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:	Final report, August 2015
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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):	31st July, 2015

The results and conclusions in this report are based on an investigation conducted over a 3-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 (single and multiplex) lateral flow tests have been used to measure field bioaerosols containing light leaf spot, powdery mildew and white blister. Each test has a diagnostic range suitable for use in disease risk studies**.**

- An in-field lateral flow test for daily testing of crop-based bioaerosols has been used successfully in conjunction with the MORPH Brassica Spot system to identify when commercial Brassica crops are at risk to White blister disease. A lateral flow for light leaf spot has also been used to monitor crop bioaerosols for light leaf spot.

- A laboratory ELISA has been developed giving participating growers a weekly disease risk of light leaf spot and white blister disease from MTIST collected field bioaerosols. Risk thresholds for both pathogens have been developed during this project. In Scotland, the system has been used in a commercial cropping system for the timed application of light leaf spot control measures. Good control has been achieved with minimal pesticide application. Significant disease was observed on plants at harvest in an adjacent varietal trial that did not receive timed fungicide applications.

- With no powdery mildew disease symptoms observed on Brassicas during trials in 2013 and 2014 it has not been possible to determine a biological disease threshold or confirm the accuracy of the test developed for this disease. However, the Brassica powdery mildew spores (*Erysiphe cruciferarum*) cannot be reliably differentiated from other powdery mildew species using the methods described in this project.

- The systems described in this report demonstrate an integrated disease management approach towards the reduction of crop inputs to control diseases of Brassicas. According to the European Union sustainable pesticide use directive (2009/128/EC) producers have had to apply general principles of integrated pest and disease management from January 2014.

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, AHDB Horticulture has provided funding for the development of systems to monitor field bioaerosols of 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 when to apply the appropriate fungicides.

Studies measuring *M. brassicicola* (ringspot) in airborne spore samples have shown that under ideal environmental conditions, high concentrations of spores are required in the air for infection to occur (2000 spores per cubic metre). This study aimed 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 have been adjusted to reflect this. Results from previous studies show that light leaf spot ascospores appear in the air in high enough concentrations 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 Brussels sprout crops. Fungicide applications can provide good control of light leaf spot in Brussels sprout crops if applied at times when the inoculum is in the air. Where routine 'blanket' crop spray programmes have been applied, control can be ineffective and resistance to control products can develop. Light leaf spot is endemic in Scotland and becoming common in Brassica production areas of northern England. Targeted application of effective fungicides in response to spore concentrations will play a vital role in controlling the disease. Inappropriate or unnecessary fungicide applications are not only costly but increase the pressure for development and selection of pathotypes able to resist previously effective control measures. Regulatory pressure is reducing the number of available pesticides which are approved for use on edible field crops.

Both powdery mildew and 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 is highly airborne and small numbers of conidia (spores) can be wind dispersed over long 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 during autumn and winter, where powdery mildew development is more likely to occur, may act as a bridge for the pathogen to occur on Brussels sprouts buttons. The application of fungicidal sprays (e.g. Nativo – trifloxystrobin and tebuconazole) is 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 at this time.

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). This study aimed to improve the white blister disease risk forecast by including information on the incidence of *A. candida* airborne spores. Monoclonal antibodies with reactivity to *A. candida* (Race 9) spores were 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 FV 033 for identification of airborne spores of light leaf spot and Brassica powdery mildew were adjusted for commercial field usage. This has enabled information to be provided indicating the need for fungicide spray applications in response to peaks in airborne spore numbers.
The expected deliverables from this project were:

- Measure spore concentrations in field aerosol samples 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 (in field tests by UK growers or consultants) or indirectly (laboratory analysis) to identify presence of these three diseases in the air. Identify the spore concentrations likely to cause disease on 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.

Summary

Diagnostic Test

Batches of lateral flow tests (rapid on-site tests) have been used to successfully measure field bioaerosols containing light leaf spot, powdery mildew and white blister. Laboratory tests using the MTIST bioaerosol sampler have also been successfully validated for this process. Each test has a diagnostic range suitable for use in disease risk forecast studies. Ideally, these tests should be used in conjunction with an environmental risk model. However, at the moment Morph Brassica Spot only provides this for White blister disease. The development and use of bioaerosol diagnostic tests has the advantage of detecting the very earliest possibility of disease occurrence which is well ahead of symptom occurrence. A biological disease threshold (spore concentration at which disease could initiate on susceptible plants) has been identified for light leaf spot and white blister. This information has been incorporated into the diagnostic tests which have been developed to provide a disease risk threshold. However, for Brassica powdery mildew this has not been possible. The test may prove more useful as a generic powdery mildew test.

Each of the lateral flow tests should deliver a shelf life of between one and two years at room temperature. A multiplex text for use in laboratory analysis has been developed for the measurement of light leaf spot and white blister spores from a single bioaerosol sample. Similarly, a multiplex lateral flow (in field) test is available for testing bioaerosols for white blister and powdery mildew.

Field Trials

Light leaf spot: It is likely that the regional harvest of oilseed rape provides the source of the light leaf spot disease plumes recorded in the Scottish field trials from July through to September. In conjunction with environmental data, the development of regional oilseed rape harvest source maps could provide a useful tool towards forecasting early light leaf spot disease risk on Brussels sprout crops in the northern regions of the UK. During these at risk periods the on-site tests could be used to confirm and measure the spore (disease) concentration. This would provide a targeted and relatively inexpensive (approx. £7 / test) way of confirming this early phase of disease potential in the crop. For the remaining part of the season, i.e. through to March, daily testing of field bioaerosols may prove too expensive in time and cost. Unlike ringspot and white blister no environmental disease forecast model is available for light leaf spot. Therefore, it would not be useful to identify specific days in a weekly sample whilst omitting others. For this reason, weekly bioaerosol sample catches (i.e. 7 days bioaerosol catch to provide a single test result) will likely prove more popular for this disease. The MTIST bioaerosol sampler (requires laboratory processing) has been shown to be reliable in this approach across the seasons tested (June through to March) and provided improved timing and use of fungicide applications, with good control of the disease. Significant disease was observed on plants at harvest in an adjacent varietal trial that did not receive timed fungicide applications. Also, the test has been used to forecast several diseases simultaneously. In this approach, identifying risk of ringspot and light leaf spot disease from the same weekly collected bioaerosol may be possible. With suitable antibody probes, the number of tests could be extended to eight diseases.

Weekly testing using a lateral flow approach can present problems. Sampling into a single air sampler tube over a week continuously has, in dry conditions, led to soil particles becoming airborne and concentrating over time in the collection tube. In wet conditions or high humidity (for example when a sampler is positioned within coastal regions with prolonged periods of sea fog) the tube can quickly become full of water and the sampling efficiency of the trap significantly compromised. Also, potential then exists for germination of previously trapped spora which may compromise the quantitative nature of the test. To prevent germination we have found it useful to pre-coat air sampler tubes with sodium azide. Also, a net placed over the sampler to prevent the entry of flies in to the tube has proved useful.

Brassica Powdery Mildew: Powdery mildew spores were identified in field bioaerosol samples during each of the trials conducted in 2013 and 2014. In July 2013, disease pressure for powdery mildew was considered high with more than 70 spores per cubic

metre of air identified in crop bioaerosols. However, no powdery mildew disease was recorded on any of the plants in the trial. It was proposed that the lack of disease may 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 continued up to the middle of July, when temperatures regularly passed 30°C (86F). The south east of the country, which included 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 was repeated in 2014 to confirm the concentration of spores required to initiate powdery mildew in the crop. No disease was observed on susceptible cultivars. Each of the tests identified powdery mildew in crop bioaerosol samples. With no powdery mildew disease symptoms observed on Brassicas during trials in 2013 and 2014 it has not been possible to determine a biological disease threshold or confirm the accuracy of the tests developed. The Brassica powdery mildew spores (*Erysiphe cruciferarum*) cannot be reliably differentiated from other powdery mildew species using the methods described in this project (FV 333). For this reason, the test may have application in protected cropping systems as a generic powdery mildew disease risk indicator.

White blister White blister became established in the trial crop during September 2013 and 2014. Over a sampling week, an average spore concentration of >20 spores per cubic metre air sampled was estimated to have provided first risk of this disease development. In 2014, when daily bioaerosol samples were also used to characterise disease risk, a spore concentration of >120 *A. candida* spores per cubic metre of air sampled (daily average) was recorded. This inoculum plume occurred in the same week that the crop was identified as being at risk to white blister spores (i.e. an average weekly spore concentration of >20 spores per cubic metre air). By using this information (disease availability) together with an environmental disease forecast model (Morph Brassica™ Spot), it was possible to accurately predict when disease would be visible on unsprayed susceptible plants. In 2014, the daily lateral flow test enabled more accurate input to the white blister model by identifying disease risk each day. This should provide increased test accuracy and economy of scale with growers testing bioaerosols only on days that are identified as at risk by the Brassica Spot White blister model. Improved management of the disease and reduced applications of the fungicides applied, should be achieved by including information on availability of *A. candida* spore load within the white blister forecast model.

Field Bioaerosol Samples

Listed below are details of the two bioaerosol samplers that were successfully used in each of the field trials to identify disease risk periods:

- A Microtitre immunospore trap (MTIST), available from Burkard Manufacturing (http://www.burkard.co.uk) at a cost of approx. £2,300 + VAT, provides a weekly field air sample in a 32 well format. However, samples require laboratory processing by ELISA (enzyme-linked immunosorbent assay). Using a postal delivery, the results can be made available within several days. The ELISA process takes no more than four hours.
- A weekly multi-vial air field sampler (provides seven daily air samples in separate tubes for testing on-site by LFDs). This field-ready air sampler with a timer can be purchased from Burkard Manufacturing for an approx. costing of £1,650 + VAT. Daily air samples are assessed once weekly by an agronomist or grower using on-site field tests (lateral flows). On-site lateral flows for other plant diseases currently retail at circa £7 per test. At present a digital lateral flow test reader would be required for measurement of spore concentrations. A reader currently retails at circa £1,000 however smart phone readers with downloadable applications are being developed for other diseases. For example: www.**novarumreader**.com/**novarum**-mobile-**reader**/

Financial benefit

There is considerable scope for benefit from this work in terms of early detection and improved spray timing. Ultimately, financial benefit will be gained through improved quality and reduced pesticide residues.

Action Points

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 (pyraclostrobin + boscalid) 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 MTIST has a disease threshold set at a weekly average of > 20 *A. candida* spores per cubic metre air sampled (Standard control, 0.02 ELISA

Absorbance). Using a calibrated *A. candida* spore concentration across a disease threshold range the daily lateral flow test has been set to record a disease risk at 100 (lateral flow Prism Graph Pad spore concentration conversion). Each test should be used in conjunction with the MORPH (Brassica Spot) white blister disease forecast model.

- The systems described in this report demonstrate an integrated disease management approach which can contribute to the reduction of crop inputs to control vegetable Brassica diseases. Also, the timed applications of targeted crop inputs should result in improved control, reduce unnecessary applications and has potential to avoid resistance build up and overall improve crop yield.

- To discuss use and application of these systems contact Alison Wakeham at NPARU for further information : a.wakeham@worc.ac.uk

SCIENCE SECTION

Introduction

Antibody probes which recognize spores 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 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 i.e. 1 vial/day (Fig. 2). The air sampler can be powered using batteries / solar power units and requires a once weekly changeover / collection of the tube(s). After sampling the tube is removed, liquid added which is then transferred to an 'in field' lateral flow device. After approximately 10 minutes the lateral flow is assessed for test line development and an 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 seeks to determine the airborne inoculum concentrations required for light leaf spot, powdery mildew and white blister 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 CP 099 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 099 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 bio-aerosol sample. Similarly, a multiplex lateral flow was developed to discriminate and measure white blister and powdery mildew spores.

