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SECTION 1 – PLANT PATHOGENS, CROP LOSS AND DIAGNOSTICS

Introduction

Worldwide and on an annual basis diseases of agriculture and horticulture cropping systems impact significantly on crop yield and quality. These losses can be quantitative and / or qualitative. Quantitative losses results from reduced productivity, leading to a smaller yield per unit area. Qualitative losses may result from the reduction of an essential substance, a change in taste, cosmetic appearance or contamination of the harvested product by mycotoxin producers. It has been estimated that an average of 20 – 30% of crop yield is lost annually from the field, even in crops where pesticides and cultivars with improved genetic resistance to pests and diseases are used. Among crops, the total global potential loss due to pests and diseases varied from 50% in wheat to more than 80% in cotton production (Oerke 2006). The perpetrators of which have been classified by their impacts into groups: reducers (damping off pathogens), photosynthetic rate reducers (pathogens), leaf senescence accelerators (pathogens), assimilate sappers (nematodes, sucking arthropods, pathogens), light stealers (weeds, some pathogens) and tissue consumers (chewing animals, necrotrophic pathogens) (Boot *et al*., 1983). A major problem for producers is that diseases are moving targets that evolve in response to agricultural practices and environmental change. Despite a clear increase in pesticide use, crop losses have not significantly decreased during the last 40 years (Oerke, 2006). Pesticide use has however enabled farmers to modify production systems and to increase crop productivity. With increasing globalization, travel and the international trade in plants the risk of disease through inadvertent introduction of pathogens is exacerbated (Brasier, 2008). The emergence and spread of new diseases, or more aggressive or pesticide-resistant biotypes is an inevitable part of the evolutionary chain. To tackle these issues effectively and, with the increasing restrictions placed by the 'green environment', requires the development and adoption of disease control systems that are sustainable with the use of lower-input farming systems (Beddington 2010; Godfray *et al*., 2010). With over five million tonnes of pesticide applied world-wide annually this poses producers with significant challenges to

- Increase yield production by 0.4% to meet the global food security requirement.
- Reduce crop protection inputs, energy and greenhouse gas emissions.
- React to the emergence of new disease threats resulting from the effects of climate change.

Pathogen Diagnosis and Integrated Disease Control

Most horticultural crops in the UK are currently produced using a variety of pesticides. In relation to the Arable market, the horticultural sector provides a limited market and accounts for only 3.7% of non-grassed cropped land. Nevertheless with more than 300 crops and, with a combined home-produced value of around £2.23 billion (farm gate value) it provides significant economic value to the UK economy. However there is a reduction in broad spectrum fungicides available to the Horticulture Industry. Despite the development of the 'off-label' system for minor uses the number of pesticides that are currently approved for use on horticultural crops is under ever increasing pressure. To utilise these efficiently and with optimal disease control performance there is a now a move away from routine pesticide application to targeted crop treatments (pesticides and biological) based on regular monitoring. This requires a greater depth of knowledge by producers and their staff to identify problems quickly. The ability to quickly diagnose many disease pathogens does still not exist and simple, yet accurate, diagnostic tools for growers should be developed. With increased restrictive legislative pressure on the use of protectants within the cropping season the diagnostic test should not necessarily be limited to a single disease but perhaps provide a more holistic approach to plant / disease pressure. To address this systems that contain an array of nucleic acid-based probes each for a different target species have been developed (Lievens *et al*., 2006, Tambong *et al*., 2006). However proper implementation of these techniques poses challenges, ranging from the significant cost of the technology to the need to understand the techniques well enough to be able to interpret and troubleshoot anomalous laboratory results (Vincelli & Tisserat, 2008).

Disease diagnosis and pathogen detection are central to the ability to protect crops and natural plant communities from invasive agents (Miller *et al.* 2009). Early diagnosis can provide the grower with useful information on optimal crop rotation patterns, varietal selections, control measures, harvest date and post-harvest handling. Pathogen detection prior to infection can reduce disease epidemics. Classical methods for the isolation and identification of crop pathogens are commonly used only after disease symptoms are observed. Such processes are often time consuming, with a reliance of the organism to be cultured and availability of expert taxonomic training for accurate identification. These processes frequently delay the application of control measures and often at potentially important periods in crop production. As a result blanket pesticide application has been common. The timely detection and identification of economically important diseases in a commercial cropping environment will provide the initial key to drive a successful and informed control strategy. It is however only part of the solution and the success of which will depend on how the information is evaluated and then incorporated within an integrated disease management system (IDMS). Once a disease is identified information about the presence of sufficient pathogen concentration coupled the

associated environmental parameters is required to determine accurate disease thresholds at which damage may occur (Scherm & van Bruggen, 1995). This information needs to be translated in a timely and accessible way to growers to for targeted and cost effective control measures to be taken to enable disease containment or eradication. For this purpose extensive ecological studies need to be conducted, studying the responses of a pathogen in relation to both biotic (microbial, plant) and abiotic factors (light, temperature, humidity etc) of its environment (Lievens & Thomma, 2005). Early detection allied to key environmental parameters to control disease at the onset can lead to an increase in production, an improvement of resource efficiency and make a substantial contribution to food security (Wakeham & Kennedy, 2011; Carisse *et al*., 2005). In successful delivery of an integrated disease management system (IDMS), sampling procedures and sample size will prove critical to account for spatial variation of pathogen incidence within a cropping system. With the development of new diagnostic technologies often the material required for analysis reduces as test sensitivity increases. This has the potential to be particularly problematic in cases where disease potential ahead of infection or pre symptomatic infection is a requirement. Equally in plant health quarantine, seed and certification of transplant stock, the sample size and sampling strategy is critical to identifying and determining an accurate disease potential. However this issue may be overcome by the isolation and concentration of the target pathogen(s) from the sample medium ahead of testing. Nevertheless the sampling plan should be performed in a manner that ensures a statistically representative sample (Ranjard *et al.* 2003).

Available Technologies for detection of plant pathogens

The increasing concern about pesticides in the environment, the long term usage for effective control of disease and the rising production costs has provided the platform for scientists to focus their attention towards the development of more rapid and accurate disease diagnostic systems. The pioneering work developed in the medical field during the latter decades of the previous century provides a plethora of techniques. Many of which have been developed and deployed commercially at a global scale to provide clinical diagnostic point of care systems to a wide audience range (clinical diagnostics, drugs of abuse testing, fertility, allergy). Equally, as a result of recent global activity, the defence industry has had to respond to the development of rapid, highly sensitive and accurate systems to deliver on-line responses to threat of biological warfare activity (RAZOR® EX Anthrax Air Detection System). These emerging technologies increasingly place emphasis towards nanobiotechnology (Jeong-Yeol & Bumsang, 2012). There is a real expectation that the mobile phone in the future is likely to provide a global laboratory platform for many of these techniques. The challenge for the plant scientist however remains the ability to identify, select and modify these systems to provide a diagnostic tool that is able to deliver useful information to the end user, appropriate

to the delivery point *i.e.* in a lab or on-site and mindful of the economies of scale in an agricultural / horticulture setting. Suitable validation of the test by the end user and robustness in use will markedly affect the deployment and successful uptake by industry, laboratories and / or inspection agencies.

Molecular techniques From the early 1980's there has been a rapid development of molecular techniques for improved pathogen detection and identification systems. Some of which will be described below:

PCR. Molecular methods, essentially based upon Polymerase chain reaction (PCR), have evolved from a complex test to become an indispensable, routine tool used widely in the diagnosis of infectious diseases. Over the past two decades PCR and quantitative PCR techniques (q PCR) have expanded to become one of the most widely used laboratory assays for the direct detection of low levels of pathogenic microbes in environmental samples (Theron *et al.,* 2010). The increasing ability to sequence pathogen genomic content provides a capability to design specific and sensitive primer sets to amplify target pathogen DNA by PCR to detectable levels. The Internal Transcribed Spacers (ITS) of ribosomal DNA is reported to be the most widely sequenced DNA region of fungi (Peay K.G *et al*., 2008) and has been recommended as the universal fungal barcode sequence (Schoch, 2012). Consisting of alternating areas of high conservation and variability it has proved universal for the development of highly specific and sensitive primer sets for use in PCR based diagnostic tests to discriminate target fungal plant pathogenic species in complex environmental samples (Klemsedal *et al*., 2008; Lees *et al*., 2012). The 16s ribosomal DNA has been used for the detection of bacteria. Quantitative PCR, a process by which DNA copy generation is monitored by conformational change of a fluorescently labelled probe, provides a platform to measure target disease concentration in a sample with reference to a standard curve. This system is often referred to as real-time PCR as the fluorescently labelled PCR products produced during each amplification cycle can be monitored as the reaction progresses.

Although widely used, routine PCR diagnostic testing is still somewhat confined to larger central laboratories where special room requirements are required to eliminate aerosol contamination (Regis *et al*., 2006). The 'closed' quantitative q PCR process can to some extent overcome this requirement and has been shown to have advantages of speed, accuracy, and sensitivity over conventional PCR-based techniques (Schaad & Frederick, 2002). However purchase costs of a 'real time' laboratory operating system is expensive (circa £45 k) and which for many provides an unaffordable option. Nevertheless where speed, specificity and sensitivity is a priority in sample analysis there is a capability to perform q PCR outside of the laboratory setting. In a Horticultural setting this could prove invaluable for disease quarantine purposes and, if cost appropriate, where a timely and accurate diagnosis is key to the successful management of a disease control strategy (IDMS). The system was originally developed for US military requirements, to provide on-site

capability to monitor bioterrorism related outbreaks of anthrax. The real time platform at that time was supported by a portable battery and packaged in a large brief case to allow movement to a field situation. The portable sampler has since been made commercially available (Cepheid Smartcycler Inc., Sunnyvale,California) and assessed for quantitative capability of infectious agents (Bélanger *et al*., 2003; Tomlinson *et al*., 2005). The fully automated sample preparation system is designed to work with a disposable cartridge that accepts up to several milliliters of an unknown aqueous sample. The sample preparation procedure is performed in less than five minutes and within the single platform provides real time detection with a limited multiplex diagnostic capability. However the molecular detection of fungal pathogens in plant material requires the pre-extraction of DNA (Schaad, 2009) so on-site molecular testing demands not only a portable real time PCR platform but also a simple and robust DNA extraction method to be performed in the field. A 2hr on-site DNA extraction process and the use of a portable real-time PCR platform for the detection of *P. ramorum* from symptomatic plant material has been developed (Tomlinson *et al*., 2010). However at this time a basic Smartcycler II Laptop platform (Fig. 1) retails in the UK at £32K with an optional £1.8k maintenance contract. A number of other portable real-time PCR platforms are commercially available: the R.A.P.I.D. system and RAZOR instrument (IdahoTechnologies, Salt Lake City, UT), and the hand held BioSeeq instrument (Smiths Detection, Edgewood, MD) for on-site molecular testing.

Figure 1. Portable Multiplex PCR platform: SmartCycler II Laptop System

LAMP. As a result simpler, less expensive technologies have been sought to allow molecular based assays to be translated from the laboratory to the field. Loop-mediated isothermal amplification (LAMP) method (Notomi *et al*., 2000) provides a novel nucleic acid amplification process under isothermal conditions (60 to 65°C). Simple incubators, such as a water bath or a block heater, are sufficient for DNA amplification. As a by product of the reaction a white precipitate of magnesium pyrophosphate is produced which enables the visual judgment of amplification by a

naked eye. It has been reported to be less affected by inhibitors (Francois *et al*., 2011) and as a result of its isothermal nature, has the potential to be deployed in the field. Because of its speed, robustness and simplicity LAMP is gaining momentum in diagnostics of human medicine (Parida *et al*., 2008) and, more recently in plant health (Kubota *et al*., 2008; Tomlinson *et al*., 2010; Buhlmann *et al*., 2013). In the United States the development of a 'grower performed LAMP PCR' has been assessed for the detection-based management of spray programmes for grapevine powdery mildew in vineyards (Mahaffee *et al*., 2011). Based on two years of results a commercial company ran a feasibility trial to offer a grower based test service. Estimates were that it would require $2100 in capital equipment, $60 dollars in reagents and 25 minutes labour with a 1.5 hr time to process 10 samples. This did not however include the cost of a sampler for collection of field aerosols. The LAMP process required several steps to include heating, centrifugation and, although could be carried out in a grower's office with desktop equipment, it was found that the participants were not consistently successful when interpreting the results. The company considered performing the LAMP service 'in-house' however opted to partner with a commercial laboratory to offer a laboratory quantitative PCR service (Reiger, 2013). As a result of test sensitivity the company reported that one of the biggest concerns in the collection of samples for a commercial DNA based testing service was the cross-contamination of samples. The spores could be easily picked up and moved on peoples clothing and hands. For this reason they instituted clean practices whereby samplers wear gloves and protective clothing, which is changed between traps. Mahaffee and his team at the United States Department of Agriculture continues to work with growers to develop a field test which is more economical and easier to use. The team is currently investigating the use of a hand-held, portable device called the Smart-DART (www.diagenetix.com/product-and-technology/smart-dart-platform) which allows the LAMP process to be performed on site and provides an application to an Android phone device for quantitative measurement of the assay process (Fig. 2). If successful the grower will still have to perform the DNA extraction process. Mahaffee estimates a grower could set the complete system up for less than $2000 in initial capital equipment with an annual operating cost of $400 for test reagents. Labour costs to operate the system were not included within the analysis. If successful this system could prove useful in a field situation where speed, sensitivity and specificity are key to a successful outcome and, with an economy of scale for use within Integrated Disease Management Systems.

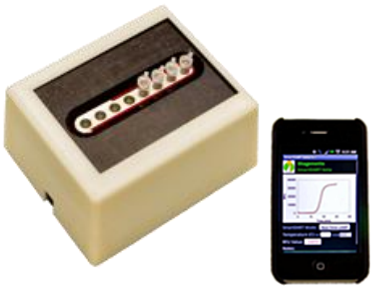

Figure 2. The Smart-DART™ platform and application for Android devices

Nevertheless the ability to perform nucleic acid–based tests in the field remains a challenging goal for complex environmental samples (plant tissue, soil) , largely due to the reliance on pre-processing of samples (nucleic acid extraction), which is a rate and skill limited step due to the relatively complex nature of current nucleic acid extraction methods (King *et al.,* 2008). If portable real-time PCR platforms are to be used successfully they should ideally consist of completely closed systems capable of performing all steps of the assay. These steps include (1) nucleic acid extraction, (2) PCR set-up, (3) amplification and (4) unambiguous calling of results (Mikidache *et al.,* 2012). A significant driver for use of these systems in the field will be ease of use and test reliability. It is likely that only those technologies that are cost-effective will be used in plant pathogen diagnostics. This is a particularly consideration to commercial agriculture and horticultural cropping systems where the profit margins and emotional attachment to crops are low. The cost of equipment, expensive reagents and a requirement of skilled staff would not be easy to justify. For these reasons it is unlikely that molecular techniques can be adapted in a cost effective way for widespread use in the field. Where legislative issues are a factor and potential of quarantine outbreaks a concern the demand for specificity, sensitivity and speed is likely to prove an overriding factor to cost.

Molecular Array. Where a laboratory / clinic environment is acceptable, advances in molecular diagnostic test technology has provided the opportunity to couple PCR with high throughput pathogen detection multiplex arrays. These array systems were originally designed for gene expression profiling, gene discovery and single nucleotide polymorphism (SNP) analysis (Lockhart & Winzeler 2000; Mei *et al*., 2000). PCR based multiplex arrays generally consist of a high density of selected and synthesised immobilized nucleic acid sequences spotted on to a solid platform such as glass microslides, beads or nylon membranes (Eptstein & Butow, 2000, Ishii *et al*., 2008). Following sample DNA extraction of the environmental sample, amplicons of a target DNA region are generated by PCR and bound with a fluorescent, biotinylated or enzyme label. Following a process of DNA hybridisation, amplicons which are able to bind selectively to immobilised target sequences of the

array, are visualised either by direct fluorescence scanning or enzyme-mediated detection to yield a semi-quantitative result (de Boer & Beurmer, 1999). In general, target amplification is based on the use of universal primers that recognize conserved sequences flanking variable domains in housekeeping genes, such as the ribosomal RNA gene. In this way, numerous targets can be amplified with a single primer pair, while target discrimination is performed afterwards on the array (Lievens *et al*., 2003, Lievens *et al*., 2011). DNA arrays have been developed for the detection of plant pathogens in a range of environmental samples (Tambong *et al*. 2006, Boonham *et al*., 2007; Mumford *et al*., 2006; Lievens *et al*., 2012). As a laboratory tool, the molecular array system can provide a highly specific and sensitive assay for the simultaneous detection of multiple diseases present in a cropping system (Robideau *et al*., 2008) and has been used to identify species with fungicide resistance (Ishii *et al*., 2008). In general macro arrays (immobilized nucleic acid sequences spotted onto reusable membranes) have been used for plant disease diagnosis as a result of cost, sensitivity and more modest equipment requirements (Lievens *et al*., 2012). Bio-art bvba (Belgium), a small company established in 2011, provides such a multiplex approach to detect a range of fungal and bacterial pathogens of designated cropping systems: DNA MultiScan® Applications for disease diagnosis of plant pathogens in horticulture and agricultural cropping systems (http://www.bio-art.org).

