Project title:	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
Project number:	CP 099
Project leader:	A Wakeham, National Pollen and Aerobiology Research Unit, University of Worcester, Henwick Grove, Worcester WR2 6AJ
Report:	Annual report, August , 2014
Previous report:	Annual report August 2013
Key staff:	G. Keane S. John G. Petch
Location of project:	University of Worcester
Industry Representative:	A Richardson, Allium and Brassica Centre, Wash Road, Kirton, Lincolnshire.
Date project commenced:	31st July, 2012
Date project completed (or expected completion date):	March ,2015

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

.Headline

- In-field lateral flow tests have been used to measure field bioaerosols for light leaf spot, Brassica powdery mildew and white blister.
- Each has a diagnostic range suitable for use in disease risk forecast studies**.**
- A spore concentration (white blister) in excess of 20 spores per cubic metre of air sampled is thought to be required for disease expression on susceptible plants under suitable environmental conditions. A trial will be repeated in 2015 to confirm these findings.
- The unusually dry and hot conditions experienced during June and July 2013 is likely to have affected Brassica powdery mildew establishment in the trial crops. Spore concentrations during this period were identified (70 spores per cubic metre of air sampled) but no disease establishment was observed. The trial will be repeated in 2015 to establish the biological disease threshold (no of spores) required for disease establishment in the crop.

Background

Background and expected deliverables

In the airborne environment many plant diseases are able to spread between and within cropping systems. In the UK, using either laboratory based analysis or a field based 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 available for the following vegetable plant pathogens: *Peronospora destructor* (onion downy mildew), *Mycosphaerella brassicicola* (ringspot), *Alternaria brassicae* (dark leaf spot), *Pyrenopeziza brassicae* (light leaf spot), *Erysiphe cruciferarum* (Brassica powdery mildew) and *Albugo candida* (white blister). 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.

Studies measuring *M. brassicicola* (ringspot) in airborne spore samples has shown that under ideal environmental conditions, high concentrations of spores are required in the air for infection to occur (2000 spores per cubic metre). The current study aims to identify bioaerosol concentrations of light leaf spot, powdery mildew and white blister spores that are required to cause disease symptoms on crops at a commercial scale. The developed diagnostic test formats for each of these diseases will be adjusted to reflect this. Results from previous studies show that light leaf spot ascospores appear in the air in large enough

levels to be a problem only during discrete periods. Light leaf spot inoculum may be present at other times but occurs at too low concentrations to become a problem in developing sprout crops. Fungicides applications can provide good control of Light leaf spot in Brussels sprout crops if applied at times when the disease is in the air. Where routine 'blanket' crop spray programmes have been applied, control can be ineffective. Light leaf spot is endemic in Scotland and becoming common in Brassica production areas of Northern England. Targetted application of effective fungicides in response to spore concentrations can play a vital role in controlling the disease. Inappropriate or unnecessary fungicide applications are not only costly but will increase the pressure for development and selection of pathotypes able to resist previously effective control measures.

Powdery mildew as, light leaf spot can infect any above ground plant part reducing plant growth and yield. Most horticultural brassicas are susceptible to infection and these include Brussels sprouts, cabbage, Chinese cabbage, kohlrabi, broccoli, kale, mustard, collards, cauliflower, radish, and horse radish. Powdery mildew disease is highly airborne and small numbers of conidia (spores) can be wind dispersed over large distances. To date there is little information on the environmental requirements for Brassica powdery mildew development although it appears to be favoured by dry conditions and, in the UK, these usually only occur during early summer. Infection of vegetable Brassica crops is unaffected by the powdery mildew occurring on oilseed rape crops. The occurrence of older tissues where powdery mildew development is more favoured, during autumn and winter, may act as a bridge for the pathogen to occur on Brussels sprouts buttons. Applications of fungicidal sprays (Nativo) are approved for control of the disease however as for light leaf spot, information about the availability of powdery mildew inoculum would be useful in control regimes. The airborne concentration of powdery mildew required for disease development in the crop is unknown but it is thought to play a vital role in the initial development of powdery mildew in brassica crops.

White blister is caused by the oomycete pathogen *Albugo candida* and is a common disease of many economically important cruciferous vegetables and oilseed crops. Significant yield losses from this disease have been reported on the oilseeds *B. rapa* and *B. juncea* and, to a lesser extent, on susceptible lines of *B. napus.* 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. To date, more than 10 distinct biological races of *A. candida* have been identified and classified based on host specificity. Race 9 infects *B. oleracea* and

management strategies to control the disease in these crops has included the development of an environmental model (Brassica spot™ – White Blister model). The present study aims to improve the white blister disease risk forecast by including information on availability of *A. candida* airborne disease. Monoclonal antibodies with reactivity to *A. candida* (Race 9) spores will be used in an immunological chromatographic test strip format (lateral flow) to provide information on *A. candida* concentration in collected field air samples. Similarly, lateral flow tests and laboratory diagnostic tests developed in HDC FV33 for identification of airborne disease of light leaf spot and Brassica powdery mildew will be adjusted for commercial field usage. Enabling provision of information for requirement of fungicide spray applications in response to peaks in airborne spore numbers.

The expected deliverables from this project are:

- Measure disease in field aerosols for light leaf spot, powdery mildew and white blister. Evaluate the effect on infection and symptom development in commercial Brassica cropping systems
- Provide tests which can be used directly by UK growers or consultants to identify presence of these three diseases in the air. Identify the spore concentrations likely to cause disease at a commercial scale.
- Ability to detect white blister, Brassica powdery mildew and light leaf spot in field bioaerosols before disease is visible in the crop.
- Improved use of fungicide applications within vegetable Brassica production systems and the reduced likelihood of tebuconazole resistance within light leaf spot populations (already reported).
- Assess the potential to develop a multiplex test to identify risk of multiple pathogens on a single test device
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Summary

Diagnostic Tests

Batches of lateral flows (rapid on-site tests) have been used successfully to measure field bioaerosols for light leaf spot, Brassica powdery mildew and white blister. Each has a diagnostic range suitable for use in disease risk forecast studies. The development and use of the tests has the advantage of detecting the very earliest possibility of disease occurrence. Each test should provide a shelf life of between one and two years at room temperature. A multiplex text has been developed for the measurement of light leaf spot and white blister spores from a single bioaerosol sample. These tests will be used in the field and their use reported on in the final report (2015).

Field Trials

Light leaf spot

It is probable that the regional harvest of oil seed rape provided the source of the light leaf spot disease plumes recorded in field trials from July through to September 2013. In conjunction with environmental data, the development of regional oilseed rape harvest source maps could provide a useful tool towards forecasting light leaf spot disease risk on Brussels sprout crops in the UK. During at risk periods the on-site tests could be used to confirm and measure the spore (disease) concentration. This would provide a targeted and inexpensive (approx £7 / test) way of determining disease risk.

Brassica Powdery Mildew

Brassica powdery mildew spores were identified in field bioaerosols during the trial period. In July 2013, disease pressure for powdery mildew intensified. More than 70 spores per cubic metre of air were identified during this period in the weekly collected crop bioaerosols. However no powdery mildew disease was recorded on any of the plants in the trial. This is likely to have been a result of the environmental conditions. The UK Met Office recorded 2013 as the seventh sunniest summer since records began in 1929. A prolonged heatwave remained in place to the middle of July, when temperatures regularly passed 30ºC (86F). The south east of the country, to include the location of the trial, recorded the lowest amount of rainfall since 1995. The unusually dry and hot conditions may have prevented establishment of the disease in the crop. The trial will be repeated in 2014 to confirm the concentration of spores required to initiate powdery mildew in the crop. The results of which will be reported in HDC report CP99 July, 2015.

White blister

Conversely, white blister disease became established in the trial crop during September 2013. A spore concentration of >20 spores per cubic metre was estimated to have provided risk of disease development. Using these criteria the in-field lateral flow test was able to detect the disease in weekly collected bioaerosols ahead of symptom development on the crop. Improved management of the disease and, reduced applications with effectiveness of the fungicides applied, should be achieved by including information on availability of spore concentration (i.e. using an on-site test) with the white blister forecast model (MORPH Brassica spot). An extension of HDC CP99 will look to confirm this in Year 3 of the project (six month extension). The results of which will be reported on in July 2015.

Financial benefit

The specific action points for growers at this stage in the project are:

- The light leaf spot in field test has a disease threshold set at 200 spores per cubic metre air sampled for provisional timing for application of Signum to vegetable Brassica crops. This is likely to improve the efficacy of this chemical especially in production of vegetable brassicas in Northern Britain.
- The white blister in field test has a disease threshold set at >20 spores per cubic metre air sampled. The tests should be used in conjunction with the MORPH (Brassica Spot) white blister disease forecast.

Action Points

- The light leaf spot in field test kit will be available to the UK horticultural industry from 2015. The Brassica powdery mildew and white blister field test will be available from 2016. Alison Wakeham at NPARU can be contacted for further information.

SCIENCE SECTION

Introduction

Antibody probes developed to disease propagules of *Erysiphe cruciferarum* (Brassica powdery mildew) *Pyrenopeziza brassicae* (Light leaf spot) and *Albugo candida* have been incorporated in to lateral flow format for 'in field' testing. The lateral flow devices have been developed to detect disease of Brassica powdery mildew, light leaf spores and white blister in collected field aerosols The tests are semi-quantitative and based upon test line depletion (visual or by electronic measurement), spore concentrations in the air can be estimated. A control line remains constant to show that the test has worked. The test is counter intuitive in that as spore concentration increases the test line decreases in colour intensity. At high spore concentrations no test line is visible (Fig. 1).

Figure 1. A competitive lateral flow assay with powdery mildew spore numbers measured between 0 – 4800.