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). Air samplers were placed 2m apart in the crop. Collected bio-aerosols were assessed for disease propagules of light leaf spot in Scotland, powdery mildew and white blister in Lincolnshire. Three types of bio-aerosol sampler were used in the field: 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 number 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 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 risk estimates. Field exposed microtitre strips at trial sites in Lincolnshire were collected at weekly intervals, stored at -20^{o}C prior to quantification of airborne 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 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 of spores / cubic metre present in the crop were calculated between the hours of 06:00 to 18:00 daily.

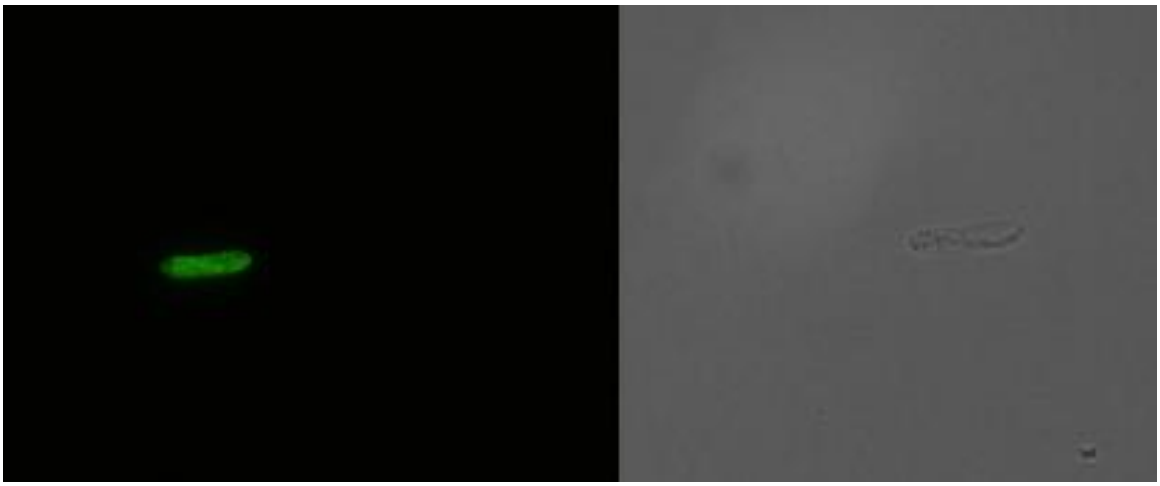

Figure. 5. Light leaf spot ascospore (airborne disease) as visualised by immunofluorescence and bright field light microscopy.

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, and is adapted for field usage with air particulates trapped in a 1.5ml microfuge tube (Fig. 2). At each of the sites the sample tube was changed weekly and prior to assessment was stored at -20^{0}C. The tubes were assessed at the end of each trial period by lateral flow.

Lateral flow process:

Each field exposed microfuge tube had 200µl of NPARU B2 buffer (included within diagnostic kit) added, which was 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 100µl aliquot of the field spore suspension (taken from the tube) was then applied to the inlet of the lateral flow sample pad (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 were provided on a weekly basis. Measurement of this was by laboratory ELISA analysis of the collected MTIST samples. 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 results. 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 collected.

An additional field study was carried out in 2014 through to early 2015. However, this time an agronomist operated the air sampling equipment and carried out the weekly light leaf spot risk assessment using a lateral flow test. Aside the commercial crop a light leaf spot resistance trial was in operation and the crop received no spray applications.

Brassica Powdery Mildew and White Blister

In 2013, 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 carried out at NPARU for the presence of *A. candida* (white blister) and *E. cruciferarum* (powdery mildew) spores. Information derived from a data logger, sited within the crop, was processed using the 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. The trial was repeated in 2014.

In 2014, three hundred Brussels sprout transplants of two cultivar types (Standard cv. 'Helemus' and a trial variety incorporating resistance, breeding line (B1539)) were planted on the 19th and 20thJune, 2014 at St Lamberts' Hall Farm, Weston (OS ref. TF 301 277). Within the trial area, 150 plants (standard commercial variety and resistant trial line) remained untreated with no fungicide applications made to the crop. Air sampling equipment (MTIST, volumetric, single and multi-vial cyclone samplers) operated within the crop throughout the trial period. Sampler vials and tapes were changed weekly. Tests were carried out on air sampler vials and tapes as in 2013. A multiplex lateral flow test was used in 2014. Ten marked 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 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.

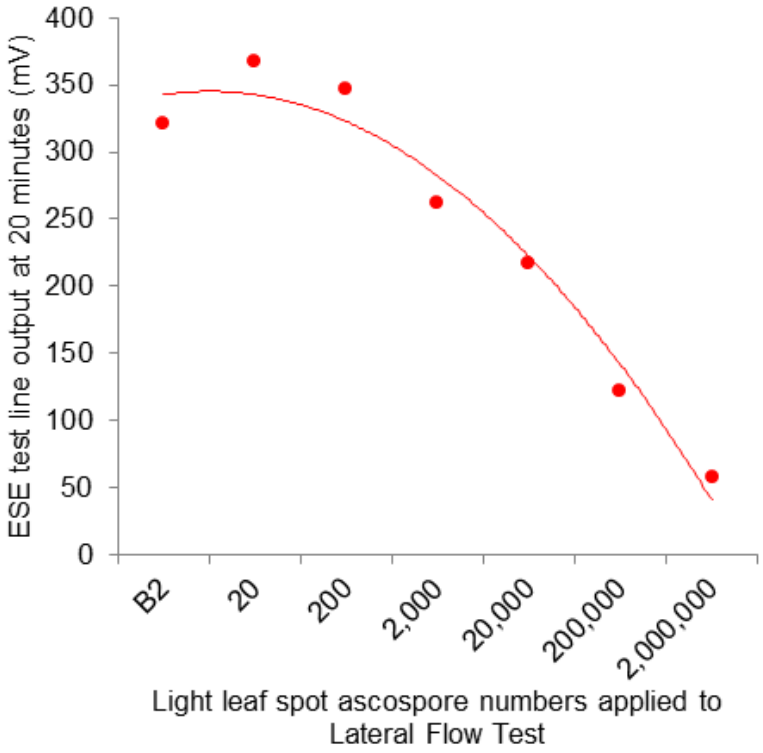

Figure 9. Light leaf spot ascospore concentration series as measured by lateral flow devices (in field test) using an ESE reader. B2 is the control and contains no spores.

Brassica Powdery Mildew

In Year 1, a correlation of r^2=0.9799 was recorded when concentrations of Brassica 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.

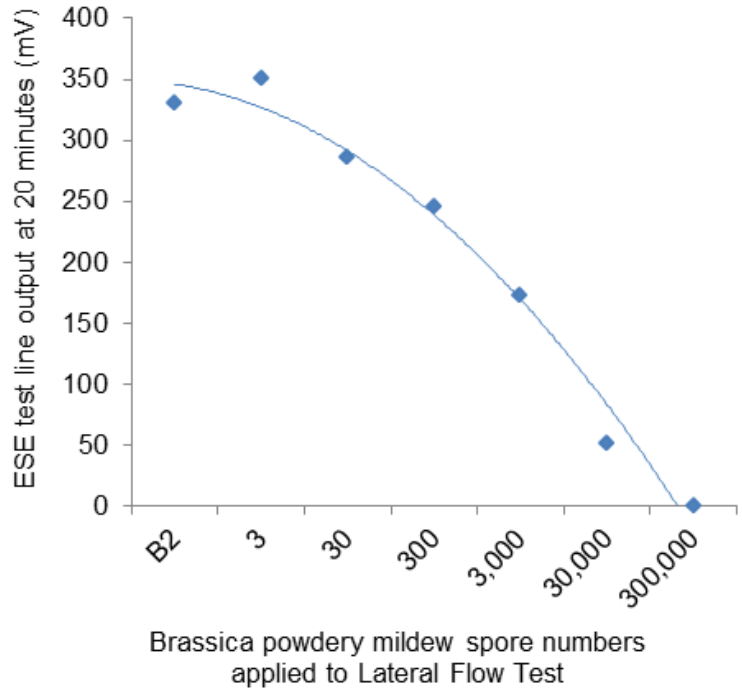

Figure 10. Brassica powdery mildew spore concentration series and quantitative measurement by lateral flow device (in field test) using an ESE reader. B2 is the control and contains no spores.

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.

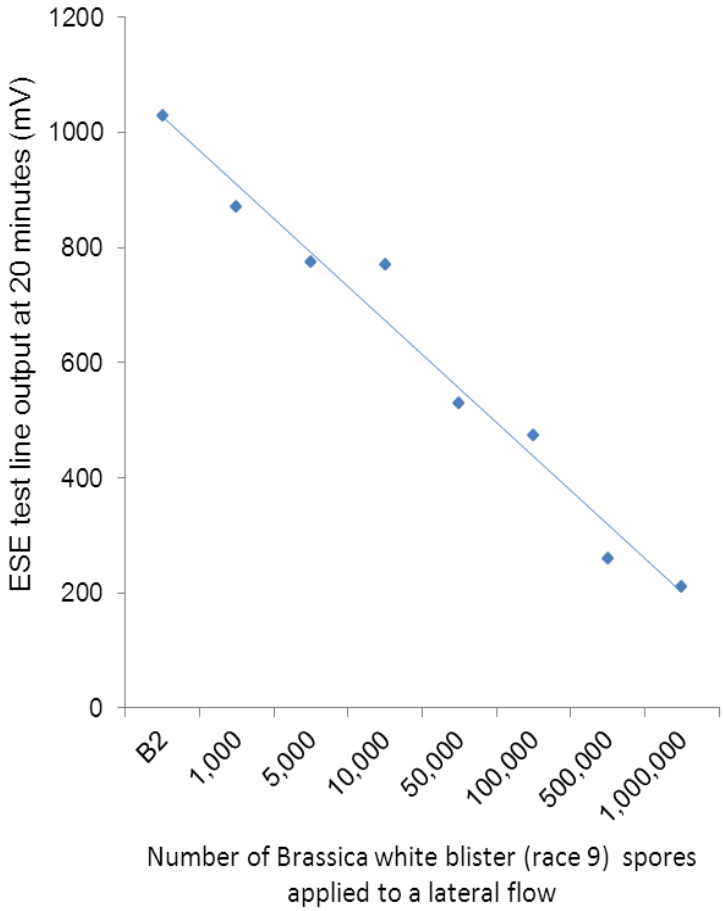

Figure 11. White blister spore concentration series applied to lateral flow devices (in field test) with the test results measured using an ESE reader. B2 is the control and contains no spores.

Assessment of the lateral flow batches for shelf life stability

Test line: Over a 21 month period, the test line antigens of the light leaf spot, Brassica powdery mildew and white blister lateral flows remained stable and biologically active.

Gold antibody conjugate pad: The incorporation of sucrose and mannitol within the conjugate application buffer improved the stability of each of the 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.

It is expected that all tests will have a shelf life in excess of 1 year although this has yet to be proven.

White blister and light leaf spot: 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.