Next Generation Sequencing. The considerable advances in areas of genomics and bioinformatics determines that more powerful molecular diagnostic methodologies continue to be developed. Next generation sequencing (NGS) offers a diagnostic tool that requires no previous knowledge of either a specific host or pathogen (Schuster, 2008). NGS is a high-throughput approach that generates thousands to millions of DNA sequences. However obtaining and making sense of these sequences involves several complex stages, both at the lab bench and at the computer desk. With more and more organisms being sequenced, a flood of genetic data is being continually made available (Liu *et al*., 2012). Distilling meaningful information (bioinformatics) from the millions of new genomic sequences and interpreting this from voluminous, noisy, and often partial sequence data presents a serious challenge. Analysis requires considerable skill and understanding to avoid potential pitfalls and challenges in the process (Dewoody *et al*., 2013). NGS has however the capability to analyse complex environmental samples and from this identify uncultured known, unknown and novel pathogen variants (Adam *et al*., 2009, Harju *et al*., 2012, Be *et al*., 2013 , Breitbart *et al*., 2008). For plant virus identification Adams (2009) reports a cost of £1000 per sample analysis but that this sum could reduce considerably in the future. For the moment however, NGS is likely to remain a sophisticated laboratory tool which will underpin fundamental genetic based studies to provide a new perspective to host pathogen interactions and ecological studies. It will provide considerable support to the development of new diagnostic molecular based technologies.

Molecular Diagnostic Challenges. The specificity that can be achieved by molecular methods remains persuasive in diagnostic choice. Target organism genomic sequences can readily be compared using DNA-similarity searches like BLAST (Altschul, 1997) and DNA and RNA sequences databases, such as the International Nucleotide Sequence Database (INSD). However some caution should be taken. For fungal species, it has been reported that less than 1% of the estimated 1.5 million viable species have been sequenced for the ITS region and, as much as 20% of all fungal sequences deposited in the INSD maybe incorrectly annotated to species level (Bridge *et al*., 2003, Nilsson et. al., 2006). There are also concerns with the classification of species solely as a result of DNA region / gene analysis. Classical identification of plant pathogens has relied heavily on morphological and biological features (van der Plaats-Niterink, 1981). These relationships are not always conveyed when compared by genomic analysis. Will (2004) reports on the myth of the DNA barcode for species classification and reasserts the requirement of morphological analysis in the identification and classification process. Interestingly in the field of medical mycology, the uptake of PCR as a diagnostic tool has been constrained by the lack of standardization, such that PCR in not an accepted diagnostic criterion for the detection of human fungal diseases according to 2008 EORTC/MSG guidelines (De Pauw *et al.,* 2008).

Aside this careful consideration should be given to a sampling plan and sample size along with a suitable extraction and / or concentration process to enable efficient and reliable amplification of low numbers of target genomic sequences. Careful optimisation and evaluation of the PCR should be made. This should include melting and annealing temperatures to prevent the formation of undesirable secondary structures such as primer dimers (Saiki *et al*., 1988; Atlas, 1991). Assess the potential of sample inhibitors such as Humic substances, pesticide residues and organic material. All of which are reported to inhibit the DNA polymerase enzyme (Kong *et al*., 2003) and colloidal matter which has a high affinity for DNA (Way *et al*., 1993, Wilson, 1997). The presence of these in field samples has the potential to affect the amplification process and test sensitivity (Lombard *et al*., 2011; Stewart-Wade, 2011). Also the test parameters should consider whether the ability to discriminate dead/inactivated species from viable disease is relevant and, at what concentration. With water conservation a priority for communities' worldwide, integrated disease management of recycled water systems provides a potential pitfall in disease diagnosis. UV radiation, pressure systems, sonication, chemical biocides and surfactants may prove effective in removing the capability of the pathogen to survive or remain pathogenic. Differentiating between the infectious and non-infectious state remains a limitation of PCR (Stewart-Wade, 2011) with DNA persisting for periods of time after the death of cells (Master *et al*., 1994). In diagnosis there is also a need to determine the detection threshold of a pathogen (if a pathogen is there) and the biological / disease threshold (the concentration at which the pathogen is of a frequency for disease potential on a host). The development and successful uptake of any test will therefore require careful planning

and with optimisation of the process for each target and with a robust validation period.

Immunological Assay Systems: With this in mind other system processes have been investigated for their use to provide simple, inexpensive and robust diagnostic tools to monitor disease epidemics. Following the work of Yallow & Berson (1959), using anti-insulin antibodies to measure hormone levels in blood plasma, immunological assay systems have provided an important contribution to analytical diagnostic test development. With an array of different labels and detection systems available, measurement of the antibody (diagnostic probe) and antigen (target analyte / disease propagule) interaction can be quantitative or qualitative. The immunoassay system is a biochemical process that is highly transferrable from a commercial centralised laboratory offering a test with high throughput, specificity and sensitivity to a simple point of care (POC) test operated by non-specialist operators. In 2005, over 6 million POC tests were performed in USA hospitals and by 2011 this was to exceed 1.2 billion (Esposito, 2008). Workers outside of the biomedical industry were at first slow to realize the potential of these powerful, robust and easy to use systems. However by the late 1970's to the early 1980s there was an increase in applications to which immunoassays were applied, including veterinary medicine, agriculture, the food industry, environmental health and forensic science (Pal, 1978; Collins, 1985; Morgan, 1985; Morris & Bolton, 1985; Morris & Clifford, 1985). This was in part due to the advent of monoclonal antibody production (Milstein and Köhler, 1975) which revolutionized the field of immunology. This technique using hybridoma technology and a myeloma cell line provides the capability to produce and select monoclonal antibody cell lines with a specificity to a particular epitope of an antigen (target analyte). This process had two important advantages: first, because of the method of selection it is possible to choose only those antibody-producing clones which have the desired specificity; second, the cell lines can be stored and the antibodies produced in large quantities as required. The successful development and commercialisation of Unipath's Clear view home pregnancy test launched in 1988 also provided validation of the antibody based system as a universal point of care diagnostic test. Using a homogeneous format (a system which is able to measure bound label without the need to separate bound and free label) the test provided evidence to a global audience of the speed, simplicity of use, specificity, sensitivity, robustness and the low cost that an immunological based diagnostic assay test could provide. Bangs Technote 303 (www.bangslabs.com) reports a production cost of $0.35 per test and can provide a long shelf life with 12 to 24 months at room temperature reported on for commercially available pregnancy tests.

In the 1970's, antibody based immunoassays were reported on for the detection of viruses and bacterial plant pathogens 'in planta' (Clark & Adams, 1977). However the poor specificity achieved using polyclonal antisera to the structurally more

complex fungal plant pathogens (Drouet, 1986) hampered the development of immunologically accurate diagnostic probes. The advent of hybridoma technology in the mid-1970s and, more recently antibody engineering using phage display technologies (Arap, 2005), has allowed the generation of highly specific monoclonal antibodies (mAbs) or single-chain antibody variable fragments (scFvs) that are able to discriminate not only between groups of organisms, but also between different genera and species of pathogenic fung (Thornton & Wills, 2013). This has resulted in a rapid expansion of MAb-based diagnostic procedures for fungal pathogen detection both in an agricultural and in a clinical setting (Dewey *et al*., 1993, Karpovich-Tate *et al.,* 1998; Wakeham & Kennedy, 2011; Wakeham *et al*., 2012; Dewey *et al*., 2013 & Thornton & Wills, 2013). The use of scFvs derived from phage display libraries has been relatively low in fungal disease diagnosis. Although a single-chain variable antibody fragment has been developed to accurately monitor a fumosinin producing pathogen (*Fusarium verticillioides*) to 10-2mg of contaminating mycelium/g maize (Hu *et al*., 2012). Although the success of an immunoassay diagnostic test will ultimately rely on the sensitivity and specificity of the antibody probe, critical for successful test uptake is consideration of the type of assay system for the diagnostic purpose and the sample matrix to be tested.

Immunoassay Diagnostic Challenges. The detection of fungal antigens in environmental samples, in particular plant and soil has its challenges. Equally in environmental water samples and, as observed with molecular probes, problems can arise from reactivity of the antibody to closely related genera, non-viable target disease propagules and inhibition of the assay by contamination of pesticide residues and organic matter (Ali-Shtayeh *et al*., 1991; Stewart-Wade, 2011; Hong *et al*., 2003a). For Pythiaceous fungi this has been overcome by the development of a zoospore trapping assay (ZTI assay). The process allows litres of irrigation water to be sampled and, with only the trapped zoospore and flagella (disease propagule and motile germ tube) identified using the attachment of a selective antibody probe and, the reaction visualised using a colour label. Pettitt (2002) compared the system with an immunological dipstick assay and two conventional assays of filtration with selective plating and baiting, for their sensitivity in detecting two important horticultural plant pathogenic species of *Pythium* and two species of *Phytophthora*. In each of the tests carried out the ZTI assay proved to be the most sensitive in the detection of viable disease propagules in horticulture nursery systems.

Efficient and simple extraction of fungal antigens from soils is however one of the biggest hurdles in the development of quick and sensitive immunoassays (Dewey & Thornton, 1995). Many of the soil-based tests developed have had to use a biological amplifications stage (soil-baiting) to provide target analytes at a concentration for readability (Yuen *et al.,* 1993 1993, Thornton *et al*., 2004,). However one benefit of this is that the tests can provide valuable information on viability of the target organism but are likely to remain as a qualitative test and, can

prove as timely as conventional media based isolation processes. Equally soil tests which require a pre-treatment i.e. drying, grinding, centrifugation and floatation processes to recover pathogen resting structures prove both laborious, lack an economy of scale and, require considerable laboratory space prior to analysis (Wallis et al., 1995; Wakeham & White, 1996; Miller et al., 1997). The ability to develop a highly specific, sensitive and inexpensive assay is somewhat irrelevant if the extraction process is lengthy, laborious and costly in time and labour. Where immunoassay systems have been used directly in the soil matrix, issues of sensitivity, specificity, non-specific binding of the probe and assay inhibitors within soil components are reported (Kageyama *et al*., 2002, Otten *et al*., 1997). Although success has been reported for the detection of *Pythium* species in infested soils and plant tissues by using a competitive immunoassay format (Lyons & White, 1992; Kageyama *et al*., 2002). Nevertheless simple and rapid processes are required to isolate and concentrate target disease propagules from soil. Separation of target cells from a suspension can be achieved by immunomagnetic capture. In the early 1990's reports were made of successful isolation, concentration and detection of pathogenic bacteria from contaminated feedstuffs (Johne *et al*., 1989; Mansfield et al., 1993), faeces (Luk & Lindberg, 1991) aquatics (Bifulco & Schaefer, 1993) and soil (Mullins *et al*., 1995). Recently this approach has been adopted to isolate and concentrate resting spores of the clubroot plant pathogen from infested UK horticultural and agricultural soils. Monoclonal antibodies specific to *Plasmodiophora brassicae* (causal agent of clubroot) and conjugated to super paramagnetic spheres 'fish' soil samples for *P. brassicae* spores. After which the labelled spores are isolated from the soil matrix and concentrated by exposure to a magnetic field. Quantification of the isolated spores is determined by quantitative polymerase chain reaction (qPCR) using *P. brassicae* specific primers (Lewis, 2011). Immunoassay systems offer a diagnostic process which can be simple rapid and robust. Critical to the test will be available target at a concentration for detection; a sample matrix which when optimised for extraction is free of inhibitors, a probe which displays a required specificity for its target and, a platform suitable for the end user and place of operation. Below, a range of immunoassay formats routinely used for environmental sampling will be discussed along with emerging methodologies which may prove useful in future diagnostic test applications of horticultural and agricultural plant diseases:

*ELISA***.** Clark and Adams introduced the use of the enzyme-linked immunosorbant assay for the quantification of plant viruses in host tissues in 1977. This system is now used routinely in laboratories worldwide to provide high throughput, quantitative measurement of contamination of viral, bacterial and fungal plant pathogens in a range of environmental samples. For viral and bacterial samples many of the commercial ELISA systems use a double antibody sandwich format (DAS ELISA). This can prove useful in capture and isolation of a target pathogen from a complex material and provide improvement of specificity with attachment of a second antigen specific labelled antibody. A second type of ELISA is

the plate trapped antigen (PTA ELISA) which are generally reported for use in the diagnosis of fungal plant pathogens. It has been reported that the glycoprotein fraction of the fungal immunogen predominantly elicits an immunodominant response from the host (Dewey *et al.,* 1997). As a result selected antibodies are often directed to this form of structure. Glycoproteins tend to bind readily to the solid phase surface of an ELISA process and so not require a capture antibody as in the DAS system. Secondly, many soluble glycoprotein structures do not lend functionally to the binding of two antibody types at one time. Nevertheless these structures can prove heat stable and, in issues of antibody cross-reactivity in complex samples, this could prove useful in sample treatment. The third type of ELISA system used routinely is the competitive ELISA (c ELISA). This format is used extensively in the detection of mycotoxins in food, pesticides in ground water and has been reported for the measurement of some soil-borne fungi in soil and plant tissues (Lyons & White, 1992; Kageyama et al., 2002). Although restricted to the laboratory and requiring and initial investment for equipment to process and analyse samples (<£5000.00, www.bio-rad.com/en-us/product/microplate-readers/imark-microplate-absorbance-reader) the ELISA often forms the basis for development of other systems e.g. immunoarray systems and point of care (on-site) testing.

*Immunoarray systems***.** Where high throughput multi-pathogen assays systems are required an immunoarray may prove useful. Unlike PCR based systems, where tedious and expensive pre-extraction processes are required to isolate pathogen nucleic acid from plant or soil samples by highly skilled workers a microsphere capture immunoassay system (Luminex MAGPIX technology) offers a new approach. The technology employs a set of 50 different fluorescence colour coded magnetic microspheres of which each coloured microsphere set can be coated either with target analyte or target pathogen specific probe. Using a 96 well ELISA format, samples for testing are aliquoted (20 to 100µl / well) and within each well there is the potential for 50 bead types to seek and find homologous target analyte to bind to. By applying a magnetic field the bound target material and beads are retained free of sample and potential assay inhibitors. After which an ELISA process is carried out and the magnetic sphere bound target analyte is identified by linking with a fluorophore (R-phytcoerythrin) conjugated detector antibody. The MAGPIX system is able to identify the colour coded magnetic bead and measure the fluorescence of the detector antibody to provide quantification of target pathogens in a sample. This approach provides a versatile multiplexing platform capable of performing qualitative and quantitative analysis of up to 50 target analytes in a single reaction volume and, in a variety of sample matrices. The assay time of the microsphere immunoassay (1hr) is much shorter that of a standard ELISA system (approx. 4hr).There have been several reports using this new technology to detect foodborne pathogens and toxin (Kim *et al.,* 2010), three potato viruses in infected host tissues (Bergervoet *et al*., 2008) and a multiplex plant pathogen assay designed for use in seed screening to simultaneously detect four important plant pathogens: a fruit blotch bacterium (*Acidovorax avenae* subsp. *Citrulli*), and three viruses (chilli vein-banding mottle

virus, watermelon silver mottle virus and melon yellow spot virus) (Charlermroj *et al.,* 2013). This approach may prove useful for the isolation and, have potential for concentration, of multiple plant pathogens from complex samples such as soil, plant and water systems.

Point of Care systems (POC - Lateral Flows): These are a type of test designed to be used at or near the site where the problem is located, do not require a permanent dedicated space and can provide results quickly. Originally these types of tests were devised for a clinical setting and used in doctors' offices, hospitals, and in patients' homes. POC diagnostic devices are used to provide quick feedback on many sorts of tests i.e. enzyme analysis, drugs of abuse, infectious agents, toxic compounds, metabolic disorders allergens, ovulation and pregnancy testing (Posthuma-Trumpie *et al*., 2009). Existing assays such as the ELISA often translate to the simplified POC format whilst retaining the tests original performance characteristics. This process eliminates the requirement for laboratory equipment, highly trained personnel and can provide a quick test turn-around time of approximately 10 minutes. The results can be qualitative (yes/no) or made quantitative by using a digital reader. The Clearblue Advanced Pregnancy POC system combines two tests within one system and an inbuilt digital reader to report to the end user a written display of "Pregnant" or "Not Pregnant". A quantitative reading of 1-2, 2-3 or 3+ is displayed to indicate by how many weeks. This integrated technology is currently limited to the pharmaceutical industry where a strong global market can support the financial investment required in test development and delivery. In agriculture and horticulture, where the financial return is limited, quantitative POC test readings are delivered using a stand-alone reader. Initially these readers were developed for use solely with a specific product line. Charm Sciences offers the ROSA-M reader system which is a hand-held instrument designed to electronically read and quantitate results from ROSA POC strips for feed, grain and wine mycotoxin tests (www.charm.com/instruments/instruments-rosa-reader). However with the surge in POC development to ever expanding markets more companies are coming on line to deliver generic POC readers which can be tailored to specific product lines. These smart readers are able from the POC test barcode to identify the correct analysis to be applied for quantitative measurement and display, print, email or download the results to a computer. The Vertu reader has been tailored to deliver POC mycotoxin testing to food and agriculture producers worldwide to protect humans and animals from potentially lethal effects of contamination (www. vicam.com/vertu-lateral-flow-reader). The reader in its generic form is an ESE-Quant Lateral Flow System and can be purchased from QIAGEN (www.quiagen.com). Skannex (http://www.skannex.com) offers the SkanSmart hand held system which can be developed to analyse POC specific test formats. It has been developed to provide ultimate flexibility by providing a capability to analyse tests in multiple design formats: single and multiplex analysis of a strip, single and multiple strip cassette strip formats.