In a field setting a cyclone air sampler is used to sample daily aerosols. The air sample can be collected in to a single tube over multiple days (i.e. a week) or as daily aerosols using a multi-vial sampler (Fig. 2). The air sampler can be powered from tractor batteries / solar power units and requires once weekly attention for changeover / collection of the tube(s). After sampling the tube is removed, liquid added and transferred to an 'in field' lateral flow device. After approximately 10 minutes the lateral flow is assessed for test line development and estimation made as to whether the target spore type is present and at what concentration. An electronic reader can be used to provide a digital print out or the assessment can be made by eye.

Figure 2. Automatic Multi-vial field cyclone sampler (www.burkard.co.uk)

Prior to this project, the airborne concentration of each of the spore types required for disease development in the crop was unknown. A previous study (Kennedy *et al.,* 2000) demonstrated that under optimal environmental conditions upwards of 2000 *Mycosphaerella brassicicola* ascospores per cubic metre air sampled were required for significant ringspot disease development on exposed susceptible Brussels sprout plants. Lateral flow tests developed for the measurement of ringspot inoculum in the air have been calibrated to reflect this information. At concentrations close to those required for crop disease establishment (2000 spores per cubic metre) the test line of the lateral flow is depleted (not visible). This study looks to determine the airborne inoculum concentrations required of the light leaf spot, powdery mildew and white blister pathogens for disease development on commercial cropping systems.

Materials and Methods

Diagnostic tests: Evaluation of light leaf spot, powdery mildew and albugo in single and multiplex format

On site tests (lateral flows) were produced for the individual measurement of Brassica powdery mildew, white blister (race affecting *Brassica oleracea*) and light leaf spores according to the protocols reported in HDC CP99 Year 1 Annual Report. Test sensitivity to the homologous spore type and the stability of the incorporated reagents were determined (shelf life) for each of the tests as previously described (HDC CP 99 Year 1 Annual Report). The construction of a lateral flow for the dual measurement of white blister spores and light leaf spot was assessed. The multiplex test was developed to discriminate and measure light leaf spot and white blister spores from the same field bioaerosol.

Field Trials: Light Leaf Spot, Powdery Mildew And White Blister

Air samplers (Burkard Manufacturing, www.burkard.co.uk) were positioned within commercial Brassica cropping systems (Brussels sprouts) in Lincolnshire and the East of Scotland (Fig. 3). At each site the air samplers were placed at 2m distance from each other. Collected bioaerosols were assessed for disease propagules of light leaf spot in Scotland, powdery mildew and white blister in Lincolnshire. To collect spores in the air, three types of bioaerosol sampler were used: a volumetric sampler to identify target spores by microscopic analysis, a microtitre air sampler (MTIST) for antibody based laboratory analysis and a cyclone sampler to provide a '10 minute' on-site field test reading. Agricultural batteries (12V) were used to power the air samplers and where possible solar panels were attached to reduce the labour of battery changes.

Figure 3. Environmental data logger and four field bioaerosol (air) samplers sited in a Brussels sprout crop in the East of Scotland

A detailed description of the air samplers, operation and spore assessment is described below:

Microtitre Immunospore Trap (MTIST). A detailed description of the MTIST device can be found in Kennedy *et al.,* 2000. In the outdoor 'field' version air is drawn thorough a manifold consisting of a plastic tube with a right angle bend placed over the sampler inlet (Fig. 4). The sampler contains four microtitre strips each containing 8 wells. The MTIST air sampler uses a suction system and particulates in the airstream are impacted on the base of each collection well of the four microtitre strips. The four coated eight well microtitre strips were coated with a combination of 2 x strips at 0.1mg ml-1 Poly-L-Lysine (Sigma P-1524) in distilled water and 0.05% sodium azide (Sigma P-1524) and 2 x strips 5:1 mixture of petroleum jelly and paraffin wax (Wakeham *et al.,* 2004). The MTIST spore trap was operated for 12H periods from 06:00 H to 18:00 daily.

Figure 4. MTIST air sampler positioned within a commercial Brussels sprout cropping system and operated from an environmental data logger

The 4x 8 well coated microtitre strips were changed weekly. For trials in Scotland the field exposed microtitre wells were posted to NPARU and processed immediately on receipt by PTA ELISA (plate trapped antigen enzyme-linked immunosorbent assay (Kennedy *et al.,* 2000)) to provide weekly light leaf spot disease alerts. Field exposed microtitre strips at trial sites in Lincolnshire were collected at weekly intervals, stored at -20^{o}C prior to quantification of aiborne inoculum of powdery mildew and white blister by PTA ELISA.

Burkard 24hr Volumetric glass slide air sampler. A Burkard volumetric air sampler which contained a Melinex tape coated with silicone (BC 380S, Basildon Chemical Co, Kimber road, Abingdon, Oxon, UK) operated at an air flow of 10 L of air per minute over a full 7 day period. Field sampled air particulates were impacted directly on to an area of the tape which corresponded to time intervals by movement of the tape over an hourly period. Following weekly field exposure the Melinex tape was removed and posted to NPARU. On receipt the tape was sectioned in to daily segments, mounted and by bright field microscopy assessed for *A. candida* and *E. cruciferarum* (white blister and powdery mildew) spores between the hours of 06:00 H to 18:00 daily. The Melinex tapes received at NPARU from the Scottish site were processed over time by immunofluorescence (Kennedy *et al.,* 1999). Ascospores of light leaf spot were identified on the tape using MAb UW 277 attached to an anti-species fluorescein conjugate (Figure 5) and the numbers calculated to spores / cubic metre present in the crop between the hours of 06:00 H to 18:00 daily.

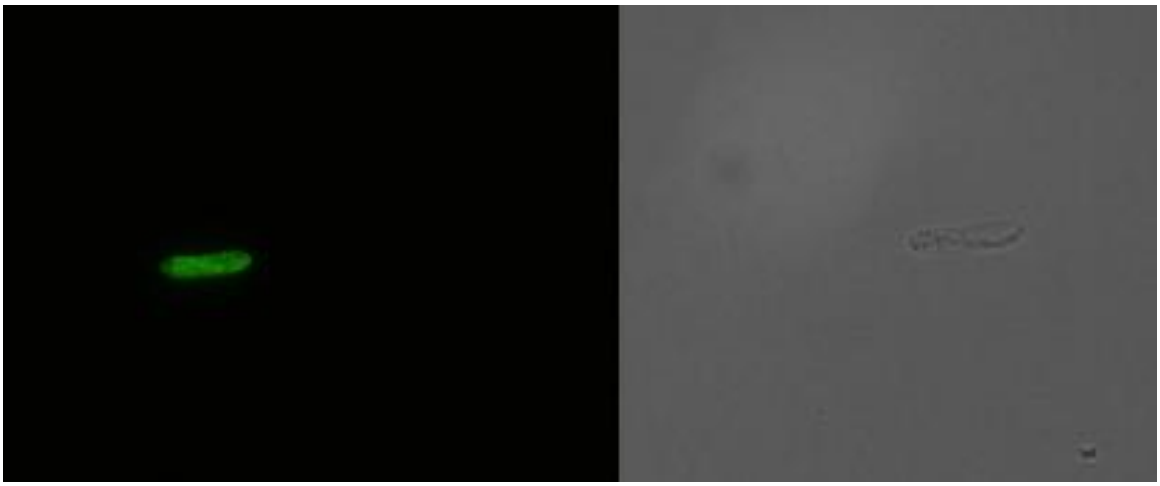

Figure. 5. Light leaf spot ascospore (airborne disease) as visualised by immunofluorescence and bright field light microscopy.

Burkard cyclone air sampler. The characteristics of a cyclone air sampler are described by Ogawa & English (1995). Air is drawn through the sampler using a vacuum pump in the form of a cyclone. The height of the cyclone and air inlet, along with the width of the air inlet, air exhaust diameter and the diameter of the cyclone within the length of the exhaust pipe influence the relative efficiency of the trap. These characteristics have been drawn together and standardised within the Burkard cyclone sampler (Burkard Manufacturing Co.). The cyclone air sampler operates at an air flow rate of 10 to 15 L air / min, is adapted for field usage and air particulates trapped in a 1.5ml microfuge tube (Fig. 2). At each of the sites the field exposed tube was changed weekly and prior to assessment was stored at -20^{0}C. The tubes were assessed for the target spore types at the end of each trial period by lateral flow.

Lateral flow process: To each field exposed microfuge tube 200µl of NPARU B2 buffer (included within diagnostic kit) was added, swirled and incubated at room temperature for five minutes (Fig 6). A lateral flow device developed for field assessment risk of one of the target spore type was identified. A 100ul aliquot of the field spore suspension (taken from the tube) was then applied to the sample pad of the lateral flow device (Fig. 7) and test line development was assessed at 10 minutes using an ESE Quant reader (Fig. 8).

Figure 6. Grower ‘in field’ light leaf spot diagnostic kit: Application of buffer (NPARU B2) to the field bioaerosol sample.

Figure 7. Grower diagnostic kit: Transfer of the field bioaerosol sample to the light leaf spot lateral flow test

Figure 8. Quantitative measurement (electronic record) of the test using a portable ESE lateral flow reader.

Field trials

Light leaf spot.

At a site on the East Coast of Scotland, bioaerosol samples were collected weekly in a crop of Brussels sprout *c.v.* Petrus from June 2013 through to February 2014. Risk periods for light leaf spot disease transmission were provided on a weekly basis. Measurement of this was by laboratory ELISA analysis of the collected MTIST wells. However during the monitoring period the collection efficiency of the MTIST air sampler was affected by limitations of power. For this reason the results could not be relied on from October 2013 to February 2014. Similarly the volumetric air sampler was affected and mechanical failure was also problematic. This impacted on the usefulness of the data collected across the season. 'In field' test readings (cyclone air sampler with lateral flow) were also affected by power failure from October onwards. Test readings of the lateral flow test (using an ESE reader) were compared where possible with results derived from the MTIST laboratory processed air samples.