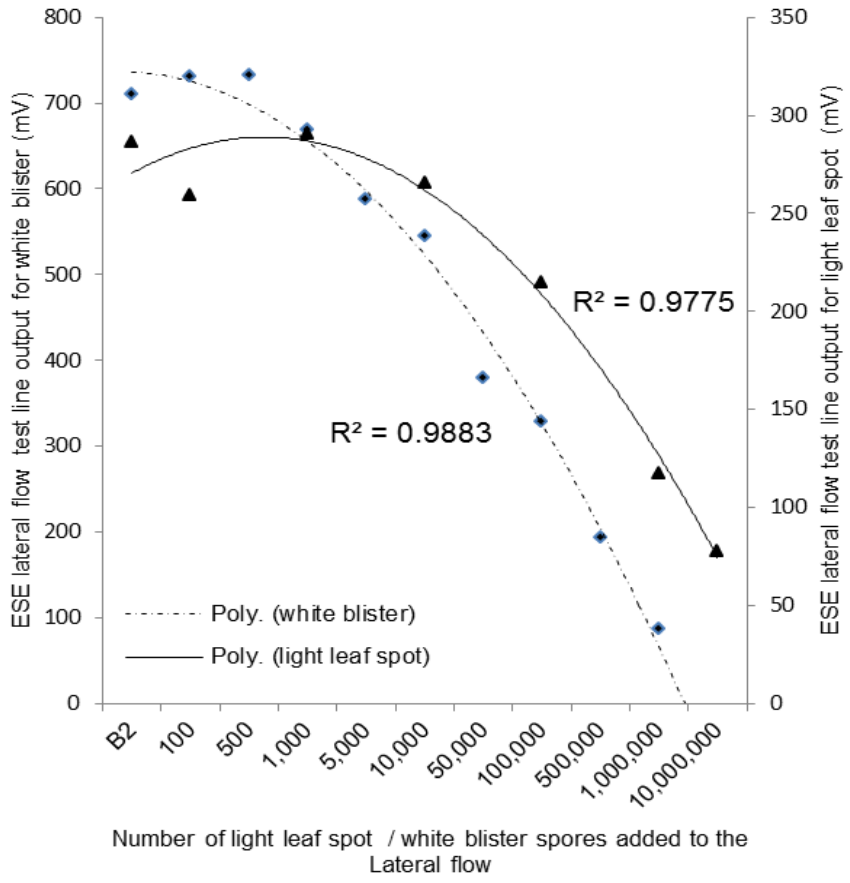

Figure 12. Mixed spore concentrations of *Albugo candida* (white blister) and *Pyrenopeziza brassicae* (light leaf spot) applied to multiplex (two test lines for independent measurement of mixed spore types in a sample) lateral flow devices

White blister and Brassica powdery mildew: Mixed spore types of *Albugo candida* and *Erysiphe cruciferarum* 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 in a mixed

sample on a single lateral flow (multiplex test) (Figure 13). A correlation was observed for each spore type and spore concentration independent of whether the test was single or multiplex (Figure 14). No reactivity / interference in test development was observed between the two spore types in multiplex format.

Figure 13. Multiplex lateral flow device for the measurement of *Albugo candida* (white blister) and *Erysiphe cruciferarum* (Brassica powdery mildew): two test lines for independent measurement of each spore type and a control line.

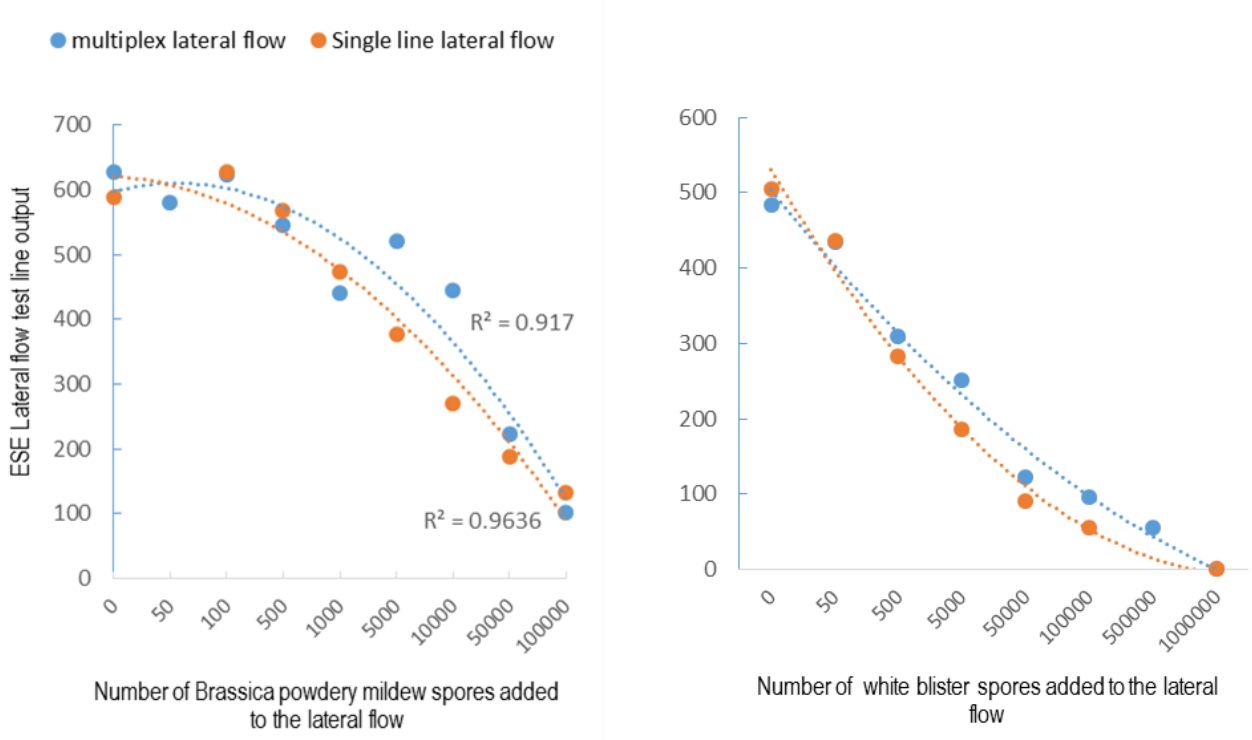

Figure 14. Measurement of mixed spore concentrations of *Albugo candida* (white blister) and *Erysiphe cruciferarum* (Brassica powdery mildew) when applied to single and multiplex lateral flow devices.

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 tape samples 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 of that month 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 collected during the week commencing 11th July 2013. From this date until the week commencing 12th September disease concentrations were predicted to be at a level which could result in the infection of the crop should environmental conditions allow (Fig. 15). This was 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 CP 099 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) agreed with those results derived by the MTIST ELISA. With the exception of one week (commencing 12th September), bioaerosols containing light leaf spot spores were predicted to be at a concentration to place the crop at risk until the middle of October 2013. Thereafter power and mechanical failure of the MTIST bioaerosol sampler prevented useful measurements to be made.

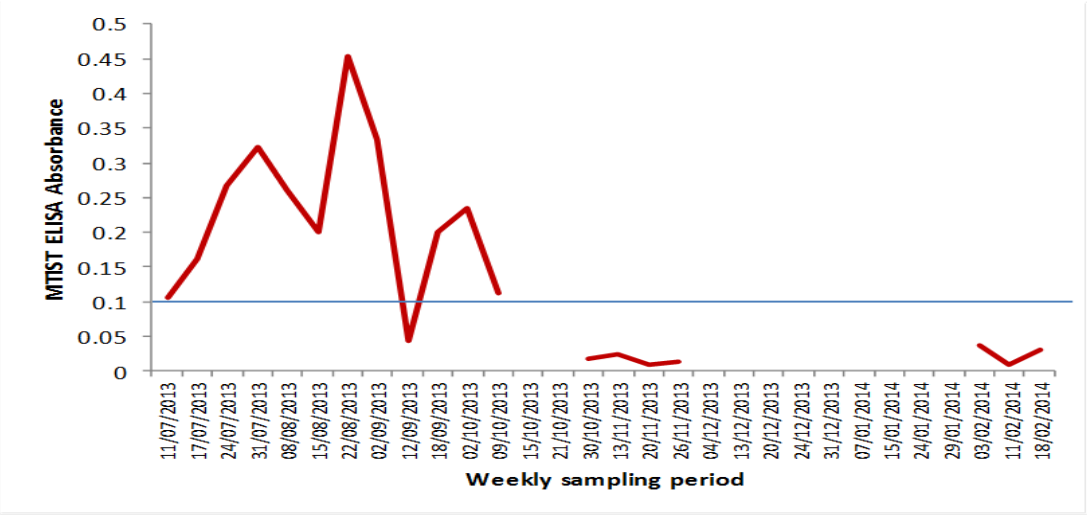

Figure 15. Monitoring MTIST collected bioaerosols for light leaf spot disease at a Brussels sprouts (cultivar Petrus) field site by ELISA.

Weekly bioaerosol samples 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 for the inlet of 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 16 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 a reduced collection efficiency observed (Fig 16b).

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

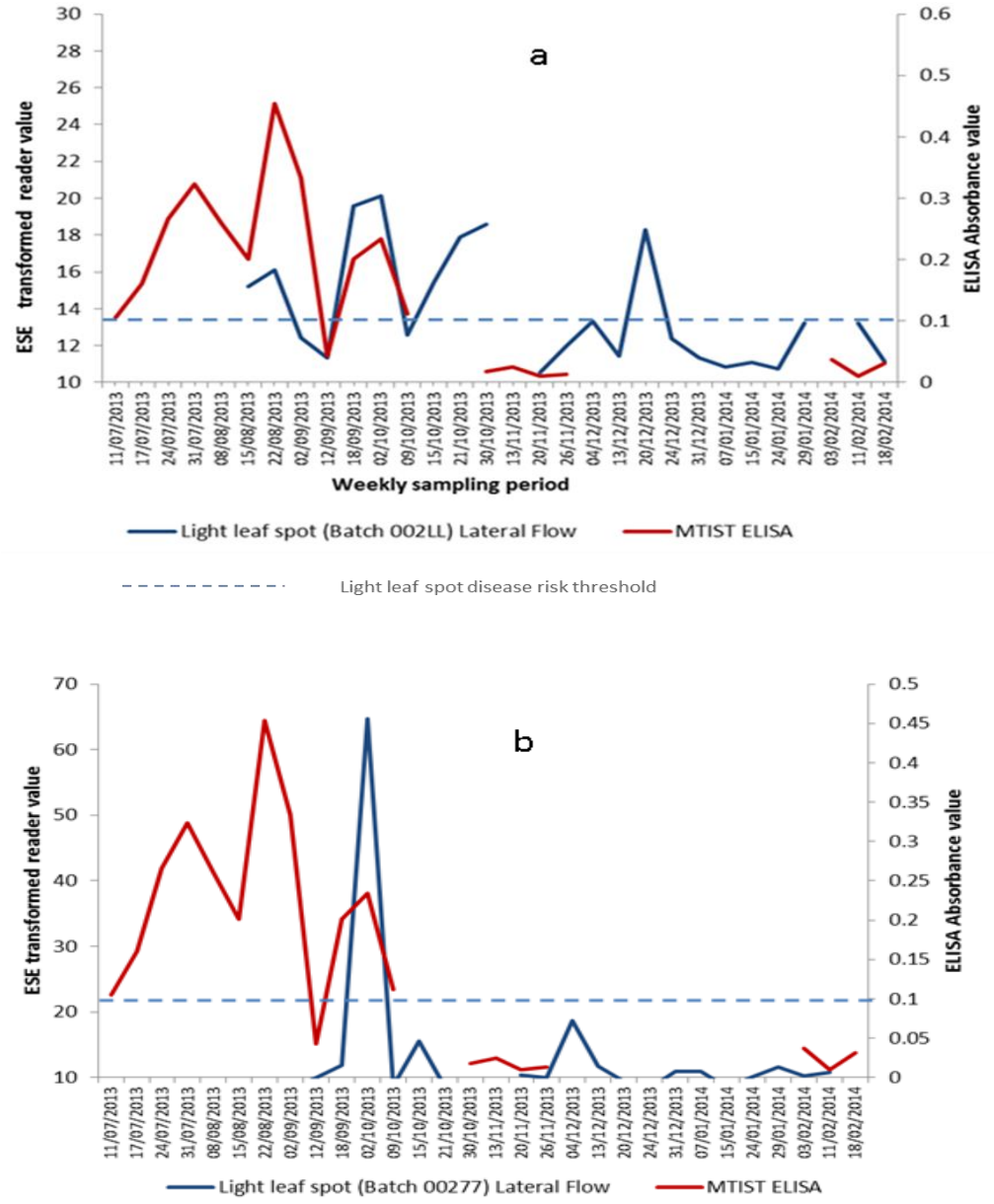

Figure 16. Monitoring light leaf spot disease 'risk' transmission periods in weekly collected field bioaerosols by (a) PTA ELISA and lateral flow (Batch 002LL) and (b) PTA ELISA and lateral flow (Batch 00277LL)

With limited data available from the 2013 to 2014 trial a follow on study was made in Scotland for the 2014 – 2015 Brassica production season. In this study, calibration of the MTIST ELISA process and standard curve (2014 standard control) provides a 'weekly' light leaf spot risk alert when a value of 0.5 is attained. This equates to 200 light leaf spot ascospores per cubic metre of air sampled. According to this value and a lateral flow standard curve (2014 batch 003 LL) an ESE transformed reader value of 80 provides risk of light leaf spot. The 'in-field' bio-aerosol risk assessments were made by an agronomist

using 003 LL batch lateral flow devices with an ESE reader and the results shown in Figure 17. Results for the period 01/10/2014 and 29/10/2014 were not available as a result of error (transient field operator). The crop bio-aerosol was tested once weekly. A battery voltage log of the air sampler was recorded weekly by the agronomist (Table 1).