Several assay formats exist in POC testing. In the early 1990s, a commercially available flow-through POC system was developed and made commercially available for the diagnosis of a range of horticultural pathogens in plant tissue (Agri Diagnostic, USA). The flow through assay system consists of a device with an absorbent core to which a specific antibody probe is attached. The assay is performed by adding reagents to the surface of the device from dropper bottles. As each solution is absorbed into the device, it passes through the surface zone of immobilized antibody allowing any target disease antigen to bind to the related specific probe. The immunoassay can be completed within 10 minutes and with a visually observable colour endpoint. Each assay device contained a negative control reference zone that was used for comparison to the sample zone. These tests were originally developed by Agri-Diagnostics Associates, (2611Branch Pike, Cinnaminson, New Jersey, USA.) and then marketed by Neogen (Lansing, Michigan, USA). More recently Neogen Europe have moved to market a lateral flow format for POC of plant diseases and supply a range of diagnostic products for the detection of viral, bacterial and fungal plant pathogens (www.plant.neogeneurope.com). Similarly Pocket Diagnostics a UK based company provide a series of easy to use, rapid lateral flow (LFD) on-site test kits for diagnosing commercially damaging plant diseases caused by fungi, bacteria and viruses (www.pocketdiagnostic.com). As with Neogene they have worldwide distributors for their products. The tests have been developed to confirm the presence or absence of a disease on plants expressing disease symptoms.

Lateral flow POC immunoassays are used for qualitative and semi-quantitative detection of target analytes. Lateral flows consist of a carrier material containing dry reagents that are activated by applying a liquid sample. Movement of this liquid allows passage across various zones where molecules have been attached that exert specific interactions with target analytes. Results are usually generated with 5 – 10 minutes with the formation of a control and test line as appropriate to the sample and the test type. They are designed for single use, can provide a multiplex test platform and, as described previously, are available commercially for a wide range of applications. The most well know of which is the Unilever Clear Blue Pregnancy Test Kit. More recently they have become increasingly important in the diagnosis of plant pathogens and can prove useful as a first line rapid defence screen. This is amply demonstrated in forestry disease management where a genus *Phytophthora* LFD device has been used in the UK by Fera Plant Health and Seed Inspectorate to monitor the spread of the Oomycete pathogens *Phytophthora ramorum* and *Phytophthora kernoviae.* Initial positive diagnosis of the pathogen has enabled the effective management of the disease by immediate quarantine and containment measures. Once a sample is identified as a potential risk from infestation confirmatory tests are undertaken in the diagnostic laboratory to fully characterize the strains involved using molecular q PCR (www.fera.defra.gov.uk/plants/publications/documents/factsheets/pramparks.pdf).

For the control of fire blight disease, a simple lateral-flow has been developed for the rapid identification and detection of *Erwinia amylovora* (Braun-Kenwick 2011)**.** The 15 minute test was validated in field and provided a valuable tool for plant inspectors and growers to improve phytosanitary management of fire blight disease. The test was compared to PCR and conventional plating techniques and reduced the provision of laboratory generated results from 2 days to 15 min. The rapid in field tested was reported to provide high-specificity and sensitivity with a lower-detection limit (5.7 CFU/ml).

Lateral-flow devices can also be used as a product quality control diagnostic tool to provide immediate on-site results of product suitability. Used commercially in the USA a lateral flow device has been developed to rapidly determine levels of stable *Botrytis* antigens in table and dessert wines (Dewey *et al.,* 2013). As a result of the specificity and sensitivity achieved, consideration of this assay for use within horticultural production systems should be assessed. Envirologix currently markets this test and supplies a reader to enable quantitative measurements to be made (http://www.envirologix.com). The importance of Fusarium mycotoxins to human and animal health is well documented and considered a serious product quality control issue. MAb-based immunoassays specific to fusarins, T-2 toxin, zearalenone (F-2 toxin) and DON, have been developed for tracking Fusarium mycotoxins in contaminated crops (Barna-Vetro´ et al., 1994; Casale et al., 1988; De Saeger& Van Peteghem, 1996; Maragos et al., 2008). These assays have improved the sensitivity, specificity and speed at which the mycotoxins can be detected in contaminated food through the development of rapid on-site immunoassays for use in farms, storehouses and factories. Envirologix, under their QuickTox label supply a range of lateral flows to provide quantitative and traceable test results for mycotoxins in commodity grains. In addition they also supply laboratory based diagnostic probes for identification of viral and bacterial plant pathogens in host material. Lateral flows assay systems have also been employed to track horticultural biocontrol agents. A rapid and user-friendly LFD incorporating a monoclonal antibody probe has been developed and available commercially to identify active propagules of *Trichoderma* species in soil samples within 15 min of antigen extraction. The LFD can also be used to detect human infections (Thornton & Wills, 2013).

However although the lateral flow POC assay has its strengths there are potential weaknesses of the test format (Poshuma-Triumphe *et al.,* 2009). As with molecular PCR the restriction on total volume of the sample that can be applied may lead to a limit on sensitivity. This may be addressed by a pre-extraction treatment such as immunomagnetic capture however additional sample processing would add a level of complexity and detract from the simplicity of the one step LFD approach. Application of the sample to the lateral flow is often dropwise and this has the potential to lead to a level of imprecision. In complex environmental samples i.e. soils, food and estuarine water there is the capacity for the lateral flow matrix to become blocked and inhibit the assay process. Suppliers of lateral flow components have to the most

extent overcome these problems by producing pre filtration materials that can be incorporated within the lateral flow format. Equally they have been quick to react to sample volume and to supply a range of sample pads that allow increased volumes to be held prior to the immunoassay stage (www.millipore.com/diagnostics; www.whatman.com/DiagnosticComponents). Measures should also be taken to determine the shelf life stability of the product over a range of environmental conditions. Often a requirement of these tests is global shipment and this may involve periods of time in transit where extreme temperatures can exist prior to the final country of destination. However specificity, sensitivity and robustness of the test over extended time periods and with global distribution and have been shown to be strength of this type of test (Unilever Pregnancy Test, malaria and HIV testing). Nevertheless the antibody probes used within each POC test format will ultimately prove key to whether the sensitivity and specificity can be attained at a level suitable for the application and commercialisation of the test. As a result new tests which combine antibody and nucleic acid molecular techniques in a lateral flow format (aptamer / nucleic acid lateral flow (NALF)) are being developed to provide highly specific and sensitive diagnostic assays for plant pathogens (Tomlinson *et al*., 2010)). However sample preparation (nucleic acid extraction and amplification) may prove a significant weakness for use as a one-step assay format in a POC system.

Diagnostic Challenges.

There are considerable biological and technical challenges in developing a suitable diagnostic probe(s) and assay system for monitoring plant pathogens in sample matrices, whether for use in a laboratory by highly skilled operators or on-site by non-scientists. As discussed earlier soils can be particularly problematic with a range of soil compositions, composed of differently sized aggregates and, with microbial populations that are not evenly distributed. This factored often with a small sample volume for test analysis can provide difficulty in identifying a sampling strategy to provide a suitable test coverage. Also there should be a known requirement of whether the test requires the capability to differentiate between viable and non-viable organisms. This may prove critical in nursery irrigation systems or soil / composting materials where treatment processes can lead to detectable pathogen presence but which no longer present a disease risk. Equally test specificity should be at an appropriate level and not jeopardise indigenous biocontrol agents. A consideration is the existence of fungal species that contain pathogenic and non-pathogenic or even beneficial strains. This is a known phenomenon for complex species such as *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* (Recorbet *et al.*, 2003). Consideration also for *Pythium*, which is one of the most frequently, described Oomycete plant pathogens and, where morphological analysis has identified more than 130 species (van der Plaats-Niterink, 1981). With a wide and diverse host range, *Pythium* has been reported as parasitic on plants, animals (such as fish or crustaceans) and humans (causative agents of arteritis, keratitis, cutaneous or subcutaneous infections). Some of these species are parasites of other fungi and

provide real potential as useful horticulture and agricultural biocontrol agents (Paulitz et al., 1990; Martin & Hancock, 1987; White *et al*., 1992). Others are primarily saprophytes and found abundantly in the environment. In horticulture, where many different *Pythium* species are present and occur across a range of cropping systems, the challenge in developing a suitably specific and sensitive probe will rely on the capability to identify and detect pathogens responsible for specific crop diseases. A HDC funded project (White *et al.*, 1998; HDC 30971) reported five key *Pythium* species responsible for disease epidemics in the UK bedding plant industry and described the preliminary development of rapid diagnostic tests targeted to these pathogens (White & Wakeham, 1995). The authors had previously reported on the deleterious effect of soil applied metalaxyl and mancozeb on the biocontrol agent *Pythium oligandrum* and were acutely aware of the requirement to differentiate between the Pythium species. This holistic approach in probe selection and development is critical if a useful test is to be delivered to the end user. Abd-Elmagid *et al*., (2013) adopted a similar process by developing a multiplex PCR test which was able to discriminate four key plant pathogenic *Sclerotinia* species (*Sclerotinia homeocarpa*, *S. minor* , *S. sclerotiorum*, and *S. trifoliorumarecommon* in a single PCR reaction. Lievens *et al*., (2006) describes the development of q PCR to measure the concentration of a number of economically important fungal pathogens of tomato in soils and plant material (*Fusarium solani*, *Rhizoctonia solani*, *Verticillium* species responsible for tomato wilt and *Pythium ultimum*). Equally, knowledge of sufficient presence of target pathogen concentration coupled to the associated environmental parameters should be characterised in order to produce accurate test disease thresholds at which damage on crops may occur in pre-symptomless detection diagnostic assay systems. Once a suitable diagnostic prototype is available it is essential that it is extensively validated against existing adopted systems (isolation of pathogens by use of selective media, culture based morphological analysis, plant baiting, Koch's postulates) and, that this process is carried out across the range of environments that the test will be used. Equally, if the test is to be carried out by non-scientists the robustness of the system should be assessed with multiple 'non-skilled test' end users. Early collaboration with design engineers to make ergonomic improvements may provide optimal test delivery and speed up commercialisation of the product.

SECTION 2 – UK HORTICULTURE CROPS, DISEASES AND AVAILABLE PLANT PATHOGEN DIAGNOSTICS

Introduction

In 2012 there were 148 thousand hectares dedicated to fruit and vegetables in the UK. The overall vegetable area is at 119 thousand hectares and the total fruit area at 29 thousand hectares. Most of these crops are produced using a variety of pesticides to control diseases. As a result of legislation the available products registered for use is limited and, despite the 'off label' system for minor use, is likely to reduce further. To utilise those actives available efficiently and with optimal disease control performance there is a move away from routine pesticide application to targeted crop treatments (pesticides and biological) based on regular monitoring. This requires a greater depth of knowledge by producers and their staff to identify problems quickly. The ability to quickly diagnose many disease pathogens still does not exist and simple, yet accurate, diagnostic tools for growers should be developed. The objective of this report was to identify key crops across sectors of the UK horticulture industry, identify diseases that are of economic importance and, the available 'rapid' diagnostic processes available to enable timely disease management strategies to be put in place for effective disease control.

The crops assessed in this work are:

Apples
Brassicas
Carrots
Leeks
Onions
Cucumbers
Tomatoes
Outdoor Lettuce
Strawberries
Raspberries
Hardy Nursery Stock
Ornamentals

UK HORTICULTURAL CROPPING SYSTEMS, DISEASES AND DIAGNOSIS

BRASSICAS

Of all the vegetable crops, Brassicas in 2012 occupied the largest area of land at nearly 28 thousand hectares. Of which a significant proportion is grown on land which is rented out, both for business and crop rotation purposes. Leaf and flower head Brassicas are very susceptible to a number of foliar diseases as well as root

diseases such as clubroot. Timely and accurate methods that can confirm presence or absence of a disease in soils ahead of rental agreements is sought. The effective control of foliar parts during the cropping period is critical, otherwise yield and quality can be compromised. There is no tolerance for any blemish on marketed produce. The storage of winter white cabbage can be affected by disease occurrence. The table below represents information taken from two main sources: Impact of changing pesticide (www.randd.defra.gov.uk) and DEFRA Basic Horticultural Statistics (www.gov.uk/government/collections/horticultural-statistics). Eight diseases are highlighted in Table 1 with the associated pathogens listed and would be considered problematic to crop yield on an annual basis (Wynn *et al*., 2010).

Table 1. Economic disease impact to the UK Brassica Horticultural Industry employing available crop disease measures.

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Brassicas 28,000 ha	Downy Mildew	*Hyaloperonospora parasitica*	5344	1
	Dark leaf spot	*Alt. brassicae/ brassicicola complex*	18703	9.8
	Light leaf spot	*Pyrenopeziza brassicae*	2672	1
	Phoma leaf spot / canker		18703	6.7
	Powdery mildew	*Erysiphe cruciferarum*	4008	1.4
	Ringspot	*Mycosphaerella brassicicola*	18703	9.8
	White blister	*Albugo candida*	21375	13
	Clubroot	*Plasmodiophora brassicae*	2210	1.06

Diseases

Brassica Downy mildew: *Hyaloperonospora parasitica* (formerly *Peronospora parasitica*) is the fungal pathogen responsible for crucifer downy mildew, one of the most important diseases of Brassica crops worldwide (Channon 1981). Although field plants are severely affected, disease epidemics are particularly important in the nursery, when the infection may kill the seedlings, retard their development or cause lack of uniformity and quality (Coelho *et al.* 1998). As the identification of P.

parasitica is based on morphological characteristics (microscopic) of spores (Dickinson & Greenhalgh 1977), downy mildew diagnosis on infected seedlings is delayed until sporulation occurs. This is problematic for the grower as the disease in effect can only be treated once it is established in the crop. Equally infected young Brassica leaves packed in sealed plastic bags for supermarket distribution may leave the nursery visually in good stage but develop sporulating lesions before reaching the consumer. The pathogen survives on diseased plant debris and transmission to previously uninfected crops is by airborne spores. For this purpose sensitive and reliable diagnostic methods are required to monitor disease concentrations in the air prior to infection of the crop. Casimiro (2004) reported the preliminary development of a PCR based diagnostic test to amplify the full ITS and ITS2 region of the pathogen however no further reports on this are available and diagnosis remains a visual assessment based on post infection and, following the production of disease transmissible spores.

Dark leaf spot: is caused by the complex *Alternaria brassicicola* and *A. brassicae* and causes cosmetic damage to the outer leaves of affected crops leading to significant yield losses. Symptoms usually occur from July on the heads of cabbage and cauliflower and on Brussels sprout buttons, but are also present on cruciferous weeds and in oilseed rape. Crop residues can be a major primary inoculum source of airborne spores. These spores can infect susceptible crops locally and travel over considerable distances. Typically a 10 – 14 day period is required for symptom appearance after favourable environmental conditions for infection have occurred. The current technique for detection and identification of dark leaf spot is isolation of the pathogen from diseased leaf material and the production of spores. Identification of the spore is made using a microscope and morphological assessment (Simmons, 2007). This process is tedious and time-consuming and can take several weeks to obtain a diagnostic result. In the UK a Brassica Alert system is operated by Syngenta to provide subscribers in Lincolnshire with weekly dark leaf spot disease transmission and infection risk warnings. The information is generated using an environmental disease forecast model (Brassica spot™) and quantitative information of disease presence / absence can be generated from collected air samples using a dark leaf spot lateral flow POC test (www3.syngenta.com /BrassicaAlert). These warnings are designed to provide growers with an integrated disease management system and allow appropriate control measures to be made at times when the crop is at risk from disease pressure.

Light leaf spot: is increasingly becoming an important problem in the UK causing blemishes on the Brussels sprout button and also in winter white cabbage. Light leaf spot is endemic in Scotland and becoming common in Brassica production areas of Northern England. Infected oil seed rape crops can be a major primary inoculum source of the pathogen to susceptible Brassica crops. Targeted application of effective fungicides in response to airborne spore concentrations can play a vital role in controlling transmission of the disease to new crops and within crops. Lateral flow

point of care tests have been developed for use with weekly collected field aerosols and are currently being trialled in Scotland against an ELISA based laboratory test and microscopic analysis of field exposed tapes of a 7 day field air sampler. Investigations are in progress to determine the relationship between airborne disease concentration and symptom expression on commercial cultivars in the field.

Phoma leaf spot /canker: Symptoms occur on the stem as light brown or purplish cankers and on the foliage as large lesions with necrotic areas. Phoma diseases (*Leptosphaeria maculans* and *Leptosphaeria biglobosa*; asexual stage *Phoma lingam),* also known as blackleg, are important worldwide in Brassica crops including the leafy and flowerhead types, turnip, Chinese cabbage and pak choi, oilseed rape and swedes and mustard. In the UK, Phoma stem canker of oilseed rape is the most economically important disease in southern, eastern and central England. In Western Europe, ascospores (long range airborne spore disease transmission stage) are released from debris of the previous season's winter oilseed rape crop from late September onwards throughout the autumn/winter period. The disease is thought to be monocyclic and the introduction of an initial epidemic into a crop is thought to be by these airborne ascospores. Severe losses can occur in cauliflower and swede, but it is mainly a leaf blemish on other vegetable Brassicas. Once leaf spots appear, the fungus can grow in the plant without causing any symptoms before causing severe stem symptoms. A laboratory DNA-based method has been developed for detecting airborne inoculum of isolates of *Leptosphaeria maculans* species associated with oilseed rape. A method for purifying DNA from spores collected using Hirst-type air samplers and PCR reaction is described. As few as 10 spores could be detected by PCR and, against a background of spores of six other species (Calderon et al., 2002). This technique may prove useful in the validation of a field based POC system for monitoring airborne ascosporic inoculum in Brassica crops. The development of a POC system could have application globally and for use in oil seed rape production. It could provide a useful early warning system for disease risk.