The crop was treated according to the growers schedule with commercially available fungicide applications to control light leaf spot disease. As no control area was available plant assessments were not made

***Brassica Powdery Mildew and White Blister*.**

One thousand Brussels sprout transplants of two cultivar types (commercial and a genetically identical variety, but incorporating intermediate resistance to *Albugo candida*) were sown between the 10th and 15th May, 2013 at Dotams Lane, Butterwick (OS ref. TF 376 458). Within the trial block, 500 plants (commercial variety and intermediate resistance) remained untreated with no fungicide applications made. Air sampling equipment (MTIST, volumetric and cyclone) operated within the crop throughout the trial period. The Melinex tape of the volumetric air sampler, the four MTIST strips and the microfuge tube of the cyclone sampler were changed on a weekly basis. Analysis of the bioaerosols was at NPARU for disease presence of *A. candida* (white blister) and *E. cruciferarum* (powdery mildew) spores. Information derived from a data logger, sited within the crop, was processed by an environmental white blister disease model (Morph Brassica Spot) to forecast white blister risk. Twenty leaves of ten tagged plants were identified in each cultivar type of the sprayed and non-fungicide treated area of the trial. A total of 40 plants were assessed at two to three week intervals for disease presence of powdery mildew and white blister.

Results

Diagnostic Tests: Evaluation of light leaf spot, powdery mildew and albugo candida lateral flows

Lateral flow sensitivity to target spore type

Light leaf spot.

In Year 1, a correlation of r^2=0.9661 (Fig. 9) was recorded using lateral flow batch test devices (coded 001LL) for the measurement of *Pyrenopeziza brassicae* spore concentrations. A detection limit of > 200 ascospores (spores associated with light leaf spot long range disease transmission) was achieved. Similarly in Year 2, a correlation of r^2=0.9771 was observed for batch test devices (coded 002LL) and with a test detection limit of >200 ascospores.

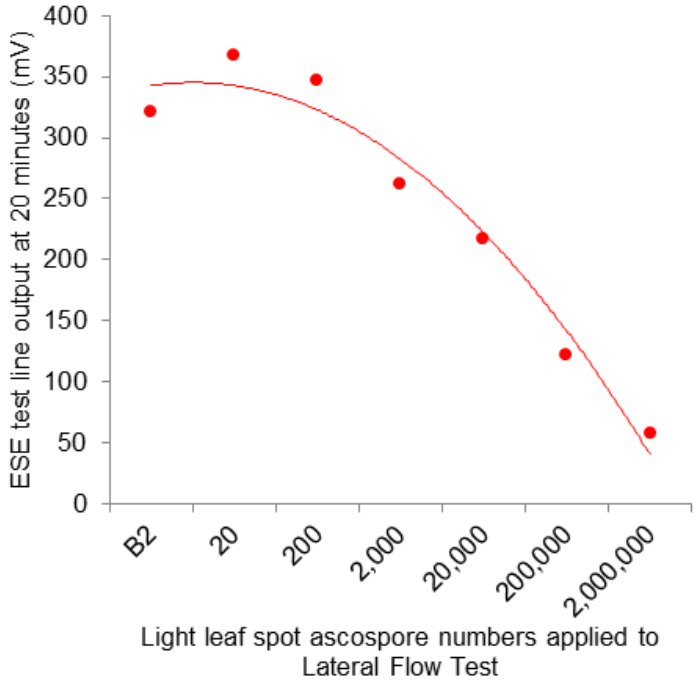

Figure 9. Light leaf spot ascospore concentration series as measured by lateral flow devices (in field test) using an ESE reader. B2 is the control and contains no spores.

Brassica Powdery Mildew.

In Year 1, a correlation of r^2=0.9799 was recorded when concentrations of powdery mildew spores were applied to lateral flows of batch 001BPM (Fig. 10). A detection limit of 30 spores was observed. In Year 2 of the study a correlation of r^2=0.9782 was reported for batch 002BPM (2014 Brassica powdery mildew lateral flow batch). A detection threshold of 30 spores was recorded.

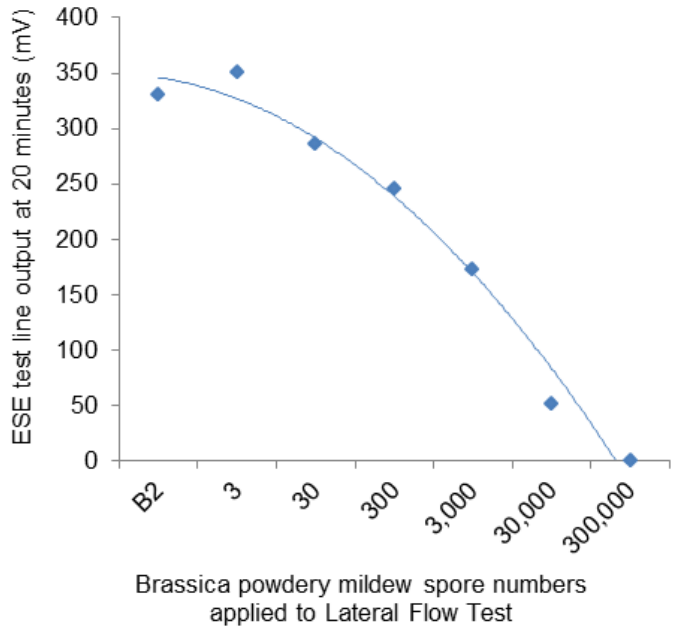

Figure 10. Brassica powdery mildew spore concentration series and quantitative measurement by lateral flow device (in field test) using an ESE reader. B2 is the control and contains no spores.

White blister.

A correlation of r^2= 0.9718 was recorded for *A. candida* spore (UK Race 9 *Brassica Oleracea*) concentrations when applied to lateral flow test batch 001AC. A detection limit of 1000 spores was observed for the test application (Fig. 11). For the 2014 white blister lateral flow batch (002WB) a correlation of r^2=0.9782 was recorded and with a test detection threshold of 1000 spores.

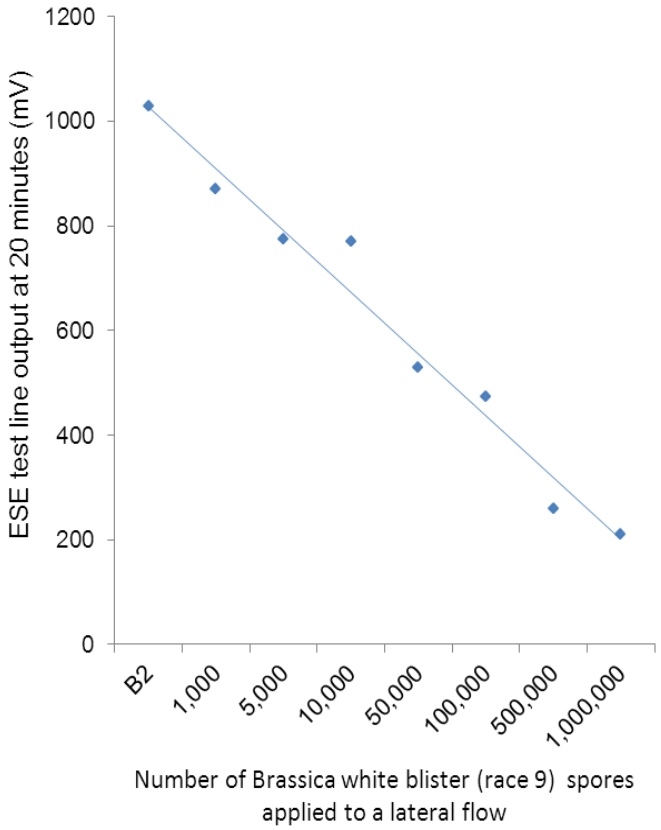

Figure 11. White blister spore concentration series applied to lateral flow devices (in field test) with the test results measured using an ESE reader. B2 is the control and contains no spores.

Assessment of the 2013/2014 Lateral Flow Batches for shelf life stability

Test line: The test line antigen of the light leaf spot, Brassica powdery mildew and white blister lateral flows remained over a 21 month period of testing stable and biologically active on the lateral flow membrane.

Gold antibody conjugate pad: The incorporation of sucrose and mannitol within the conjugate application buffer improved the stability of each of the disease lateral flow devices. The lateral flows record conjugate pad stability at: light leaf spot + 9 months, Brassica powdery mildew + 21 months and white blister + 5 months.

Testing remains on-going. It is expected that all tests will have a shelf life in excess of 1 to 2 years.

Preliminary studies to investigate the potential of a multiplex lateral flow

Mixed spore types of *Albugo candida* and *Pyrenopeziza brassicae* were applied in a liquid volume to a multiplex lateral flow. Inhibition of the respective test lines were observed as the homologous spore concentration increased. This provided measurement and discrimination of the two spore types on a single lateral flow. A correlation was observed between the spore type and concentration applied to the multiplex lateral flow (Fig. 12). No reactivity / interference in test development was observed between the two spore types in multiplex format.