Light leaf spot risk periods (ESE transformed >80) were predicted during July and August (30/7 through to the 26th August, 2014), October (15th to 21st October) and December (24th to the 6th January). Where possible (19/112014 to 14/01/2015) light leaf spot ascospore concentration was estimated on the base of the MTIST microtitre well by bright field microscopy (Figure 18a). On all other occasions the spore load was so high that identification of light leaf spores was impossible to establish by microscopic analysis (Figure 18b). A relationship between the weekly lateral flow test and light leaf spot spore concentration is shown in Figure 19.

Light leaf spot disease was recorded on the unsprayed variety trial plants (Table 2).

Table 1. Cyclone single vial air cycler battery voltage

Date	Battery							
09/07/2014	12.5		03/10/2014	12.03		10/12/2014	12.07
15/07/2014	12.96		07/10/2014	12.05		17/12/2014	12.02
23/07/2014	12.45		12/10/2014	11.9		24/12/2014	12.47
30/07/2014	12.96		16/10/2014	11.98		31/12/2014	12.33
06/08/2014	12.44		20/10/2014	11.94		07/01/2015	12.18
13/08/2014	12.36		24/10/2014	11.84			
20/08/2014	12.28		29/10/2014	10.72			
27/08/2014	12.31		05/11/2014	11.55			
03/09/2014	12.53		12/11/2014	6.87			
11/09/2014	12.36		19/11/2014	12.92			
18/09/2014	12.23		26/11/2014	12.35			
25/09/2014	12.14		04/12/2014	12.3			

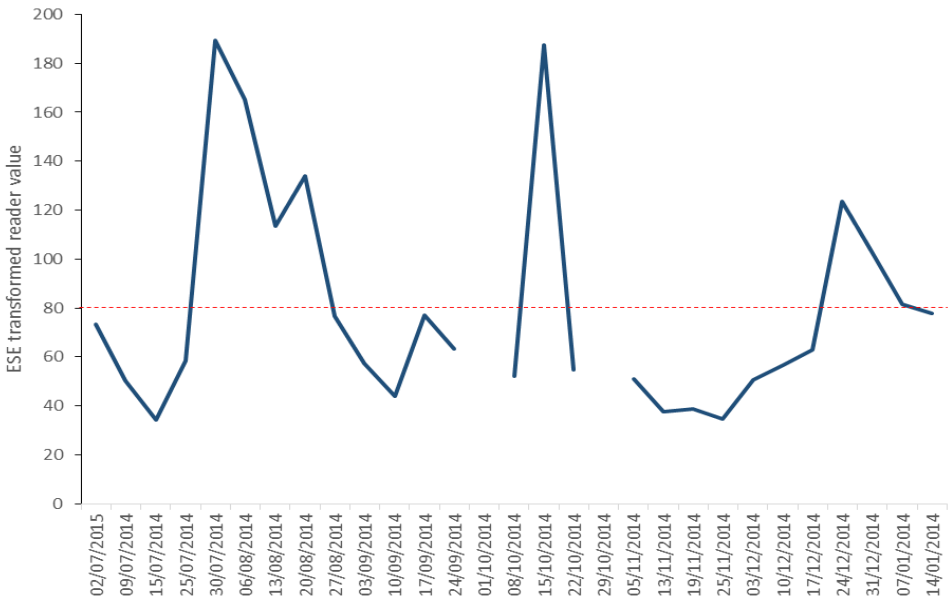

Figure 17. Grower monitoring of light leaf spot disease 'risk' transmission periods using in-field lateral flow kit (Batch 003LL) in weekly collected field bioaerosols.

Table 2. Light leaf spot incidence on unsprayed varietal trials

Plot ID	Variety	Harvest date	growing days	LLS
140008	A	01/09/14	116	7
140016	B	25/10/14	170	7
140019	C	01/10/14	140	5
140034	D	01/12/14	201	7

- The scoring is based on a visual 1-9 scale where 9 is no LLS and 1 is heavily infested. Disease data was supplied by the seed trial company.

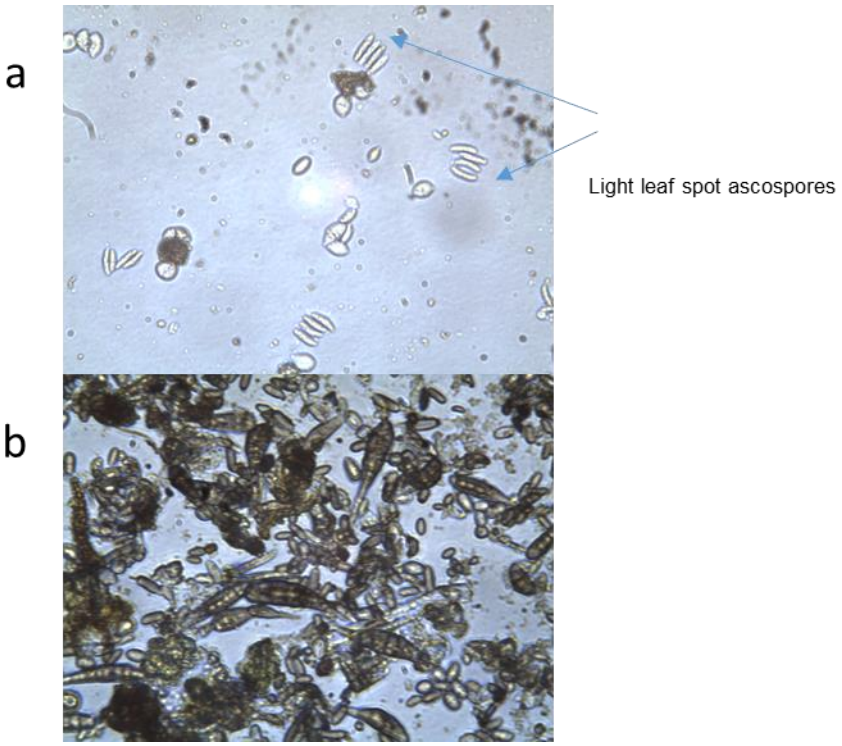

Figure 18. Microscopic analysis of field exposed microtitre well base (a) primarily light leaf spot ascospores (b) high spore load (20/08/2014).

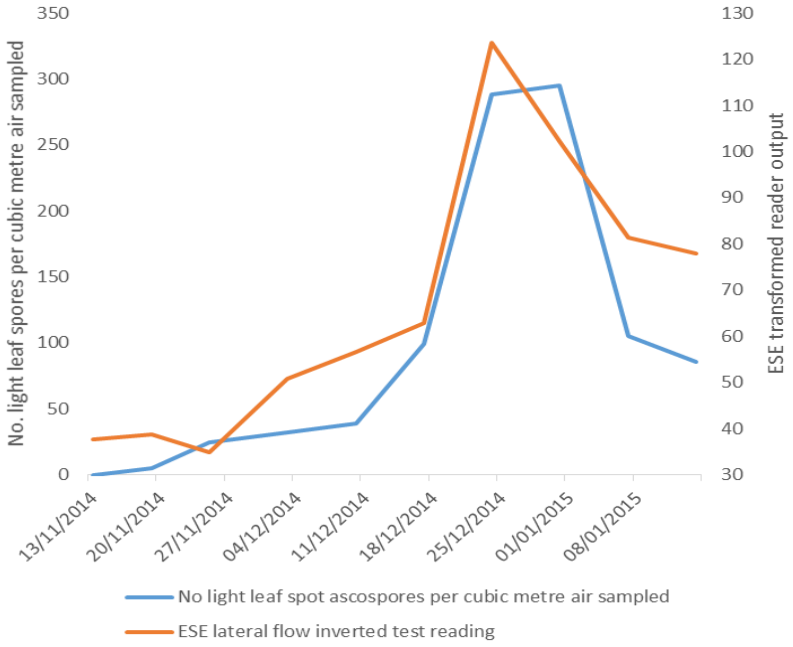

Figure 19. Relationship between the number of light leaf spot ascospores trapped in collected bio-aerosols and the test line absorbance reading of the weekly lateral flow test.

Brassica Powdery mildew and White blister (2013)

The Melinex tape (volumetric air sampler), 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 sent weekly to the NPARU and processed for the measurement of Brassica powdery mildew and white blister inoculum.

Volumetric bioaerosol sampler: Low concentrations of spores which resembled *Albugo candida* (white blister) and *Erysiphe cruciferarum* (powdery mildew) spores were recorded in the airborne environment of the crop for most of the trial period. Although, a sharp increase of powdery mildew spores was observed in July (>70 spores per cubic metre of air sampled daily, Figure 20). This increase in powdery mildew spore concentration was also observed using the lateral flow devices (Figure 21). However, no Brassica powdery mildew symptoms were observed on the sprayed or unsprayed plants during the trial period.

Note: Bright field microscopy would not be able to differentiate the different races of *Albugo candida* present in a bioaerosol sample. For example, race 4 is the causative agent of white blister of shepherd's purse and race 9 occurs in Brassicas.

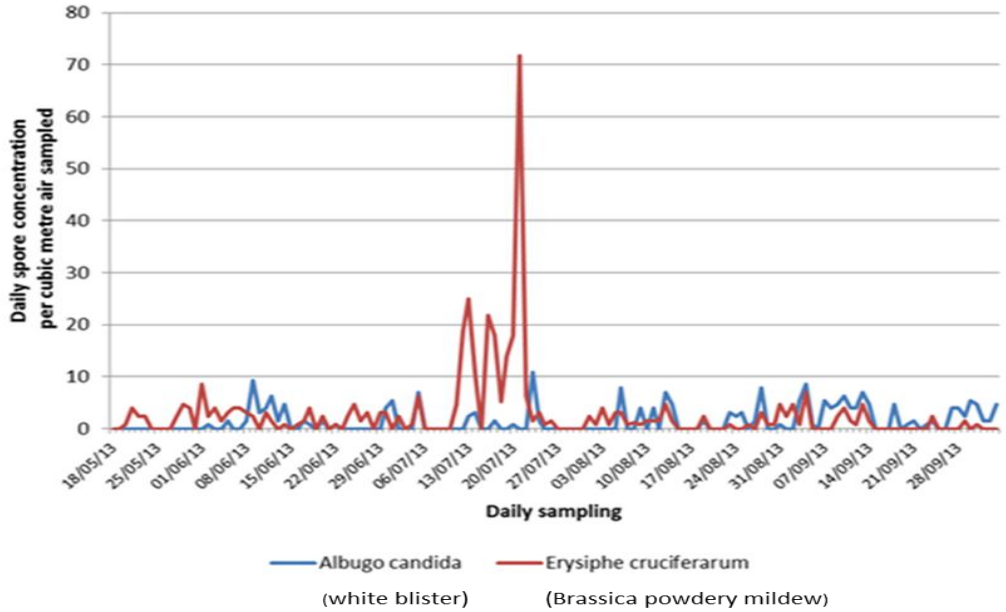

Figure 20. Estimated daily spore concentrations of *Albugo candida* and *Erysiphe cruciferarum* on field exposed Melinex tape (volumetric 7day spore trap) as recorded by microscopic analysis