Ringspot: caused by *Mycosphaerella brassicicola*, is a major foliar disease of vegetable brassicas and is very common in coastal and high rainfall locations such as the southwest and northwest of the UK. Ringspot can spread easily from heavily infected crops (over-wintered cauliflowers favouring spore production) to recently transplanted crops. The ability of the pathogen to spread from heavily infected over-wintered crops to sequentially transplanted crops in the same area has a major impact on control of the disease during the season. As for dark leaf spot an environmental disease risk forecast and a POC infield lateral flow has been developed (HDC FV233) to predict and identify airborne field disease transmission events (Kennedy *et al.,* 1997; Wakeham & Kennedy, 2011). The system is available commercially (www3.syngenta.com /BrassicaAlert).The POC test has been set to

identify spore concentrations that will initiate disease symptoms on exposed susceptible Brassica hosts (Kennedy *et al*., 2000).

Brassica Powdery mildew: caused by the fungus *Erysiphe cruciferarum*, is one of the most common foliar diseases of vegetable Brassicas in the UK. It is an important cause of blemish on Brussels sprout buttons and on cabbage. When swedes and turnips are severely affected it causes loss of yield and quality. Brussels sprout quality is markedly affected by the appearance of blemishes on the buttons. As with the other Brassica leaf pathogens, the fungus completes its life cycle on the surface of the plant and spores are then produced transferred in the wind and rain to infect other Brassica hosts. For this process to occur a time period of 10-14 days exists between infection and disease symptoms. Under HDC R & D (FV 333) a POC lateral flow device to measure airborne concentrations of these spores has been developed for weekly collected field air samples. The test is currently being evaluated for commercial use at locations in the UK and should be available to growers in 2015 (HDC CP 099).

White blister: is caused by the fungal plant pathogen *Albugo candida* and is a common disease of many economically important cruciferous vegetables and oilseed crops. Affected vegetables include broccoli, Brussels sprouts, cauliflower, radish, mustard, Chinese cabbage and turnip. The impact of disease in these crops is of a cosmetic nature and can render crops unmarketable. There are more than 10 distinct biological races of A*. candida* which have been identified and classified based on host specificity. In the UK and Australia, POC lateral flows have been developed (HDC CP99) to detect and measure spore concentrations in the air of the spore type which infect Brassica vegetable crops. The test is currently under evaluation at commercial vegetable sites in the UK and should be available to growers in 2015. The test aims to improve the environmental white blister disease risk forecast (www3.syngenta.com/BrassicaAlert) and provide information on disease concentrations in field air samples.

Clubroot: is caused by the soil borne organism *Plasmodiophora brassicae*. Mild clubroot infections lead to slowed growth and delayed harvesting. Severe infections result in total crop failure. Infection is easily recognisable by swelling of root tissue causing galls and club shaped structures. Once soils are infested with Clubroot the resting spores can remain viable and are capable of inducing disease in vegetable Brassica crops for up to 18 years after the initial disease outbreak. Information on the presence or absence of clubroot in soils has been achieved by growing bait plants and observing symptom development on root systems. However this approach is slow (six weeks), labour intensive and expensive requiring glasshouse facilities. New detection methods based on molecular q PCR (Faggian *et al.,*1999) have been developed and are now being tested for applicability in UK soil types

(HDC FV 349, CP 099). Within this work sampling procedures over hectare sites is assessed and the extraction efficiency of the DNA in different soils types examined. Alongside this work a laboratory immunoassay (Wakeham & White, 1996) and a POC lateral flow in field test (HDC FV 349) have been developed for the quantitative measurement of resting spores in soil. As part of the HDC funded work, the POC and the laboratory based molecular test are being evaluated over a two year period prior to release as a commercial test system (HDC CP 099a) in 2015.

CARROTS

By value (£129 Million) carrots are the most important vegetable crop in the UK and grown virtually all year round on some 11,000ha. In UK production, four diseases are highlighted with the associated pathogens listed and, would be considered problematic to crop yield on an annual basis (Table 2). If 100% disease control could be attained an increase in value to the UK industry would record at above £4 M for *Alternaria* leaf blight, £3M cavity spot, and £2 M for powdery mildew and £8M *Sclerotinia* (Wynn *et al.,* 2010)

Table 2. Economic disease impact to the UK Carrot Horticultural Industry employing available crop disease measures.

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Carrots 11,028 ha	Alternaria leaf blight	*Alt. dauci*	8822	3.8
	Cavity spot	*Pythium violae / sulcatum*	2206	2.9
	Powdery mildew (Cercospora spot)	*Erysiphe heraclei*	4411	1.9
	Sclerotinia	*Sclerotinia sclerotiorum*	8822	8.1

Diseases

There are some 10 fungal, bacterial and viral diseases which could have an impact on carrot crops. The crop is vulnerable to root diseases from an early stage onwards, to foliage diseases and to those pathogens which affect the crop during long-term field storage. Much of the recent progress in crop yield, quality and season extension is directly due to an improved understanding of disease control and the use of

fungicides. Their use has enabled the carrot crop to retain a healthy canopy for longer and as a consequence marketed yields have improved to keep pace with falling prices (Wynn et al., 2010). The four main diseases of economic yield impact are discusses along with current diagnostics:

Cavity spot: In the UK this soilborne disease on carrot roots is caused by *Pythium violae* and *Pythium sulcatum* (Hiltunen & White, 2012). Once infected carrots can quickly develop cavities which are sunken, circular to elliptical lesions usually less than 10 mm across, and sometimes surrounded by a pale halo. The cavities develop rapidly on roots that are close to harvest; severely affected carrots are unmarketable. Infection and progress of the disease is affected by environmental conditions during the season. Both of these Pythium spp. are able to survive in soil as thick walled oospores, and in the absence of a carrot crop can survive for some years. Both are pathogenic on a wide plant host range so crop rotation as a disease measure can be problematic. They produce zoospores (motile infectious agent) in wet soil, so that disease severity can increase rapidly in wet weather. A laboratory disease diagnostic ELISA has been developed to monitor oospore concentrations in soils (White *et al*., 1195, 1996b, 1997) however it is reported as of limited use as Pythium oospore concentrations do not correlate well with cavity spot incidence as a result of environmental factors (Wynn *et al*., 2000). This may be a result of the assay system consisting of polyclonal antisera (PAbs). A wide range of Pythium species are found naturally occurring in soil and the closely related genus of *Phytophthora.* It would be important to optimise the existing system using monoclonal antibodies (MAbs) specific for the oospore structure. Kageyama (2002) reports the development of a MAb cell line for *P. sulcatum* and its use to diagnose the pathogen in infested soils and plant tissue. The use of immunomagnetic fishing to capture the oospores directly from soils could improve the efficiency of the assay system and remove the inhibition and non-specific assay factors associated with different soil types i.e. clay and peat (high humic and fulmic acid). Using suitable MAb probes this format has the potential to be used within a multiplex laboratory ELISA format (XMAP technology) to multiplex and screen soils for a wide range of crop pathogens in soil. Equally once 'fished' and concentrated from the soil the diagnosis of the pathogen complex could be by POC lateral flow (multiplex to include information on other soilborne pathogens). LAMP or quantitative laboratory PCR. The development of suitable primer has been reported for P. violae (Wang & White, 2003) and, in Norway where they report five species of *Pythium* to be involved in the disease complex molecular primers have been developed (Klemsdal *et al*., 2008).

Alternaria blight (*Alternaria dauci*)**:** This disease is primarily spread by spores in the air and is favoured by warm and wet conditions. The disease is also reported as being seed-borne. Early epidemics reduce yield. Leaves of severely diseased crops appear burned as a result of coalescence of dark brown lesions, sometimes

surrounded by a chlorotic halo, under favourable conditions. Alternaria blight is controlled through integrated use of pathogen-free seeds, crop rotations, fungicide applications and partially resistant cultivars. However, none of these measures is completely efficient under high disease pressure. An environmental disease forecasting system is available and has a small number of users in the Netherlands but this has not been validated under UK conditions (Bouma 2003). Molecular specific primers have been developed to include discrimination of *A. dauci* within seed and plant tissue (Konstantinova *et al*., 2002) and more recently a quantitative PCR for quantitation of the pathogen in infected plant material (Boedo *et al*., 2012). Adoption of the q PCR methodology for collected field aerosols may prove useful in monitoring disease transmission events of the airborne spore. This approach has the potential to be developed as a multiplex laboratory molecular microarray format for a range of target spore types. Monoclonal antibodies have been developed to other Alternaria species but none reported specifically to spores of *Alternaria dauci*.

Powdery mildew: on carrots is caused by the fungal plant pathogen Erysiphe *heraclei*. This pathogen also causes powdery mildew on celery and parsley. The disease is spread by airborne spores and is often found with Alternaria blight despite its preference for drier conditions. As with powdery mildew on other crops, the initial infection sites are small and often not easily seen. However, under favourable disease development conditions the fungus will continue to grow and produce massive numbers of spores, which will in turn initiate more infections to rapidly escalate disease development. Currently other than visual assessment on the plant followed by confirmatory laboratory identification using conventional plant pathology techniques no molecular or antibody based diagnostic systems are available for use. Webling and Panstruga (2012) have however reported the development of DNA based primers for the rapid quantification of plant powdery mildew spores of *Golovinomyces orontii* . This pathogen causes powdery mildew on Arabidopsis thaliana, a small flowering plant native to Europe and Asia.

Sclerotinia (*Sclerotinia sclerotiorum*)**:** Symptoms on foliage can be obvious from mid-August and subsequently the disease can cause rotting of roots in the field. *Sclerotinia sclerotiorum* is one of the most damaging pathogens, which can infect more than 400 plant species (Boland & Hall, 1994). It is of global economic importance being a pathogen on oil seed rape crops. The fungus produces black resting structures in the soil, called sclerotia. In favourable environmental conditions the sclerotia may germinate to produce tiny mushroom-like bodies, apothecia, from which millions of spores are released in to the air. In the UK fungicide applications to commercial carrot crops may commence based on information from commercially-funded Sclerotinia apothecial germination monitoring sites. There is some interest in using sprays at planting of the biological control agent *Coniothyrium minitans* (Contans WG) for control of sclerotia in the soil, but the treatment is expensive. Molecular DNA probes have been developed to detect the pathogen in infected oil seed rape plant tissue (Yin *et al.,* 2009). Rogers (2009) describes the development a

diagnostic technique to quantify airborne inoculum of *Sclerotinia sclerotiorum* and discusses its potential use in oil seed rape disease-forecasting schemes. DNA was extracted from wax-coated plastic tapes, such as those used in Burkard spore traps and rotating-arm traps and able to detect DNA representing as few as two spores. The technique was insensitive to DNA of the host plant, Brassica napus, and other plant pathogens, including *Sclerotinia minor*, *S. trifoliorum* and *Botrytis cinerea*, and common airborne fungal genera such as *Cladosporium* and *Penicillium*. However no indication on the number of spores required for disease expression on susceptible host plants was provided. More recently an investigation to quantify airborne inoculum of *Sclerotinia sclerotiorum* and the integration of this with a Sclerotinia rot of carrot (SRC) forecast model has been published (Parker, 2012). A quantitative polymerase chain reaction (qPCR) assay has been developed to specifically detect and quantify DNA from airborne ascospores of *S. sclerotiorum*. The qPCR assay was evaluated on air samples collected using a Burkard air sampler, and showed that ascospores of *S. sclerotiorum* were specifically detected among a pool of foreign DNA. The concentration of detected ascospores was related to the observed incidence of SRC to suggest a preliminary threshold of 2 to 4 ascospores m^{-3} of air for SRC development. Comparative sampling and detection of airborne ascospores of *Sclerotinia sclerotiorum* for forecasting risk of Sclerotinia rot of carrot, and assessment of induced resistance for disease management is discussed.

An immunological ELISA based assay has been developed to detect *S. sclerotiorum* from infected oil seed rape petals (Jamaux & Spire, 1994) however the use polyclonal antisera did not provide the specificity required. Development of monoclonal antibodies to the ascosporic stage of the pathogens life cycle may prove useful in developing a system to monitor the pathogen in aerosol samples ahead of infection. This approach has been adopted successfully for another ascosporic fungi (Kennedy *et al*., 1999; Kennedy *et al*., 2000; Wakeham & Kennedy, 2011) and is used commercially in the UK. This system can be operated as a POC lateral flow field test or a laboratory ELISA test and when used in conjunction with an environmental forecast provides an early disease warning prior to symptom development on the plant.

LEEK

The leek crop in 2009 was valued at £35M and grown on 1647 ha. The policy is zero tolerance of disease because of risk of reduced quality leading to additional market preparation costs and the possibility of crop rejection where retailer/processor size grade specifications are not met. If 100% disease control could be achieved for rust a potential increased of £2.6 million could be achieved (Wynn *et al.,* 2010). Two diseases are highlighted with their associated pathogens and would be considered problematic to crop yield on an annual basis (Table 3).

Table 3. Economic disease impact to the UK Leeks Horticultural Industry employing available crop disease measures.

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Leeks 1647 ha	White tip	*Phytophthora porri*	659	1.3
	Rust	*Puccinia allii*	988	2.1

Diseases

White tip: White tip, caused by *Phytophthora porri*, is a devastating disease in the autumn and winter production of leek (*Allium porrum*) in Europe. A soil-borne disease favoured by wet weather in late summer and autumn, leading to water-soaked lesions and dieback which requires increased stripping costs to meet market specifications. The pathogen produces resting structures in the soil called oospores and these can survive up to four years in the absence of a susceptible crop. In wet weather the oospores germinate to produce motile zoospores which can survive at temperatures between 0 and 24°C for at least 7 weeks. A life cycle has been proposed whereby oospores germinate in puddles, and zoospores reach the leaves by rain splash and survive in water in the leaf axils, from where they infect the plant by direct penetration. A PCR DNA based diagnostic approach has been reported for the quantitative detection of P. porri in soil using zoospore induction (Van Poucke *et al.,* 2010). As with cavity spot a similar approach could be applied using species specific monoclonal antibodies to isolate the oospores directly from increased soil volumes and quantitate in the laboratory using a multiplex soil test for target pathogens using either DNA or an antibody based ELISA system. Equally this process could be developed to provide an infield soil based diagnostic system using POC lateral flow as under evaluation for *Plasmodiophora brassicae* in soil samples utilising super-paramagnetic capture beads.

Rust (*Puccinia allii*)**:** In the UK this is the most important disease of leeks and can be found from July onwards, although in cold frosty weather plants usually grow away from the disease. Leek rust results in yield loss and appreciable extra stripping costs to obtain a marketable product. A disease infection model has been reported for *Puccinia allii* (Gilles & Kennedy, 2003). The effects of an interaction between

inoculum density and temperature on germination of *Puccinia allii* urediniospores and leek rust progress was assessed. The model has not been evaluated in the UK under field conditions but demonstrates the potential for use as a disease management tool.

ONIONS

Bulb onions grown on 9000 ha and is currently worth in the region £456 M. If improved control could be achieved (to 100% control) then an additional £3.5 million for control of downy mildew, £2.5 M for Botrytis neck rot could be achieved (Wynn *et al*., 2010). Five diseases are highlighted in Table 4 with the associated pathogens land would be considered problematic to crop yield on an annual basis.

Table 4. Economic disease impact to the UK Onion Horticultural Industry employing available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Onions 8575 ha	Leaf spot	*Botrytis squamosa*	8575	1
	Neck rot	*Botrytis allii*	5574	2.5
	Downy mildew	*Peronospora destructor*	8575	3.5
	Leaf Blotch	*Cladosporium allii cepae*	2573	0.3
	White rot	*Sclerotium cepivorum*	1715	0.4

Diseases

The control of both foliar and soil-borne diseases in bulb onions is extremely important for a number of reasons. Onion bulbs with high levels of foliar disease are more susceptible to fungal and bacterial storage pathogens and are also less able to take up any sprout suppressant which is used to prolong the storage period. Skin retention can be affected, which may result in downgrading from pre-packing to processing with a consequent drop in value of circa £100/tonne. Crops with internal disease problems in excess of 2% after grading may be unmarketable

Botrytis leaf spot: is caused by the fungal plant pathogen *Botrytis squamosa*: Following infection small white circular or elliptical white spots with water-soaked margins appear on the host leaves, particularly during wet or humid periods, and can

spread rapidly causing collapse of foliage. In cooler climates it is thought that initial inoculum arises from spore aerosols produced from sclerotia that overwintered in soil, on onion debris, or on onion bulbs in cull piles. In most onion production areas, recommendations for managing the disease include a crop rotation of 2 to 3 years with non-susceptible crops, the removal of volunteer plants and onion cull piles, the use of less susceptible cultivars, and the application of fungicides to reduce disease progress. Several environmental disease forecasts are reported (Lacy & Pontius, 1983; Sutton *et al*., 1986; Vincelli *et al*., 1989;) and recently a laboratory DNA-based method for quantifying airborne inoculum of *Botrytis squamosa* has been developed. In field trials the diagnostic system was found to be reliable for quantifying aerosols of *B. squamosa* inoculum in commercial onion fields. Previous studies on the aerobiology of this disease showed that monitoring the presence and quantity of airborne spores could provide a direct measure of vulnerability of onion to infections (Carisse *et al*., 2005; Carisse *et al*., 2008). An action threshold of 10 to 15 conidia/m^3 has been shown to be reliable in determining the need for fungicide application (Carisse *et al*., 2005). By combining information on airborne disease concentration and the use of an environmental disease forecast there is the potential to develop a risk management system for Botrytis leaf blight infection and optimise the use of fungicides more effectively. However the DNA based method remains a laboratory test. The application of specific monoclonal antibodies as utilised for other airborne diseases of vegetable crops in the UK (light leaf spot, onion downy mildew, dark leaf spot, ringspot, Brassica powdery mildew and white blister) may prove optimal in delivering a rapid multiplex crop specific test for delivery of timely and effective crop protectant measures.