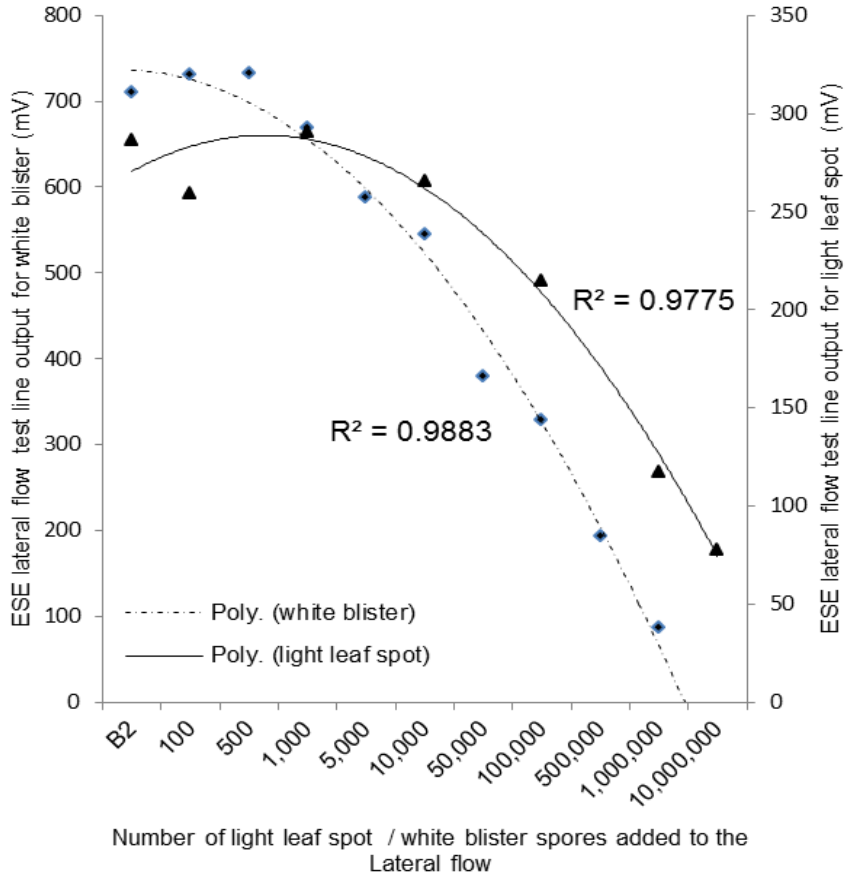

Figure 12. Mixed spore concentrations of *Albugo candida* (white blister) and *Pyrenopeziza brassicae* (light leaf spot) applied to multiplex (two test lines for independent measurement of mixed spore types in a sample) lateral flow devices

Field Trials: Light leaf spot, powdery mildew and white blister

Light leaf spot.

Ascospore concentrations of light leaf spot were visually identified by immunofluorescence, when weekly collected Melinex tapes of the Burkard volumetric spore were viewed by UV microscopy. Light leaf spot spore concentrations varied over the monitoring period. In the first few weeks of assessments (18th June – 1st July) low numbers were observed (5 to 80 per cubic metre air sampled). From the beginning of July until 25th July 2013 no further assessments of spore concentration could be made due to mechanical failure of the air sampler. However from the 25th July increased ascospore concentrations were observed (1000 – 2000 ascospores per cubic metre air sampled). From the second week in August concentrations fell to below 1000 spores per cubic metre air sampled. For the remaining period of the trial both mechanical and power failure of the volumetric spore sampler limited useful monitoring of the crop for light leaf spot bioaerosols.

The first set of MTIST air samples available for light leaf spot risk assessment by ELISA were from the week commencing 11th July 2013. From this date until the week commencing 12th September disease concentrations were predicted to be at a level for infection to occur should environmental conditions allow (Fig. 13). This is estimated to be at or near 200 ascospores per cubic metre. The MTIST ELISA provides a risk alert of 0.1 when this value is met (HDC CP099 Report, Year 1). The peak in ascospore concentrations measured by the volumetric air sampler from the 25th July to the 8th August, followed by a decline during the week commencing 15th August, (to below 1000 spores per cubic metre) conferred with those results derived by the MTIST ELISA. With the exception of one week (commencing 12th September), disease bioaerosols were predicted to be at a concentration to place the crop at risk until the middle of October. Thereafter power and mechanical failure of the MTIST bioaerosol sampler prevented useful measurements to be made.

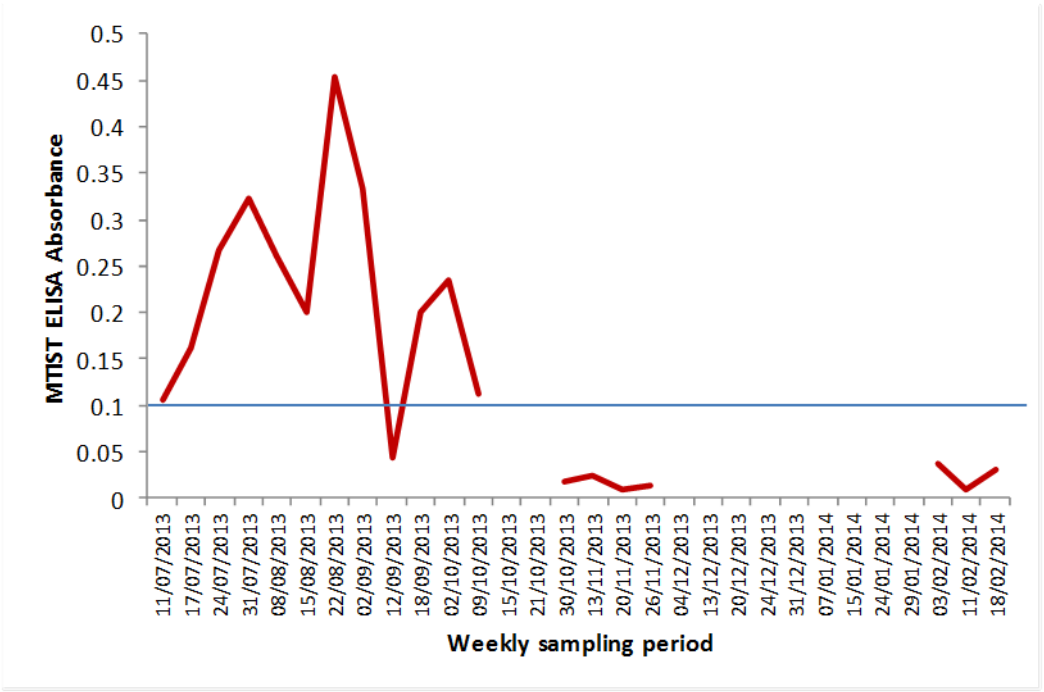

Figure 13. Monitoring MTIST collected bioaerosols for light leaf spot disease at a Brussels sprouts (cultivar Petrus) field site by ELISA.

Weekly bioaerosols were also collected into 1.5ml microfuge tubes using two cyclone air samplers. However as a result of technical and contamination issues the sampling commenced proper on the 15th August and 2nd September 2013 respectively. Problems of insect contamination were addressed by the addition of a nylon mesh to cover each bioaerosol sampler. Two lateral flow prototypes (Batch 002LL and Batch 00277LL) were used to test the weekly collected air samples for light leaf spot disease transmission periods. Where possible these were compared to the MTIST ELISA data (Fig 14 a, b). Both cyclone samplers were affected by power issues (solar power and tractor battery engagement) from October. However it is likely that the bioaerosol sampler used with lateral flow Batch 00277LL was most compromised by power and reduced collection efficiency observed (Fig 14b).

Fungicide applications (full details of commercial name and active withheld due to confidentiality restriction) were applied throughout the trial.

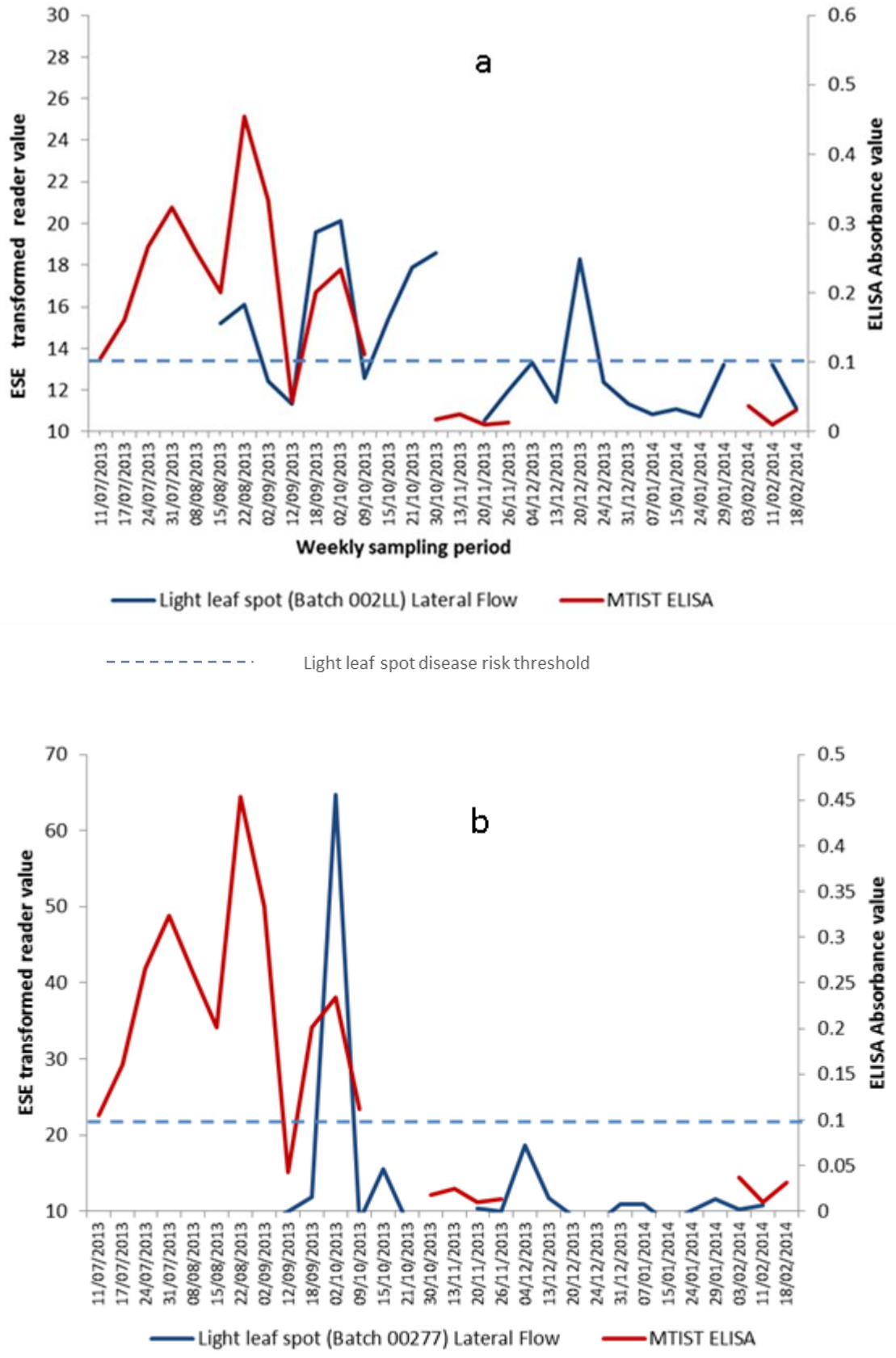

Figure 14. Monitoring light leaf spot disease 'risk' transmission periods in weekly collected field bioaerosols by (a) PTA ELISA and lateral flow (Batch 002LL) and (b) PTA ELISA and lateral flow (Batch 00277LL)

Brassica Powdery mildew and White blister.