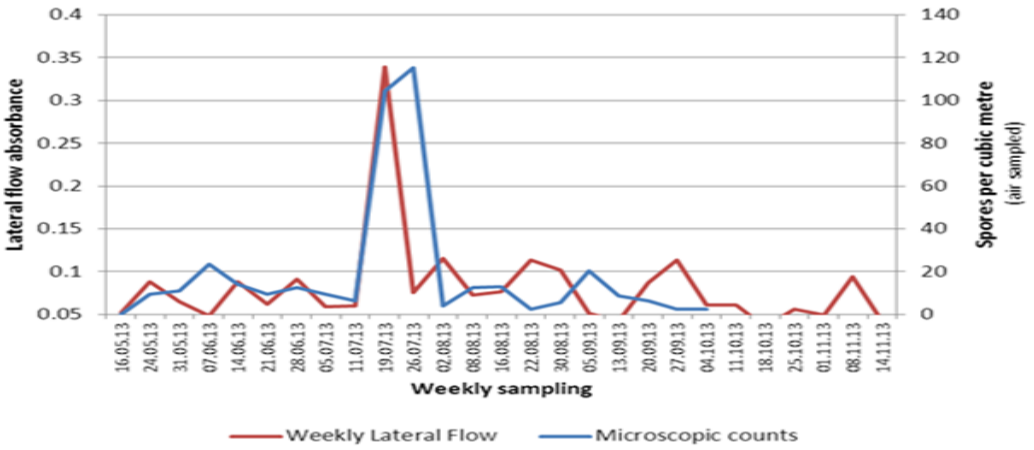

Figure 21. Assessment of bioaerosols on a weekly basis for Brassica powdery mildew by microscopic analysis and using 'in field' lateral flow tests

MTIST ELISA bioaerosol samples: Assessment of the four microtitre 8 well strips by MTIST ELISA analysis (laboratory antibody based test) indicated that white blister spore numbers were low (<0.2 absorbance) throughout the period of the trial. Nevertheless, an association was observed between the spore concentration (per cubic metre of air sampled weekly) as estimated by bright field microscopy and the corresponding ELISA result (Figure 22). This occurred on two occasions (weeks commencing 16/8 and 5/9). The ELISA values exceeded a value of 0.15 and indicated risk periods for white blister (Fig 22).

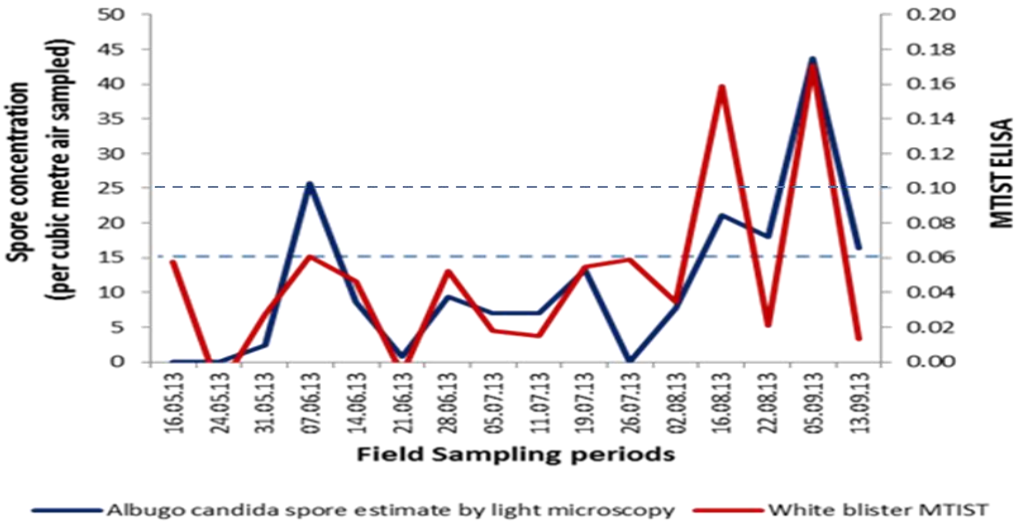

Figure 22. Monitoring weekly *Albugo candida* spores (white blister) in field bioaerosols by bright field microscopy and laboratory ELISA

Single tube cyclone sampler: The 'in-field' lateral flow test detected a high concentration of white blister spores during the week of the 22nd August (Fig. 23). 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 risk model (Morph Brassica Spot) used to forecast white blister risk was activated on the 22nd August as a result of the positive lateral flow result (Fig. 24). 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 2013. 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 2013 (Fig. 25). 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 observed in the collected field bioaerosol samples.

The values in Figure 23 were derived from the white blister standard curve (Figure 14, *Albugo candida* single test line lateral flow) and using prism graph pad software (www.graphpad.com/scientific-software/prism). The daily lateral flow outputs were log transformed and a four parameter logistic regression made. The results were interpolated against the onion downy mildew standard curve and an inverse log transform carried out to provide the predicted spore concentrations.

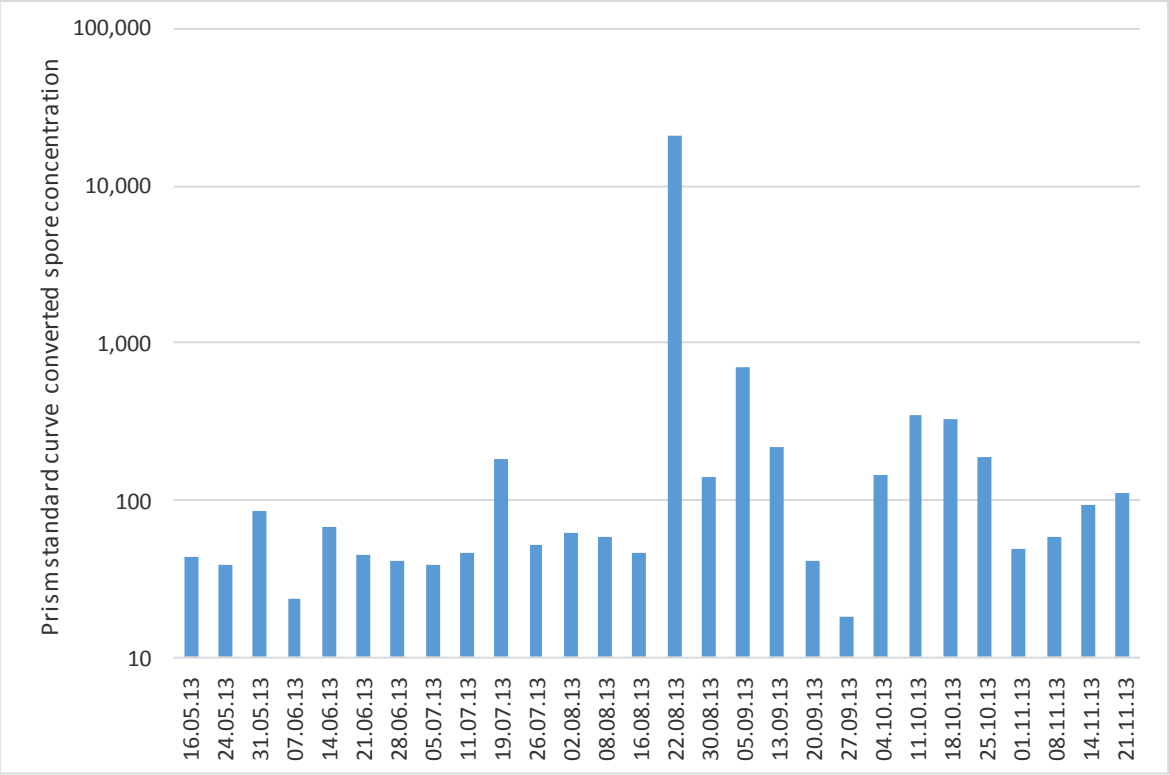

Figure 23. Monitoring weekly *Albugo candida* spores (white blister) in field bioaerosols using 'in field' lateral flow tests.

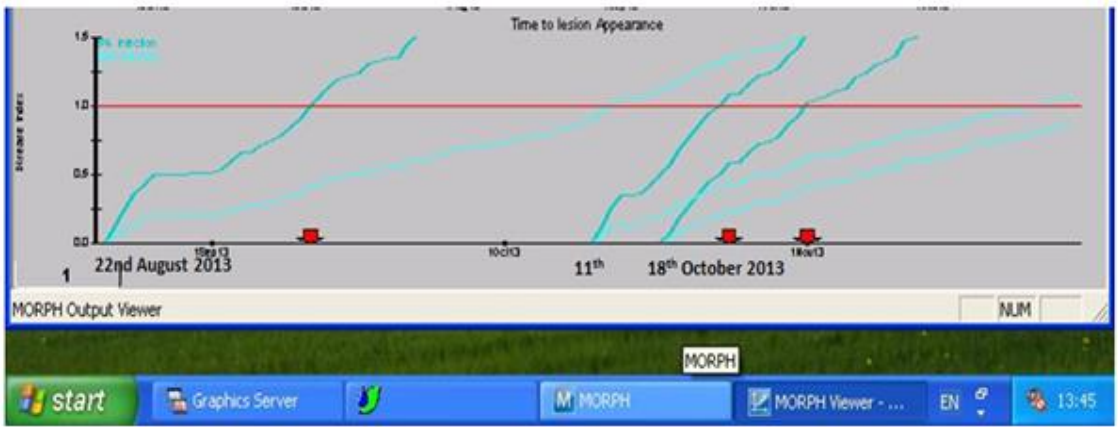

Figure 24. Prediction of white blister lesion appearance on trial plants based on *Albugo candida* inoculum availability as predicted by the lateral flow test on the 22nd August 2013.

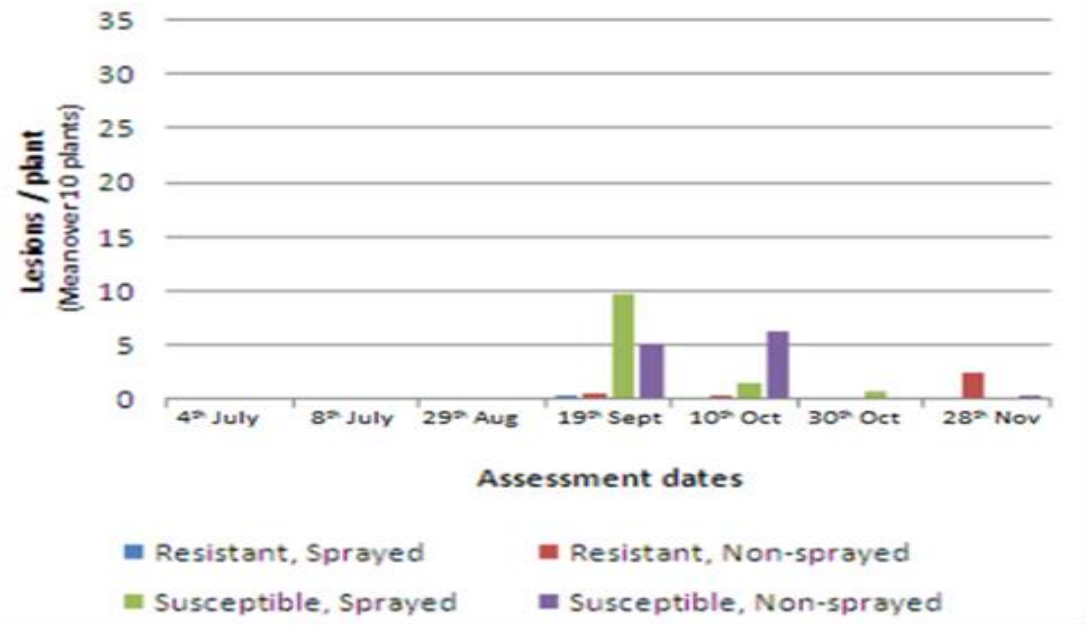

Figure 25. Assessment of 40 tagged plants identified in each cultivar type (susceptible and intermediate resistance) for white blister lesion development within the sprayed and non-fungicide treated area of the trial

Brassica Powdery mildew and White blister (2014)

The same samplers were used in 2014 to obtain field bioaerosol samples.