Botrytis neck rot: Three species are considered the primary causal agents of neck rot of onion, namely *B. aclada*, *B. allii,* and *B. byssoidea.* Following infection by severe losses can occur in store with affected bulbs becoming soft with a brownish rot of the scales. Symptoms of neck rot typically develop only after onion bulbs have been harvested and placed in storage, even though infection occurs in the field. Seed can be a source of infection as can soils. Diagnosis using conventional techniques (isolation and culturing the fungi on agar media, and/or measurement of conidial dimensions using a compound microscope) can be difficult due to the presence of other Botrytis species (*B. cinerea* differs from neck rot in that infection is typically limited to the outermost dry scales of the bulb, resulting in a cosmetic brown blemish on the surface of the bulb). A real-time fluorescent PCR assay has been developed to quantify the amount of neck rot Botrytis spp. (*B. allii, B. aclada*, and *B. byssoidea*) present in onion seed (Chilvers *et al*., 2007). The assay appears to be more sensitive than plating onion seed onto selective agar media, as the PCR assay enabled DNA of Botrytis neck rot species to be detected from seed lots that tested negative using conventional seed plating assays. Linfield (1995) developed polyclonal antisera for an indirect ELISA assay to detect latent *B. allii* (undifferentiated from *B. aclada*) infection in stored onion bulbs. The ELISA enabled

earlier detection of *B. allii* in bulb tissue compared with conventional isolations onto agar media. The antisera did not cross-react with *B. byssoidea*, *B. porri*, or *B. squamosa*, but showed some cross-reaction with *B. cinerea*. Linfield also demonstrated that non-uniform infection of onion neck tissue by *B. allii* necessitates sampling whole slices across the neck of each bulb for more accurate detection of the incidence of bulbs with latent infection. Neogen Europe Ltd. (Auchincruive, UK) offers a commercial, non-quantitative polyclonal ELISA IDENTIKIT for *B. allii* .

Downy mildew: caused by the plant pathogen *Peronospora destructor* is a common, geographically wide-spread and serious disease on onion crops that can result in major yield losses in bulb and salad onions and in onion seed production. Actual yield losses in bulb onions of 60 to 75% have been recorded. These losses mainly result from severe infections in bulb onion crops causing early defoliation, reduced bulb sizes, and poor storage quality of bulbs. The disease is spread by airborne spores which are produced during the night and only when favourable environmental conditions occur. The control of downy mildew in onions relies mainly on the prophylactic application of fungicides, as frequently as every10 days. However, to reduce the impact of fungicides on the environment, an integrated disease management (IDM) system has been developed and operates in the UK. The development of MILLIONCAST (Gilles *et al.,* 2004) provides an improved model for predicting downy mildew sporulation on onions and POC lateral flow tests have been developed (Kennedy & Wakeham, 2008) to monitor in field spore disease transmission events (HDC FV356). A reduction in the number of fungicide applications, while maintaining disease control has been achieved by applying fungicides only at times when conditions are favourable for disease development.

Leaf blotch: caused by *Cladosporium allii-cepae* is a common but relatively minor foliar disease on onions. Following infection by airborne spores the lesions begin with elliptical white or pale brown spots which darken with age. The lesions are difficult to differentiate between white tip and purple blotch. There are currently no diagnostic assays available for monitoring disease spread or infection.

White Rot: is caused by the soil-borne fungus *Sclerotium cepivorum*. The disease can cause severe losses and produces resting bodies (sclerotia) which can persist for many years. Once soils are infected they can become so damaging as to lead to complete crop loss and prevent future production of Allium sp on that site. The disease can cause large economic losses in most temperate areas of the world including Europe, Americas and Australasia. Affected plants show yellow leaves and wilting followed by root rotting, and the base of the bulb becomes covered with white or grey fungus which produces new sclerotia. The only known source of spread is by infected plant material (onion transplants) or sclerotia. Dispersal of sclerotia in irrigation water has been demonstrated in the USA and in wind-blown plant remains and infested soil. Sclerotia have also been shown to survive ingestion by sheep and goats when infected plants have been used as fodder. Detection of sclerotia has

been by wet sieving of soils however this is has proved a laborious process. A method for isolation of sclerotia from soil using a dilution plate technique has been developed but this again has proved an intensive and tedious process with variable results. In the late 1990s Wakeham & White described the development of an immunological assay for the detection of *Sclerotium cepivorum* in onion bulbs and soil. However the assay relied on a polyclonal antiserum which is no longer available. Nevetheless a correlation was observed between the mycelial stage of the pathogens life cycle and the developed assay in artificially inoculated soils. More recently DNA primers have developed to confirm the presence of the fungus in onion host tissue at an early stage of infection when disease symptoms were apparent (Haq *et al*., 2003).

CUCUMBERS

In 2012, Cucumbers were grown under approximately 105 ha of glass in the UK. Disease control is achieved by a combination of tolerant varieties (where available), fungicides, glasshouse and environment control (esp. humidity control), good hygiene and crop management. The major causes of loss are gummy stem blight (*Mycosphaerella melonis*) (£3M), powdery mildew (£1.5M) and Pythium root rot (£1.5M). 105 ha are currently affected by Gummy stem blight, 50 acres by Powdery Mildew, and 10 acres by Pythium root rot. Ten diseases are highlighted with the associated pathogens and which would be considered problematic to crop yield on an annual basis (Table 5).

Table 5. Economic disease impact to the UK Cucumber Horticultural Industry employing available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Cucumber 105 ha	Gummy stem blight	*Mycosphaerella melonis*	105	3
	Powdery mildew	*Podosphaera fusca Golovinomyces cichoracearum*	50	2
	Root rot	*Pythium spp.*	10	2
	Black root rot	*Phomopsis sclerotioides*		0.1
	Grey mould	*Botrytis cinerea*		0.1
	Downy mildew	*Pseudoperonospora cubensis*		0.1-0.2
	Verticillium wilt	*Verticillium*		0.15

	Fusarium wilt	*Fusarium oxysporum f.sp. cucumerinium*	0.2
	Sclerotinia stem rot	*Sclerotinia sclerotiorum*	0.02
	Penicillium stem rot	*Penicillium oxalicum*	0.04

Diseases

Gummy stem blight (Black stem rot of cucumber)**:** is caused by *Mycosphaerella melonis* syn. *Phoma cucurbitacearum* (syn. *Didymella bryoniae*) and is of worldwide importance, causing significant economic damage of cucumber & other cucurbits. It causes extensive stem & leaf infections which when severe can debilitate or even kill plants. Airborne spores are produced by the pathogen and infection of flowers & developing fruit leads to fruit end rot which are sometimes not visible until the fruit is marketed. This leads to rejection and reduced retailer & consumer confidence in the product. Fungicides are used routinely in an attempt to suppress the disease and prevent plant and fruit losses. Unfortunately, the fungicides that are available in the UK for use on cucumber (primarily for powdery mildew control) provide only a partial suppression or reduction of the disease. Under a HDC funded project (PE 001) a range of alternative fungicides are currently being reviewed for their efficacy in controlling the disease. The project has developed monoclonal antisera and a laboratory based ELISA to monitor glasshouse aerosols for *M. melonis* spore disease transmission events and, the timed application of control measures made during periods of peak spore production (>2000 spores m^3). Ideally this information should be utilised within a fully integrated disease management system. In Holland, an environmental model is under evaluation for control of the disease. Future work should look to integrate the environmental disease forecast with information generated from the air sampling and antibody based diagnostic test. This would provide information on when airborne disease was available at a concentration required for infection of the crop and whether the environmental conditions were conducive to infection. In this way an informed decision could be made to apply the appropriate control measure in an effective and targeted way. To provide this information in a more timely way the laboratory based immunoassay test should be transferred to a lateral flow format. The current test provides a weekly monitoring period however studies should look to determine whether daily risk periods are appropriate.

Botrytis grey mould: is caused by the ubiquitous plant pathogen *Botrytis cinerea* and attacks leaves, stems, flowers and fruits of various plant crops grown in greenhouses. Grey mould is considered a global problem and of economic impact to the horticultural industry worldwide. The frequent development of *B. cinerea* isolates resistant to common fungicides and the desire to reduce pesticide should lead to

efforts to improve disease control measures. Diagnosis is generally made in the glasshouse when visible symptoms are available and disease progression is established. Aerial dissemination of the disease within the glasshouse and propagation system is by the production of spores. Workers (Meyer & Dewey, 2000) reported the development of monoclonal antibodies to *Botrytis cinerea* and the incorporation with an ELISA based system and a POC lateral flow. The latter test has recently been commercialised and is available for use on site at vineyards in the rapid determination of levels of stable *Botrytis cinerea* in table and dessert wines (Dewey *et al*., 2013; http://www.envirologix.com). A POC lateral flow for *B. cinerea* detection in plant tissue is available in the UK (www.pocketdiagnostics.com). A similar approach has been used to develop and monitor *Botrytis cinerea* in glasshouse aerosols using an MTIST air sampler ((DEFRA project HH3611SPC), Kennedy *et al*., 2000; Wakeham *et al*., 2004). This approach should be adopted by the UK horticultural industry to provide an early disease warning grey mould system in glasshouse collected aerosols.

Powdery mildew: is probably the most common, conspicuous, widespread and easily recognizable disease of cucurbits. Like other powdery mildew diseases, its symptoms are characterized by the whitish, talcum-like, powdery fungal growth that develops on both leaf surfaces, petioles and stems but rarely on fruits. The disease is spread to other plants by the release of spores from the talcum like lesions in to the air. The disease can be caused by either *Podosphaera fusca* or *Golovinomyces cichoracearum*. Powdery mildews are comparatively difficult fungi to work with. Their obligate, biotrophic, parasitic nature and consequent inability to grow on artificial culture medium significantly hamper research. However there is a need to develop specific monoclonal antibodies to the airborne stage of each of the pathogens life cycle to enable the development of rapid on-site and laboratory assay systems to discriminate and diagnose spore concentrations affecting cucurbits in glasshouse propagation systems. A similar approach could be developed as previously described. Ideally this would be included within a multiplex crop specific diagnostic assay to provide early warning disease alert for a range of target pathogens in glasshouse aerosols of cucurbits production. This system could be developed as a laboratory microarray system or as a POC lateral flow system for testing aerosol samples on site.

Pythium root and stem base rot (*Pythium aphanidermatum* and other *Pythium* species)**:** This is a major disease of cucumber and affects plants in all substrates. As a result of their ability to produce motile zoospores, the use of hydroponic systems favours the production and spread of *Pythium* species. Recirculatory systems can provide a rapid increase of disease and infection of plants across the propagation unit. *Pythium* species can survive in hydroponic systems saprophytically between croppings of susceptible hosts. A study in Norway identified 64 *Pythium* isolates from cucumbers and of these 12 different *Pythium* species were identified. Isolates

of *Pythium ultimum* and *P. aphanidermatum* were by pathogenicity tests determined as being highly aggressive on cucumber seedlings (Herrero *et al*., 2003). Commercial diagnostic antibody based tests are available for on-site use in nurseries (www.plant.neogeneurope.com) for detection of *Pythium* species. However as previously reported the available assay systems are unable to discriminate specific species or differentiate between viable and non-viable disease. This provides an opportunity to develop probes of improved specificity to target *Pythium* species which affect horticultural cropping systems. This could be in the form of an array to identify to a species level and preferably to discriminate between viable and non-viable propagules as described by Wakeham *et al*., (1997).

OUTDOOR LETTUCE

In 2008, 5,592 ha was recorded for outdoor lettuce propagation in the UK and have little changed in the intervening period. Disease control is achieved by the use of fungicide programmes, together with the use of resistant varieties. If improved control of Downy mildew could be achieved financial gains of £10M could be achieved. For *Sclerotinia* £5.4 million. In the absence of pesticides major losses would occur in production i.e. downy mildew at 40% reduction (Wynn *et al*., 2010). Table 6, identifies seven diseases and the associated pathogens that would be considered problematic to crop yield on an annual basis (Table 6).

Table 6. Economic disease impact to the UK Outdoor Lettuce Horticultural Industry employing available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Outdoor Lettuce 5592 ha	Grey mould	*Botrytis cinerea*	5592	5
	Downy mildew	*Bremia lactucae*	5592	10
	Ringspot	*Microdochium panattoniatum*	1398	1.2
	Sclerotinia	*Sclerotinia sclerotiorum* and *minor*	5592	5.4
	Lettuce Mosaic	Virus	4194	1.5
	Beet Western	Virus	4194	1.5
	Cucumber mosaic	Virus	4194	1.5

Diseases

Downy mildew: The most important disease of outdoor lettuce, which can attack all lettuce types in UK horticultural production is caused by the plant pathogen *Bremia lactucae*. This foliar disease affects crop yield and quality. Disease symptoms manifest as white cotton-like fungal growth, on the undersides of leaves. Infection occurs under conditions of high relative humidity and temperatures from 5 to 24°C. The disease can be transmitted by seed, spores or through contact with an infested lettuce crop. Once established, spores are released in to the air from and will spread to other lettuce plants. Two environmental disease forecast models have been developed for Downy mildew on lettuce and evaluated for use by a team of scientists in Norway (Nordskog et al., 2004). The two systems were PlantPlus (PP) and Modell-Analys (MA), which is based on a model developed in California. Disease was detected on lettuce plants following forecasts issued by both models in 5 of 8 fields. However, forecasted infection periods were not always followed by disease occurrence. Both PP and MA usually called for fewer fungicide applications than a calendar schedule of sprays at 10-day intervals, with no
difference in disease suppression among treatments. The integration of this system with an airborne *B. lactucae* disease monitoring network may prove useful and discriminate between environmental non-limiting periods and disease availability to provide an accurate disease forecast model.

Sclerotinia (*Sclerotinia sclerotiorum, S. minor*)**:** An economically important problem on lettuce in the UK. Due to long-term survival of sclerotia in soil, it is difficult to control this disease by rotation.

Grey mould (*Botrytis cinerea*)**:** A fungal plant pathogen which is ubiquitous within horticultural systems. It can be found under a wide range of conditions but is particularly favoured by relative high humidity and leaf wetness. *B. cinerea* tends to be problematic on damaged or senescing tissue, or following infection by another pest or pathogen. The pathogen affects a wide host range and all parts of the growing crop. It is also a cause of postharvest fruit rot and this can prove particularly problematic with supermarket returns in fruit and lettuce. Fieldclimate.com provides an environmental model for infection risk of Botyrtis grey mould in lettuce. The model takes in to account leaf wetness periods and the temperature (http://www.metos.at/tiki/).

Ringspot (*Microdochium panattoniatum*)**:** Most prevalent on Cos / Romaine lettuce types, causing loss of quality. Also known as shot hole or ring spot, anthracnose. It initially causes small (less than 0.125 inch or 3 mm), water-soaked spots on outer leaves. Spots enlarge, turn yellow, and are usually irregular and angular in shape. Under cool, moist conditions, white to pink spore masses of the fungus will be visible in the centre of the lesions. Spores are spread by water-splash, so the disease is

particularly severe in wet seasons. To prevent disease development, planting of early spring lettuce in fields having a history of the disease should be avoided. Rotations with any crop other than lettuce will help reduce soil inoculum levels, though such rotations will not eliminate the pathogen unless lettuce is not planted for over four years (www.ipm.ucdavis.edu, UC IPM Pest Management Guidelines: Lettuce). The pathogen is able to survive in soils in the absence of a susceptible host in the form of microsclerotia (30 to 60µm) and this can provide the primary inoculum source (Patterson & Grogan, 1991).

Sclerotinia stem rot (*Sclerotinia sclerotiorum*)**:** This disease is most commonly found on shoots in the upper canopy, although it can affect fruit and stems. Control is by removal of affected plants or plant parts. Fungicides are rarely used to control the problem. Molecular probes have been developed for *S. sclerotiorum* (see report section on diseases of carrots) and a multiplex PCR for four species of the *Sclerotinia* (Abd-Elmagid et al., 2013)

Viruses: The main viruses in UK lettuce production are Beet Western Yellows, Lettuce Mosaic Virus (LMV) and Cucumber Mosaic Virus (Wynn *et al*., 2010). Each of these is aphid-borne so control depends largely on the use of aphicides. LMV can also be seed-borne. In the UK commercial antibodies and PCR based diagnostics are available for diagnosis of a wide range of viruses affecting plant horticultural and agricultural cropping systems (www.lynchwooddiagnostics.co.uk; www.foresitediagnostics.com; www.plant.neogeneurope.com)

Downy mildew (*Pseudoperonospora cubensis*)**:** This non-indigenous disease tends to occur in a few crops each year, especially on the outside row of heated crops or in unheated crops.

Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*)**:** An occasional but very damaging disease that tends to occur on certain nurseries each year.

Penicillium stem rot (*Penicillium oxalicum*)**:** Occasionally this disease occurs and can be very damaging, spreading rapidly at high humidity, but is usually only seen on a small number of isolated plants.

