthomonas euvesicatoria* in pepper seeds	Emilio Stefani (UNIMORE)
10:10-10:30	Pathoscreen, a new approach in non-destructive quantitative detection	Els Verstappen (DLO)
	Session D: Diagnostic method validation	**Chair: Michel Ebskamp (Naktuinbouw)**
10:30-10:50	Culture-free rapid molecular detection of *Clavibacter michiganensis* subsp *michiganensis* in seeds of tomato	Harrie Koenraadt (Naktuinbouw)
10:50-11:20	*Coffee break*	
11:20-11:40	Comparison of detection methods for *Ditylenchus* in alfalfa and Fava Bean seed lots and method validation	Valérie Grimault (GEVES)
11:40-12:00	Validation of a direct PCR for detection of pospiviroids in tomato seeds	Maaike Bruinsma (Naktuinbouw)
	Session E: Seed treatment	**Chair: Marie-Agnès Jacques (INRA)**
12:00-12:15	Validation study of alternative seed treatments for *Xanthomonas campestris pv. campestris* on Brassica	Gloria Mandiriza (UP)
12:15-12:30	Validation study of alternative seed treatments for *Ditylenchus* on alfalfa	Edgar Mangwende (UP)
12:30-13:00	Project outputs and conclusions	Françoise Petter (EPPO), Valerie Grimault (GEVES)
13:00	**Close of TESTA sessions**	
13:00-14:00	*Lunch*	

Tuesday 01 December afternoon

	Session One - New molecular and bio-analytical identification tools	**Chair: Nathalie Franquet**
14.00-14.10	Welcome	EPPO (or invited guest)
14.10-14.30	Multiplex detection of plant pathogens	Jan Bergervoet (Wageningen University, NL)
14.30-14.50	Development of a polyvalent detection method for Begomoviruses presenting a threat to the European tomato industry	Pascal Gentit (ANSES, FR)
14.50-15.10	Generic RT-PCR tests for detection and identification of Tospoviruses	Marcel Westenberg (NPPO, NL)
15.10-15.30	Detection and identification of *Meloidogyne enterolobii* in complex DNA-backgrounds using LNA-probe based real-time PCR assays	Sebastian Kiewnick (Agroscope, CH)
15.30-15-50	New diagnostic tools for improved diagnostics of grapevine phytoplasmas	Natasa Mehle (NIS, SI)
15.50-16.20	*Coffee break*	
16.20-16.40	Improvements in challenging diagnostic of Pepino mosaic virus and Potato spindle tuber viroid in tomato seeds for better sensitivity	Natasa Mehle (NIS, SI)
16.40-17.00	Developing a molecular diagnostic tool for *Fusarium oxysporum* f.sp. *cubense* tropical race 4 through Diversity Array Technology genotyping	Cor Schoen (Wageningen UR, NL)
	Session two - New tools for better morphological identification	**Chair: Géraldine Anthoine**
17.00-17.20	Quick identification of commonly intercepted Tephritidae in Europe: How does molecular identification help the morphology?	Valérie Balmès (ANSES, FR)
17.20-17.40	Application of digital microscopy for the remote identification of invertebrate pests	Dom Collins (Fera, GB)
17.40-17.50	**Group photo**	

19:30 - Departure by bus from Angers Centre de Congres for the Conference meal at the Château de la Perrière, buses will bring participants back to the Conference Centre for 23:30.

Wednesday 02 December

	Session three - Improved approaches for early detection	**Chair: Rick Mumford**
08.40-09.00	Proposed EPPO validation of plant viral diagnostics using next generation sequencing	Ian Adams (Fera, GB)
09.00-09.20	Development of high-throughput sequencing (HTS) based sentinel tools for the detection and early warning of airborne fungal pathogens	Jaime Aguayo (ANSES, FR)
09.20-9.40	Tree disease surveillance: metabarcoding to identify fungi in spore traps	Ian Adams (Fera, GB)
9.40-10.00	NGS applications in plant pathogen diagnostics	Annette Dullemans (Wageningen UR, NL)
10.00-10.20	Biological and epidemiological studies of *Pseudomonas syringae* pv*. actinidiae* and *Pseudomonas syringae* pv*. actinidifoliorum* pathogenic on kiwifruit to improve their detection	Amandine Cunty (ANSES, FR)
10.20-10.50	*Coffee break*	
10.50-11.10	Unravelling the *Synchytrium endobioticum* genome; working towards reliable and rapid molecular pathotype identification.	Bart Van de Vossenberg (NPPO, NL)
11.11-11.30	Improved pathogen management in crops using rapid in-field diagnostics	Catherine Harrison (Fera, GB)
11.30-11.50	Deployment of loop-mediated isothermal amplification (LAMP) assays for rapid pest and pathogen identification in the UK	Ian Adams (Fera, GB)
11.50-12.10	Assessment of performance criteria of DNA amplification methods for detection of '*Candidatus* Liberibacter solanacearum'	Marianne Loiseau (ANSES, FR)
12.10-13.10	*Lunch*	

	Session three *(continued)* - Improved approaches for early detection	**Chair: Geraldine Anthoine**
13.10-13.30	New approaches for detection and characterization *of Xanthomonas arboricola* pv. *pruni* on stone fruits and almond	Maria López (IVIA, ES)
13.30-13.50	New approaches for the early detection of tree pests and pathogens	Rick Mumford (Fera, GB)
13.50-14.10	*Xylella fastidiosa* outbreak in Europe: new genotypes in Corsica.	Valérie Olivier (ANSES, FR)
14.10-14.30	Potential of spiral plating and digital real-time PCR for improved seed health testing	Manca Pirc (NIB, SI)
14.30-14.50	Enrichment procedures to improve detection of *Clavibacter michiganensis* subsp. *michiganensis* in seed extracts with a dilution plating, a TaqMan PCR and a LAMP assay	Flavia Vieira Lelis (Wageningen UR, NL)
14.50-15.10	Beyond the science - Collaborative approaches to early detection of pests	Rehema White (St Andrews University, GB)
15.10-15.40	*Coffee break*	
	Session four - Other topics of importance in diagnostics	**Chair: Nathalie Franquet**
15.40-16.00	A flexible scope on phytosanitary diagnostics	Arjen Werkman (NRC, NPPO, NL)
16.00-16.20	Experiences of the Dutch National Plant Protection Organization in obtaining an ISO17025 accreditation for PCR-sequencing (DNA barcoding)	Bart van de Vossenberg (NPPO, NL)
16.20-16.40	Comparison and implementation of detection tests for '*Candidatus* Liberibacter solanacearum' (CLso) in plant tissue samples.	Leon Tjou-Tam-Sin (NVWA, NL)
16.40-17.00	Results of the Euphresco- SENDO project: improving diagnostics in *Synchytrium endobioticum*	Gerard van Leeuwen (NPPO, NL)
17.00-17.20	Results of the Q-collect project	Peter Bonants (Wageningen UR)
17.20-17.40	Euphresco	Baldissera Giovani (Euphresco Co-ordinator at EPPO)
17.40-17.45	Close	Françoise Petter (EPPO)

Session A: Introduction and sampling
Chair – Christine Henry

Roy Macarthur and Valerie Cockerell

(Please note that this abstract covers the first and second presentation)

Seed sampling: one plan or many? & Practical sampling of seed for phytosanitary and quality testing

Valerie Cockerell[1], Roy Macarthur[2], Jane Thomas[3,] Mauro Dal Pra[4],Maaike Bruinsma[5], Harrie Koenraadt[5], Ilaria Alberti[4]
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TESTA studied the presence of four seed-transmitted pathogens (*Ditylencus gigas* and *Ditylenchus dipsaci* in *Vicia faba, Xanthomonas campestris* pv*. campestris* in *Brassica* spp*., -Fusarium graminearum* and *F. poae* and *Tilletia caries* in *Triticum* spp.) in a minimum of three infected seed lots, for each pathogen, to determine both between location variation and within location variation of the individual pathogens. The information from these studies was used to produce statistical models that defined the minimum number of primary samples to be taken from the seed lot, the working sample size and the number of sub-samples to be tested for detection of the pathogen to meet a specified target for limit of detection.

We will describe (A)Practical sampling plans for these pathogens under different targets for limit of detection; (B) How to run studies to examine sampling from lots; (C) and some suggestions for Quality Assurance in sampling for pests and pathogens. The influence of detection method used in the laboratory, and hygiene methods used during sampling will also be discussed.

Session B: Seed transmission
Chair – Peter Bonants

Marie-Agnès Jacques

Efficiencies of bacterial transmission from plants to seeds and from seeds to plantlets

Jacques Marie-Agnès (1), Guimbaud Jean-François, Darrasse Armelle .
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Protection of seed production crops is a key issue for seed health management. Understanding mechanisms of bacterial pathogen transmission from mother plants to seeds and measuring efficiencies of transmission from seeds to plantlets were the objectives of our work. We will present results of experiments aiming at (i) analysis the role of look-alikes in transmission of *Xanthomonas fuscans* subsp. *fuscans* to bean seeds, (ii) following gfp-tagged strains of *X. fuscans* subsp. *fuscans* colonizing seeds on mother plants using confocal scanning-laser microscopy, and (iii) efficiencies of bacterial transmissions from seeds to plantlets.

Jean Martin van der Wolf

Colonization routes of *Xanthomonas campestris* pv. *campestris* in Brassica plants

Jan van der Wolf(1), Pieter Kastelein(1), Tadeu Tadeu Antônio Fernandes da Silva Junior (1,2) and Flávia Vieira Lelis (1) 1 Wageningen UR, P.O. Box 16, 6700 AA, Wageningen, the Netherlands. E Jan.vanderWolf@wur.nl. 2 Faculdade de Ciências Agronômicas, UNESP - Botucatu-SP, Brazil

Xanthomonas campestris pv. *campestris* (Xcc), a seed-borne bacterial pathogen, is the causative agent of black rot in Brassicas. In our study we aimed to improve knowledge on how Brassica seeds become infected with Xcc and where it is located in the seed. Multiple spray-inoculations of flowers of Rapid Cycling Brassica plants with a GFP-tagged strain of Xcc, in a density 10^7 - 10^8 cells/ml resulted in contamination of flowers and subsequently in a systemic and symptomatic infection of siliques as shown by dilution plating and epifluorescence stereomicroscopy. Highly infected seed lots were harvested from inoculated plants; ca. 7% were externally infected and 2% remained positive after a warm water treatment. Xcc could be observed inside some of the seed (in endosperm and embryo) using confocal laser scanning microscopy, indicating internal infections. We further showed that bumble bees can transmit Xcc from infected to non-infected plants resulting in symptomatic siliques and infected seeds. Xcc could persist for 23 days in a colony of bumble bees.

Marie-Agnès Jacques

Diversity of seed-borne bacteria and consequences for detection strategies

Jacques Marie-Agnès, Guimbaud Jean-François, Darrasse Armelle INRA, UMR1345 Institut de Recherches en Horticulture et Semences, SFR4207 QUASAV, F-49071, Beaucouzé, France. (1) e-mail: marie-agnes.jacques@angers.inra.fr

The first strategy to use for controlling any disease is to eliminate or reduce the amount of the pathogen available to initiate a disease. Seed health testing is one of these exclusion methods used to control seed-transmitted pathogens. We will illustrate the importance of a detailed understanding of the diversity of the microbial community associated to seeds in regards to detection of seed-associated pathogens. The agent of bacterial canker of tomato *Clavibacter michiganensis* subsp. *michiganensis* share the same niches as non-pathogenic relatives, which interfere in detection tests. Look-alikes were able to efficiently colonize the plant vasculature, but it was not possible to detect transmission to seed for any look-alikes. In contrast, transmission to seeds of pathogen was efficient even when mixtures of pathogenic and look-alike strains were inoculated on mother plants. These results emphasise the need for a sound knowledge of the seed-associated microbiota, its structuration, its impact on plant growth and on pathogen behaviour.