The Melinex tape (microscopic analysis), microfuge tube (cyclone sampler) and 4 x 8 well microtitre strips were collected from each of the samplers operating at the Lincolnshire trial site. These were received weekly by the NPARU and processed for the measurement of Brassica powdery mildew and white blister disease propagules. Microscopic measurement determined a low concentration of *Albugo candida* spore (white blister) and *Erysiphe cruciferarum* (powdery mildew) spores in the airborne environment of the crop for most of the trial period. Although during July the daily spore concentrations of *Erysiphe cruciferarum* peaked to >70 spores per cubic metre of air sampled (Figure 15). This increase in powdery mildew spore concentration was also observed using the lateral flow devices (Figure 16). However no Brassica powdery mildew disease was observed on the sprayed or unsprayed plants during the trial period.

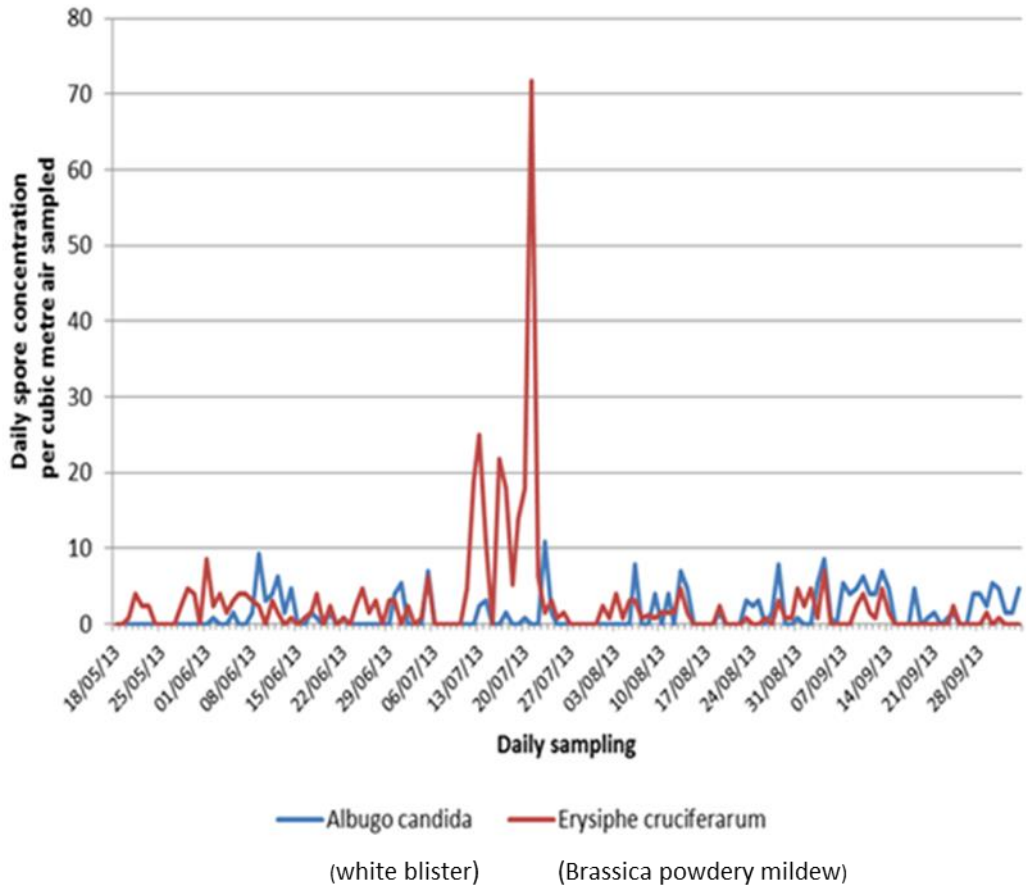

Figure 15. Daily spore concentrations of *Albugo candida* and *Erysiphe cruciferarum* recorded by microscopic analysis of the field exposed Melinex tape (volumetric 7day spore trap).

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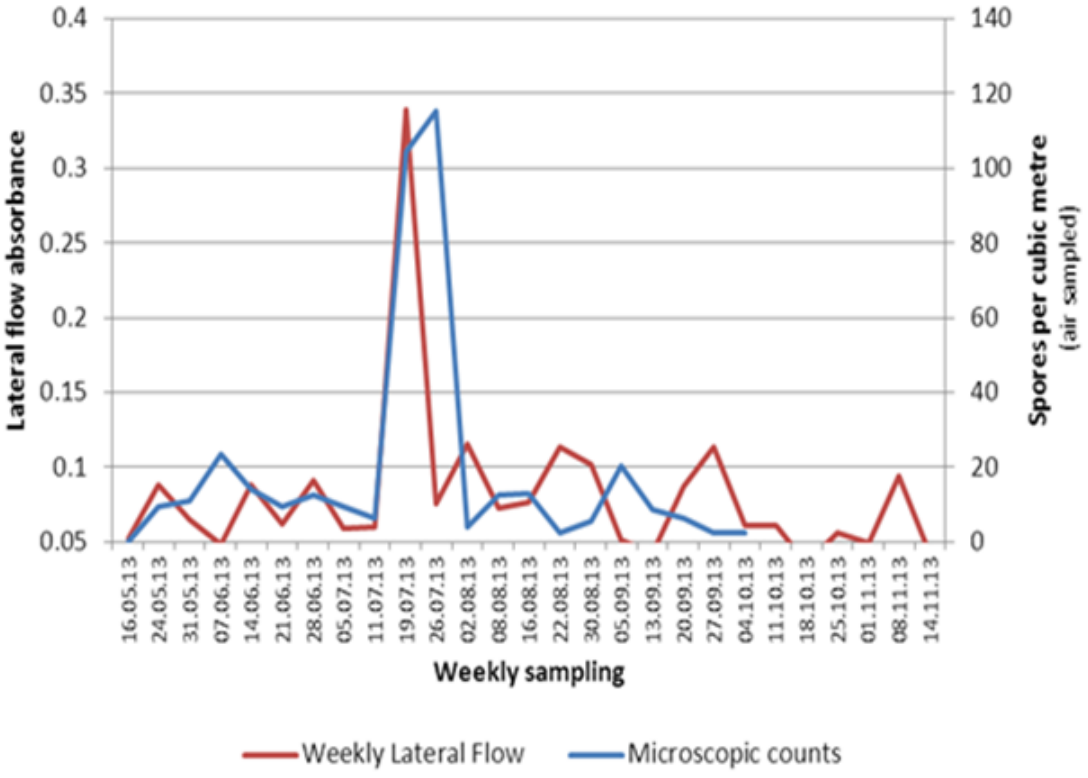

Figure 16. Assessment of bioaerosols on a weekly basis for Brassica powdery mildew by microscopic analysis and using 'in field' lateral flow tests

Assessment of the four microtitre 8 well strips by MTIST ELISA analysis (laboratory antibody based test) provided confirmation also of white blister presence throughout the period of the trial. The ELISA values correlated with the weekly *A. candida* spore counts (microscopic analysis). Two main spore peaks were identified (weeks commencing 16/8 and 5/9) as potential disease risk periods for white blister (Fig 17).

