Institution: University of Nottingham

Unit of Assessment: 6; Agriculture, Veterinary and Food Science

Title of case study: Development of diagnostic systems for plant diseases

1. Summary of the impact

Diseases of plants impact upon global food production and the environment, necessitating careful control. University of Nottingham (UoN) research has contributed to new lab-based and in-field tests that are extensively used by plant health inspectors and overseas organisations. The research has produced validated, accurate pathogen detection systems for use by plant health inspectorates and quarantine services as part of their testing services. The methods have been adopted by the Food and Environment Research Agency (Fera) in the UK for routine testing, and also by the Swiss diagnostics company Bioreba as part of their diagnostic services.

2. Underpinning research

Key researcher at UoN:
Prof. Matt Dickinson: Professor of Plant Pathology (June 1992 – current)

Phytoplasmas are bacterial plant pathogens that have been found associated with diseases in over 200 plant species. Amongst the more economically important diseases are the aster yellows type that occur on crops such as potatoes, carrots, maize, tomatoes, onions and flowers worldwide, the apple proliferation / pear decline diseases of Europe, the potato witches' broom and maize bushy stunt diseases of the Americas, and the coconut lethal yellowing-type diseases of the Caribbean, Central America and Africa. New phytoplasma diseases are constantly emerging in new hosts and at new locations, and in 2005, Defra perceived that these quarantine organisms were becoming an increasing threat to UK agriculture due to climate change and the increased import of plant material from countries where such diseases are endemic. As a result, they funded a Defra Plant Health Fellowship in 2005 at UoN in collaboration with Rick Mumford and Neil Boonham (Fera), with the aim of training a diagnostician in phytoplasma diseases **[a]**. The Fellowship employed a PhD student at Nottingham, who developed a range of diagnostic approaches culminating in the validation of a real-time polymerase chain reaction (PCR) method that was able to efficiently detect all known phytoplasmas and show no significant cross-reaction with other related bacteria **[6]**. Built into the real-time universal diagnostic system was the capacity to develop group specific probes for particular phytoplasma 'species' and proof of concept of this technology was confirmed.

This initial Plant Health Fellowship led to additional joint funding with Fera and co-supervision of more PhD students registered with Prof. Dickinson at UoN **[d,e]**. Two of these, have developed and validated real-time PCR detection tests for a range of potato and wheat viruses, that are now used for routine diagnostics testing at Fera. In addition, we have been developing rapid 'in-field' diagnostic systems for a range of plant pathogens, including sudden oak death (*Phytophthora ramorum*) and *Botrytis cinerea*. These methods are based on the LAMP (loop-mediated isothermal amplification) technology **[1,2]**, and further work to develop this technology for phytoplasmas has been funded through BBSRC and a Royal Society Leverhulme Africa Award (2009-2012) with Ndede Yankey (a PhD student from Ghana registered and working at Nottingham) and Robert Quiacoe (CSIR coconut research programme, Ghana) **[b,c]**. The technological developments have also involved an informal collaboration with the UK Company, Optigene, who have been developing and commercialising reagent mixes and hardware for real-time detection of LAMP products. In consultation with Optigene, UoN proposed the development of a lightweight, portable real-time LAMP detection machine that could be battery operated and therefore used with minimal additional equipment for detection of plant and other pathogens in the field in remote locations. Such a machine, the Genie II, was subsequently developed by Optigene, and has been piloted as part of the Royal Society Leverhulme funding in Ghana in combination with a rapid 2-minute plant DNA isolation technique developed by UoN **[3,4,5]**.

3. References to the research

Evidence for the international quality of the research is indicated through publication of outputs in appropriate peer-reviewed journals. These journals have been selected on the basis of having

intermediate to high impact factors, and more importantly, as being the journals most likely to be accessed by researchers in plant disease diagnostics worldwide – i.e. applied microbiology and plant pathology orientated international journals.