Session C: Diagnostic methods
Chair – Françoise Petter

Geoffrey Orgeur

Detection of viable spores of *Tilletia caries* in a seed lot and a PCR protocol to study their transmission to soil and plants

ORGEUR Geoffrey (1), CHAMAILLE Audrey (1), SERANDAT Isabelle (1), DUPUY Aurélie (1), ROLLAND Mathieu (1), GOMBERT Julie (2),VALADE Romain (3) and GRIMAULT Valérie (1) 1GEVES, 25 rue Georges Morel, 49071 Beaucouzé, France 2FNAMS, Impasse du Verger, 49800 Brain sur l'Authion – France 3ARVALIS, Domaine AgroParisTech, Bât. INRA Bioger-cpp, 78850 THIVERVAL-GRIGNON – France

Current protocols to study transmission of *T. caries* is based on sowing naturally or artificially contaminated seeds in the field and recording of bunted ears, which is time consuming. The aim of our study was to develop a protocol which was not dependant on environmental conditions, not time consuming and could give an early result. Artificial seed and soil contamination (adapted from CEB method N°42[2]) with different contamination rates of viable spores and non-viable spores were done to evaluate the threshold level for damage caused by *T. caries*. The assays were conducted in climatic chamber according to Eibel *et al.* (2005) and a PCR test was developed for an early plant stage. For the plants transferred in field and greenhouse, results showed that a low contamination rate of seeds by *T. caries*, was able to induce symptoms in ears the next year and confirmed the capacity of transmission of *Tilletia* caries at low concentration. Results showed that the early detection by PCR showed a better correlation with the symptoms observed on ears when PCR was done in stem six weeks after sowing compared to four weeks after sowing. Correlation between PCR detection and symptom expression will be presented to show the efficiency of the protocol to study transmission of *T. caries* from seed to plantlets, from soil to plant and its usefulness to access efficiency of seed treatments, in a faster way than the current protocol in the field.

1) [2] CEB, Méthode N° 42, (2000) Méthode d'essai d'efficacité pratique de fongicides destinés à combattre les champignons parasites transmis par les semences de céréales à paille.

Peter Bonants

Novel molecular diagnostic methods for seed transmitted pathogens of tomato

Peter Bonants, Els Verstappen, Annette Dullemans, Cor Schoen, José van Beckhoven, René van der Vlugt, Ilse Houwers, Jan Bergervoet, Jan van der Wolf and Theo van der Lee
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Within TESTA we developed generic methods for detection of pathogens in tomato seeds using real-time PCR so that detection can be performed using standard conditions. In an ideal situation one would like to detect all different pathogens in a DNA/RNA extract (multiplex detection). The Luminex technology has been investigated for simultaneous detection of viruses, viroids and bacteria within tomato seeds. The targets chosen were the bacterium *Clavibacter michiganensis* subsp *michiganensis* and the viral pathogens Potato Spindle Tuber Viroid (PSTVd) and Pepino Mosaic Virus (PepMV). Generic amplification was performed on the DNA/RNA extracted and amplicons were added to primer extension (PE) reactions where target specific primers (labelled with specific Tags) have been elongated. PE reactions products were analysed on the Luminex platform using target specific beads, on which the Tag sequence has been bound. Within the last couple of years many new sequence technologies have been developed (Next Generation and Third Generation Sequence Technology) to determine DNA/RNA sequences in a short time period and with high amounts of sequence data for low costs. We investigated NGS possibilities on DNA and RNA extracts from tomato seed lots used within the project. Results of all these new methods will be presented and discussed.

Emilio Stefani

Comparison of three DNA extraction methods suitable for PCR-based detection of *Acidovorax citrulli* in watermelon and melon seeds

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Acidovorax citrulli is the causal agent of the bacterial fruit blotch of cucurbits. The bacterium is seed-borne: thus, seed analysis and seed certification are essential to ensure phytosanitary seed quality. Seed is often a difficult matrix for DNA extraction and purification, due to the presence of inhibitors and contaminants. Therefore, an extraction and purification procedure ensuring high quality DNA is a critical issue to ensure a robust PCR analysis. During our study, we implemented pathogen detection in seed lots by comparing two types of bacterial extraction (overnight soaking and dry hammering) and three DNA extraction methods (DNeasy Blood and Tissue and DNeasy Plant Mini Kit, both by Qiagen and Wizard Magnetic 96 DNA Plant System by Promega), for both watermelon and melon seeds. Each DNA sub-sample obtained has been analysed with two different primers sets, SEQID3/SEQID4 and WFB1/WFB2. Results showed that a DNA extraction and purification procedure, based on soaking the seeds, followed by the use of the DNeasy Plant Mini kit (Qiagen) on the washing fluids gave the highest amount of purified, bacterial DNA, sufficient to increase the detection threshold of the pathogen. This was calculated to be 1 contaminated seed out of 1000. This procedure is under validation and will allow the improvement of current detection procedures.

Rene Van der Vlugt

"Dead or alive" that is the question, with examples of CGMMV

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Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus which is currently causing significant problems worldwide. The virus is mainly restricted to Cucurbitaceae and is seed transmitted which is the suspected cause for recent virus outbreaks in the USA and Australia. For CGMMV detection several serological and molecular tests are available, each with their own characteristics with respect to sensitivity and specificity. The challenge with CGMMV detection on seeds is to distinguish between viable ('infectious') and non-viable ('non-infectious') virus. This distinction is currently only possible using elaborate, expensive and relatively insensitive bioassays. The viability of the virus depends on the presence of its intact RNA genome. We designed a new method, based on the simultaneous detection of different regions of the viral RNA. This test employs the significant multiplex capabilities of the Luminex xTAG technology to detect intact CGMMV RNA in cucumber seeds. The test successfully detected different CGMMV isolates and was validated with respect to specificity, selectivity, sensitivity, repeatability and reproducibility. A standard dry-heat treatment on different batches of CGMMV-infected cucumber seeds reduced all xTAG signals to nearly background levels, indicating a significant breakdown of the viral RNA. Current bio-assays will need to confirm the abolishment of virus infectivity.

Mogens Nicolaisen

Comparison of next generation sequencing and VideometerLab for pathogen detection on cereal grain

Nicolaisen Mogens (1), Boelt Birte (1), Knudsen Søren (2), Carstensen Jens Michael (3)
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Cereal seeds carry a high number of fungi, including pathogens such as *Fusarium graminearum, F. culmorum, F. poae, F. avenaceum, Tilletia caries* and *Pyrenophora teres. Fusarium*. is a widely distributed fungal genus causing yield reduction in a range of agricultural crops and many species in the genus produce mycotoxins responsible for serious quality deterioration. In malting barley, *Fusarium* also has a negative effect by causing gushing in beer. In this project we used next generation sequencing (metabarcoding) to evaluate contamination on single cereal grains. The seed mycoflora was examined by sequencing the ITS region from total DNA. We compared the metabarcoding results to spectral images of the seeds obtained using the VideometerLab system. Approximately 2-4000 sequences were obtained from each seed, and these were identified to species level. The main fungal genera identified were *Fusarium, Pyrenophora, Epicoccum, Didymella, Alternaria, Bipolaris* and *Microdochium*. The fungal composition on each seed varied significantly. In general, there was a good correspondence between metabarcoding results and spectral images.

Emilio Stefani

Implementation of the detection protocol for *Xanthomonas euvesicatoria* in pepper seeds

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Bacterial spot is a destructive disease of pepper. Its causal agent *Xanthomonas euvesicatoria* is seed-borne and regulated. The EPPO diagnostic protocol for seed analysis suggests performing direct isolation on seed extract, plus one additional test chosen among ELISA, IF or PCR. During our study, we compared ELISA, with the direct isolation and identification of the pathogen, and with a specific molecular detection (simplex-PCR), but following two different DNA extraction and purification procedures in parallel, after seed soaking and crushing with a Stomacher: 1) heat shock or 2) use of DNeasy Plant Mini Kit columns (Qiagen). During a season, thirteen ELISA positive pepper seed samples were found: from those ELISA positive seed samples, 5000 seeds for each extraction method were taken and extracted according the two procedures mentioned above. ELISA results were comparable to those obtained by PCR, but the most sensitive and reliable pathogen detection was by seed extraction with the DNeasy Plant Mini Kit, followed by simplex-PCR. Isolates of *Xanthomonas euvesicatoria* from those positive seed samples were subject to genotyping through rep-PCR, using the BOX, REP and ERIC primers. Results highlighted that *Xanthomonas euvesicatoria* is quite a uniform population, taxonomically very close to *Xanthomonas perforans*, but clearly distinguished from the other two xanthomonads.

Els Verstappen

PathoScreen, a new approach in non-destructive quantitative detection

Verstappen Els, Bonants Peter, Zijlstra Carolien and van der Lee Theo Wageningen UR, BU Biointeractions & Plant Health, PO. Box 16, 6700 AA Wageningen, The Netherlands, e-mail: els.verstappen@wur.nl and theo.vanderlee@wur.nl

For many studies the correct assessment of the presence of a particular pathogen as well as the stage/spread of the infection in plants is critical. Currently, assessment of infection of pathogens is complicated and scoring often relies on manual recording of visual symptoms. However, this scoring is subjective, time consuming, results in extreme data reduction whereby the original data are lost making reanalysis of the data impossible. In addition plant pathogen interactions often involve a latent infection phase for which no visual symptoms can be recorded. Consequently, there is a strong need for development of new procedures. In the last decades we have been experimenting with different procedures and identified that correct colour interpretation (chlorosis, necrosis), measurement of plant stress by chlorophyll fluorescence (fv/fm), quantifying the amount of chlorophyll and direct visualization of pathogens labelled with GFP or RFP can be extremely useful to monitor the various stages in the infection process. In addition, as plant pathogens often show localized infection with limited pathogen biomass, a high resolution combined with a high sensitivity is required. Based on these requirements we have initiated the development of the PathoScreen platform that allows fast multi-dimensional Digital Phenotyping of plant pathogen interactions. Examples of its use will be shown.

Session D: Diagnostic method validation
Chair – Michel Ebskamp

Harrie Koenraadt

Culture-free rapid molecular detection of *Clavibacter michiganensis* subsp. *michiganensis* in seeds of tomato

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Clavibacter michiganensis subsp*. michiganensisis* (Cmm) is the causal organism of bacterial canker of tomato. Cmm is seed transmissible and dilution plating of seed extract is widely used to determine its presence in seeds. In this assay Cmm-suspected colonies are identified by two PCRs and one (time–consuming) pathogenicity assay. Dilution plating was improved in recent years but the recognition of suspected colonies can be difficult due to variable morphology of Cmm and the presence of lookalikes colonies. The objective was to develop and validate a reliable and rapid Cmm specific molecular detection method for the matrix seed. Two complementary Cmm specific real-time PCR tests (Taqman) are used to detect the target pathogen directly in purified DNA from concentrated seed extract. A *Clavibacter michiganensis* subsp*. tesselarius* (Cmt) specific Taqman was developed as an internal amplification control (IAC). In the new triplex Taqman assay seed extracts are spiked with Cmt to monitor DNA isolation and PCR inhibition. The new screening method generates results in two days. Validation data of the new molecular detection method and pros and cons in comparison with the dilution plating assay will be presented.