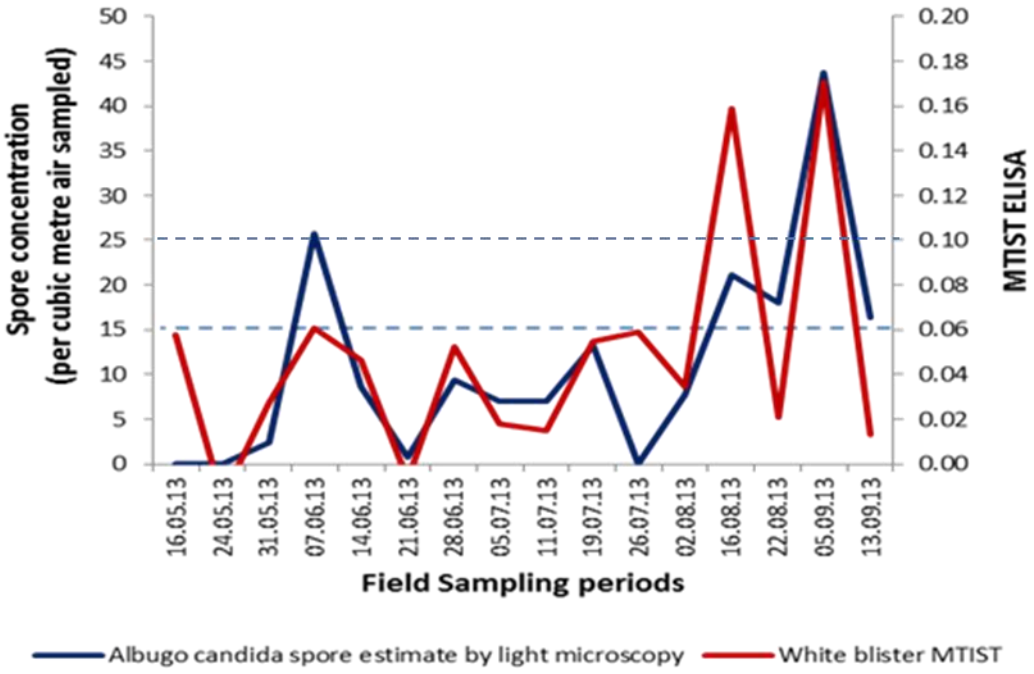

Figure 17. Monitoring weekly *Albugo candida* spores (white blister) in field bioaerosols by light microscopy and laboratory ELISA

The 'in-field' lateral flow test detected a high concentration of white blister spores during the week of the 22nd August (Fig. 18). Reduced spore cycles thereafter were recorded but with peaks identified on the weeks commencing 5th September, 11-18th October and 28th November. An environmental white blister disease model (Morph Brassica Spot) to forecast white blister risk was activated on the 22nd August as a result of the positive lateral flow result (Fig. 19). Based on available disease for this date, the model predicted that white blister disease would be visible on susceptible unsprayed plants from the second week in September. Of the twenty leaves of ten tagged plants identified in each cultivar type of the sprayed and non-fungicide treated area of the trial, white blister was first observed in the crop on the 19th September (Fig. 20). A total of 40 plants were assessed at two to three week intervals for disease presence throughout the trial. The model predicted two further white blister sporulation dates from the 11th and 18th October. The weekly lateral flow assessments from the week commencing 4th October demonstrated evidence of this with increased spore concentrations in the collected field bioaerosols. A spore cycling process was evident during this period and again in November (Figure 18).

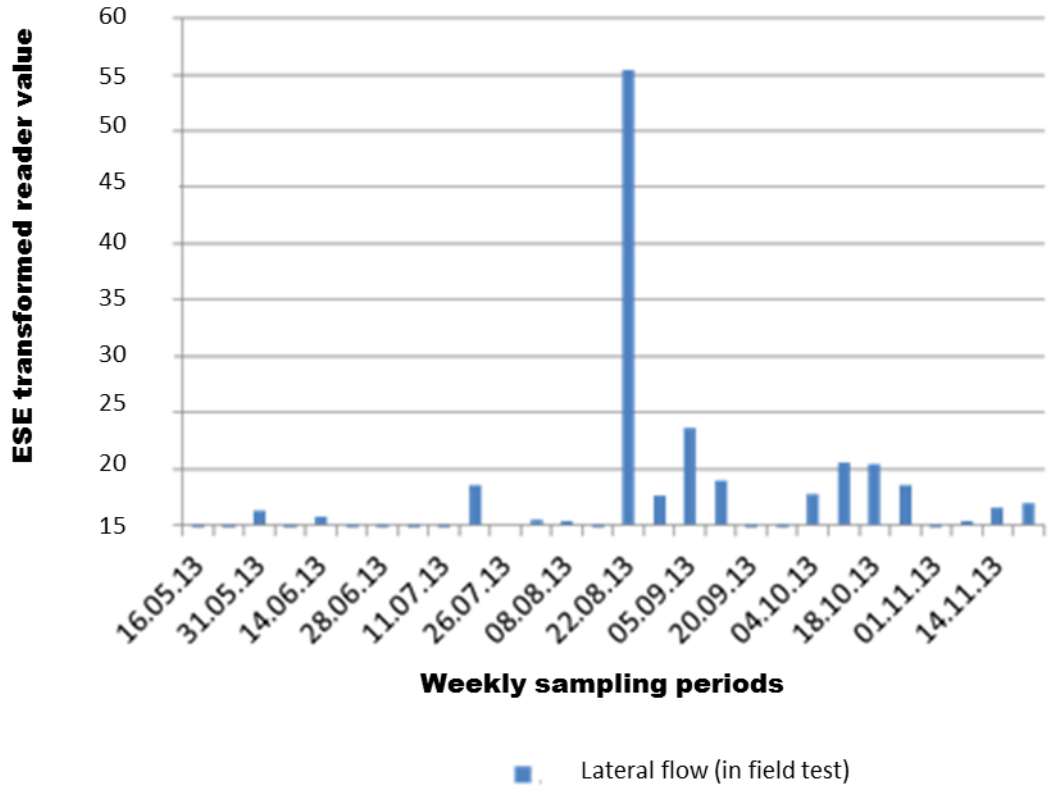

Figure 18. Monitoring weekly *Albugo candida* spores (white blister) in field bioaerosols using 'in field' lateral flow tests.

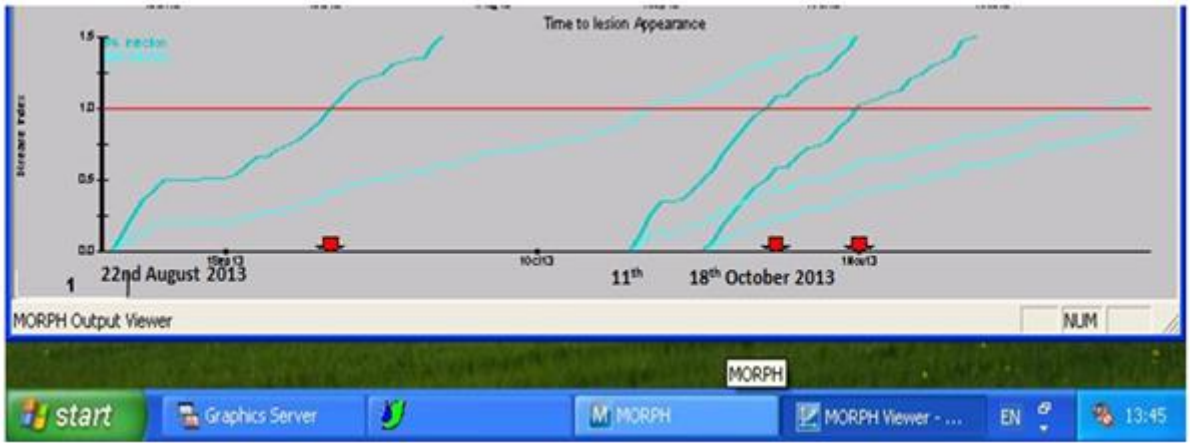

Figure 19. Prediction of white blister lesion appearance on trial plants based on *Albugo candida* inoculum availability as predicted by the lateral flow test on the 22nd August 2014.

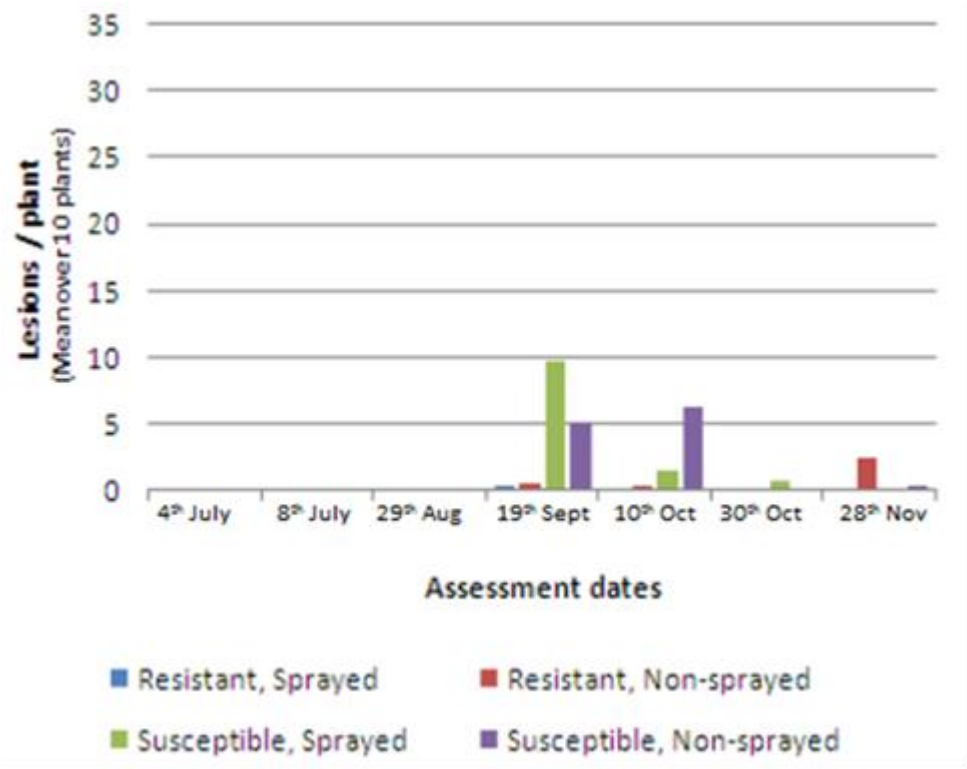

Figure 20. Assessment of 40 tagged plants identified in each cultivar type (susceptible and intermediate resistance) for white blister lesion development within the sprayed and non-fungicide treated area of the trial

Discussion

Diagnostic tests

Batches of lateral flows have been used to measure field bioaerosols for light leaf spot, Brassica powdery mildew and white blister. Results to date indicated that the tests have a diagnostic range suitable for use in disease risk forecast studies. Test batches provide measurement of light leaf spot ascospores between 200 and two million; Brassica powdery mildew concentrations of between 30 and three hundred thousand; white blister spores at concentrations of 1000 to 1 million. Each test should provide a shelf life of between one and two years at room temperature. A multiplex test has been developed for the measurement of light leaf spot and white blister spores from a single bioaerosol sample. These tests will be used in the field and their use reported on in the final report (HDC CP 99 2015).