Verticillium wilt (*V. albo-atrum*)**:** This disease affects both soil-grown and substrate grown crops. Affected plants invariably die.

TOMATOES

In 2008, 212 ha of tomatoes were cultivated in the UK. According to a report published on the impact of changing pesticides availability on horticulture (Wynn *et al*., 2010), improved control of Botrytis could achieve (£5.5M), Fusarium diseases (£2.5M) and Verticillium wilt (£1.4M). In the absence of pesticides Botrytis (£22M) powdery mildew (£11M) and Pythium root rot (£5.5M) could have a serious financial impact. Twelve diseases with the associated pathogens that would be considered problematic to crop yield on an annual basis are shown in Table 7.

Table 7. Economic disease impact to the UK Tomatoes Horticultural Industry employing available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Tomatoes 212 ha	Grey mould	*Botrytis cinerea*	53	5.5
	Fusarium wilt Fusarium crown rot	*Fusarium oxysporum f.sp lycopersci* *F.oxpysporum f.sp radices-lycopersci*	6	2.5
	Powdery mildew	*Oidium neolycopersici* *Golvinomyces orontii*	106	3.9
	Verticillium wilt	*Verticillium*	11	1.4
	Phytophthora root and stem rot	*Phytophthora spp.*	2	0.4
	Pythium root rot	*Pythium spp.*	21	0.5
	Leaf mould	*Fulvia fulva*	6	0.6
	Late blight	*Phytophthora infestans*	4	0.2
	Black root rot	*Thielaviopsis basicola*	21	0.5
	Stem rot	*Didymella lycopersici*		0.4

Diseases

Botrytis (*Botrytis cinerea*)**:** A wide range of fungicides are available and used on tomato for control of Botrytis. An environmental risk forecast and diagnostic processes have been described earlier in the report.

Fusarium wilt (*F. oxysporum* f. sp. *lycopersici*)**:** and Fusarium crown and root rot (*F. oxysporum f. sp. radicis-lycopersici*): Fusarium wilt is generally controlled by use of resistant varieties, but several recent outbreaks in varieties listed as resistant to Fusarium wilt indicate a new race is present in the UK. Fusarium crown and root rot is controlled very effectively by resistant varieties in the presence of established Fusarium races, but outbreaks continue in 'heritage' varieties.

The genus Fusarium currently includes over 100 species of ubiquitous soil fungi and contains two species, *F. graminearum* and *Fusarium oxysporum*, ranked fourth and fifth, respectively, in terms of scientific and economic importance in a 'Top 10' list of fungal plant pathogens (Dean *et al.,* 2012). Some species cause root and stem rots (for example *F. solani*), while others cause vascular wilts (for example tropical race 4 of *F. oxysporum* on banana); fruit rots (numerous) or ear diseases (e.g. *F. graminearium* head blight of wheat). In some species, host-specific pathogenic strains are distinguished as formae speciales (e.g. the root and stem rot pathogen of pea *F. solani f.sp. pisi*) which otherwise do not differ from saprotrophic strains of the same species. As for Pythium, this can prove problematic in the development of suitable diagnostic probes. Neogen Europe market a Fusarium species antibody based ELISA however this is for use as a confirmatory test on infected plant tissue. Molecular DNA tests have been developed to discriminate specific race types of Fusarium (Ying-Hong *et al.,* 2013) and species specific tests are available commercially (*F. graminearum* www.2020seedlabs.ca/fusarium-tests-and-how-use-them). However Fusarium species are not only important as plant pathogens, but also as producers of mycotoxins (Jelinek *et al.,* 1989). Many commercially available tests are used in the testing of food products for mycotoxin presence (www.neogeneurope.com; www.mycotoxins-rapid-tests.eu). Of the 100 or so species of Fusarium, roughly 12 are considered human pathogens. The most common pathogen is *F. solani* accounting for 50% of reported cases of human infections, and is also responsible for emerging diseases in wild and domesticated animals (Jain *et al.,* 2011).

Powdery mildew: The powdery mildews are a diverse group of pathogenic fungi that can infect a large number of plant species. On tomatoes the disease can be caused by the plant pathogens *Oidium neolycopersici* and Golovinomyces *orontii* (*Erysiphe rotini)*. A widespread and common disease in the UK horticulture production of tomatoes however it is partially controlled by the use of tolerant varieties. In the USA an environmental model has been developed and used by the

industry to identify periods when the crop could be at risk from the disease (http://www.ipm.ucdavis.edu/MODELS/TOMPM/usingtomatopm.html). As with other powdery mildew diseases the disease is spread by airborne spores. Although protectants can be applied according to the risk model they may be unnecessary if the disease is not present within the cropping system. Development of an on-site POC test would be useful to routinely test glasshouse aerosols for disease presence. In this way the disease could be identified at an early stage and preventative measures put in place before allowing the disease to progress on infected plant material. The powdery mildews are a group of pathogens that are obligate parasites so to complete their life cycle and produce spores they must infect a compatible and susceptible host material.

Verticillium wilt: of tomatoes and potatoes can be caused by two different soil-borne fungi, *Verticillium albo-atrum* or *Verticillium dahliae*. Both pathogens have a very broad host range and can infect a host range of nearly 400 species of plants. These include cucumber, eggplant, pepper, rhubarb, watermelon, artichoke, beet, broad bean, strawberries, raspberries, and a number of weedy plants. They may also infect several woody species such as maple, ash, lilac and roses. The fungi are able to infect a susceptible host through wounds in the roots caused by cultivation, nematodes or the formation of secondary roots. This disease is considered a cool-weather disease and is favoured by moist soils and a temperature range of 21-27° C (70-81°F). For survival between crops Verticillium is able to persist in the soil for long periods by producing either small black resting bodies known as microsclerotia (*V. dahlia* (38-150µm) or as melanised mycelia (V. albo-atrum). Germination of these resting structures is stimulated by root exudates of both host and non-host plants. Once a plant has succumbed to the disease diagnosis can be made visually. Confirmation of the disease can be made in the laboratory by selective agar medium and, after a period of approx. four days. A new HDC project (46097100) is utilising a quantitative PCR test developed by Biodeau (2012) and incorporating a soils extraction process developed in HDC SF97 to develop a DNA based soil assay for microsclerotia.

In the UK isolates have been able to overcome host resistance and are becoming increasingly common on UK nurseries. Control is currently achieved by use of grafted plants to increase root vigour .There is room for improvement in the control of Verticillium wilt in tomato. Switching from own-roots to grafted plants increases costs from 80p to £1.20/plant.

Late blight: caused by the Oomycete plant pathogen *Phytophthora infestans* is increasing as a problem, particularly unheated or poorly heated glasshouses. The disease is difficult to control with fungicides, more readily controlled by use of heat, ventilation and removal of the affected tissues. Monitoring glasshouse aerosols and

irrigation systems for the airborne and waterborne disease transmission stages may prove useful if developed as a species specific POC lateral flow test. This test could be developed in collaboration with the Potato Council. *P. infestans* is a serious airborne foliar pathogen of potatoes and accounts for losses of approximately 1 billion Euros (cost of control and damage) to the almost 6 Mha of potatoes grown annually in the European Union (Haverkort et al., 2008).

Phytophthora foot rot and **stem rot** (*Phytophthora cryptogea*)**:** Primarily a problem of soil-grown crops, but can be severe in nutrient film technique (NFT) or hydroponic crops where the irrigation waste solution is recycled. This disease could become increasingly important if growers are required to recycle irrigation solution in substrate crops, though losses can be mitigated by an effective solution disinfestation treatment (e.g. heat, UV light, slow sand filter). A number of POC lateral flow tests are commercially available for confirmation of the disease in host plants exhibiting disease symptoms. Diagnosis of the pathogen is at the genus level. As rationale provided earlier in the report, a multiplex POC for target pathogenic species of *Phytophthora* and *Pythium* is required in the Horticulture Industry to discriminate and identify target species in water supplies and to provide information on viability. This will prove particularly useful in confirmatory tests that disinfestation treatments remain effective and continue to prevent infection outbreaks. An on-site test could provide growers with the capability to carry out routine inexpensive checks as part of a weekly sanitisation programme. Equally the development of an on-line antibody based sensor could be developed to screen water samples continuously, both prior to and after sanitisation processes, to identify disease contamination issues of the recirculated system. EU environmental legislation is moving towards closed recycling systems and disease containment within these systems has the potential to be problematic with significant yield losses.

Pythium root rot (pathogenic Pythium spp.)**:** Pythium root rots can be troublesome in hydroponic systems, especially where drainage is poor and at the plant establishment stage. As described earlier, a POC test is required to identify pathogenic *Pythium* species of horticultural crops prior to an infection outbreak.

MINOR DISEASES

Black root rot (*Thielaviopsis basicola*) A common disease in NFT crops and occasionally found in crops grown in soil or on substrates. NFT crops appear to tolerate infection to some extent.

Leaf mould (*Fulvia fulva*)**:** An occasional problem, especially on older varieties with incomplete resistance.

Stem rot (*Didymella lycopersici*)**:** A rare but potentially devastating disease due to its high infectivity.

Technologies to monitor these pathogens are considered within HDC PC 281a (Tomato: Application of DNA based microarray and LAMP assay for the improved detection and understanding of root diseases). In phase 1 of the project a Laboratory DNA microarray is under development to detect and provide quantitative information on 50 bacteria and fungi associated with the root zone of the tomato crop. Phase 2 of the project (2013 -2015) will continue to optimise the laboratory based microarray and develop a portable DNA LAMP system to provide a diagnostic test for up to 12 pathogens simultaneously. However for the reasons discussed in Section 1 of the report and, as reported by Reiger (2013), the LAMP based system will remain for the foreseeable future a relatively expensive process and require staff to extract DNA from samples, process and analyse the results.

STRAWBERRY

In the UK approximately 4770 ha area is cropped on an annual basis. Two main diseases are identified as of significant economic impact on strawberry production (Table 8). Botrytis grey mould is the major limiting factor in strawberry production.

Table 8 . Economic disease impact to the UK Strawberry Industry using available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Strawberry 4,770 ha	*Grey mould	*Botrytis cinerea*	4770	33.3
	*Powdery mildew	*Podosphaera aphanis*	3339	24.7

*routine application of protectant fungicides is norm.

Diseases

Botrytis grey mould: Crop losses as a result of *Botrytis cinerea* infection, both before and especially after harvest, remain problematic. In 2008, it was suggested that if 100% control was achieved an additional £33M could be saved by the industry (Wynn *et al*., 2010). A high proportion of the picked fruit carry latent infection which manifests during distribution or sale as visible aerial sporulating material. Routine spray applications is a normal practice. After harvest, the fungus is able to survive in the soil as small, black, inactive sclerotia or on infected leaf and fruit material. *Botrytis cinerea* as previously described is ubiquitous and can be found on decomposing, dead organic matter of many plant species in and around the growing area. A *Botrytis* infection risk model has been developed (www.ipm.ucdavis.edu) that allows growers to define an infection period as light to severe based on field temperature and wetness data. No fungicide is recommended when conditions are

determined not suitable for infection. Ideally this model should be implemented with information on disease availability. This has been shown to be useful in monitoring disease epidemics on Brassica, Onion and Cucumber crops (HDC FV HDC FV233, FV356, PE001; Kennedy & Wakeham, 2008; Wakeham & Kennedy, 2011; Wakeham *et al*., 2012) and, could be adapted to include airborne spores of *Botrytis cinerea* (Kennedy *et al*., 2000; Wakeham *et al*., 2003).

Powdery mildew: caused by the fungal plant pathogen *Podosphaera aphanis* (previously known as *Sphaerotheca macularis*) is a serious problem with 3,339 hectares estimated to be affected (Wynn *et al*., 2010). This disease is of considerable and increasing importance as it infects foliage; stolon's, flowers and fruits. The pathogen inhibits the photosynthetic ability of the plant, reducing growth and cropping potential. Mildew-infected fruits are unmarketable. Even where tunnel management is good enough (appropriately ventilated) to achieve temperatures and relative humidity less conducive to this disease, routine application of fungicides is the norm, especially for crops which are being harvested during the period July – September.

Both pathogens produce spores which disseminate the disease over distances. An environmental risk model has been developed for *Botrytis cinerea* and powdery mildew (*Erysiphe necator*) on grapes (www.ipm.ucdavis.edu). These models could be expanded to cover strawberries. The development of a multiplex POC lateral flow could be used in conjunction with an air sampler to determine whether the diseases were in the environment during periods conducive to infection and at a concentration which cause risk of disease. This could also be expanded to cover raspberry crops.

RASPBERRY

In the UK approximately 1,634 ha of raspberries is cropped on an annual basis. Four main diseases are identified as of significant economic impact on raspberry production (Table 9). As in Strawberry production Botrytis grey mould is the major limiting factor in raspberry production.

Table 9 . Economic disease impact to the UK Raspberry Industry using available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Raspberry 1,634 ha	Cane and Fruit	*Botrytis cinerea*	1634	4.7
	Cane spot	*Elsinoe veneta*	817	2.4

	Cane blight	*Leptosphaeria coniothyrium*	817	2.3
	Powdery Mildew	*Sphaerotheca macularis*	817	2.3

Diseases

Cane and fruit Botrytis (*Botrytis cinerea*)**:** Considerable fruit loss can be experienced particularly during wet springs and summers. In addition, yield loss can occur due to the infection of the nodal buds of primocanes, which may die or become weakened at an early stage of their development or die later in the following spring/early summer when the primocane becomes floricane. Cultivars vary greatly in their susceptibility to cane Botrytis e.g. Glen Ample and Tulameen are particularly susceptible, whilst Malling Juno and Leo are tolerant or resistant to infection. Most growers however continue to apply a routinely programme of fungicides from the early spring up until the onset of (and many during) flowering of the crop. Monitoring aerosols within the crop would allow detection of Botrytis *cinerea* spores and determine the concentration changes of the pathogen during the season. This may assist spray timing events and management of the disease.

Cane spot: Caused by the fungal plant pathogen *Elsinoë veneta*. It is of worldwide distribution but particularly in temperate areas of North America, W. Europe and Australia. The pathogen releases ascospores from diseased tissue (spores involved in local and long range dissemination of the disease) usually continuing during spring-early summer or until autumn. Localised spread of the disease is by rain-splashed conidia from mycelium in overwintered lesions. There are currently no diagnostic assays available for monitoring disease spread or infection. Development of a POC lateral flow test and monitoring of aerosols would enable disease transmission periods to be monitored and informed disease management strategies to be made.

Cane blight: This fungal disease caused by *Leptosphaeria coniothyrium*. The fungus invades through wounds, such as those resulting from fruit catching plates of mechanical harvesters, pruning, and insect damage. Dark brown to purplish cankers form on new canes near the end of the season. Localised disease transmission occurs when spores are produced (pycnidia) on the surface of the necrotic tissue (canker) and are spread by wind, splashing water and insects. The release of ascospores (second spore type produced by the pathogen) in to the air provides opportunity for the pathogen to disperse over a long range. Removal of the necrotic

tissue assists in reducing available inoculum for disease dispersal. There are no diagnostic assays available to monitor disease spread or infection. Development of a POC lateral flow test and monitoring of aerosols would enable disease transmission periods to be monitored and informed disease management strategies to be made.

Powdery mildew: Is caused in raspberries by the fungal plant pathogen *Sphaerotheca macularis*. As with other powdery mildews it is an obligate parasite that produces airborne spores. Most crop loss is from fruit infection rendering them unmarketable. A routine programme of fungicides is usually applied where susceptible cultivars are being grown, and protected by, tunnels, commencing at the first signs of infection on primocane shoot tips or foliage in the spring or just prior to the onset of flowering. Primocane-fruiting crops are usually also sprayed during flowering and harvest to provide control of this disease. Development of a POC lateral flow test and monitoring of aerosols would enable disease transmission periods to be monitored and informed disease management strategies to be made.

HARDY NURSERY STOCK

In the UK, container grown stock in 2008 was recorded at 2,380 ha and 7,139 ha of field grown cropping. This figure remains little unchanged. The diseases identified as of significant economic impact on HNS production are shown in Table 10 and, as identified by Wynn *et al*., 2010. As in strawberry and raspberry production Botrytis grey mould is the major economic limiting factor.

Table 10 . Economic disease impact to the UK HNS Industry in 2008 using then available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
HONS* (container stock) 238 ha	Grey mould	*Botrytis cinerea*	238	4.3
	Downy mildew		238	1.7
	Powdery mildew	*Podosphaerea spp. Oidium spp. Erysiphe spp.*	238	4.3
	Black root rot		71	0.5
	Rhizoctonia		238	1.7
	Bacterial Leaf spot			0.4
	Fungal leaf spot		119	0.4
	Rust		167	0.6
	Verticillium wilt	*Verticillium spp.*	119	0.8

* Container production only as data for field grown stock not available

Diseases (Container – grown and Field)

Botrytis (*Botrytis cinerea*)**:** Botrytis can potentially infect most plants as a true pathogen or as a secondary pathogen, establishing on dead or dying tissue and then infecting healthy tissue. Botrytis spores require moisture to penetrate plant tissue, therefore the disease is more problematic when humidity is high or growing conditions are damp. Cultural measures and manipulation of the growing environment are employed where feasible to limit the severity of this and other foliar pathogens that require these conditions. Such measures include ensuring adequate plant spacing, good air movement and the avoidance of watering late in the day. Protectant fungicides are still used. Detecting early disease levels, a key to good control, is difficult due to the large areas grown in blocks. An environmental risk model and diagnostic approach to monitor airborne spores has previously been discussed.