Valérie Grimault

Comparison of detection methods for Ditylenchus in alfalfa and Fava Bean seed lots and method validation

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The detection and identification of *Ditylenchus dipsaci* and *D. gigas* in seed lots are an obligatory part of the sanitary control in Europe. After a comparison of the performance of the biological and molecular protocols currently used in Europe, the objective was to validate a method that enable the detection of the two pathogens and propose it for inclusion in ISTA and EPPO protocols. Two procedures, filtration and decantation, were compared to detect nematodes after soaking of seeds (to enable extraction and migration of nematode). Both methods allowed nematodes to be detected in infested seeds (Alfalfa and Faba bean) but the filtration protocol using a sieve to collect nematodes allowed a better estimation of the infestation level. It enables identification based on morphological characters as well as the quantification of nematodes which is needed to provide infestation levels of the seed. Confirmation is allowed through a PCR method. Three PCR protocols were compared (Kerkoud, Esquibet and Wood) in order to evaluate their performances (trueness, analytical sensitivity and analytical specificity repeatability, reproducibility). Results have shown better performances in trueness and sensitivity for Kerkoud whereas repeatability and specificity were better with Wood primers. This sieving method with confirmation by PCR with Kerkoud and Wood primers is currently being validated in an interlaboratory test. Results of the validation are to be presented.

Maaike Bruinsma

Validation of a RT Taqman PCR for detection of pospiviroids in tomato seeds

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Potato spindle tuber viroid (PSTVd) is a quarantine pathogen of tomato and several other hosts. PSTVd-contaminated seed is a potential route for dispersal of the pathogen. A PSTVd-detection method was developed and validated for tomato seeds a few years ago. New phytosanitary regulations for additional pospiviroids demanded a test for a broader range of pospiviroids. Therefore, a generic pospiviroid seed assay for CEVd, CLVd, PCFVd, PSTVd, TASVd, TCDVd, and TPMVd was developed. In this assay, subsamples are extracted in a bagmixer. After subsequent RNA extraction, four real-time RT-PCRs based on TaqMan® technology are carried out for detection of the pospiviroids. Dahlia latent viroid (DLVd), a non-target pathogen, is spiked in the extraction buffer as an internal amplification control to monitor RNA extraction and PCR inhibition. Results of the validation study of the assay as well as test results of tomato seed lots, including seed lots that were produced more than 20 years ago, will be presented.

Session E: Seed treatment
Chair - Marie-Agnès Jacques

Gloria Mukwirimba Mandiriza

Validation study of alternative seed treatments for *Xanthomonas campestris* pv*. campestris* on Brassica

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Black rot disease, caused by *Xanthomonas campestris* pv*. campestris,* presents a major challenge to brassica vegetable production by smallholder farmers in Africa. This study evaluated the potential of selected plant extracts and hot water as seed treatments for the control of black rot under greenhouse conditions. Acetone extracts of *Agapanthus caulescens* (15 mg/ml), *Cymbopogon citratus* (10 mg/ml) and hot water at 50°C 30 minutes were applied as seed treatments to seeds artificially inoculated with the pathogen. Seedling emergence, disease incidence, severity and dry mass were recorded. Seed treatments with plant extracts and hot water significantly increased seedling emergence. Hot water treatment proved most effective as it increased seedling emergence by 28 % when compared to the untreated control. Treatment with *A. caulescens* (15 mg/ml) significantly reduced the incidence of black rot to 4.5 % when compared to the untreated control (27.5 %). Hot water at 50°C 30 minutes and *A. caulescens* (15 mg/ml) significantly reduced the severity of black rot. This study showed that alternative seed treatments with plant extracts and hot water have the potential to control black rot disease. Smallholder farmers may adopt the use of such methods since they are non-toxic and cheaper compared to chemical control.

Edgar Mangwende

Validating a thermotherapy seed-treatment against *Ditylenchus dipsaci* associated with alfalfa seeds

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The objective of this study was to evaluate efficacy of steam thermotherapy to validate it as a method for seed sanitation of alfalfa (*Medicago sativa*) seed lots against *Ditylenchus dipsaci.* Greenhouse experiments were conducted to determine the efficiency of the method based on transmission from seed to seedlings. Alfalfa seed lots treated with steam thermotherapy conditions 1, 2, 3 and 4 were compared to non-treated nematodes infested alfalfa seed lots of cultivars A, B and C. All thermotherapy conditions tested recorded 100% efficiency against transmission of nematode numbers except for thermotherapy condition 1 on cultivars A and B, from which 3 and 2 *D. dipsaci* were transmitted into seedlings. Since plant densities of 1000 seedlings/treatment showed low nematode transmission increased seedling density (6000 seedlings/treatment) were used. No nematodes were transmitted into seedlings raised from thermotherapy treated seeds, except for few *D. dipsaci* (2 and 3 nematodes) recorded on seedlings raised from seeds of cultivars C and B, respectively, treated with thermotherapy condition 1. Therefore, a steam thermotherapy condition (>condition 1) can be approved as an official seed sanitation method for the control of *D. dipsaci* on alfalfa seed lots.

Session 1 - New molecular and bio-analytical identification tools
Chair - Nathalie Franquet

Jan Bergervoet

Multiplex detection of plant pathogens

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ELISA is widely used in routine analysis to detect plant pathogens in plant material, while if no antibodies are available alternatively real-time PCR (TaqMan) may be used. However, the majority of these tests detect one pathogen per sample, while multiplex tests would be more cost-effective and convenient. Wageningen UR developed multiplex assays using the Luminex microsphere technology The use of two different bead types; MagPlex xTAG beads and MagPlex xMAP beads allows specific nucleotide (DNA or RNA) sequence detection and detection of plant pathogen antigens respectively. The MagPlex-xTAG beads were used to develop a multiplexed diagnostic method for the detection and identification of nine pospiviroid species in one assay, which can be completed in a single day. Multiplex antibody based MagPlex-xMAP assays, for a wide range of viruses were also developed and these can be completed within four hours instead of the commonly two days it takes, for an ELISA. The sensitivity of the xMAP assay is comparable to ELISA but significantly reduces the use of consumables such as microtiter plates and reagents. It can be concluded that the Luminex microsphere technology can be used for both serological and molecular based methods, with comparable assay sensitivity and similar laboratory workflow, while considerable savings are made on labour and consumables.

Pascal Gentit

Development of a polyvalent detection method for Begomoviruses presenting a threat to the European tomato industry

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One of the most harmful members of the genus Begomovirus is the TYLCV. This virus is widespread worldwide and associated with tomato yellow leaf curl disease. It seems to have spread from infected plants and with the dissemination of its insect vector: *Bemisia tabaci*. Although there is some genetic variability, all isolates found in France belong to two species: TYLCSV or TYLCV. Three other Begomoviruses, not present in France, should be considered as a potential threat for the tomato production: ToMoV, ToLCV and ToLCNDV. Being regulated as quarantine pest in Europe, their introduction and dissemination should be monitored to avoid their spread. After an evaluation of different antisera directed against TYLCV was performed, results showed a large heterogeneity for their performance criteria (18% to 80% sensitivity) and their inability to distinguish the different species. Consequently, a new set of primer pair in conventional PCR was evaluated for the detection of strains of TYLCSV and TYLCV and the three other Begomoviruses. The sensitivity of this set of new primers reaches 100% and allowed all targets to be distinguished. This method could be used in a conventional detection scheme either as a detection test or as a method to confirm positive and/or indeterminate samples obtained by ELISA.

Marcel Westenberg

Generic RT-PCR tests for detection and identification of Tospoviruses

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Detection and identification of tospoviruses by serological tests is often hampered by cross-reactions of antisera and the majority of described molecular tests target just one or a few species. Therefore a set of tests for generic detection and identification of tospoviruses has been developed. Based on an alignment of 28 nucleotide sequences of different species, primers were designed for all currently recognised clades, i.e. American clades 1 and 2, Asian clades 1 and 2, the Eurasian clade and the remaining independent species. These primers target part of the nucleocapsid gene and 3'-UTR of the S-RNA segment. Testing the primers on twenty isolates from different species from the aforementioned clades showed that all these species could be detected. Moreover, the nucleotide sequences of the amplicons appeared suitable for provisional identification of the species. In the final design, the primers have been applied successfully in a setting of five RT-PCR tests for identification of seven tospoviruses in diagnostic samples so far. In addition, they enabled detection and preliminary identification of a non-described tospovirus species in alstroemeria plants. These results demonstrate that the newly developed generic RT-PCR tests provide a useful tool for detection and identification of tospoviruses in plant diagnostic laboratories.

Sebastian Kiewnick

Detection and identification of *Meloidogyne enterolobii* in complex DNA backgrounds using LNA-probe based real-time PCR assays

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Rapid and reliable tools for detection and identification of plant parasitic nematodes are needed to prevent the introduction and spread of quarantine nematodes. The presence of *M. enterolobii* in greenhouses in Switzerland and France clearly demonstrated that pathways for introduction do exist. To identify these pathways and to ensure that appropriate phytosanitary measures are applied, reliable detection and identification tools are needed. Therefore, Locked Nucleic Acid (LNA) Probe based real-time PCR assays were designed for the specific detection, identification and potential quantification of *M. enterolobii*. Furthermore, the tests were evaluated in a test performance study involving seven laboratories. Validation included test performance in terms of accuracy, analytical specificity, analytical sensitivity, repeatability, and reproducibility. All positive and negative results for detection, identification and specificity were consistent between different laboratories despite different equipment being used. Accuracy of real-time PCR was 100%. Analytical sensitivity results also matched between laboratories independent of the equipment used. The smallest amount of target DNA tested, two second-stage juveniles of *M. enterolobii* in a background of 500 non-target nematodes, was reliably detected by all labs. In addition, the repeatability and reproducibility of test results between laboratories was 100%, even at the limit of detection. Thus, the robustness of the developed methods could be confirmed.

Natasa Mehle

New diagnostic tools for improved diagnostics of grapevine phytoplasmas

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The detection of '*Candidatus* Phytoplasma solani' (BNp), the causal agent of Bois noir and Flavescence dorée phytoplasma (FDp), which causes the most serious phytoplasmal disease of grapevine is difficult due to their uneven distribution within the host and low titre. We developed a reliable and sensitive diagnostic procedure easily applicable to high-throughput diagnosis of FDp and BNp which is composed of a simple and quick homogenization step of crude extracts with DNA extraction based on the binding of DNA to magnetic beads and a real-time PCR detection system (Mehle *et al.,* Springer Protocols: Methods in Molecular Biology, 2013, 938: Chapters 12 and 22). For the on-site detection as well as for the screening detection in laboratories, loop-mediated isothermal amplification (LAMP) that gives results in 30 minutes including hands-on work, has been developed for FDp (Kogovšek *et al.,* Plant Pathol, 2015, 64: 286-296) and for BNp. Existing LAMP assays were optimized to allow direct testing of crude homogenates without the need for DNA extraction. For quantification without the need of calibration curve and quality control of DNA based on in-house reference materials typically used in diagnostics and metrological laboratories a droplet-digital-PCR was developed (Mehle *et al.,* Phytopathogenic Mollicutes, 2014, 4:9-15).