Field Trials

Light leaf spot.

Over an eight month period a commercial Brussels sprout crop *c.v.* Petrus was monitored for bioaerosol concentrations of light leaf spot disease (*Pyrenopeziza brassicae* ascospores). An earlier study deemed a Brussels sprout crop risk to light leaf spot disease when a spore concentration was recorded at or above 200 per cubic metre of field air sampled (HDC CP99 Year 1 Annual Report, 2013). The MTIST sampler by ELISA provides a risk warning for this when a criteria value of 0.1 is met. In the 2013 field trial this value was achieved in the first week of sampling (week commencing 11th July). However the environmental conditions were likely not met for development of the disease on plants. With the exception of one week the crop remained at risk to disease bioaerosols until the middle of October. Thereafter power and mechanical failure of the air samplers prevented useful measurements to be made. In combination with light leaf spot disease threshold information a commercial fungicide spray program was operated throughout the life of the crop for treatment of the disease. The crop remained relatively disease free and was marketed for commercial supply through to February 2014.

It is probable that the regional harvest of oil seed rape provided the source of the light leaf spot disease plumes recorded from July through to September (Fig. 21). In conjunction with environmental data the development of regional oilseed rape harvest source maps could provide a useful tool towards forecasting light leaf spot disease risk on Brussels sprout crops in the UK. During at risk periods the on-site tests could be used to confirm and

measure spore concentration. This would provide a targeted and inexpensive (approx. £7 / test) way of determining actual risk. Equally, as in this project, the tests can be used weekly.

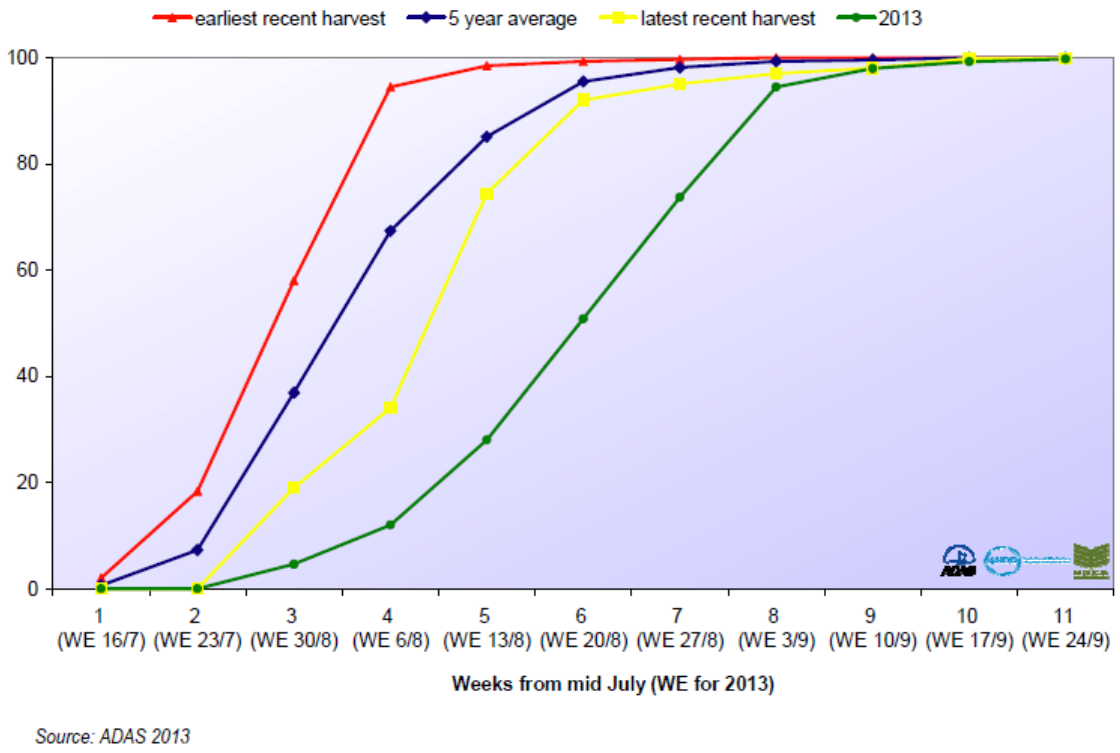

Figure 21. Cumulative % winter oilseed rape harvest progress comparison (ADAS 2013)

Brassica Powdery Mildew and White blister.

Both Brassica powdery mildew and white blister spores were identified in field bioaerosols during the trial period. In July, the disease pressure for powdery mildew intensified and more than 70 spores per cubic metre of air were identified in the weekly collected crop bioaerosols. This peak in spore numbers was confirmed using the powdery mildew lateral flow. However no disease was recorded on any of the plants in the trial. The potential exists for disease to be at a concentration suitable for infection of susceptible hosts (biological disease threshold). However if the environmental conditions are not suitable the disease is unlikely to progress to symptom development. Although Brassica powdery mildew is thought to require relatively dry conditions the environmental parameters for infection are not yet well described. For this reason it is not possible from this trial to determine whether the disease threshold for Brassica powdery mildew has been met. The study will be repeated in 2015 under an extension of HDC CP 99.

The MTIST and the volumetric air samplers identified white blister spores in the crop throughout most of the trial period. During July the *Albugo candida* spore concentration was

recorded at or near 15 spores per cubic metre. For most of these days the model predicted the crop to be at moderate or high risk to white blister infection (Figure 22). However no evidence of disease was observed on the plants until the 19th September.

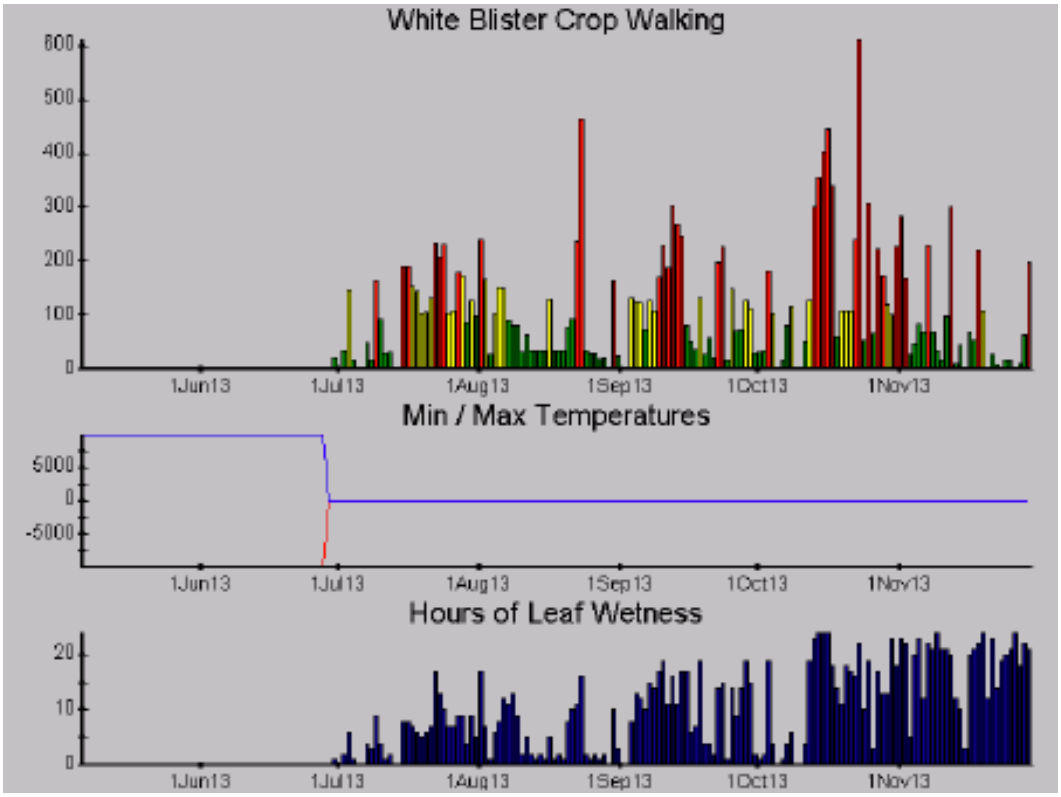

Figure 22. White blister model output for the trial sited at Dotams Lane, Butterwick

Conversely, the lateral flow test for white blister did not estimate risk of disease until the week commencing the 22nd August. For this period the environmental model (Morph Brassica spot TM) predicted a high risk of white blister sporulation. A disease alert was activated as a result of the lateral flow test result and the white blister forecast. Using the 22nd August as a disease start point (i.e. high spore presence detected in the crop bioaerosols) the model predicted that white blister disease would be visible on susceptible unsprayed plants from the second week in September (Figure 19). White blister was first observed in the crop on the 19th September. The model then predicted two further white blister sporulation dates from the 11th and 18th October. The weekly lateral flow assessments from the week commencing 4th October demonstrate evidence of this with increased spore concentrations identified in the collected field bioaerosols. A spore cycling process was evident during this period and again in November. It is therefore proposed that

the white blister disease threshold is likely to be in excess of 20 *Albugo candida* spores per cubic metre of air sampled. However the environmental conditions under which the crop is grown will prove important in the development of the disease to symptom expression on plants. Further observations will be made in a repeat of this study in 2015 under an extension of HDC CP99. Improved management of the disease and the reduced applications with effectiveness of the fungicides applied should be achieved by including information on availability of *A. candida* spore load (on-site test) with the white blister forecast model.