1. Tomlinson, J.A., **Dickinson, M.J.** and Boonham, N. (2010) Detection of *Botrytis cinerea* by Loop Mediated Isothermal Amplification. *Letters in Applied Microbiology* 51: 650-657 doi: 10.1111/j.1472-765X.2010.02949.x.
2. Bekele, B., Hodgetts, J., Tomlinson, J., Boonham, N., Nikolic, P., Swarbrick, P. and **Dickinson, M.** (2010) Use of a real-time LAMP isothermal assay for detecting 16SrII and 16SrXII phytoplasmas in fruit and weeds of the Ethiopian Rift Valley. *Plant Pathology* 60: 345-355. Doi: 10.1111/j.1365-3059.2010.02384.x
3. Tomlinson, J.A., **Dickinson, M.**, Hobden, E., Robinson, S., Giltrap, P.M. and Boonham, N. (2010) A. five-minute DNA extraction method for expediated detection of *Phytophthora ramorum* following pre-screening using *Phytophthora* spp. lateral flow devices. *Journal of Microbiological Methods* 81: 116-120. doi: 10.1016/j.mimet.2010.02.006
4. Tomlinson, J.A., **Dickinson, M.** and Boonham N. (2010) Rapid Detection of Phytophthora ramorum and P. kernoviae by Two-Minute DNA Extraction Followed by Isothermal Amplification and Amplicon Detection by Generic Lateral Flow Device. *Phytopathology* 100: 143-149. doi:10.1094/PHYTO-100-2-0143.
5. Tomlinson, J.A., Boonham, N. and **Dickinson M.** (2010) Development and evaluation of a one-hour DNA extraction and loop-mediated isothermal amplification assay for rapid detection of phytoplasmas. *Plant Pathology* 59: 465-471. Doi: 10.1111/j.1365-3059.2009.02233.x
6. Hodgetts, J., Boonham, N., Mumford, R. and **Dickinson M.** (2009) Panel of 23S rRNA Gene-Based Real-Time PCR Assays for Improved Universal and Group-Specific Detection of Phytoplasmas *Applied and Environmental Microbiology* 75: 2945-2950. doi:10.1128/AEM.02610-08

Underpinning research projects:

a. The taxonomy of phytoplasmas – a molecular approach. Jan 2006 - Dec 2009. Defra Plant Health Fellowship £74,000. M Dickinson (UoN) (COI) and R Mumford (Fera) (PI)
b. Development and in-field testing of improved phytoplasma molecular diagnostic techniques in Ghana. Sept 2009 - Sept 2013: Leverhulme / Royal Society Africa Award; £140,400 M Dickinson (UoN) (PI) and R Quiacoe (CSIR-OPRI, Ghana) (COI).
c. Phytoplasma diseases of coconuts: Understanding their transmission, and the sustainable breeding of resistant and tolerant varieties. April 2008 - March 2011. BBSRC-DFID SARID Grant. £419,472. M Dickinson (UoN) (PI) and R Quiacoe (CSIR-OPRI, Ghana) (COI).
d. The taxonomy, detection and barcode identification of non-EU strains of potato viruses. Oct 2009 - Sept 2012 DEFRA Plant Health Fellowship. £118,000. M Dickinson (UoN) (COI) and N Boonham (Fera) (PI).
e. Identification, prevalence and impacts of viral diseases in UK winter wheat crops. Oct 2009 – Sept 2012 HGCA PhD studentship project RD-2009-3625. £60,000. M Dickinson (UoN)(COI) and N Boonham (Fera (PI).

4. Details of the impact

Plant pathogens are estimated to cause losses in potential agricultural yield of around 10-15% per annum (Oerke and Dehne, Crop Protection 23 (2004) 275-285), and can also have damaging environmental impacts (see http://www.bbc.co.uk/news/uk-england-somerset-12134772 for the recent impact of sudden oak death in the UK). For example, the potato blight pathogen alone is estimated to cause $3 billion annual losses in developing countries (Schumann and Darcy 2012, Hungry Planet, APS Press, USA). Early, accurate and rapid diagnosis of the causal agents of plant disease is important for ensuring timely and appropriate control measures to minimise these potential losses. Protecting plant health in the UK is a shared responsibility between government, growers, traders and the general public. There are two main elements of control; 1) quarantine measures to keep foreign pathogens out of areas where they could cause damage to crops, trees and wild plants, and; 2) surveys and publicity to increase the chances that outbreaks are detected early when they can still be eradicated. Fera is responsible, on behalf of Defra, for implementing

the plant health regulations in England and Wales and is a key player in the UK working with other EC Member States and the European Commission to agree appropriate plant health rules for Europe and co-ordinate their implementation.

Work at UoN on developing improved plant pathogen diagnostics was initiated in 2005 through a Defra Plant Health Fellowship (see corroborating **Source 5** for details of the objectives and outcomes of this fellowship). The key benefits of this fellowship (apart from training a scientist with expertise in molecular diagnostics who is now employed at Fera) were the development and validation of real-time PCR diagnostics for phytoplasma diseases, which were recognised by Defra as a potential threat to UK agriculture because of their prevalence on crops such as apples, pears, tree species and numerous ornamentals throughout Europe. Currently these diseases are rare in the UK, so the aim was to develop protocols that could be used to test imported materials and prevent entry of these pathogens. This protocol is now used routinely by Fera for testing suspected phytoplasma infected material and is provided as part of their statutory testing **(Source 1)**. In addition, the technology has been incorporated into the accredited phytoplasma testing diagnostic service offered by the Swiss-based Agro-Diagnostics Company, Bioreba **(Source 2)**. This commercial service has been used already on hundreds of samples (mainly from fruit trees) for customers in Switzerland and Europe. Prof. Dickinson has subsequently developed specific real-time PCR based assays for detection of a broad range of potato and cereal viruses, including potato virus S, potato virus M, arracacha virus B, potato black ringspot virus, barley yellow dwarf virus variants, cocksfoot mottle virus, cocksfoot streak virus, cynosurus mottle virus, oat chlorotic stunt virus, ryegrass mosaic virus, soil borne wheat mosaic virus and wheat spindle streak mosaic virus **(Sources 1, 6)**. These assays have been incorporated into the diagnostic protocols used by Fera as part of their statutory duties. The overall financial value of these diagnostic services is difficult to quantify since they are being implemented principally as preventative measures (as quarantine / early detection systems), but should these phytoplasma and viral diseases become endemic in UK fruit trees, potato and cereal crops, the annual losses are likely to be in the order of millions of pounds. The social and environmental impacts associated with a failure of plant health protection strategies would also be significant.