Natasa Mehle

Improvements in complex diagnostics of Pepino mosaic virus and Potato spindle tuber viroid in tomato seeds for better sensitivity

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Pepino mosaic virus (PepMV) and Potato spindle tuber viroid (PSTVd) constitute a serious threat for tomato production. Both are very stable and easily transmissible either mechanically or by contaminated tools, therefore even a single infected plant, grown from a single infected tomato seed, can rapidly spread the infection to neighboring plants. To assess the presence of PepMV in tomato seeds we use a reverse transcription real-time PCR (RT-qPCR) assay that detects as low as one naturally PepMV infected seed among 5000 uninfected seeds (Gutierrez-Aguirre et al., J Virol Methods, 2009: 46-55). To screen for all PepMV genotypes we use a 'universal' RT-qPCR combined with additional RT-qPCR assays targeting other genes of PepMV, which serve as confirmatory test and at the same time allow for genotype characterization. In the case of PSTVd infected seeds we experimentally confirmed that the RT-qPCR assay by Boonham et al. (J Virol Methods, 2004: 139-146) can detect one artificially infected seed among 3000 uninfected seeds. For reliable confirmation of PSTVd in the sample, sequencing of the conventional RT-PCR product is required. RT-PCR is much less sensitive compared to RT-qPCR, therefore we developed an efficient easy-to-use method for concentration of viroids from the seeds based on binding to charged beads.

Cor Schoen

Development of a Loop-Mediated Isothermal Amplification assay for rapid detection of *Fusarium oxysporum* f.sp. *cubense* tropical race 4 through Diversity Array Technology sequencing

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Early and reliable detection of *Fusarium oxysporum* f.sp. *cubense* (Foc) tropical race 4 (TR4) is required for effective monitoring of this banana (Musa) pathogen. To date, TR4 - originally identified in Taiwan - has already spread throughout Southeast Asia and was recently discovered outside this region. Traditional morphological Foc characterization is not feasible, as microscopic structures are similar within strains belonging to the *F. oxysporum* complex. The largely consistent Vegetative Compatibility Group (VCG) analyses, are time consuming and not always possible. Rapid molecular based methods are preferential due to their accuracy. The detection of unique genomic regions is a vital step for development of strain-specific probes. In this study, the Diversity Array Technology Sequencing (DArTseq) approach was used for the discovery of unique genome regions for primer design. DNA was extracted from 29 strains representing the overall VCG diversity of Foc. Using a stringency of call rate >0.66, we selected 15 900 markers that included unique DArTseq markers for three TR4 strains-VCG01213. Alignments of the DArTseq sequences with the reference genome of Foc TR4 II-5 allowed up- and downstream extension of the ~69 base pair sequences that enabled the design of Foc TR4 specific LAMP primers for the rapid detection of TR4 strains in banana samples from a range of geographically different locations.

Session 2 - New tools for better morphological identification
Chair - Geraldine Anthoine

Valérie Balmès

Quick identification of commonly intercepted Tephritidae in Europe: How does molecular identification help the morphology?

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Tephritidae (fruit flies) are a family of Diptera which includes important pests of agriculture worldwide. Therefore, all the non-European Tephritidae are listed on the quarantine list (EU annex I/A1 - 2000/29/EC). Imported plants are inspected by NPPO officers at airports and ports and they regularly find Tephritidae larvae. Additionally, IPM (Integrated pest management) in orchards can require identification of larvae at an early stage. But accurate morphological identification at species level is always a challenge. Therefore, our laboratory is working on a morphological key, supported by molecular analysis, for the 3rd instar larvae of 9 commonly intercepted species. Both techniques are performed on the same larva in order to determine and validate the morphological criteria to discriminate species while confirming the identification of the sample by DNA barcoding. Such a validation approach, based on synergies between morphological and molecular methods could be used for the development of diagnostic tools for other quarantine pests. Even if morphological identification may not allow the same level of reliability as molecular, it is fast, inexpensive and sufficiently reliable. Moreover, for detection in a known environment or pathway, it remains the easiest method to use.

Dom Collins

Application of digital microscopy for the remote identification of invertebrate pests

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Remote microscopy allows a camera attached to a microscope to transmit a live video image over the internet. This can then be viewed by a remote diagnostician using a standard web browser, who can direct specimen manipulation in real-time via telephone or voip. Using lessons learnt during a prior study tour to Australia and New Zealand, where remote microscopy systems were set up by their plant health services, a pilot study was undertaken that sought to lay the initial foundations of a network for use in specialist-specialist interactions between the different constituent bodies that together make up the plant health services in the UK. Concerns about IT security issues proved to be the main logistical impediment to speedy set up, with varying degrees of reluctance shown by the various IT departments involved. Once set up, the study demonstrated that the technology works; the main factors to be subsequently considered are how best, and when, remote microscopy can benefit the work of the plant health services.

Session 3 - Improved approaches for early detection
Chairs - Rick Mumford and Geraldine Anthoine

Ian Adams

Proposed EPPO validation of plant viral diagnostics using next generation sequencing

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Next generation sequencing is increasingly being used in plant viral diagnosis but being a non-targeted molecular test, the standard EPPO validation scheme for molecular tests is not appropriate. We have developed a series of validation procedures and controls which mirror those of the targeted molecular tests in terms of sensitivity, specificity, reproducibility and repeatability but are more appropriate for next generation sequencing and analysis. Data on the proposed validation scheme will be presented for non-targeted screening of plant material for virus infections. We have also continued to experiment with new sequencing technology and have used the Oxford Nanopore MinION to sequence a series of plant viruses.

Jaime Aguayo

Development of high-throughput sequencing (HTS) based sentinel tools for the detection and early warning of airborne fungal pathogens

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The advent of high throughput sequencing methods such as those offered by next-generation sequencing (NGS) techniques has opened a new era in the study of fungal diversity. Faced with changes associated with globalization and climate, NGS tools have the potential to be widely used in large scale monitoring of invasive fungi due to the non-specific character of this technology. The aim of this study was to optimize and validate a monitoring system that combines spore trapping and high throughput sequencing to study the whole fungal diversity present in a sample. To do this we tested 20 different protocols which include the type of the spore trap and the preparation of the samples (spore recovery and DNA extraction) before sequencing. High throughput sequencing was performed with the Illumina MiSeq platform targeting the ITS1 and ITS2 regions of the fungal Internal Transcribed Spacer. The method shows a strong potential to be widely used in large scale monitoring, taking into account experimental biases inherent to NGS.

Ian Adams

Tree disease surveillance: metabarcoding to identify fungi in spore traps

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Developing plant disease surveillance systems is a key part of the UK government plant health strategy. We are developing methods to allow the use of existing air sampling networks such as the UK pollen network to monitor for fungal spores. Using next generation sequencing we are identifying the species present on such traps using a metabarcoding approach by amplifying the ITS1 region. Our existing methods have been successfully transferred from the Roche 454 FLX which will soon be discontinued in 2016 and replaced by the Illumina MiSeq. The methods have first been tested on a mock community of fungal pathogens on trees allowing the development of appropriate primers, polymerase mixes, quality control and analysis methods.

Annette Dullemans

NGS applications in plant pathogen diagnostics

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Routine diagnostics of plant pathogens often involve methods such as ELISA and real-time (TaqMan) PCR. These methods only detect known pathogens. Next Generation Sequencing (NGS) is a valuable method to identify new pathogens. We developed a (semi) automated pipeline to use HiSeq Illumina Sequencing to detect pathogens in different hosts. Total DNA or RNA was isolated from symptomatic plant tissue to generate sequence libraries. Individual samples were labelled with tags to pool multiple samples in one run. The sequence reads generated from a single sample were analyzed in a CLC Genomics Work Bench pipeline. Host reads were removed and the remaining reads were used in a de novo assembly. The resulting contig sequences were compared with sequences in public and in-house databases to identify the origin of the non-host sequences. General knowledge on plant pathogens is essential to identify the pathogen contigs. The read coverage of the pathogen genomes expressed as the Reads per Kb per million reads, and therefore the analytical sensitivity, depends on number of reads, genome size, and the abundance of the pathogen in the host. To investigate whether NGS can be a useful tool in routine screening, RNA samples from an inspection service were sequenced. Samples infected with known pathogens, detected by real-time (Taqman) PCR could be confirmed by NGS. Several examples of NGS will be presented.

Amandine Cunty

Biological and epidemiological studies of *Pseudomonas syringae* pv. *actinidiae* and *Pseudomonas syringae* pv. *actinidifoliorum* pathogenic on kiwifruit to improve detection

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Pseudomonas syringae pv. *actinidiae* (Psa), the causal agent of bacterial canker on kiwifruit, was first detected in Europe in Italy in 2008 and later on in France in 2010. Over the last 5 years, 300 strains were isolated and characterized using phenotypic, pathogenic, PCR-based tests and Multilocus Sequence Analysis (MLSA) based on four housekeeping genes (gapA, gltA, gyrB and rpoD). Strains causing leaf spots, die-back, and canker were identified as Psa biovar 3 and strains causing only leaf spots were identified as *Pseudomonas syringae* pv. *actinidifoliorum* (Psaf). In order to track the spread and the origin of the epidemic of Psa biovar 3 in France, we developed a Multilocus Variable-Number of Tandem Repeats (VNTR) Analysis (MLVA). Eleven polymorphic VNTR loci were selected and used to type 340 strains of Psa biovar 3 isolated in Chile, China, France, Italy and New Zealand. MLVA and Discriminant Analysis in Principal Components revealed that strains isolated in Italy and France are genetically closely related and may share the same origin. Based on the comparison of Psa and Psaf genomes, we are developing a Taqman real-time PCR assay, as a detection tool for Psa and Psaf.

Bart Van de Vossenberg

Unravelling potato wart disease; working towards reliable and rapid molecular pathotype identification

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Potato wart disease, caused by the soil-borne obligate parasitic fungus *Synchytrium endobioticum*, is a quarantine disease of potato with a high economic impact. Over the last decades a multitude of pathotypes emerged that break resistance; we hypothesize this is the result of an arms race between *S. endobioticum* avirulence (Avr) genes and potato resistance (R) genes. To better understand the obligate parasitic lifestyle of the fungus and its interaction with potato, we determined the *S. endobioticum* genome sequence from winter spore solutions. After removal of host derived sequences, over 2000 scaffolds originating from *S. endobioticum* but also from fungal and bacterial contaminants were obtained. Optimisation of scaffold selection was achieved with a comparative genomics (ZOO) approach resulting in 808 scaffolds with a total size of 21 Mbp. Putative effectors, which will be tested for their ability to trigger cell-death in potato, are currently being identified. Ultimately this will allow us to identify Avr genes whose products induce effector triggered immunity. Identifying *S. endobioticum* Avr and potato R-genes will provide insight into the coevolution of this biotrophic fungus and its host, and, using SNP information in trait associated loci, will allow the development of pathotype specific diagnostics.

Catherine Harrison

Improved pathogen management in crops using rapid in-field diagnostics

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Mycosphaerella graminicola (*Septoria tritici*, leaf blotch) is the most common foliar disease of wheat in Europe and most fungicides used on wheat are targeted against its control. Mutations at the active site of the fungicides result in resistance and the ability of the pathogen to spread unchecked. Controlling disease relies on early and accurate diagnosis informing timely and targeted intervention strategies. Good resistance management is based on minimising the levels of exposure of the target pathogen to the pesticide, only spraying where the risk warrants treatment. Knowledge of the resistance status of a field population will allow specific targeting of products avoiding use of ineffective treatments, maximising efficacy and longevity of active compounds. Fera are working with several partners to deliver a rapid, in-field test for real-time monitoring of fungicide resistant strains of *M. graminicola* within crops. To enable the level of multiplexing and discrimination of sequence polymorphisms required for this, a novel probe chemistry is being developed for LAMP. This will enable us to discriminate multiple resistance genotypes and to detect small DNA sequence changes. The discrimination of resistance mutations is critical to enable spray programmes to be tailored to specifically target the genotype of the pathogen present in the crop.