Volumetric bioaerosol sampler: Microscopic measurement determined white blister spores not to be prevalent until the final week in August (Figure 26). For this period a high concentration of spores which morphologically fitted the characteristics of white blister were identified by bright field microscopy (< 100 spores per cubic metre of daily air sampled). Thereafter, concentrations were often recorded but remained below 20 spores per cubic metre (daily) until the final week in October. White blister was first observed on plants on the 9th September 2014 (Figure 27). White blister disease development in 2014 was low and more than 50% lower than recorded in 2013.

Fungal spores with characteristics of powdery mildew were identified throughout the season and often at daily concentrations above 10 spores per cubic metre of air sampled. On peak days up to 40 spores per cubic metre were observed (15 -17th July and 21st October, 2014). As in 2013, no Brassica powdery mildew disease symptoms were observed on the sprayed or unsprayed plants during the trial period.

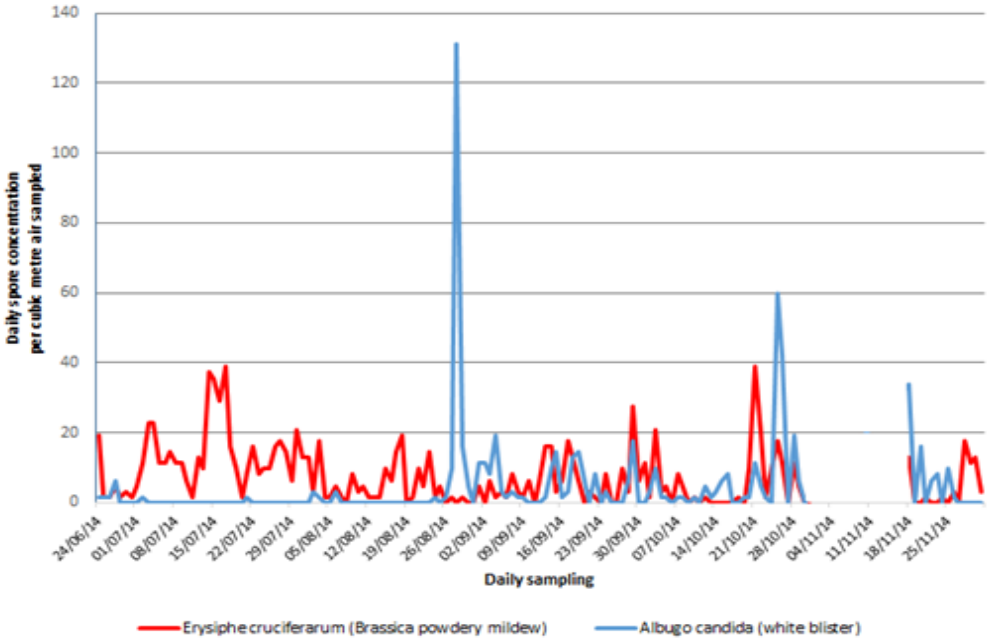

Figure 26. Estimated daily spore concentrations of white blister and powdery mildew spores on the field exposed Melinex tapes (volumetric 7 day spore trap) as recorded by bright field microscopic analysis

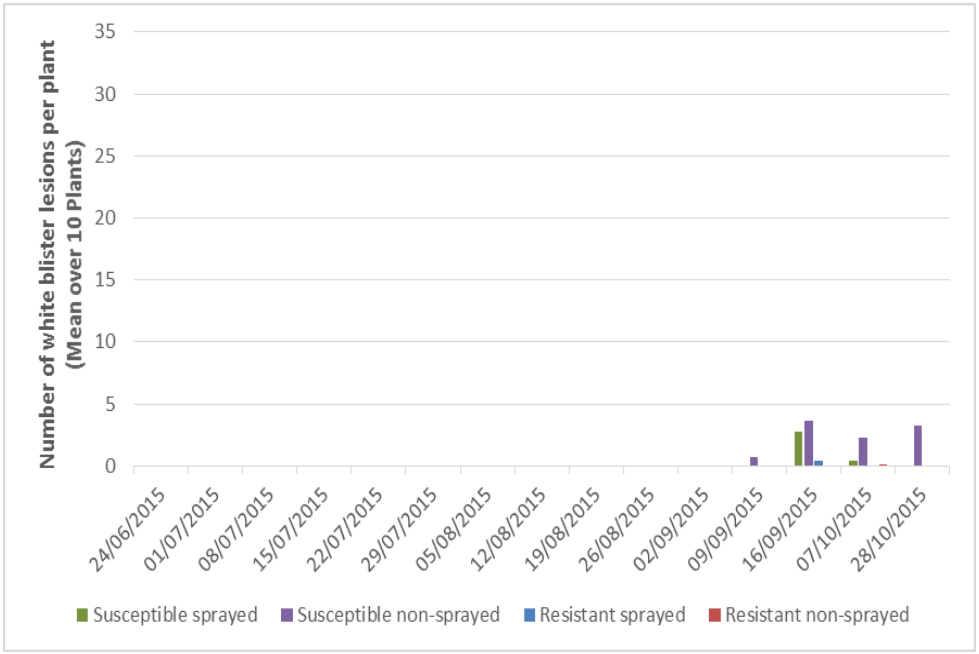

Figure 27 Assessment of 40 tagged plants identified in each cultivar type (susceptible and intermediate resistance) for powdery mildew and white blister lesion development within the sprayed and non-fungicide treated area of the trial

MTIST bioaerosol sampler: As in 2013, the absorbance values recorded by the ELISA process suggested a low concentration of white blister spores present in or around the crop (ELISA value <0.2). Nevertheless, according to a provisional MTIST risk criteria set in 2013 (weekly spore concentration of 20 spores per cubic metre air sampled, MTIST ELISA 0.08) risk for white blister disease was identified on 19th to 25th August 2014. Three smaller peaks were also noted. The sampling period of the 15th to the 21st July 2014 and 16th to the 22nd September 2014 fell close to the threshold whilst a much reduced peak was observed from the 17th to the 23rd October 2014 (Figure 28).

Powdery mildew spore peaks and troughs were observed across the period when estimated by MTIST ELISA and bright field microscopy (Figure 29). White blister spore concentrations when spread over a weekly period peaked at around 20 spores per cubic metre. No disease was observed in the crop. Bright field microscopy would not be able to differentiate many of the powdery mildew species present in outdoor bioaerosols. Also, as outlined in HDC FV 333, the antibody probe used to detect Brassica powdery mildew (MTIST and lateral flow) also reacts with other family members of the *Erysiphaceae* i.e. powdery mildew isolated from thistle, oil seed rape, garden weed and tomatoes.

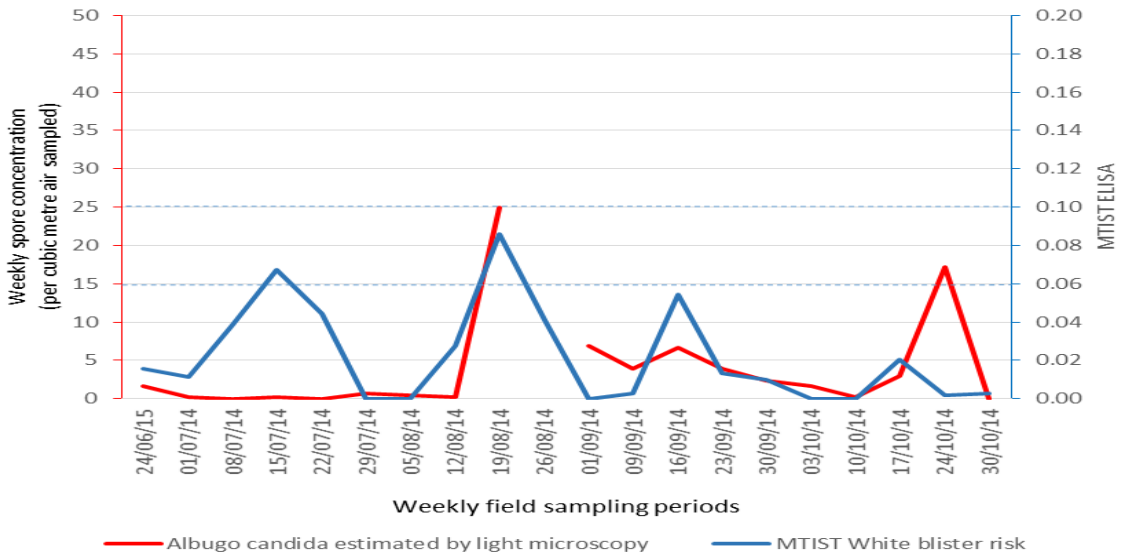

Figure 28. Monitoring weekly *Albugo candida* spore concentrations (white blister) in field bioaerosols by MTIST ELISA and bright field microscopy

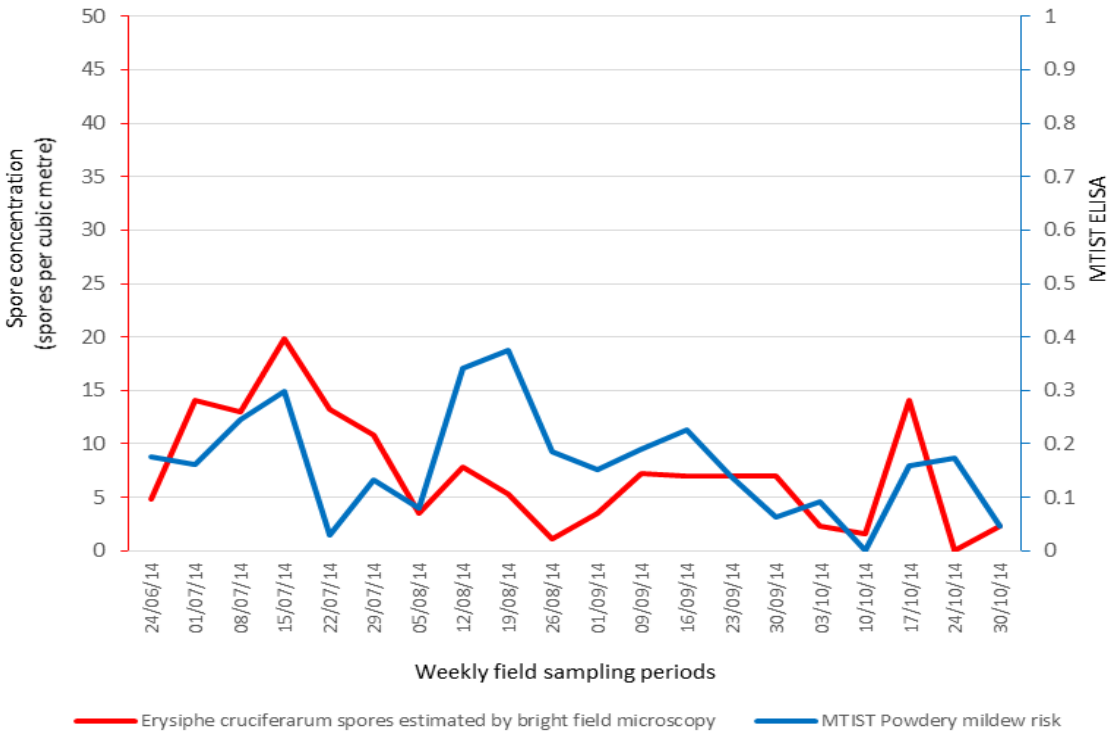

Figure 29. Monitoring weekly powdery mildew spore concentrations in field bioaerosols by MTIST ELISA and bright field microscopy

Cyclone bioaerosol sampler.