Downy mildew: Caused by the Oomycete in the family of *Peronosporaceae* which occurs on a wide range of plants in HNS production. Downy mildew pathogens include species of *Peronospora*, *Bremia*, *Plasmopara*, and *Basidiophora*. Most of the Downy mildew fungi are host specific and infect only one plant family. As previously described the downy mildews produce spores which are airborne. They present a challenge to growers because the disease can be asymptomatic for long periods of time due to growth stage effects. Downy mildews are difficult to control with fungicides once established. The pathogens are very different from Powdery Mildews as they attack plants under very different environmental conditions, and are controlled by different classes of fungicides.

Powdery Mildew (Various species)**:** Powdery mildews are caused by species of fungi in six, closely related genera: *Erysiphe, Microsphaera, Phyllactinia, Podosphaera, Sphaerotheca, and Uncinula.* Each species tends to have a very narrow host range. On HNS and ornamentals there are a range of powdery mildew species involved and these are host dependent. Powdery mildews cause significant economic losses by disfiguring and blemishing leaves and flowers. The reduction in marketability, often caused by relatively low levels of infection, necessitates stringent control measures, which are currently achieved by intensive spray programmes. Fungicide spray programmes do not always control the disease satisfactorily due to poor timing or choice of fungicides and may accelerate the selection of mildew strains that are resistant to fungicides. An HDC project (HNS 165) has developed a prototype environmental model for prediction of rose powdery mildew. Field data showed that the model accurately predicted the overall trend of epidemic development. The development of an onsite POC lateral flow powdery mildew test to monitor airborne disease would prove useful because it could detect early onset of disease.

APPLES

Apples are subject to a variety of diseases that can cause minor cosmetic damage or more significant damage, such as reduced yields and even tree death. Apple scab is one of the most common and most serious diseases that afflict apple trees in the UK. It usually appears in early to mid-spring and is more prevalent during rainy weather. The disease is caused by the fungus *Venturia inequalis.* Another important disease of apples in the UK is apple canker is a disease caused by a fungus, *Neonectria galligena*, which attacks the bark of apples and some other trees, causing a sunken area of dead bark and, eventually, death of the branch. New cankers form from mid-spring, and once formed are present all year. Fire blight is a bacterial disease that runs rampant in many parts of the U.S. and also occurs in the UK where it can be difficult to control. Trees infected with fire blight may have water stained, brown blossoms and brown leaves. Fire blight caused by the pathogen *Erwinia amylovora*. Powdery mildew is caused by the fungus *Podosphaera leucotricha* is a serious disease of apples in the UK. Like other powdery mildews it develops first as white, felt-like growths on the undersides of leaves. It over-winters as latent infections of buds on the tree so often once the apple tree is infected it is difficult to eradicate. As the disease spreads, it causes wilted leaves, stunted growth and black pinpoint specks on the leaves and twigs. *Phytophthora* rot thrives in wet, heavy soils. Trees infected with this disease show a decline in vigor and growth and may have yellowish leaves that turn purple in the fall. Brown rot is a fungal disease of top fruit, caused by the fungi *Monilinia laxa* and *M*. *fructigena*. It appears related to fruit damage from other factors. Apple is a perennial crop (with orchards grown over many years) with many diseases present from year to year. As many of the diseases are cosmetic a different approach for control of these diseases must be formulated for control to be successful. For this reason they do not offer the best crop pathogen system for use diagnostics. A better system may be to test trees individually for the presence of over wintering diseases.

PROTECTED ORNAMENTALS

Approximately **940** ha (excludes protected cut flowers) of protected ornamentals are grown in the UK. This figure excludes protected cut flowers. Improved control of Botrytis could achieve an additional £1.1M to the Industry. In the absence of pesticides, major losses in ornamentals would be observed (£15M) by Botrytis and (£5.1) downy mildews (Wynn *et al*., 2010). The diseases identified as of significant economic impact on protected ornamentals are shown in Table 11. As in strawberry, raspberry and HNS production, Botrytis grey mould is the major economic limiting factor.

Table 11 . Economic disease impact to the UK HNS Industry in 2008 using then available crop disease measures

Host	Disease	Pathogen(s)	Area affected (ha)	Cost to Industry (£M)
Protected Ornamentals **939 ha**	Botrytis	*Botrytis cinerea*	141	1.06
	Downy mildew	*Plasmopara obducens, Peronospora grisea Bremia lactucae, Peronospora violae*	94	0.2
	Powdery mildew		28	0.03
	Black root rot		71	0.5
	Root rots	*Pythium* and *Phytophthora spp.*	47	0.1
	Bacterial Leaf spot		19	0.02
	Fungal leaf spot		9	0.002
	Rhizoctonia			0.002
	Rust		19	0.009
	Sclerotinia		19	0.002

Diseases

Any sign of disease on ornamental plants often results in the whole consignment of crop being rejected by retailers and garden centres at delivery; therefore high levels of pest and disease control are important to maintain plant quality and customer satisfaction.

Botrytis cinerea (grey mould): This pathogen remains a challenge for the sector and is able to attack a wide range of plant species. Routine fungicide applications are required to supplement cultural and environmental measures.

Downy mildews: These pathogens are host specific and generally infect only one plant family. Downy mildews infect almost all ornamental plants, as well as, some indoor plants. Greenhouse crops reported to have downy mildew diseases include Snapdragon, Salvia, Alyssum, Pansy, Rose, rosemary, primula, osteospermum, impatiens wallerana, coleus, verbena, Ornamental cabbage, Basil, and Cineraria. Perennial hosts include Aster, Buddleia, Coreopsis, Geranium (not Pelargonium), Geum, Gerbera, Lamium, Delphinium, Veronica and Viola.

In the UK Downy mildew (*Peronospora grisea*) has become the major disease affecting Hebe and can affect the production of many varieties. The disease once

established can cause significant crop loss and in some cases complete crop loss can occur. Recent records show that *Peronospora grisea* is widespread being regularly isolated from infected Hebe nursery stock throughout the UK and Ireland (www.nbn.org.uk). In a report produced for the HDC (HNS 79), the author details that the environmental conditions and the timing of spray applications as potentially critical for the control of downy mildew on Hebes. Blanket spraying has been shown to be ineffective and provide the potential for change in the pathogens sensitivity to the fungicide actives.

The use of disease forecasting systems (based on models describing favourable environmental conditions for disease development and airborne disease transmission events) has become a prevalent approach for controlling fungal diseases of vegetable Brassica crops (HDC FV 233,333,189a, 356). Detecting airborne disease transmission events has the advantage in that the disease can be controlled before the crop becomes infected. This can be effectively achieved by monitoring the presence of pathogenic inoculum in air samples. A generic 'family ' POC lateral flow could be developed to include the main downy mildew species and / or tailored to deliver a multiplex test format able to differentiate between the key species involved in ornamentals production.

Powdery mildews: The disease results from infection by species of fungi in six, closely related genera: *Erysiphe, Microsphaera, Phyllactinia, Podosphaera, Sphaerotheca, and Uncinula.* Each of which tend to have a very narrow host range. On HNS and ornamentals there are a range of powdery mildew species involved and these are host dependent. An individual plant may sometimes be affected by more than one powdery mildew e.g. Poinsettias. As with the downy mildews it would prove useful to monitor glasshouse aerosols for powdery mildew spore presence. This could be in the format of a generic 'family' POC test or a multiplex lateral flow to differentiate key species.

SECTION 3 – ANALYSIS OF PATHOGEN SPECIFIC POINT OF CARE (POC) DIAGNOSTICS FOR UK HORTICULTURE CROPS

The following analysis has been produced based on the economic impact of specific pathogens and the most appropriate diagnostic technique for detection of these. The diseases have been identified based on their economic importance to the UK horticultural industry and to their prevalence across horticultural sectors.

TARGET PATHOGENS AFFECTING CROSS-SECTORAL HORTICULTURAL CROPS

(1) Single disease diagnostic array systems

***Botrytis cinerea*:** In 2009, Botrytis infection provided the second greatest cause of crop loss to the Horticulture Industry by reducing harvest yields and marketability. A total cost to the industry of £54 million was recorded. Primarily affecting strawberries (£33M) but also impacting on onions, lettuce, raspberries, tomatoes, hardy nursery stock and ornamentals. POC lateral flows are available commercially to confirm presence of the pathogen in plant tissues but only once exhibiting disease symptoms. This results largely from a sampling issue. The large numbers of samples required to detect Botrytis in plant tissues is uneconomic and erroneous. These tests have been discussed in earlier sections of the report. However the disease is able to spread quickly between and within cropping systems by the production of airborne spores. In the UK, using either laboratory based analysis (ELISA) or a field based POC pregnancy style test, HDC funded work has provided the development of systems to monitor field aerosols for target disease inoculum either on a daily or weekly basis. Air sampling systems and tests are used in vegetable Brassicas. By identifying disease (spores) in field air samples growers can time sprays more effectively and make informed decisions on which type of fungicide application to make. The development of a test to monitor *B. cinerea* in air samples could have a major impact on the implementation of effective and timely disease control measures to prevent initial disease development and, if present, assist in the containment of the disease. Routine monitoring of crop aerosols for disease may prove useful in establishing the effectiveness of environmental conditions used as part of an integrated disease management system. Table 12, lists the horticultural sectors where a generic *B. cinerea* POC lateral flow test may prove useful and the potential cost implications using current disease control measures.

Table 12 . Economic disease impact of *Botrytis cinerea* to the UK Industry in 2008 using then available crop disease measures

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Strawberry	Grey mould	*Botrytis cinerea*	4770	33.3
Raspberry	Grey mould	*Botrytis cinerea*	1634	4.7

Protected Ornamentals	Grey mould	*Botrytis cinerea*	141	1.06
HONS	Grey mould	*Botrytis cinerea*	238	4.3
Cucumber	Grey mould	*Botrytis cinerea*		0.1
Tomatoes	Grey mould	*Botrytis cinerea*	53	5.5
Lettuce	Grey mould	*Botrytis cinerea*	5592	5
Total				**53.96**

Powdery Mildew: In 2009, Powdery mildew was recorded to cost the industry £39 million. The withdrawal of key downy mildew fungicides such as mancozeb increased losses in outdoor lettuce to £29 million and onions at £26 million (Wynn *et al*., 2009). Similarly as for Botrytis a POC lateral flow test could be developed to monitor crop aerosols for disease transmission events of the Powdery Mildew pathogens. It would be recommended that a 'family' specific Powdery Mildew be developed for general monitoring with the capability to identify and differentiate at genus level: *Erysiphe*, *Microsphaera*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca*, and *Uncinula*. At a crop specific level it may be more appropriate to develop a multiplex disease crop POC test (i.e. for the key airborne diseases) but include the specific Powdery Mildew species involved. Table 13, identifies the current powdery mildews involved and their associated crop losses in UK Horticultural production systems.

Table 13 . Economic disease impact of *Powdery mildew species* to the UK Industry in 2008 using then available crop disease measures

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Strawberry	Powdery mildew	*Podosphaera aphanis*	3339	24.7
Raspberry	Powdery Mildew	*Sphaerotheca macularis*	817	2.3
HONS / Ornamentals	Powdery mildew	*Podosphaera spp* *Oidium spp* *Erysiphe spp*	238	4.3

Tomatoes	Powdery mildew	*Oidium neolycopersici Golvinomyces orontii*	106	3.9
Cucumber	Powdery mildew	*Podoshaera fusca (xanthii) Golovinomyces cichoracearum*	105	2
Ornamentals	Powdery mildew		28	0.03
Carrots	Powdery mildew (Cercospora spot)	*Erysiphe heraclei*	4411	1.9
Brassicas	Powdery mildew	*Erysiphe cruciferarum*	4008	1.4
TOTA L				**40.53**

Although grape (*Erysiphe necator*), apple and pear (*Podosphaera leucotricha*) crops have not been covered in this review the economic importance and the widespread distribution of powdery mildew to these crops is significant. For these crops there is the potential to develop a specific lateral flow (grape) or to be included as part of the powdery mildew 'family' lateral POC aerosol test.

Downy Mildews: Improved disease surveillance methods are vital if the industry is to provide early warnings of disease epidemics to control diseases efficiently, in a sustainable way and consistently with minimal pesticide usage. Although downy mildews are an important disease affecting many crops (Table 14), the occurrence of this type of disease is greatly influenced by the cultivars grown. The disease action threshold would vary for different cultivars. Equally where downy mildew resistance is established (i.e. *Bremia lactucae* in lettuce production) this would necessitate the development of diagnostic systems at the molecular level. A single POC lateral flow has however been developed for monitoring field aerosols of *P. destructor*. This test will in 2014 – 2015 be validated as part of an Integrated disease management system (IDM) for control of onion downy mildew in UK horticultural cropping systems.

Table 14 . Economic disease impact of Downy Mildew species to the UK Horticulture Industry in 2008 using then available crop disease measures.

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Brassicas	Downy Mildew	*Hyaloperonospora parasitica*	5344	1
Ornamentals	Downy mildew	*Plasmopara obducens, Peronospora grisea Bremia lactucae, Peronospora violae*	94	0.2
HONS	Downy mildew		238	1.7
Lettuce	Downy mildew	*Bremia lactucae*	5592	10
Onions	Downy mildew	*Peronospora destructor*	8575	3.5
Total				**16.4**

Sclerotinia: *Sclerotinia* sp. infects over 100 species of plants to include numerous economically important crops of which some are shown in the table below (Table 15). The development of a POC lateral flow could have potential use in both soil and aerosol analysis. An aerosol test to identify disease risk of ascosporic inoculum of Sclerotinia species would have applications across sectors and be readily incorporated within the existing sampling technologies. The development of soil tests would require studies to determine an optimal sampling methodology and extraction processes for quantification of sclerotia from all UK soil matrices.

Table 15 . Economic disease impact of Sclerotinia to the UK Horticulture Industry in 2008 using then available crop disease measures

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Lettuce	Sclerotinia	*Sclerotinia sclerotiorum* and	5592	5.4

		minor		
Onions	White rot (not related to Sclerotinia but produces large numbers of soil borne sclerotia)	*Sclerotium cepivorum*	1715	0.4
Protected Ornamentals	Sclerotinia		19	0.002
Cucumber	Sclerotinia stem rot	*Sclerotinia sclerotiorum*		0.02
Carrots	Sclerotinia	*Sclerotinia sclerotiorum*	8822	8.1
Total				**13.9**

Alternaria: The Alternaria genus provides a number of fungal plant pathogens which are considered to be of worldwide economic importance. Alternaria is also the most important source of fungal allergen affecting human health. An '*in field*' POC test is available commercially to monitor dark leaf spot in collected aerosols. This test format could be expanded to include *Alternaria dauci* on carrot. It may prove useful in the development of a lateral flow to key horticulture *Alternaria* pathogens (Table 16) to include a genus specific POC lateral flow that is able to identify presence of aeroallergenic *Alternaria* species. This could prove useful in marketing the POC to a wider audience of global aspect.

Table 16 . Economic disease impact of Alternaria to the UK Horticulture Industry in 2008 using then available crop disease measures

Hosts	Disease	Pathogen	Potential acreage affected	Loss (£M)
Carrots	Leaf blight	*Alternaria dauci*	8822	3.8
Brassicas	Dark leaf spot	*Alt. brassicae/ brassicicola complex*	18703	9.8
Total				**13.6**

Pythiaceous fungi: Generic POC lateral flow devices utilising polyclonal antibody technology exist commercially for the detection of *Pythium* and *Phytophthora* species. However these fungi are ubiquitous in the environment and, as discussed earlier, there is no capability to differentiate between the saprophytic and mycoparasitic i.e. the useful naturally occurring biological control agents of *P.oligandrum*, *P.nunn*, *P. perioplocum* and *P. acanthicum* species. In irrigation systems it would be useful to differentiate target viable pathogenic Pythium and Phytophthora species from non-viable propagules and, the closely related genus of Saprolegnia. For this purpose it is important that monoclonal antibodies are developed to key Pythium species (Pythium HS group, *P. ultimum*, *P. aphanidermatum*, *P. intermedium*, *P. irregulare*, *P. sulcatum*, *P. sylvaticum* and *P. aphinidermatum* and *P. violae*) and which can differentiate useful mycoparasites. Equally this would apply to the development of a *Phytophthora* POC lateral flow assay e.g. *P. cryptogea, P. cacotorum, P. infestans, P. ramorum* etc. The successful development of monoclonal antibody cell lines to target species would allow the development of a generic and species specific POC lateral flows for use in soil, water, plant tissues and air. However the deployment and incorporation in to test formats would depend on the medium and whether the test required differentiation between viable and non-viable propagules. In irrigation water the potential exists to deliver an on-line continuous immuno-biosensor to monitor for presence of disease propagules of target *Pythium* and *Phytophthora* species.

Table 17 shows the UK horticultural cropping systems affected by Pythium and Phytophthora species, the acreages affected and the losses using incurred using available disease control measures in 2008 (Wynn *et al.*, 2010)

Table 17 . Economic disease impact of Pythium and Phytophthora species to the UK Horticulture Industry in 2008 using then available crop disease measures

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Cucumber	Root rot	*Hort. pathogenic Pythium spp.*	5	2
Tomato	*Late blight	*Phytophthora infestans*	4	0.2
Tomato	Root and stem rot	*Hort. pathogenic Pythium spp.*	2	0.4
HONS	Root rot	*Hort. pathogenic Pythium spp.*	21	0.5
Ornamentals	Root rot	*Pythium* and *Phytophthora*	47	0.1
Leeks	*White tip	*Phytophthora porri*	659	1.3

Carrots	Cavity spot	*Pythium violae / sulcatum*	2206	2.9
Brassicas	Transplant damping off	*Hort. pathogenic Pythium spp.*	*	*

*Airborne monitoring early warning system

(2) Muliplex diagnostic assay systems for crop specific pathogens.