Ian Adams

Deployment of loop-mediated isothermal amplification (LAMP) assays for rapid pest and pathogen identification in the UK

Ian Adams, Sioban Ostoja-Starzewska, Catherine Harrison, Jenny Tomlinson, Neil Boonham and Jennifer Hodgetts

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Loop-mediated isothermal amplification (LAMP) allows rapid detection of pathogens in non-laboratory settings by non-specialist staff using simple, inexpensive equipment. These features mean that the technique has been widely utilised for the detection of plant pathogens. Within the UK plant health services LAMP has been deployed to enable on-site detection for rapid identification of numerous pathogens, including quarantine species. In addition the technique has been used in ‘citizen science’ projects to engage with members of the public and raise awareness of plant diseases. Fera has worked extensively with LAMP, developing assays and simple extraction methods to allow the technique to be used across a wide range of matrices and testing scenarios within plant health.

Marianne Loiseau

Assessment of performance criteria of DNA amplification methods for detection of '*Candidatus* Liberibacter solanacearum'

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'*Candidatus* Liberibacter solanacearum' (Lso) is a phloem-limited, Gram-negative, unculturable bacterium that is spread by psyllid insect vectors. Lso is associated with diseases in solanaceous crops in New Zealand and the Americas and with diseases in apiaceous crops in Europe and North Africa. This study reports the evaluation of diagnostic methods for the detection of Lso in host plants and in vectors. Three DNA extraction methods were assessed: CTAB method and two different commercial kits. The analytical sensitivity of each extraction methods was compared. Three DNA amplification methods were also assessed: two real-time PCR methods, both targeting 16S rDNA (Li *et al*., 2009; Teresani *et al.,* 2014) and one conventional PCR (Ravindran *et al.,* 2011) targeting 16S-23S rDNA intergenic region. For each amplification method, using a range of target and non-target samples, we evaluated five criteria: analytical sensitivity, analytical specificity, diagnostic sensitivity, diagnostic specificity and repeatability according to the EPPO Standard PM7/98. Under our conditions, all three DNA extractions methods were reliable with slight differences in the analytical sensitivity. All three DNA amplification methods reached 100% for the diagnostic specificity but the real time PCR developed by Li *et al.* showed the best analytical sensitivity and diagnostic sensitivity.

Maria Lopez

New approaches for detection and characterization of *Xanthomonas arboricola* pv. *pruni* on stone fruits and almond

P. López-Soriano[1], J. Garita Cambronero[2], J. Peñalver[1], P. Noguera[3], E. Marco-Noales[1], A. Palacio-Bielsa[4], Vernière C[5,6], J. Cubero[2], M. M. López[1], O. Pruvost[5]

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The accurate diagnosis and detection of *X. arboricola* pv. *pruni* in symptomatic and asymptomatic plant material is essential for preventive control of this emergent pathogen in many countries of the European Union. In the absence of specific serological techniques, a prototype of a lateral flow device, that can be used for rapid diagnosis and identification, has been developed using two antisera and samples conjugated with carbon nanoparticles and antibodies. A new real-time PCR protocol based on sequences of the virulence effector gene *xopE3***,** has been designed combined with magnetic immunocapture to improve sensitivity and specificity. In addition, comparison of DNA extraction protocols and several enzymes has been made in order to optimise amplification efficiency in diagnosis protocols based on real-time PCR (Palacio-Bielsa *et al.,* 2011 and Garita-Cambonero *et al.,* 2013) and LAMP (Bühlmann *et al.,* 2013). Analytical sensitivity and specificity has been evaluated for the different techniques.

Regarding characterization of *X. arboricola* pv*. pruni*, the diversity of 239 Spanish strains has been evaluated by developing a scheme based on Multilocus variable number of tandem repeats analysis (MLVA) Useful data about the dissemination pathway of the pathogen through propagative plant material among nurseries and from them to the orchards has been obtained by the developed methodology. This information could be used for tracking strain source in other countries.

Rick Mumford

New approaches for the early detection of tree pests and pathogens

Mumford, Rick
Fera, Sand Hutton, York, YO41 1LZ, UK

The 'New approaches for the early detection of tree health pests and pathogens' project started in 2014. It brings together an interdisciplinary team of UK researchers, in order to develop better methods for detecting the growing number of alien pests and diseases threatening our biosecurity. These threats are currently monitored by trained inspectors carrying out visual inspections of imported plants and plant products. However, given the sheer volume of inspections required and the fact that symptoms are often not present, this is a very difficult task to perform effectively. The project's aim is to exploit technical advances in fields such as genomics and remote sensing, translating these into new detection tools that can enhance phytosanitary inspection. It focuses on five different technologies: 1) the detection of disease–specific volatile organic compounds; 2) the use of hyperspectral imaging; 3) DNA-based methods for identifying air-borne pathogens; 4) improved pest trapping approaches; and 5) surveillance for water-borne phytophthora pathogens. A unique feature is that these technologies are being developed through an embedded, co-design approach, utilising a 'Learning Platform' to ensure early, effective and ongoing engagement between technologists and stakeholders, to shorten the time taken from concept to development and ensure effective deployment.

Valerie Olivier

***Xylella fastidiosa* outbreak in Europe: new genotypes in Corsica**

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Xylella fastidiosa is a xylem-limited bacterium, widely spread in the Americas and infecting a large range of hosts. Despite the fact that *X. fastidiosa* is a regulated quarantine bacterium for the European Union, it is now emergent in Europe and Middle-East. In 2012 in France different strains of *Xylella* from Coffea sp. were isolated and have allowed the improvement of the knowledge of this bacterium and its detection scheme (Jacques *et al.,* 2015; www.anses.fr French official method MA039). In July 2015, *X. fastidiosa* was detected for the first time in Corsica by real-time PCR after DNA extraction with a commercial kit (Bio-Nobile). In October 2015, a new outbreak was detected in the South-East part of France. The identification of the subspecies of *X. fastidiosa* was performed by multiplex PCR (Martinez-Hernandez *et al.,* 2006), directly from plant extracts of Harper-positive samples. The subspecies identification was confirmed by MLSA (Yuan *et al.,* 2010). The results of the survey in Corsica show the presence of 2 genotypes of the subspecies multiplex. These strains were detected mainly on *Polygala myrtifolia* but also on other plant species as well as on some non-yet reported hosts.

Manca Pirc

Potential of spiral plating and digital real-time PCR for improved seed health testing

Pirc Manca, Ravnikar Maja, Dreo Tanja
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Contaminated seeds remain a major source of infection for some bacterial diseases. Seeds also represent one of the most difficult matrices for laboratory analysis, containing only minute amounts of pathogens but an abundance of other bacteria and a plethora of potent PCR inhibitors. These characteristics can lower the reliability and impair the sensitivity of commonly used testing methods, isolation on media and molecular methods. To address some of the difficulties encountered in seed testing we have assessed two approaches, spiral plating and digital PCR on a model system of *Xanthomonas campestris* detection in Brassica seeds. Spiral plating (Eddy Jet 2, IUL Instruments) of extracts on FS and mCS20ABN media allowed analysis of continuous dilutions of sample on single plates. This is helpful in identifying the dilution where microflora effects are lower, and the possibility of the pathogen detection the highest. Digital PCR, a variation of real-time PCR but more resilient to PCR inhibitors, was performed in a droplet format (QX100, Bio-Rad) using primers and probe developed by Berg *et al*., 2006. In addition to detection, the digital format allows absolute quantification of the target copies thus providing a tool for generating data on the disease thresholds.

Flávia Vieira Lelis

Enrichment procedures to improve detection of *Clavibacter michiganensis* subsp*. michiganensis* in seed extracts with a dilution plating, a TaqMan PCR and a LAMP assay

Vieira Lelis Flávia M. (1), Pierro Roberto (1), van der Zouwen Patricia S. (1), Schoen Cor D. (1) and van der Wolf Jan M. (1)
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Tomato seed lots infected with *Clavibacter michiganensis* subsp*. michiganensis* (Cmm) often contain low densities of the pathogen, which are hard to find with the currently applied detection methods. In order to increase sensitivity of detection procedures, an enrichment method for Cmm was evaluated. For this, a combination of antibiotics and antifungal agents were added to the filtered tomato seed extract (SE), incubated for 3 days at 25°C and analysed by dilution plating, TaqMan and LAMP assays. For the optimization of the procedure internally contaminated seed was generated and spiked to seed lots with different levels of microbial backgrounds. Enrichment of SE prepared with 0.33% infected seeds with a low or high density of Cmm, or by addition of Cmm suspensions, resulted in an increase of Cmm densities, which became detectable with molecular techniques. Eight naturally-infected seed lots were used for evaluation of the enrichment. Cmm was detected in all 8 lots by TaqMan before or after enrichment, although Cmm was only detected by plating in 4 lots. Enrichment resulted in an increase in Cmm population densities in 2 out of 4 of these lots. The enrichment was less effective for seed lots with a high microbial background.

Rehema White

Beyond the science - Collaborative approaches to early detection of pests

White Rehema, University of St Andrews, North St, St Andrews, Scotland, UK KY16 9AL, rehema.white@st-andrews.ac.uk Marzano, Mariella, Social and Economic Research Group, Forest Research, Northern Research Station, Roslin, Midlothian, Scotland, UK EH25 9SY

Excellent science is required to develop new and emerging technologies for the early detection of tree pests and pathogens. Theoretically, engaging with other stakeholders can enhance the efficacy of new technologies, increase buy in and improve relationships and understanding between groups. We are part of a large research project aiming to bring five cutting edge detection technologies closer to Technology Readiness Level (TRL) 9 (take off!). As the social scientists involved, our aims are both pragmatic and intellectual: to underpin the science packages, facilitate stakeholder engagement, ensure project legacy and address research questions regarding socio-technological innovation. We sought a more collaborative model of science, using the theory and principles of participation, stakeholder analysis and impact. We are building a Learning Platform through annual creative workshops (including a TV series inspired 'Dragons' Den' activity), involving researchers, end users, commercial companies and policy makers. Scientists have struggled to increase stakeholder engagement due to limitations of time, confidence and opportunity. It was concluded that collaborative approaches are critical in stimulating effective technology development, but they need to be managed well to support scientists within the current societal context. We suspect that social scientists will continue to be involved in facilitation and design of such approaches through interdisciplinary projects for the foreseeable future.

Session 4 - Other topics of importance in diagnostics
Chair: Nathalie Franquet

Arjen Werkman

A flexible scope on phytosanitary diagnostics

Werkman Arjen, Van der Blom Marianne, Edema Mariëtte
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At the moment the National Reference Centre (NRC) of the NPPO of the Netherlands is ISO 17025 accredited according to a 'fixed scope'. However, in our experience and opinon, this fixed scope is not easily applicable for our work as a phytosanitary lab. For example only 5 organism-host combinations are accredited while over 1000 different species are identified every year in samples sent to the NRC. The process of accreditation adding the new and relevant organisms is time-consuming and by the time of accreditation an organism might have lost its relevance. Over the last two years we have been working on a scope with higher flexibility to be able to bring more of our phytosanitary work under accreditation. In October 2015, the Dutch Accreditation Body will visit the NRC for an audit regarding the flexible scope. In this presentation we will discuss our ideas on such a flexible scope and the possibilities and challenges in this process.