Conclusions

Control of plant pathogens could be improved if inoculum could be detected quickly in the field directly by the grower. Airborne inoculum plays a vital role in the development of epidemics caused by Botrytis leaf blight on onion crops (Carisse *et al.,* 2005). In this work, a linear relationship was found between number of lesions on plants and the airborne *Botrytis* spore concentration. Bioaerosol concentrations of 25 to 35 conidia m^{-3} of air were associated with 2.5 lesions per leaf. When detection of Botrytis inoculum was used as a control criterion under field conditions it led to a reduction in fungicide usage of 75 and 56% in 2002 and 2003. A similar relationship between spore number and disease intensity has been reported for *Cercospora apii* on celery. In both these studies, microscopes were used to determine spore numbers from air samples. In vegetable Brassica crops, detecting pathogenic spores before they can infect crops has also been shown to be a useful approach in controlling airborne diseases (Kennedy *et.al*. 2006, Wakeham & Kennedy, 2010). This study details the development of air sampling systems (laboratory) and 'in field' tests for monitoring disease transmission events in commercial crops of Light leaf spot, Brassica powdery mildew and white blister. If the technique is to be of value practically *i.e.* targeted and effective usage of crop protectants, the method of detecting spores in the field should be combined with information derived from forecasts based on environmental risk of infection (Brassica™ Spot). The current study assesses the use of weekly estimates of target disease in air samples as this has been reported for other diseases of field crops (Wakeham and Kennedy, 2010). Where daily environmental risk forecasts are available it may be of use to consider daily air sampling regimes. This process is now being used to predict ringspot disease risk alerts in the UK (http://www.syngenta-crop.co.uk/brassica-alert) and is now being trialed across Europe. The field air samplers used for this are based on the single cyclone sampler. However an automated system can provide the capture of daily air samples in to different tubes over a week period (www.burkard.com). In developing a daily or weekly system it is necessary to determine the inoculum concentration required for

infection in conjunction with the effect of environmental parameters on this process. The current study assesses the use of weekly sampling but daily air sampling microscopic counts have been recorded using the volumetric air sampling device.

The development and use of 'in field' diagnostics described in this report has the advantage of detecting the very earliest possibility of disease occurrence. The grower or consultant is able to estimate real disease risk and the appropriate control approach. The system could enhance the activity of protectant fungicides or even biological control agents. By applying fungicides quickly the grower will be in a situation where he can use protectants to control disease inoculum before it is established within the crop. Use of protectant fungicides could help reduce residues in the crop at harvest. The requirement for zero or low fungicide residues is of increasing importance in crop production. In addition, using crop protectant measures in a timely, efficient and effective way should assist in the prevention of the breakdown of plant resistant varieties and the potential for increase of virulent pathotypes. A reduction of chemical usage with an informed targeted approach towards disease control will prove popular not only with the producer user on a cost / benefit case but also with with the supermarkets and the end user i.e. the consumer.

Control of light leaf spot on Brussels sprout crops also varies with cultivar. In Scotland very susceptible cultivars such as *c.v.* Millenium are not used successfully in control regimes. This has major implications in the control of light leaf spot in Brussels sprouts where a range of cultivars are grown with differing maturity dates during the season. Often cultivars are grown side by side in the same locality or field. Use of cultivars with high susceptibility to light leaf spot increases the risk of light leaf spot epidemics as the season progresses. Even when effective chemicals are used to control infection and these are applied when light leaf spot inoculum is present. The use of partially resistant cultivars such as *cv*. Petrus has been successful in producing disease free crops later in the growing season and allow a final harvest in the following year. Consideration should be given to separating cultivars into different areas to reduce short range disease transmission events. This risk was demonstrated in 2012/2013 at the field trial in Fife, Scotland where infected plants (fungicide interaction trial of susceptible and resistant crop varieties) were in close proximity to the commercial crop and, provided an inoculum source for disease transmission to occur in December 2012. Results from this period indicate that light leaf spot airborne disease was at 200 spores per cubic metre of air sampled. After which low level infection was then observed in mid-January on the partially resistant cultivar (*c.v.* Petrus) and, at an increased level on less resistant cultivars. The threshold for low level disease risk has since been set at 200 spores per cubic metre. It has been reported that under optimal conditions the

disease threshold for significant ringspot disease was at 2000 spores per cubic metre (Wakeham & Kennedy, 2010). This work was carried out in controlled environment chambers and with a susceptible ringspot cultivar.

The present study also aims to develop useful systems to monitor disease risk of susceptible Brassica vegetable field crops to white blister and powdery mildew. 'In field' diagnostic tests have been developed to each of these two pathogens and trialed at commercial sites in the UK. The tests have been set to identify spore concentrations that are likely to cause disease risk. For white blister spore concentrations of >20 spores per cubic metre has been estimated to provide risk for disease development. Using these criteria the in-field lateral flow test was able to detect the disease in weekly collected bioaerosols ahead of symptom development on the crop. Improved management of the disease and, reduced applications with effectiveness of the fungicides applied should be achieved by including information on availability of *A. candida* spore load (on-site test) with the white blister forecast model. An extension of HDC CP99 will look to confirm this in Year 3 of the project (six month extension). The results of which will be reported on in July 2015.

It is thought that occurrence of powdery mildew on Brassicas is favoured by dry conditions. In the UK this is only likely to result during the summer months. Development of the disease in the crop during this period (June to August) will prove pivotal on the damage that this pathogen will cause over the production season. If established on the crop by autumn, the older tissues where powdery mildew development is more favoured, will act as a bridge for the pathogen to then occur on the Brussels sprouts buttons. Although it is also possible that powdery mildew penetration into axillary buds may also lead to button infection. This is thought more likely to occur if large amounts of spore bioaerosols are present within crops. In 2013, no powdery mildew disease was observed on the trial crop. This is likely to have been a result of the environmental conditions. The UK Met Office recorded 2013 as the seventh sunniest summer since records began in 1929. A prolonged heat wave remained in place to the middle of July, when temperatures regularly passed 30ºC (86F). The south east of the country, to include the location of the trial, recorded the lowest amount of rainfall since 1995. Although a plume of inoculum (70 powdery mildew spores per cubic metre air sampled) was recorded in the trial area during a period in July the unusually dry and hot conditions may have prevented establishment of the disease in the crop. The trial will be repeated in 2014 to confirm the concentration of spores required to initiate powdery mildew in the crop. The results of which will be reported in HDC report CP 99 during 2015.

For each of these diseases work will be required on how to use these air sampling devices to schedule fungicide usage. For example will sprays be applied to a threshold detection level or will they be applied when each diseases is first detected using the device. For light leaf spot sprays of tebuconazole could be applied in response to peaks in airborne numbers of light leaf spot. Tebuconazole is one of the few active ingredients available to vegetable brassica growers which have activity against light leaf spot. For this reason tebuconazole (Nativo) is widely used by vegetable Brassica growers to combat the potential for light leaf spot development within their crop. However, tests on isolates, taken from vegetable brassica crops have shown the prevalence of isolates that could grow in the presence of 10ppm tebuconazole. Fungicides with the active ingredients of boscalid and pyraclostrobin (Signum) have relatively recently been given approval for use on vegetable Brassica crops. Additionally the fungicide Rudis (which contains triazolinthione) has also given good control of light leaf spot. Signum gave good control of light leaf spot infection in Brussels sprout crops provided that it is applied at the time when light leaf spot ascospores were present in the air. The development of diagnostic devices that can identify inoculum in the air at a concentration that is able to initiate disease will prove critical if fungicides are to be targeted for minimum input with maximum effect and to prevent disease resistance for long term and widespread usage.

The light leaf spot in field test kit will be available to the UK horticultural industry from 2015. The Brassica powdery mildew and white blister field test will be available from 2016. Alison Wakeham at NPARU can be contacted for further information on these.

Knowledge and Technology Transfer

Roy Kennedy, Alison Wakeham, Gary Keane (2014) HDC/BGA Brassica Technical Seminar and lateral flow demonstration, Edinburgh 28th January 2014

Gary Keane (2014) HDC/Duchy college brassica variety trial, LFD demonstration/seminar St Ives, 16th January 2014

Alison Wakeham (2013). Forecasting disease potential ahead of symptom development: Field Vegetables - a model to follow. BHTA exploratory day, 19th November 2013, Warwick Crop Centre, Wellesbourne

Roy Kennedy (2013) Crop disease forecasting: future prospects?
Campden BRI "Sustainable Crop production. Are we there yet? 19th November 2013 at Campden BRI.

Alison Wakeham, Gary Keane, Mary Lewis (2013) "Lateral flow demonstration"
HDC/BGA Brassica Technical seminar, 14th November 2013, in Lincolnshire

Roy Kennedy and Alison Wakeham (2013). Use of diagnostics in disease control in Horticulture BPOA technical seminar. 6th February, The Belfry, Oxfordshire

Roy Kennedy (2012). Detection, forecasting and control of vegetable diseases. Presentation: Vegetable, Salad & Herb Growers Technical Update meeting, 7th Feb-2012, Pershore College. UK.

Roy Kennedy Vegetable (2012), Salad & Herb Growers Technical Update meeting, 7th Feb 2012, Pershore College. UK.

Roy Kennedy (2012). Detection and control of clubroot and other diseases. Presentation and Discussion group, Grower mini conference, Lancashire 14th February 2012

Alison Wakeham (2012). Detection and Control of airborne/ soilborne disease of brassicas. Presentation: Getting to the Heart of Horticulture 13th January 2012 NFU and Wychavon Council

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Wakeham, A. J. and Kennedy, R (2010). Risk Assessment methods for the ringspot pathogen *Mycosphaerella brassicicola* in vegetable brassica crops. Plant Disease: 94:7 pp851-859.