White blister: Assessment of the single cyclone air sampler (weekly collected bioaerosol into one tube) by lateral flow test provided few weeks where white blister spore concentrations were predicted (by Prism graph pad) to be above 10 spores and with no weeks at >100 spores (Figure 30). These figures were derived from a standard control curve (laboratory produced concentration range of white blister spores) that is run at the time of the field lateral flow tests (Figure 14, white blister multiplex lateral flow). The standard curve is used to account for variation in test running conditions (temperature, humidity, and operator) and lateral flow batches. The Prism calculated spore concentration is constructed from the standard curve. Based on the results from 2013, it was proposed that spore concentrations in excess of 100 (standard curve derived) would be required for white blister disease establishment in the field. However, of the weekly sample periods tested in 2014, the 12 - 25th August and 10th - 16th October were significant in terms of white blister disease later observed in the crop (Figure 30). On these dates total spore concentrations of upwards of 50 spores were predicted. The disease expressed on plants arising for this period was low however and < 50% of that observed in 2013 (Figs. 25, 27). Interestingly, of the daily aerosol collected samples (multi-vial cyclone (7 tubes)) processed by lateral flow, white blister spore concentrations were predicted at > 100 on two occasions

(25th August and the 10th October). The daily value exceeded the respective cumulative weekly cyclone test for that period (Figure 31).

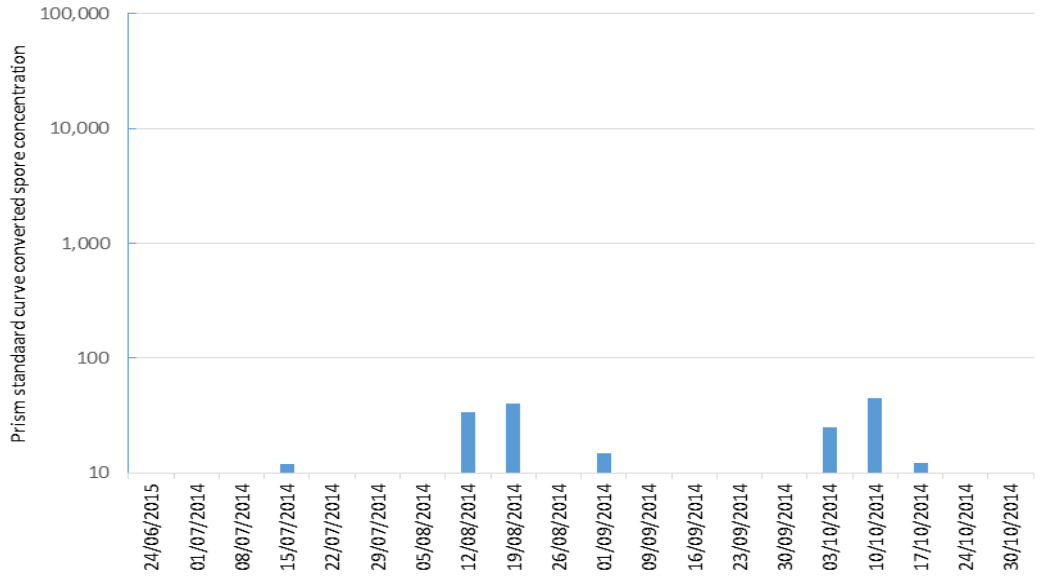

Figure 30 Monitoring weekly collected Albugo *candida* spores (white blister) in field bioaerosols using 'in field' lateral flow tests

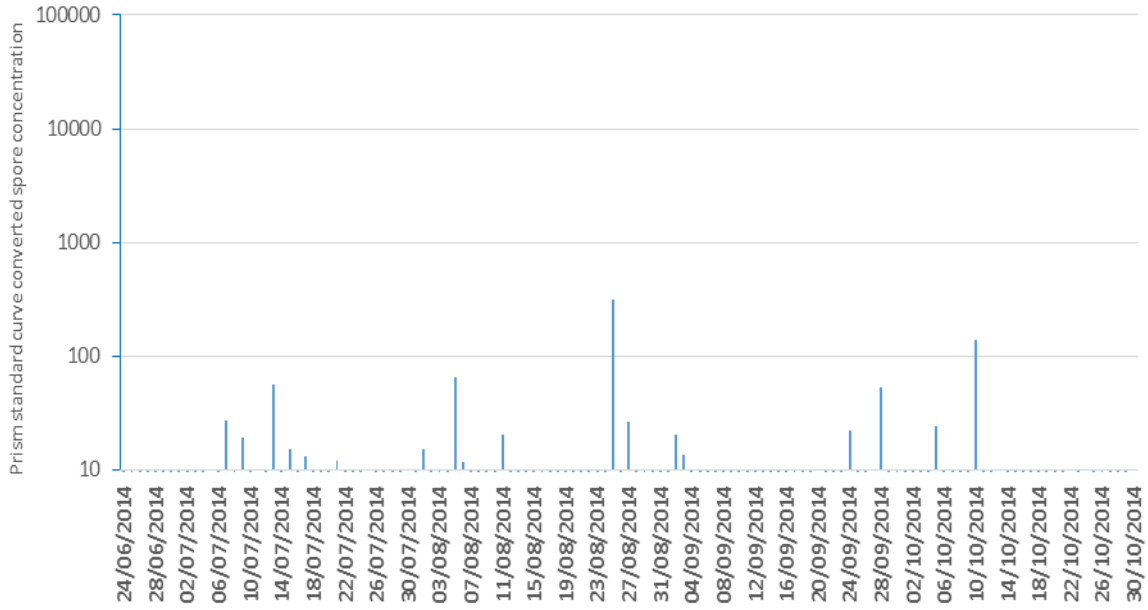

Figure 31. Monitoring daily collected Albugo *candida* spores (white blister) in field bioaerosols using 'in field' lateral flow tests

White blister Disease Forecast Model

Using the criteria developed in the 2013 trials (weekly average of white blister spores per cubic metre air sampled, MTIST ELISA at 0.08 Absorbance, > 100 spores lateral flow Prism

graph pad standard curve) each of the sampling systems provided the first risk of white blister symptom in field bioaerosols for the period of 19th to 26th August (MTIST ELISA, microscopic analysis) and more specifically the 25th August (daily lateral flow test). Using this information an environmental white blister disease risk model (Morph™ Brassica Spot) was activated on the 25th August, 2014. For this period the white blister crop walking model describes high and moderate infection risk periods (Figure 32, red and yellow bars). Based on white blister inoculum (*A. candida*) being present on the 25th August, the model predicts 5% disease expression on plants (white pustules on leaf or button surface) will occur around the period 4-6th September 2014 and 50% infection visible on plants around the 18th to 20th September 2014 (Figure 32, Time to lesion Appearance).

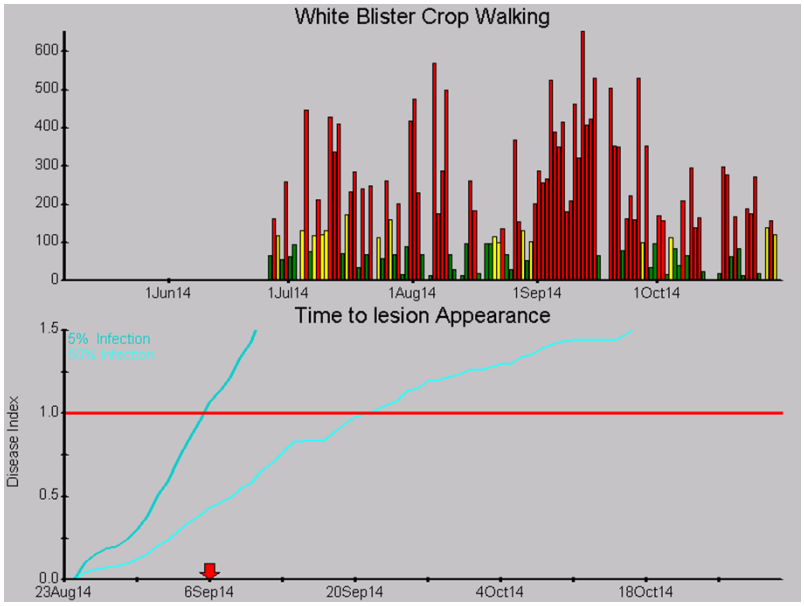

Figure 32. MORPH™ Brassica Spot: White blister lesion appearance according to an infection start of the 25th August, 2014.

Crop assessments

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 9th September, 2015 (1 lesion per unsprayed plant). No disease was apparent when the crop was previously assessed on the 2nd September or prior to that. By the 16th September, 4 and 3 lesions were visible per plant assessed (mean over 10 plants) on both the unsprayed and sprayed susceptible trial respectively.

Brassica powdery mildew: Assessment of the single cyclone air sampler (weekly collected bioaerosol into one tube) predicted powdery mildew spore concentrations for all weeks tested (Figure 33). Also, when the daily collected bioaerosols were tested by lateral flow most days predicted above 1000 and often 10,000 spores (powdery mildew standard curve, prism graph pad). Each of the bioaerosol sampling systems identified powdery mildew in field bioaerosols throughout the season. Although, a clear relationship between the lateral flow and the other bioaerosol systems could not established (Figure 33). As in 2013, no disease was observed on any of the plants assessed or visible within the wider crop.

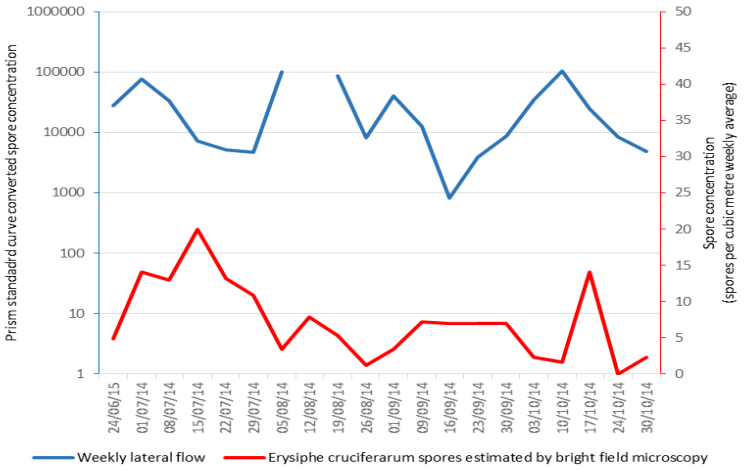

Figure 33. Monitoring weekly powdery mildew spore concentrations in field bioaerosols by lateral flow and bright field microscopy

Discussion

DIAGNOSTIC TESTS

Batches of lateral flows have been used to measure field bioaerosols for light leaf spot, Brassica powdery mildew and white blister. The developed tests have a diagnostic range suitable for use in disease risk forecast studies. Lateral flow batches tested with known spore standards 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 50 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 simultaneous measurement of:

- light leaf spot and white blister spores
- white blister and powdery mildew spore

Field Trials

Light leaf spot

Over an eight month period a commercial Brussels sprout crop *cv.* Petrus was monitored for bioaerosol concentrations of light leaf spot disease (*Pyrenopeziza brassicae* ascospores). An earlier study proposed a Brussels sprout crop at biological risk to light leaf spot disease when a spore concentration was recorded at or above 200 per cubic metre of field air sampled (HDC CP 099 Year 1 Annual Report, 2013). In the 2013 field trial, this value was achieved in the first week of sampling (week commencing 11th July). However the environmental conditions were unfavourable for the development of the disease on plants. With the exception of one week the crop remained at risk from disease until the middle of October. Power and mechanical failure of the air samplers resulted in some disruption of the trial, although, it remained possible to identify potential disease thresholds. Using this information a fungicide spray program was operated throughout the life of the crop for treatment of the disease. The crop remained relatively disease free and was able to be marketed for commercial supply to February 2014.

To provide clarity, the trial was repeated later in 2014 to 2015. This time the end user (agronomist / grower) processed the weekly lateral flow test and made battery voltage checks on a weekly basis. Bright field microscopic analysis of light leaf spot ascospore concentration correlated with the weekly lateral flow test. Disease ‘risk’ peaks in excess of

200 light leaf spot ascospores were observed. Light leaf spot disease was recorded during the trial period on unsprayed Brassica varietal trial plants.