Bart van de Vossenberg

Experiences of the Dutch National Plant Protection Organization in obtaining an ISO17025 accreditation for PCR-sequencing (DNA barcoding)

B.T.L.H. van de Vossenberg(1), M. Westenberg(1), M. Botermans(1)
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The Dutch National Plant Protection Organization is in the process of getting accredited for all diagnostic methods used for plant pest detection and identification. This includes PCR sequencing according to Sanger. There are examples of diagnostic laboratories and service providers that are accredited for generating sequence data, but accreditation of sequence-based species identification is new to the plant health field. One of the challenges to fulfil ISO17025 accreditation requirements for PCR sequencing is validation. Performance criteria as defined in EPPO Standard PM7/98(2) should not only be determined for the PCR tests but also for the analysis of derived sequence data. Where validation of PCR tests is straightforward, validation of sequence analysis is more challenging because the content of sequence databases changes over time. As a consequence, the analytical specificity of a "locus–species" combination can change when analysed at different moments. Under ISO17025, performance criteria have to be evaluated on a regular basis to determine if they are still valid. The use of constantly expanding sequence databases challenge the way we look at validation status evaluation. Therefore, instead of determining the analytical specificity of a sequence once, it is defined each time an analysis is performed.

Leon Tjou-Tam-Sin

Comparison and implementation of detection tests for '*Candidatus* Liberibacter solanacearum' (CLso) in plant tissue samples.

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To analyse samples from the national survey on '*Candidatus* Liberibacter solanacearum' (CLso) in tomato and carrot crops a comparative study was done to select options for implementation of a detection test to check samples that were found during field inspections showing suspect visual symptoms. Based on the work done, the Crude plant sap extraction following Munyaneza 2010 (NVWA optimized), "QuickPick" DNA extraction plant kit + PVPP purification", the Bio-Rad (CFX96) platform, and TaqMan Universal Master Mix (life technologies) was selected for use in the diagnostic laboratory of "NRC" (National Reference Center) from NPPO-Netherlands. No CLso was detected using the implemented method described above in the national survey in NL. Suspect symptoms were seen in carrot but was found to be caused by '*Candidatus* Phytoplasma asteris'. Using this method, some carrot samples originating from abroad and received by NRC with the request for diagnosis, were found to be infected by CLso.

Gerard van Leeuwen

Results of the Euphresco-SENDO project: improving diagnostics in *Synchytrium endobioticum*

van Leeuwen Gerard
Netherlands Plant Protection Service (NPPO-NL), Geertjesweg 15, 6706 EA Wageningen, the Netherlands

Potato wart disease, caused by the fungal pathogen *Synchytrium endobioticum*, is a major quarantine pathogen in potato worldwide. The disease causes wart-like outgrowths at growing points, e.g. on buds on tubers, in leaf axils, and also on stolons. Different 'forms' of the pathogen occur in potato fields, so called 'pathotypes' or 'races'. These pathotypes differ in their ability to infect cultivars of potato. Nowadays, many cultivars of potato are fully resistant to the originally introduced pathotype 1(D1). In the EPPO Diagnostic protocol a list of differential cultivars is named, to be used by countries in the EPPO-region when identifying pathotypes. In the Euphresco-project "Diagnostic methods for *Synchytrium endobioticum*, especially for pathotype identification (SENDO)" those cultivars were tested in an interlaboratory test using the Glynne-Lemmerzahl method. New cultivars were tested as possible replacements for the cultivars Miriam, Ulme and Belita. The German cultivar Talent proved to be a good alternative for cv Miriam, and the Polish cultivar Gawin for cv Ulme/Belita. Another part of the project focused on a Test Performance Study with PCR-tests described in the literature (ten labs participating), and development of a PCR-test to discriminate between isolates of pathotype 1(D1) and other pathotypes (e.g. 2(G1) and 18(T1)).

Peter Bonants

Results of the Q-collect project

Peter Bonants[1], Jean-Claude Streito[2], John Elphinstone[3], Perrine Portier[4], Vincent Robert[5], Sylvia Blümel[6], Marianne van der Blom[7], Pascal Gentit[8], Paul de Vos[9] and Francoise Petter[10]
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Due to increased international trade and travel the rate of introduction and establishment of new, economically or environmentally damaging plant organisms and invasive species has increased steadily. National Plant Protection Organizations (NPPOs) need to detect and identify quickly and reliably these organisms. This is critical for effective phytosanitary measures to be taken and for ensuring safe transnational movement of plants and plant products. Laboratories are increasingly working under quality assurance systems (including accreditation) and need to have access to validated tests using reference material. In order to ensure proper development, validation and effective and reliable use of tests, it is essential that maintenance of that material in reference collections is ensured. A significant number of plant pathogens and pest collections are still present within Europe but they are dispersed, widespread and of very variable quality. Within Europe there is a need to improve the infrastructure supporting phytosanitary important collections so as to more efficiently use the available infrastructure and improve collaboration in the field of phytosanitary infrastructure with regard to means, knowledge, expertise on taxonomy and development of detection methods. Within the EU project Q-collect we addressed several important issues related to collections: inventory, quality, access, information and network of collections.

Baldissera Giovani

Euphresco: Phytosanitary Research Coordination

Baldissera Giovani(1), Géraldine Anthoine(2), Sylvia Blümel(3), Maria Leonor Cruz(4), Ana Isabel de la Peña(5), Linda F.F. Kox(6), Martine Maes(7), Elspeth Steel(8), Jens G. Unger(9)
(1)European and Mediterranean Plant Protection Organization, Paris, France, bgiovani@euphresco.net; (2)French Agency for Food, Environmental and Occupational Health and Safety, Angers, France; (3)Austrian Agency for Health and Food Safety, Vienna, Austria; (4)National Institute for Agriculture and Veterinary Research, Oeiras, Portugal; (5)National Institute for Agricultural Research and Food Technology, Madrid, Spain; (6)National Plant Protection Organization, Wageningen, The Netherlands; (7)Institute for Agricultural and Fisheries Research, Merelbeke, Belgium; (8)Department for Environment Food & Rural Affairs, London, United Kingdom; (9)Julius Kühn Institute, Braunschweig, Germany

The network Euphresco 'European phytosanitary research coordination' was initiated in 2006 with funding from the FP6 EU Framework Programme. As an ERA-NET, Euphresco contributed to bringing together the main actors from different European countries in order to reduce the existing fragmentation of national research programs, to optimize the use of limited resources of the sector and to promote exchange and collaboration to face the loss of knowledge and expertise. Since April 1 2014 Euphresco has become an international long-term network of programme owners and managers; 31 organisations (ministries, funding agencies, national plant protection organisations, research institutes) are currently members and the network is looking for enlargement in Europe and abroad. The 2016 Euphresco funding process started in November 2015. Members are currently identifying the national priorities which will be proposed by each country. Research topics will be drafted based on selected national priorities and funding will be committed in 2016. The Euphresco coordinator, Mr Baldissera Giovani (bgiovani@euphresco.net), is available to provide information about Euphresco and support organisations in participating in the network’s activities or in collaborating at the level of the research projects.

Organisers/Presenters for Sessions on Detection of tomato pathogens by molecular methods

Mr	**Bonants**	Peter
Mr	**Bergervoet**	Jan
Mr	**Stol**	Willem
Ms	**Van Beckhoven**	Josee
Ms	**Verstappen**	Els

Organiser/Presenter for Sessions on Detection of *Ditylenchus dipsaci* on alfalfa and fava bean by sieving method and confirmation by PCR

Ms	**Grimault**	Valérie

Organiser/Presenter for Sessions on Detection of *Phoma lingam* on Brassicas by blotter and media methods and confirmation by PCR and pathogenicity test

Mr	**Orgeur**	Geoffrey

Participants – Group One

Ms	**Battini**	Celine
Ms	**Besheva**	Ani
Ms	**Beugelsdijk**	Debby
Ms	**Bokuma**	Gunita
Mr	**Browne**	Damien
Ms	**Constant**	Carole
Mr	**Douanla-Meli**	Clovis
Ms	**Elbakidze**	Tinatin
Ms	**Gaganidze**	Dali

Participants - Group Two

Ms	**Götz**	Monika
Ms	**Gurielidze**	Manana
Ms	**Henry**	Christine
Ms	**Khalil**	Amal
Ms	**Laurenson**	Lynn
Ms	**Lee**	Boo Ja
Mr	**Lim**	Wonseok
Ms	**Mandiriza**	Gloria
Ms	**Mehle**	Natasa
Ms	**Pirc**	Manca

Participants – Group Three

Mr	**Mangwende**	Edgar
Ms	**Mcewan**	Marian
Ms	**Poliakoff**	Françoise
Mr	**Prodi**	Antonio
Mr	**Ratti**	Claudio
Ms	**Roux**	Amandine
Ms	**Sherokolava**	Natalia
Ms	**Soukainen**	Mirkka

Participants – Group Four

Ms	**Staneva**	Denitsa
Mr	**Stefani**	Emilio
Mr	**Tegel**	Jukka
Mr	**Tjou-Tam-Sin**	Napoleon
Mr	**Van De Vossenberg**	Bart
Ms	**Vibio**	Monica
Mr	**Wagner**	Stefan
Mr	**Westenberg**	Marcel
Ms	**Zubini**	Paola

Timetable for Workshops

THURSDAY 3rd December 2015				
07:45	Bus departure (Angers conference center-> GEVES)			
8:15 - 8:30	Registration and coffee			
8:30 - 9:00	Welcome speech (Arnaud Deltour), room G21-22			
	Group 1: Detection of tomato pathogens by molecular methods	**Group 2: Detection of tomato pathogens by molecular methods**	**Group 3: Detection of Ditylenchus dipsaci on alfalfa and fava bean by sieving method and confirmation by PCR**	**Group 4: Detection of Phoma lingam on Brassicas by blotter and media methods and confirmation by PCR and pathogenicity test**
9:00 - 9:30	Introduction Lecture (Peter Bonants) (room A09)		Lecture (Valérie Grimault) (room A08)	Lecture (Geoffrey Orgeur) (room G38)
9:30 - 10:00	Lecture Luminex (Jan Bergervoet) (room A09)	Demo Pract Extraction (room A10-A11)	Demonstrations and practicals (Valérie Grimault) (room D22)	Demonstrations and practicals (Geoffrey Orgeur) (room G38)
10:00 - 10:30	Coffee break			
10:30 - 11:00	Demo Pract Extraction (room A10-A11)	Lecture Luminex (Jan Bergervoet) (room A09)	Demonstrations and practicals (Valérie Grimault) (room D22)	Demonstrations and practicals (Geoffrey Orgeur) (room G38)
11.00 - 11:30	Lecture LAMP (Peter Bonants) (room A09)	Demo Pract Luminex (room A10-A11)		
11:30 - 12:00	Demo Pract Luminex (room A10-A11)	Lecture LAMP (Peter Bonants) (room A09)	Visit laboratory	
12:15 - 12:30	Group photo			
12:30 - 13:45	Lunch break			
	Group 1: Detection of tomato pathogens by molecular methods	**Group 2: Detection of tomato pathogens by molecular methods**	**Group 3: Detection of Phoma lingam on Brassicas by blotter and media methods and confirmation by PCR and pathogenicity test**	**Group 4: Detection of Ditylenchus dipsaci on alfalfa and fava bean by sieving method and confirmation by PCR**
13:45 - 14:15	Lecture TaqMan (Jan Bergervoet) (room A09)	Demo Pract LAMP (room A10-A11)	Lecture (Geoffrey Orgeur) (room G38)	Lecture (Valérie Grimault) (room A08)
14:15 - 14:45	Demo Pract LAMP (room A10-A11)	Lecture TaqMan (Jan Bergervoet) (room A09)	Demonstrations and practicals (Geoffrey Orgeur) (room G38)	Demonstrations and practicals (Valérie Grimault) (room D22)
14:45 - 15:15	Coffee break			
15:15 - 15:45	Lecture NGS / Live-Dead e.o. (Peter Bonants) (room A09)	Demo Pract TaqMan (room A10-A11)	Demonstrations and practicals (Geoffrey Orgeur) (room G38)	Demonstrations and practicals (Valérie Grimault) (room D22)
15:45 - 16:15	Demo Pract TaqMan (room A10-A11)	Lecture NGS / Live-Dead e.o. (Peter Bonants) (room A09)		
16:15 - 17:00	General Discussion (Peter Bonants/Jan Bergervoet) (room A09)			Visit laboratory
17:15	Bus departure (GEVES-> Angers conference center)			