The second aspect of this work was development of nucleic-acid based detection systems for 'in-field' or 'point-of-care' testing, and this work has been undertaken in a number of countries, including the UK, Ghana and Ethiopia, and has been published in international peer-reviewed journals. Previous methods for in-field testing have generally been antibody-based (i.e. use of lateral-flow devices), but there are many pathogens for which it has been impossible to develop the necessary specificity or sensitivity in such tests. UoN research developed the DNA-based technique of LAMP for detection of a range of pathogens **(Sources 1-4, 7)**. This was a collaboration between UoN, Fera, CSIR Ghana and the company Optigene, UK, who, following discussions with UoN in 2009, have developed and are now marketing a small battery operated portable real-time LAMP product detection machine, the Genie II (http://optigene.co.uk/) **(Source 4)**. UoN piloted use of this machine in Ghana, where the machine is now being routinely used for field detection of phytoplasma diseases in coconut **(Sources 3, 7)**. These diseases have devastated plantations in much of Africa resulting in 100% losses in many areas and severe economic hardships in communities where coconut is the main source of livelihood for several disadvantaged groups (e.g. women and landless poor). In a statement (2013) the Director of CSIR Oil Palm Research-Ghana said "*The most significant advantage of using LAMP assays by the coconut research team in Ghana is the ability to use the method in-field. The machine can be run on a rechargeable battery, is portable and therefore easy to carry to field location in very remote areas. Our staff are now able to provide diagnostic services to farmers in different parts of the country using the Genie II. The whole process of DNA extraction to sample analysis is done within 30 minutes and this is enhancing the speed and amount of work that is done on field visits. The LAMP assays are thus revolutionising diagnosis of lethal disease in Ghana and helping us to do so much more than we could do with the traditional methods*" **(Source 3)**.

In the UK, Fera is piloting the use of these real-time LAMP machines by their field inspectors and for detection of diseases such as sudden oak death. Optigene are also marketing the machine to the Animal Health and Vet Labs Agency (AHVLA) and overseas. Continuing work is now aimed at commercialising diagnostic kits, and licensing agreements are being agreed between Optigene and Eiken in Japan for 'plant health' diagnostic rights **(Source 4)**. Because of the worldwide

distribution of the pathogens for which diagnostic tests have been developed, UoN research is of major significance in the UK, continental Europe and the rest of the world.

5. Sources to corroborate the impact

1. The Head of Plant Science, Food and Environment Research Agency. *Will give corroborating evidence for the use of diagnostics developed in this research for routine testing at Fera and as part of the work of the Plant health Inspectorate. Also, evidence of the use of the LAMP technology by the Plant Health Inspectors.*
2. Research and Development Scientist, Bioreba AG, Reinach, Switzerland. *Will provide corroborating evidence on the use of the phytoplasma real-time diagnostics by Bioreba*.
3. The Director, CSIR Oil Palm Research Institute, Ghana. *Corroborating evidence for the use of Lamp technology in the field in Ghana*. 2013.
4. The Marketing and Sales Manager, Optigene UK. *Provides corroboration for discussions surrounding development of the Genie II machine, and also for the deployment of this technology worldwide*. 2013.
5. Defra Plant Health Fellowship project final report on developing molecular diagnostics for phytoplasmas: (http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=13554#RelatedDocuments) 2009. *Confirms outcomes of Defra funded UoN work.*
6. Defra Plant Health Fellowship on diagnostics for potato viruses (project description) at (http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=15463#Description) 2009. *Confirms Defra involvement with UoN development of tests for plant pathogens.*
7. The field use of Genie II and Lamp technology in Ghana. *Provides corroboration for the use of the Genie II and Lamp technology in Ghana as well as the use of the technology worldwide.*
   http://www.optigene.co.uk/applications/field-use-of-genie-ii-in-ghana/
   http://edition.myjoyonline.com/pages/news/201303/102707.php