It is probable that the regional harvest of oil seed rape provided the source of the light leaf spot inoculum plumes recorded from July through to September (Fig. 34). In conjunction with environmental data the development of regional oilseed rape harvest source maps could provide a useful tool for forecasting the onset of light leaf spot disease risk on Brussels sprout crops in the UK. During these 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 risk.

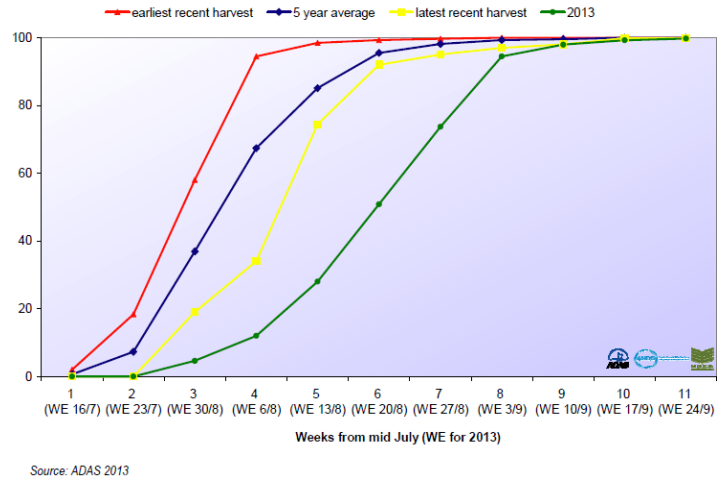

Figure 34. Cumulative % winter oilseed rape harvest progress comparison (ADAS 2013)

Brassica Powdery Mildew

Powdery mildew spores were identified in field bioaerosol samples during each of the trial periods of 2013 and 2014. In 2013, microscopic analysis of air samples suggested that for most of the period powdery mildew risk was low (<10 spores per cubic metre). Although in July numbers were seen to sharply rise with upwards of 70 powdery mildew spores per cubic metre of air identified. This rise was also observed using the powdery mildew lateral flow. However, no disease was recorded on any of the plants in the trial. In 2014, daily powdery mildew concentrations often reached 20 spores per cubic metre and remained relatively constant across the period. On a number of days, concentrations did rise to near 40 spores per cubic metre. As in 2013, no powdery mildew disease was recorded on any of the plants in the trial.

The potential exists for disease to be at a concentration for infection of susceptible hosts (biological disease threshold) *but* if environmental conditions are not suitable the disease is

unlikely or unable to progress to symptom development. Brassica powdery mildew is thought to require relatively dry conditions but as yet the environmental parameters for infection are not yet well described. With no disease symptoms observed during each of these trials it is not possible to determine if the biological disease threshold had been met. Also, another factor may be the accuracy of the test. The Brassica powdery mildew spores (*Erysiphe cruciferarum*) cannot be reliably differentiated from other powdery mildew species using the methods described in this project. For this reason, it is possible that the fluctuation in spore concentrations during the period of the project is a result of other powdery mildew species. This being the case, the test may have better application in protected cropping systems as a generic powdery mildew disease risk indicator.

White blister

White blister spores were identified in field bioaerosols during each of the trial periods of 2013 and 2014. In 2013, the MTIST and the volumetric air samplers identified white blister spores in the crop throughout most of the trial period. In July of that year, the white blister spore concentration was recorded at a weekly average of near 15 spores per cubic metre of air sampled. For most of these days the white blister model predicted the crop to be at moderate or on occasion at high risk to white blister infection (Figure 35). However, no evidence of disease was observed on the plants until the 19th September 2013. The lateral flow test predicted white blister to be present throughout the monitoring period. However, with the exception of the week commencing the 19th July 2013, spore concentrations remained low until the 22nd August 2013. White blister weekly lateral flow test regularly provided values in excess of 100 (Prism standard curve generated spore value). The environmental model (Morph Brassica spot TM) for this period (22nd August 2013) indicated a high risk for white blister infection. 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 bioaerosol samples) the model predicted that white blister disease would be visible on susceptible unsprayed plants from the second week in September 2013 (Figure 24). White blister was first observed in the crop on the 19th September 2013 (Figure 25). The model then predicted two further white blister sporulation dates from the 11th and 18th October 2013. The weekly lateral flow assessments from the week commencing 4th October 2013 demonstrated 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 2013.

Also, from the week commencing 15th August 2013 an increase in white blister concentration was observed by microscopic analysis and absorbance values of the MTIST ELISA. From this trial it is therefore proposed that the white blister disease threshold is likely to be in excess of 20 white blister spores per cubic metre of air sampled (bright field microscopy - weekly average) and for the MTIST ELISA an absorbance value 0.08 (defined by the use of ELISA standard controls)

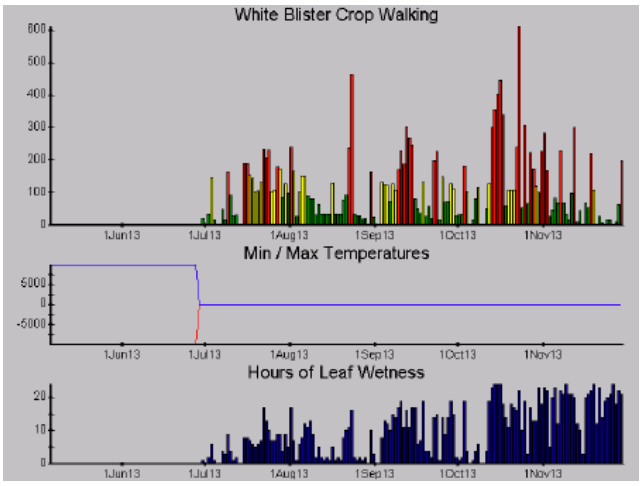

Figure 35. White blister model output for the trial sited at Dotams Lane, Butterwick

In 2014, the field trial was repeated. Between June and August 2014 very little disease was observed in the collected bioaerosols when examined by microscopic analysis. However, on one day, a spike in white blister inoculum (>120 spores per daily cubic metre air sampled, microscopic analysis) was seen to breach the proposed at risk value. For this period, the weekly MTIST ELISA bioaerosol sampler (19 to the 26th August, 2014) also broke the threshold value identified in 2013 (average concentration of 20 spores per cubic metre air sampled over a week and 0.8 ELISA value). The daily cyclone air sampler (multi-vial sampler) also for the first time during the monitoring period exceeded the proposed lateral flow risk value of >100 spores (Prism graph pad) and was recorded on the 25th August 2014 with a value of > 500. The weekly cyclone trap for this period (19th to 26th August 2014) provided its greatest lateral flow signal for the period but did not reach the threshold set. The weekly sampler provided a much reduced signal overall compared to 2013. No mechanical issues were recorded for the air sampler and the batteries which supplied the instrument were checked weekly. It is possible that the collection efficiency of the sampler may have been affected over the period but this cannot be confirmed. Another reason may result from interference of the lateral flow assay by windborne soil collected over a weekly

sampling period into a single tube. Studies in 2014 (HDC CP 099C) also found that weekly bioaerosol collections for the measurement of onion downy mildew spores (*Peronospora destructor*) were similarly affected when compared to daily collections. For the onion downy mildew trial (June – August, 2014) soil was observed regularly in the weekly collection tubes.

As in 2013, a concentration of white blister spores was measured by each of the air samplers to identify a period when the crop was first at risk to a white blister 'biological threshold'. Using this information with an environmental disease forecast model (Morph Brassica™ Spot) it was possible to accurately predict when disease would be visible on unsprayed susceptible plants. By including information on availability of white blister spore load (using either the laboratory MTIST or on-site daily lateral flow test) it has been demonstrated in each of the two years that in conjunction with the white blister forecast model improved management of the disease with reduced applications and effectiveness of the fungicides applied can be achieved.

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 cubic metre 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) to monitor disease thresholds in commercial crops for light leaf spot, 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 where possible be combined with an environmental risk model for each disease (Morph Brassica™ Spot). Also

for consideration is the use of daily or weekly estimates to determine presence of target disease in air samples. This approach has reported for other diseases of field crops (Wakeham and Kennedy, 2010, Wakeham et al., 2011). Where daily environmental risk forecasts are available it may prove more accurate to use daily air sampling regimes. This process is being used to predict risk alerts of ringspot disease in the UK (http://www.syngenta-crop.co.uk/brassica-alert) and also trialled successfully by Syngenta in Europe. However in developing a daily or weekly system it is necessary to determine the biological disease threshold at which infection will occur and the necessary environmental parameters on this process.

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. *According to the European Union Sustainable Pesticide Use Directive (2009/128/EC) professional producers will have to apply general principles of integrated pest and disease management from January 2014. The systems described in this report demonstrate an integrated disease management approach towards the reduction of crop inputs to control disease.*

Light leaf spot test *:* Control of light leaf spot on Brussels sprout crops varies with cultivar. In Scotland, highly susceptible cultivars such as *c.v.* Millenium are not used successfully even with fungicide control regimes. This has major implications in the control of light leaf spot in Brussels sprouts where a range of cultivars are grown for 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. 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 aptly during the field trials 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. For example in 2012 (HDC FV 333), 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 observed in mid-January on the partially resistant cultivar (*c.v.* Petrus) and, at an increased level on less resistant cultivars. Through this project the threshold for low level disease risk has been set at 200 spores per cubic metre air sampled. An MTIST spore (www.burkard.co.uk) trap can be used to provide weekly measurement of disease potential. Analysis requires that the microtitre well strips are removed and sent by post to a testing laboratory. Results should be available within 24 hours of receipt at the laboratory. The test format (32 wells) provides capability to test for multiple diseases. A lateral flow device has also been developed to identify light leaf spot risk periods.

White blister. The study has also characterized the usefulness of two other tests for monitoring crop bioaerosols for risk of susceptible Brassica vegetable field crops to white blister and powdery mildew disease. 'In field' diagnostic tests have been developed to each of these two pathogens and trialled 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 a spore concentration of >20 spores per cubic metre (weekly average) has been estimated to provide risk for disease development. Using this criteria the in-field lateral flow test was able to detect the disease in weekly collected bioaerosols ahead of symptom development on the crop (2013 trial). For the 2014 field trial, a daily bioaerosol 'in field' lateral flow test was introduced. This allows the grower to assess the white blister model on a daily basis and then make a decision as to whether it is necessary to test air bioaerosols for that date. This should provide increased accuracy of the test and targeted testing of daily air bioaerosols. 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 (infield on-site test) with the white blister forecast model.

Brassica Powdery Mildew: 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 and 2014, no powdery mildew disease was observed on the trial crop. In 2013, it was considered likely that no disease development was as 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. A plume of inoculum (70 powdery mildew spores per cubic metre air sampled) was however recorded in the trial area during a period in July. But the unusually dry and hot conditions may have prevented establishment of the disease in the crop. This scenario has been reported for Grapevine powdery mildew spores which can be killed at temperatures above 33°C (Gubler et al. 1999). When the trial was repeated in 2014, powdery mildew concentrations often reached 20 spores per cubic metre on a daily basis. On a number of days, concentrations did rise to near 40 spores per cubic metre. With no disease symptoms observed during each of these trials (2013, 2014) it has not been possible to determine if the biological disease threshold had been met. Also, another factor may be the accuracy of the test. The Brassica powdery mildew spores (*Erysiphe cruciferarum*) cannot be reliably differentiated from other powdery mildew species using the methods described in this project. For this reason, it is possible that the fluctuation in spore concentrations during the period of the project is a result of other powdery mildew species. This being the case, the test may have better application in protected cropping systems as a generic powdery mildew disease risk indicator.

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. For example, 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. To prevent further resistance build up it is important that fungicide applications are made only when required and at times when they will have maximum effect on the pathogen. Signum (pyraclostrobin + boscalid) also 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. Additionally the fungicide Rudis (which contains triazolinthione) has also given good control of light leaf spot.

The light leaf spot and white blister tests will be available to the UK horticultural industry to trial from 2015. Alison Wakeham at NPARU can be contacted for further information on these and their use with bioaerosol samplers (a.wakeham@worc.ac.uk).

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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