Timetable for Workshops

FRIDAY 4th December 2015				
08:00	Bus departure (Angers conference center-> GEVES)			
	Group 1: Detection of Ditylenchus dipsaci on alfalfa and fava bean by sieving method and confirmation by PCR	**Group 2: Detection of Phoma lingam on Brassicas by blotter and media methods and confirmation by PCR and pathogenicity test**	**Group 3: Detection of tomato pathogens by molecular methods**	**Group 4: Detection of tomato pathogens by molecular methods**
8:30 - 9:00	Lecture (Valérie Grimault) (room A08)	Lecture (Geoffrey Orgeur) (room G38)	Introduction Lecture (Peter Bonants) (room A09)	
9:00 - 9:30	Demonstrations and practicals (Valérie Grimault) (room D22)	Demonstrations and practicals (Geoffrey Orgeur) (room G38)	Lecture Luminex (Jan Bergervoet) (room A09)	Demo Pract Extraction (room A10-A11)
9:30 - 10:00			Demo Pract Extraction (room A10-A11)	Lecture Luminex (Jan Bergervoet) (room A09)
10:00 - 10:30	Coffee break			
10.30 - 11:00	Demonstrations and practicals (Valérie Grimault) (room D22)	Demonstrations and practicals (Geoffrey Orgeur) (room G38)	Lecture LAMP (Peter Bonants) (room A09)	Demo Pract Luminex (room A10-A11)
11:00 - 11:30	Visit laboratory		Demo Pract Luminex (room A10-A11)	Lecture LAMP (Peter Bonants) (room A09)
	Group 1: Detection of Phoma lingam on Brassicas by blotter and media methods and confirmation by PCR and pathogenicity test	**Group 2: Detection of Ditylenchus dipsaci on alfalfa and fava bean by sieving method and confirmation by PCR**	**Group 3: Detection of tomato pathogens by molecular methods**	**Group 4: Detection of tomato pathogens by molecular methods**
11:30 - 12:00	Lecture (Geoffrey Orgeur) (room G38)	Lecture (Valérie Grimault) (room A08)	Lecture TaqMan (Jan Bergervoet) (room A09)	Demo Pract LAMP (room A10-A11)
12:00 - 13:00	Lunch break			
13:00 - 13:30	Demonstrations and practicals (Geoffrey Orgeur) (room G38)	Demonstrations and practicals (Valérie Grimault) (room D22)	Demo Pract LAMP (room A10-A11)	Lecture TaqMan (Jan Bergervoet)
13:30 - 14:00			Lecture NGS / Live-Dead e.o. (Peter Bonants) (room A09)	Demo Pract TaqMan (room A10-A11)
14:00 - 14:30	Coffee break			
14:30 - 15:00	Demonstrations and practicals (Geoffrey Orgeur) (room G38)	Demonstrations and practicals (Valérie Grimault) (room D22)	Demo Pract TaqMan (room A10-A11)	Lecture NGS / Live-Dead e.o. (Peter Bonants) (room A09)
15:00 - 15:30			General Discussion (Peter Bonants/Jan Bergervoet) (room A09)	
15:45	Bus departure (GEVES-> Angers train station)			

POSTERS (listed in the order they were submitted)

Salima Berkani

Detection of "*Candidatus* Liberibacter solanacearum" using AmplifyRP® isothermal amplification

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"*Candidatus* Liberibacter solanacearum" (Lso) is a gram negative; phloem limited bacterium which has become economically significant. It has been attributed to an estimated 62 million USD loss in Texas and New Zealand. Lso is transmitted via psyllids and induces symptoms that often leave infected crops commercially unacceptable. Currently, five different haplotypes based on the ribosomal protein gene have been identified; LsoA and LsoB, infecting solanaceous crops in the Americas and New Zealand, LsoC infecting carrots in Finland, LsoD isolated in the Canary Islands and Spain and LsoE in Spain, the Canary Islands, as well as Morocco. Agdia Inc. has developed a rapid molecular detection method for "*Candidatus* Liberibacter solanacearum" (Lso) using the Accerler8 format of AmplifyRP. The process works using isothermal DNA/RNA amplification technique. The assay developed matches the sensitivity of conventional PCR and real-time PCR assays. Initial data has shown no cross reactivity to other Liberibacter or common solanaceous pathogens. Agdia has been able to verify use with psyllids, carrot seeds, tubers and infected plants. Work is being done to ensure accurate, reliable detection of all five haplotypes and harmonization with internationally accepted protocols.

Marleen Botermans

Q-bank for sharing data and information on plant virus and viroid isolates in collections

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The availability and accessibility of well characterised reference isolates of plant viruses and viroids is crucial for research and diagnostic laboratories. To ensure the availability of isolates and reference materials, it is of utmost importance to collaborate at an international level. Seeing the general tendency of decreasing budgets and loss of experienced staff, future efforts should focus on sharing data as well as expertise on reference collections. Q-bank, the comprehensive databases on plant pests and diseases, offers an excellent platform to share data on plant virus collections (http://www.q-bank.eu/Virus). It includes biological, serological and molecular characteristics of available virus and viroid isolates and indicates the collection from which they can be obtained. In addition, the database provides easy access to overviews of regulated species, various test protocols and a sequence BLAST tool to assist diagnostic laboratories in species identification. Q-bank offers the opportunity to share data of characterised isolates that are physically available for public use. Moreover, curators ensure that quality standards are met and conservation is safeguarded. To further strengthen the Q-bank database and collections, the EUPHRESCO project VirusCollect aims to extend the network of reference collections for regulated and other important viruses and viroids at the European level.

Charles Lane

Skills pipeline - developing future plant health professionals

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The UK's Tree Health and Plant Biosecurity expert task force identified the need to 'address key skills shortages'. 'The task force noted with concern that there had been an erosion of capability, in the UK and internationally, to deal with some aspects of tree and other plant pests and diseases. In addition, there has been a decline in some of the underpinning natural and social science expertise essential to inform and implement policy. The poster will report on progress in identifying needs and opportunities for developing skills and creating a recognised profession for plant health diagnosticians, consultants and researchers from both the public and private sectors. It will report on the following critical success factors: 1: inspire the next generation of plant health scientists by identifying and developing engaging teaching resources for both curricula and extra-curricular activities. 2: inspire graduate entry into plant health careers through raising awareness and developing new work-based learning opportunities and new modular training resources for Higher Education Institutions (HEIs). 3: identify and develop formal professional qualifications and continual professional development opportunities for plant health professionals

Lynn Laurenson

Validation and Verification of new methods at Fera

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Ensuring methods are robust and fit for purpose by completion of validation or verification is an important task for all laboratories working under quality standards. Prior to 2014, validation and verification at Fera was carried out in isolation within each discipline. This resulted in inconsistencies and differing levels of uptake. Using the EPPO Standard for validation of methods (PM 7/98) as a foundation, a standardised process has been established within the plant health program. Details of this process will be displayed. Alongside this a working group had been setup, consisting of both diagnostic and R&D staff. This allows the focus on validation to be consistent and supported by experienced staff. The process has been designed to ensure all aspects of the EPPO standard are fulfilled in the most efficient way. Template documents and SOPs have been devised and implemented to provide consistency to validation data. In addition a peer-review of data allows all stakeholders to agree on the suitability of the method. Furthermore, data can be fast tracked allowing rapid accreditation under flexible scope along with deployment into routine use . The use of this process and working group has seen validation activities within the program increase along with a greater understanding of the importance of method validation within the plant health program.

Lynn Laurenson

Implementation and optimisation of *Gremmeniella abietina* detection using real-time PCR

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Gremmeniella abietina is a pathogenic ascomycete responsible for Brunchorstia dieback. Infection with this pathogen produces stem cankers and shoots dieback within many conifers species including genera *Abies, Picea, Pinus, Larix, Tsuga* and *Pseudotsga*. It has been reported in Europe, eastern North America and Japan and although is not considered as a quarantine pest by EPPO, it is quarantine for NAPPO and other regions. Detection of fungal pathogens from infected material by molecular techniques is commonly used for diagnostic purposes. A new extraction method using KingFisher and a real-time PCR assay (TaqMan®) have been implemented at FERA in order to reliably detect *G. abietina*. The real-time PCR was verified using assays described by Isabella Borja *et al.,* (2006). The limit of detection using infected tissue was established at 0.6pg/µl. Specificity tests were also performed against 24 closely-related organisms and no cross-reactions were seen. This method has been verified and optimised at FERA to produce a rapid and reliable method to provide routine diagnosis of *G. abietina*.

Borja, I., Solheim, H., Hietala, A. M. and Fosdal, C. G. 2006. Etiology and realtime polymerase chain reaction-based detection of *Gremmeniella*- and *Phomopsis*-associated disease in Norway spruce seedling. *Phytopathology* 96:1305-1314.

Lynn Laurenson

Downy mildew of sunflower (*Plasmopara halstedii*) - first UK finding and screening by real-time PCR for continued exclusion.

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Plasmopara halstedii is an oomycete pathogen of sunflowers. Infection can result in a severe impact on the yield of sunflower seed. It is listed as an II/A2 quarantine pest in Europe and trade of sunflower seeds is subject to phytosanitary controls. An outbreak was detected in the UK in 2010, since then continued exclusion and screening is carried out through testing of seed to prevent the introduction and spread of more virulent or fungicide-tolerant *P. halstedii* strains in Europe. A duplex real-time PCR for detection of *P. halstedii* using a specific *P. halstedii* primer-hydrolysis probe (qPHAL-F/-R/-P) (Ioos *et al*., 2012) in sunflower seeds has been verified and implemented at Fera, offering specific and sensitive detection in high throughput setting.

Ioos R, Fourrier C, Wilson V, Webb K, Schereffer J-L & Tourvieille de Labrouhe D (2012) An optimized duplex real-time PCR tool for sensitive detection of the quarantine Oomycete Plasmopara halstedii in sunflower seeds. *Phytopathology* 102, 908–917.