In this section the report looks to identify where the development of POC lateral flow multiplex diagnostic assay systems could be developed to assess environmental samples (soils, water plant or air) for crop specific pathogens. Only those cropping systems where a multiplex disease approach is considered economic will be discussed.

Cucumber: HDC PE 001 has provided the development of a laboratory based immunoassay system (MTIST air sampler and ELISA) to monitor airborne spore disease transmission events of Mycosphaerella *melonis* in commercial cucumber cropping systems. This process could be expanded to incorporate a multiplex format to monitor airborne spore inoculum of the pathogens described in Table 18. Other pathogens affecting cucumber are too minor to be considered. Equally, this approach could be extended to allow assessment of the collected air sample on site by growers using a multiplex POC lateral flow. As discussed earlier and, for each disease, information about the presence of sufficient pathogen concentration coupled to the associated environmental parameters should be determined to provide accurate disease thresholds at which damage may occur on the crop. In this way growers should be able to make informed, targeted and potentially cost effective control measures to contain or eradicate disease(s).

Table 18 . Economic impact of disease to the UK Cucumber Horticulture Industry in 2008 using then available crop disease measures

Aerial sampling

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Cucumber	Gummy stem blight	*Mycosphaerella melonis*	32	3
Cucumber	Powdery mildew	*Podosphaera*	105	2

		fusca(xanthii) Golovinomyces cichoracearum	
Cucumber	Sclerotinia stem rot	*Sclerotinia sclerotiorum*	0.02
Cucumber	Grey mould	*Botrytis cinerea*	0.1
Total			**5.12**

Lettuce: As described for cucumber, the concentration of pathogens (Table 19) in collected aerosols of Lettuce crops could be assessed by the development of a multiplex POC lateral flow test. For each disease, information about the presence of sufficient pathogen concentration coupled to the associated environmental parameters should be determined to provide accurate disease thresholds at which damage may occur on the crop. In this way growers should be able to make informed, targeted and potentially cost effective control measures to contain or eradicate disease(s). For *Bremia lactucae* it may prove necessary to provide additional information depending on the cultivar grown. Where *B. lactucae* resistant isolates are considered important it may be more appropriate to utilise a laboratory molecular system for analysis. If however an on-site or plant clinic approach is preferred the development of a LAMP and / or combined with a nucleic acid lateral flow (NALF) system may prove less costly and able to quantify disease potential.

Table 19 . Economic impact of disease to the UK Horticulture Lettuce Industry in 2008 using then available crop disease measures

Aerial sampling

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Lettuce	Ringspot	*Microdochium panattoniatum*	1398	1.2
Lettuce	Sclerotinia	*Sclerotinia sclerotiorum* and *minor*	5592	5.4
Lettuce	Grey mould	*Botrytis cinerea*	5592	5
Lettuce	Downy mildew	*Bremia lactucae*	5592	10
Total				**21.6**

Strawberries and Raspberries: In the UK nearly 6500 ha of strawberries and raspberries are cropped on an annual basis. Routine fungicide applications are made in an effort to control disease. As discussed earlier, an environmental model has been developed for *Botrytis cinerea* and powdery mildew on grapes. This model could be expanded to cover these two cropping systems. The development of monoclonal antibody cell lines to specifically label the airborne stage of each of the pathogens listed in Tables 20 and 21 could provide the opportunity to monitor airborne disease concentrations during the production season. This may lead to the improved timing of fungicides and increase the efficacy of the products when applied. Improved control early in the season and knowledge of disease transmission periods could prove useful in determining a suitable harvest schedule and the control of post storage disease development.

Table 20. Economic impact of disease to the UK Horticulture Strawberry Industry in 2008 using then available crop disease measures

Aerial sampling

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Strawberry	Grey mould	*Botrytis cinerea*	4770	33.3
	Powdery mildew	*Sphaerotheca macularis*	3339	24.7
Total				**58**

Table 21. Economic impact of disease to the UK Horticulture Raspberry Industry in 2008 using then available crop disease measures

Aerial sampling

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Raspberry	Cane spot	*Elsinoe veneta*	817	2.4

	Cane blight	*Leptosphaeria coniothyrium*	817	2.3
	Powdery Mildew	*Podosphaera aphanis*	817	2.3
	Grey mould	*Botrytis cinerea*	1634	4.7
Total				**11.7**

HNS and Protected Ornamentals:

Developing a POC lateral flow multiplex to monitor airborne disease concentrations of economically important HNS and Protected Ornamentals is problematic given the breadth of powdery and downy mildew species involved (Table 23). However there is the capability from an air sample to split across multiple POC lateral flow devices (Fig. 3). In this way it would be possible to identify key plant pathogenic fungi at a genus level and, as appropriate identify to a species level those organisms which are of significant economic impact or interest. In this way the tests become interchangeable and can be used as a single test or if additional information is required the additional tests can be bolted on to provide added value. Within this setting it would need to be decided whether a qualitative test (presence / absence) was appropriate or a quantitative test. This may prove different depending on the pathogen. Where information about the presence of sufficient pathogen concentration is required, the associated environmental parameters should be determined to provide accurate disease thresholds at which damage may occur on the crop.

Figure 3. Multiplex lateral flow

Equally there is a need to provide diagnostic capability to quickly identify pathogen type on infected plant tissue. This can be particularly problematic for the protected edible crops, HNS and the ornamental industries. Table 22, provides a list of fungi identified that should be included within a multiplex POC lateral flow to provide growers with a one stop test to quickly evaluate disease symptoms on infected plant tissues.

Table 22. Economic impact of disease to the UK HNS and Ornamentals Industry in 2008 using then available crop disease measures

Aerial sampling

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Ornamentals and HONS	Powdery mildew	*Podosphaera spp* *Oidium spp* *Erysiphe spp*	238	4.3
	Downy mildew	*Plasmopara obducens,* *Peronospora grisea* *Bremia lactucae,* *Peronospora violae*	332	1.9
	Sclerotinia	*Sclerotinia spp.*	19	0.002
	Black root rot	*Thielaviopsis basicicola*	*	*
	Grey mould	*Botrytis cinerea*	379	5.36
Total				**11.6**

*No data available

Table 23. Economic impact of diseases to the UK HNS and Ornamentals Industry in 2008 using then available crop disease measures

Crowns, lower stems and Root rots - Multiplex

Host	Disease	Pathogen	Potential acreage affected	Loss (£M)
Protected Crops / HONS	Plant tissue	Pythium and Phytophthora (Pathogenic)	75	3.0*
	Plant tissue	*Fusarium*		
	Plant tissue	*Rhizoctonia solani* (Pathogenic)	238	1.7
	Plant tissue	*Thielaviopsis basicicola*	*	*
	Plant tissue	*Verticillium*	*	*

*Brassica transplant data not included

SECTION 4 – SUMMARY

Diseases of UK Horticultural cropping systems impact significantly on crop yield and quality on an annual basis. A major problem for producers is that diseases are moving targets that evolve in response to agricultural practices and environmental change. With more than 300 crops and, with a combine home-produced value of around 2.23 billion (farm gate), effective disease control remains a priority. With a reduction in broad spectrum fungicides available for use and, limited 'off label' approval, there is a real urgency to utilise these efficiently and with optimal disease control performance. This means a move away from routine pesticide application to targeted crop treatments and improved horticultural practices i.e. good sanitisation, crop rotation.

Early disease diagnosis and pathogen detection are central to the ability to protect crops. The success of this approach will depend on how the information is evaluated and then incorporated within an integrated disease management system. Using diagnostics in crops for detection of pathogens requires some information on sampling requirements. Air samples have been used successfully both in protected and outdoor crops for disease detection. Individual thresholds have been determined and the detection of many pathogens in air samples can be easily calibrated to determine spray/economic damage thresholds. Much of this however is influenced by the cultivar grown, the environment and cropping conditions. The detection of pathogens in soil or water samples is more difficult and has its challenges. Soil provides a diverse matrix which can alter considerably within a sampling area and influence pathogen distribution. Issues of sensitivity, specificity , non-specific binding of the diagnostic probe and soil inhibitors are well documented in assay development. Efficient and simple extraction of pathogen material is one of the biggest hurdles in the development of a quick and sensitive test. Sample size is an issue with nano technology demanding smaller and smaller sample volumes. This will likely not reflect well on a large cropping acreage. Often biological amplification is required by soil baiting, and although this can provide information of viability of the target organism it makes quantitative readings more difficult. Soil tests which require a pre-treatment i.e. drying, grinding, centrifugation and floatation processes to recover pathogen structures prove laborious and expensive in time and space. These are not suitable for "on-site" tests. Experience with soilborne pathogens means that economic thresholds are very different even when affected by the same disease e.g. clubroot affecting vegetable Brassicas in comparison to clubroot affecting oilseed rape. Carrying out the diagnostic test in soil and water samples will require information on optimal sampling and sample processing. While some of this information is available the format for new tests will depend on the pathogen(s) and may require additional experimentation and validation studies.

The system should be evaluated on a cost return basis and suitability of use. Growers are unlikely to invest in equipment that is expensive and requires an annual maintenance contract, staff to operate and the equivalent to a small laboratory. The report focuses on those techniques which do not require these approaches.

CONCLUSIONS

(1) Develop new rapid (in field) diagnostic tests for major cross-sectoral horticultural pathogens as single tests.

Consideration should be given to the development of single “on site “ tests which could be used on major pathogens which are cross-sectoral. A good example of this would be the development of a test for *Botrytis* spores. This test would be mostly used within protected cropping and ornamental production. Botrytis affects a wide range of crops and the usage of the test would result in economies of scale in production. The results of the test would be widely applicable (e.g. glasshouse production) in situations where more than one crop was present. Application of optimal sampling periods in air would ensure that spores detected were recently produced or viable. There is ample evidence to suggest that sampling periods should be adjusted in relation to wind speeds outside the glasshouse. There was a significant relationship when *Botrytis* in air samples was compared to wind speeds in glasshouse tomato production (See DEFRA project HH3611SPC). The widespread scale of tomato and ornamental production makes air sampling the only realistic approach that can determine the occurrence of *Botrytis* at low levels pre symptomatically. Recently the approach has been proven in the control of *Mycosphaerella melonis* in glasshouse cucumbers where the occurrence of the disease in crops was detected before symptom appearance. Provided that effective control treatments were available, action thresholds can be determined from these air samples that would enable control measures to be taken in an effective manner. Another major cross-sectoral airborne pathogen is powdery mildew where this approach could be used. Soilborne pathogens exist in horticultural crops as major cross-sectoral problems e.g. *Sclerotinia*, *Verticillium* and Pythium species. In soil the accuracy of the test is influenced by the sampling regime used and also the processing of the samples. However generic approaches used in the development of the clubroot (*Plasmodiophora brassicae*) test in vegetable Brassica growing areas could be used in the development of diagnostic approaches.

(2) Develop and integrate existing rapid tests for airborne pathogens of horticultural Brassicas, protected crops and bedding plants into one rapid test format (multiplex).

Where more than one important pathogen occurs on the crops diagnostic test requirements would mean that one test should be developed which could be used to detect more than one pathogen. In outdoor crops the best examples of this are in vegetable Brassicas and onions where single tests have been developed but in certain areas the main pathogen are different. For example in Scotland the most important pathogen is light leaf spot (*Pyrenopeziza brassicae*). However this pathogen is of secondary importance in England to white blister (*Albugo candida*)

and ringspot (*Mycosphaerella brassicicola*). Scotland is too cold for white blister to be a major threat. Therefore Brassica pathogen multiplex “on site” detection tests would be different for Scotland compared to England. Within England there may also be differences such as the importance of powdery mildew in drier warmer climates. To assist this the format shown in Figure 3 (multiplex lateral flow) may prove optimal in development and would allow tests to be interchangeable depending on the users requirements.

Within glasshouse crops diseases such as powdery mildew could be combined with *Botrytis* or for cucumbers *Mycosphaerella melonis* for detection in air samples. Combined tests would also be useful for air samples where only one sample collection device is available for one time period. However if several pathogens were to be detected individually from the same collected air sample it is important to determine each spore types characteristics. For example *Alternaria* and ringspot in vegetable Brassicas can be detected in the same air sample vial. However ringspot spores remain attached to the tube while *Alternaria* spores in solution are easily removed. The individual tests can be carried out provided they are in the correct sequence. Where combinations of pathogens need to be detected an approach which can allow multiple testing will need to be developed. The example shown in Figure 3 may prove useful for this purpose. Air samples can be combined as dual tests because the same sampling issues do not exist as observed for soil and water. Multiplex “on site“ tests for existing vegetable Brassica lateral flow tests could act as the first range of tests to be combined in this way.

(3) Develop on site testing for soil samples which includes rapid and simple steps enabling processing and concentrating pathogen content in samples for more sensitivity in detection.

Using diagnostics in crops for detection of pathogens requires some information on sampling requirements. Air samples have been used successfully both in protected and outdoor crops for disease detection. Individual thresholds have been determined and detection of many pathogens in air samples can be easily calibrated to determine spray/economic damage thresholds. Much of this is influenced by the spacing in outdoor crops or cropping conditions in combination with the type of glasshouse and its control characteristics for protected crops. Detecting pathogens in soil or water samples is more difficult due to the specific distribution of the pathogens in soil samples for example. Experience with soilborne pathogens means that economic thresholds are very different even when different crops are affected by the same disease e.g. clubroot affecting vegetable Brassicas in comparison to clubroot affecting oilseed rape. Carrying out the diagnostic test in soil and water samples will require information on optimal sampling and sample processing. This is particularly important in molecular diagnostics where different DNA extraction kits processing variable soil sample sizes exist. The pre-processing of samples for some

soilborne diseases may be necessary to improve sensitivity of the POC tests. Integration of existing information and techniques from experience in the detection of clubroot in soils can be adapted for other soilborne diseases to form an easy extra step in the usage of the “on site” tests.

(4) Develop laboratory testing facilities for multiple testing of air, plant, soil and water samples from horticultural crops. Identify from laboratory testing those combinations which could be developed as on site tests to incorporate new technological developments.

Developing molecular information which could be used in the development of “on site” tests would be important for soil pathogens. The clubroot lateral flow was successfully developed and validated by cross referencing LFD reactions with the results from a molecular diagnostic test for contaminated soil samples. Further development of molecular approaches for soil borne pathogens affecting horticultural crops would be necessary. Existing markers for some soil pathogens exists (*Verticillium*) but these have not been used in a wide range of sample types. The approach might be particularly useful where there is a disease complex with many potential pathogens present such as with *Pythium* and *Phytophthora* in hydroponic systems. Here it may be important to integrate the information to the growth stage of the crop.

Development of sustainable disease management approaches for glasshouse and field crops which utilise cultivar resistance also requires information on the genetic variation of the pathogen. Utilising race specific molecular markers which could recognise field races of some downy mildew pathogens (*Bremia lactucae*) would be a major advance in the deployment of resistance at the site specific level. At the moment differentiating between races of *Bremia lactucae* is carried out by specific reactions on a differential set of cultivars. Lettuce growers currently rely on the IBEB (The International *Bremia* Evaluation Board) system, which attempts to assign isolates to a race according to the presence or absence of growth on a set of differential cultivars. Although this helps breeders to determine the prevailing isolates for next season, it is time consuming and it does not help growers to make a rapid informed decision. In addition, using this conventional technique, investigations on epidemics are impossible and generating rapid diagnostics to determine the pathogen spread would be difficult. A diagnostic laboratory test would be considerably more useful in every sense, especially to the grower, allowing them to rapidly deploy cultivars resistant to the prevailing *Bremia* isolates or for other downy mildews. As more genetic information become available the possibility exists where by molecular screening of downy mildew pathogen isolates can be developed for other crop pathogen complexes. The information could be used by growers to deploy any available resistance in cultivars more effectively. It is possible that in the future these molecular laboratory based tests for this purpose could be used “on site”.

The challenge for the scientist in developing “on site” molecular approaches remains the ability to identify, select and modify the molecular approach to provide a simple diagnostic tool. This tool must deliver useful information to the end user, be robust and mindful of the economies of scale. On development of a test it is essential that it is extensively validated against existing adopted systems. This should include testing across the industry, by the end user and over successive seasons to ensure consistency and accuracy of the results based on crop quality and yield. In the United States the development of a grower ‘LAMP PCR’ was found not to be feasible when participants were not successful in interpreting the results and sensitivity of the DNA assay proved problematical with cross-contamination of samples. However further developments in technology will make this type of application more easily achievable in the future.

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HDC CP 099A: Validation of the clubroot lateral flow in UK commercial Brassica cropping systems.

HDC CP 099: Diagnostics; Validation of the lateral flow detection devices for the light leaf spot and powdery mildew vegetable Brassica pathogens and testing of white blister detection test prototypes.

HDC FV 349: Further development of in field tests for resting spores of clubroot and development of clubroot control based on detection.

HDC FV 356: Onions; further development and calibration of detection tests for conidia of onion downy mildew in combination with the Morph forecast model MILIONCAST.

HDC PE 001: Cucumber, Improving control of gummy stem blight caused by Mycosphaerella melonis (Didymella bryoniae).