Anna Skelton

The application of Next-Generation Sequencing for screening seeds for viruses and viroids

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Currently the majority of test methods used in screening seeds for viruses and viroids are based around targeted test methods, such as ELISA and real time PCR. Non-targeted molecular based approaches are being increasingly used in plant pathology for the identification of novel pathogens, however, the application of this technology in routine screening work has yet to be fully explored. Preliminary work to validate Next Generation Sequencing (NGS) has been carried out through testing serial dilutions of *S. lycopersicon* seed samples infected with Potato spindle tuber viroid (PSTVd), Columnea latent viroid (CLVd) and Pepino mosaic virus (PepMV). The limit of detection of NGS compared to real time PCR is discussed. Seed was from both natural infections (CLVd) and from artificial inoculations (PSTVd and PepMV). A control sample from a commercial seed lot was also used for the serial dilution of test samples and was shown to contain PepMV, Tobacco mosaic virus, Cucumber mosaic virus, Tomato bushy stunt virus, Potato leafroll virus and a novel member of the genus Cavemovirus. The viroids from the test samples and the viruses from the control/dilution seed lot were detected simultaneously, the first time this has been demonstrated for botanical seed. Work is ongoing to further validate this technology for seed screening.

Claudio Ratti

Multidisciplinary approach for seed quality assessment and certification

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Quality testing, aimed at ensuring the desired genetic, physical, physiological and health properties of seed, is an important mean to achieve successful agricultural production. The increasing demand for certification of seedlots moving all over the world calls for appropriate scientific and technical infrastructures to assess seed quality, and capable to perform routine analyses as well to adjust the procedures to the legislation of importing countries. In our Department areas with different expertise on seed-borne pathogens and other aspects of seed quality are being combined to provide a qualified, wide-spectrum support to the stakeholders of the Italian seed production chain. Testing activity is particularly focused on traditional and molecular approaches to detect pathogens, saprophytes, weeds, other contaminants and the unintended presence of GMO in seed lots. The species concerned belong to all agronomic groups but, in particular, to vegetable crops of the families Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Fabaceae, Lamiaceae, Liliaceae and Solanaceae. The pathogens most frequently detected are *Fusarium oxysporum* (different formae speciales), *Didymella bryoniae*. Lettuce mosaic virus, Pepper mild mottle virus, Tomato mosaic virus. Currently our interests are focused also on organisms producing mycotoxins (*Fusarium* spp.) on cereals and garlic seeds as well on seed transmission of *Peronospora belbahrii* in basil.

Aude Chabirand

Appropriate sampling for an efficient pathogen detection in plants: The example of the detection of *Xanthomonas axonopodis* pv*. allii* in onion seeds

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With the rapidly expanding world trade of seeds, the risk of spreading seedborne pathogens has drastically increased. In this context, the healthy quality of seeds becomes an essential issue to minimize the spread of plant diseases. *Xanthomonas axonopodis* pv*. allii* (Xaa) is the causal agent of bacterial blight of onion (BBO), a seed transmitted disease. This bacterium is an EPPO listed pest (A1 list i.e. absent from the EPPO region). In the case of Xaa, a seed lot with a contamination rate of 0.04% was shown sufficient to cause BBO outbreaks in a tropical environment (Roumagnac *et al.,* 2004). Consequently it is of crucial importance to develop efficient detection methods combined with sampling procedures which allow the detection of seedborne pathogens for which the level of inoculum can be very low in very large seed lots. Recently, two molecular diagnostic tests have been developed for the detection of Xaa in onion seeds: a duplex nested-PCR (Robène-Soustrade *et al*. 2010) and a triplex real-time quantitative PCR (Robène *et al*., 2015). Given the detection performance of these two methods (evaluated through inter and intralaboratory collaborative studies), different sampling protocols were proposed according to the targeted detection level and pvalue.

Geoffrey Orgeur

TESTA project: Validation of detection methods for *Phoma lingam* by blotter and media protocols

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The aim of the TESTA project was to validate the replacement of the toxic herbicide 2,4-D with a deep freezing step in the validated ISTA method 7-004. Both are known to suppress germination of seeds during the blotter test allowing better assessment of the infecting pathogen. After some pre-tests, the performance of the methods (media and blotter) did not show any significant difference in the capacity to detect *Phoma lingam* on seeds. Comparison between the 2,4-D and deep freezing on blotter showed efficiency of both. In parallel, different confirmation methods (Pathogenicity test and PCR) were developed. According to Liu *et al.* (2006), primers LmacR and LmacF and different conditions for the pathogenicity test were tested on a collection of *Phoma lingam* isolates. After improvement, the PCR protocol was tested in one lab only, during the comparative test. The best method was chosen through observation of the expected symptom expression: inoculation at cotyledon stage by injection. All the isolates suspected to be *Phoma lingam* were positive and showed characteristic symptom expression. A comparative test of the three methods (media, blotter 2,4-D and blotter deep freezing) is currently on going. Results of the comparative test and performance criteria of the methods are to be presented.

EPPO activities on diagnostics

http://www.eppo.int/QUARANTINE/diagnostic_activities.htm

EPPO activities on diagnostics

Since 1998, EPPO has established a work programme in the area of diagnostics to harmonize procedures across the EPPO region. This programme mainly focuses on the diagnostics of pests which are regulated as quarantine pests. The different activities conducted in this framework are described below.

EPPO diagnostic protocols

The preparation of protocols involves close collaboration between different EPPO Panels involved in diagnostics. These Panels are composed of diagnostic experts nominated by the NPPOs of the EPPO member countries. The EPPO Panels involved in diagnostics are the following (click on the links to view their composition):

Panel on Diagnostics and Quality Assurance
Panel on Diagnostics in Bacteriology
Panel on Diagnostics in Entomology
Panel on Diagnostics in Nematology
Panel on Diagnostics in Virology and Phytoplasmology
Panel on Diagnostics in Mycology

Each draft diagnostic protocol is initially prepared by an individual expert according to a **common format** which ensures that the draft contains all necessary information to detect and positively identify a particular pest. Whenever available, validation data is also provided for the different tests included in the diagnostic protocols. The draft protocols are reviewed by the relevant Panels and submitted to a consultation phase among all EPPO member countries to ensure their wide acceptance. As it is the case for all EPPO Standards, diagnostic protocols are officially approved by the EPPO Council (at its yearly Session in September) and then published in the EPPO Bulletin. Over 100 diagnostic protocols have been approved.

View and download currently approved EPPO diagnostic protocols

Draft protocols: the following protocols are under preparation by the various Panels:

- *Pest specific protocols*
  - *Spodoptera* spp.
  - *Andean potato latent virus* and *Andean potato mild mosaic virus*
  - *Xanthomonas axonopodis* pv. *allii*
  - *Acidovorax citrulii*
  - '*Candidatus* Liberibacter solanacearum'
  - *Phytophthora lateralis*
  - Generic Standard on the detection of pospiviroids
- Horizontal Standards
  - Standard on ELISA test for viruses
  - Standard on Immunosorbant Electron Microscopy
  - DNA barcoding as an identification tool for regulated plant pests
  - Guidelines for the Authorisation of laboratories
  - Guideline on flexible scope in plant health
  - Guidelines on National Reference Laboratories

A survey on the use of the protocols was conducted in 2008 on a selection of 58 protocols in all disciplines of plant health diagnosis (**Petter & Suffert, 2010**). Laboratories registered in the EPPO database on Diagnostic Expertise (see below) were asked to indicate the number of samples that they tested in 2007 and which test they used. From this survey it can be concluded that many of the tests for detection mentioned in EPPO diagnostic protocols are widely used in laboratories in the EPPO Region. This survey was repeated in 2013 on 68 protocols. The results from this will be used to identify the widely used tests as these can be used under accreditation following PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* without providing data on repeatability and reproducibility as these are considered as giving appropriate confidence regarding repeatability, reproducibility and selectivity.

The EPPO Technical Document no. 1056: Pictorial Glossary of Morphological Terms in Nematology lists all nematological terms used in the EPPO diagnostic protocols on plant parasitic nematodes with their definitions.

Accreditation and quality management

So far two EPPO Standards on quality assurance have been approved:

- **Basic requirements for quality management in plant pest diagnosis laboratories**
- **Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity**

The latter of these standards is currently under revision by the Panel on Diagnostics and Quality Assurance.

A joint communiqué between EPPO and EA (European Co-operation for Accreditation, the European network of nationally recognised accreditation bodies) states that *'EA will recommend that assessors from Accreditation Bodies take note of EPPO documents when evaluating plant pest diagnostic laboratories'*. Since 2011-01 EPPO is a 'recognized stakeholder' for EA.

EPPO database on diagnostic expertise

In 2004, EPPO Council stressed that the implementation of phytosanitary regulations for quarantine pests was jeopardized by decreasing knowledge in plant protection (**Madeira declaration**). The Panel on Diagnostics proposed that an inventory should be made of the available expertise on diagnostics in Europe. The database on Diagnostic Expertise was created (**Roy *et al.*, 2010**) to allow identification of experts who can provide diagnosis of regulated species and those who can help in the identification of new or difficult to identify species. EPPO member countries were contacted and as of Nov 2013, 86 laboratories from 35 countries have provided data corresponding to more than 500 experts. These results are available in a searchable database on the EPPO website. The database can also help national accreditation bodies identify auditors for pest diagnostic laboratories for accreditation.

Consult the EPPO database on diagnostic expertise

Workshops and Conferences

Since 1985, EPPO has organized a series of workshops and conferences on diagnostics.
Summaries of the recent conferences and workshops can be viewed here.

- Conferences on diagnostics (1985, 1994, 2000 and 2012 in NL - 2009 in GB)
- Workshops on Quality Assurance (2007 in DK - 2009 in GB - 2014 in GB)
- Workshop for heads of laboratories (2011 in AT - 2013 in TN)
- Workshop on 'cut-off data' for real-time PCR (2013 in FR)

Sources of EPPO information useful for diagnostics

PQR: is the EPPO database on quarantine pests (geographical distribution and maps, host plants, pathways, pictures).

EPPT: is the EPPO Plant Protection Thesaurus, a database which contains scientific and common names, elements of taxonomy, and computer codes, for more than 58 000 species of plants and pests of agricultural importance.

EPPO Reporting Service: is a free monthly report on events of phytosanitary concern (new geographical records, new host plants, new pests, new diagnostic methods).

Links to other initiatives on diagnostics

EUPHRESCO: is a European Research Area Network (ERA-NET) project for research policy development and implementation in the field of statutory and emerging plant pests. In 2013 the EPPO Council decided that from early 2014 the EPPO Secretariat should co-ordinate the EUPHRESCO network.

IPPC Phytosanitary resources: Diagnostic protocols on diagnostics

QBOL: is a project financed by the 7th Framework Program of the European Union that makes collections containing plant pathogenic quarantine organisms available. Informative genes from selected species on the EU Directive and EPPO lists are DNA barcoded from vouchered specimens.

Q-Detect: is a multi-disciplinary research network focused on developing innovative tools that enhance the capacity of phytosanitary inspectors to protect European agriculture and forestry sectors from invasive pests.

Q-bank: is a database on diagnostic tools for quarantine pests.

Q-collect: is a project financed by the 7th Framework Program of the European Union. It aims to improve the status of reference collections important to plant health.

TESTA: is a project financed by the 7th Framework Program of the European Union on Seed health: development of seed treatment methods, evidence for seed transmission and assessment of seed health. EPPO is a partner in this project and outcomes will be used to develop or revise EPPO Standards as appropriate (Diagnostic Protocols, Treatment Standards).