

Project title: Evaluation of an integrated disease management system to ascribe risk of downy mildew disease on commercial salad and bulb onion crops in the UK

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The results and conclusions in this report are based on an investigation conducted over a two-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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GROWER SUMMARY

Headline

- This project has successfully demonstrated the huge potential of using an integrated disease management approach to reduce crop protection inputs to control downy mildew in onion crops, whilst maintaining or even improving yields.
- In four out of six field trials carried out at commercial grower sites between April 2014 and October 2015 crop protection inputs were reduced and provided either similar or improved levels of onion downy mildew disease control. Whilst these results are promising, the system was not fully robust over the period.
- In the system trialled, the application of fungicide (oomycetocide) treatments was timed according to predicted 'potential disease thresholds' determined using weekly measured bio-aerosol concentrations of onion downy mildew inoculum above crops combined with environmental measurements (parameters) in a computer model (MILIONCAST).
- This study showed that there are still refinements needed in the calibration of downy mildew inoculum detection to take environmental, annual and seasonal changes in spore maturation, viability and diversity of responsiveness into account.

Background

Onion downy mildew (*Peronospora destructor*) is geographically widespread and is a serious disease in bulb onions, salad onion sets and in seed production. Downy mildew infects all of the main onion types grown in the U.K. including the common onion (*Allium cepa*), shallots (*A. cepa* var. *ascalonicum*) and Welsh onion (*A. fistulosum*). Welsh onion is particularly susceptible to downy mildew infection. Many commercial varieties of salad onion are crosses between *A. cepa* and *A. fistulosum* types. Yield losses in bulb onions of 60 to 75% have been recorded. These losses mainly result from severe infections causing early defoliation, reduced bulb sizes and poor storage quality of bulbs. In salad onions, yield losses can be as high as 100%, with whole crops being discarded as downy mildew symptoms on the plant make them unmarketable. Losses to seed production are frequently caused by the collapse of infected seed stalks and poor germination of seeds collected from infected stalks.

The onion downy mildew pathogen can overwinter as mycelium in onion bulbs and sets and, as oospores in debris from diseased foliage. It has also been shown to be seed-borne. When either sets or seeds are transplanted the mycelium grows within the foliage of the plant. Under favourable conditions onion downy mildew will produce large numbers of infective spores. This is a diurnal process requiring periods of both light and darkness. Spore production is mainly during the night under high relative humidities (> 94 - 95%) at temperatures of 6 - 22°C provided there is no rainfall. However, high day temperatures (> 24°C) have been found to inhibit sporulation during subsequent nights. Spore discharge is triggered when relative humidity falls below 59%. These spores can be transported by the wind over considerable distances and have been detected at heights of >450 metres. Once deposited on susceptible host leaf surfaces, they can germinate and infect within hours, and, under favourable conditions, disease symptoms are visible within 7 to 10 days.

The control of downy mildew in onions relies mainly on the frequent prophylactic application of fungicides (every seven to ten days), since fungicides are only effective if they are applied before or immediately after the first appearance of symptoms in a crop. To reduce the impact of fungicides on the environment, integrated disease management (IDM) approaches have been developed using predictive models run with climatic data to time sprays. Of these models, MILIONCAST (an acronym for 'MILdew on onION foreCAST'), is probably one of the best of this type of model for predictions of onion downy mildew. However, forecasts based on environmental factors alone are unable to take the presence/absence of the pathogen in the crop or locality into account and so are likely to over-estimate potential disease risks. To overcome this, methods of detecting and quantifying downy mildew spores in crop aerosols have been developed (AHDB Horticulture FV 356). Information on pathogen inoculum concentration within cropping systems can be used in conjunction with environmentally-based forecasting models to improve the precision of fungicide application and disease control. The purpose of this study was to evaluate this combined approach, as an integrated disease management system, to control downy mildew in UK commercial onion cropping systems.

Summary

The timed application of downy mildew control regimes according to bio-aerosol concentration reduced crop protection inputs by 50% and provided either similar or improved levels of disease control in four out of the six field trials assessed.

Field bio-aerosol testing was carried out using two air sampling systems:

- 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 for risk of downy mildew disease. However, samples require laboratory processing by ELISA (enzyme-linked immunosorbent assay). By postal delivery the results can be available within several days. Results indicate that a downy mildew bio-aerosol disease risk warning is provided when ELISA results of ≥ 0.6 optical density are recorded. However, doubts over the robustness of the MTIST ELISA were raised in this study when the test failed to identify risk of downy mildew disease during the final weeks of one field trial in salad onions.
- A weekly multivial air field sampler (daily air samples provided in seven tubes for testing on-site by lateral flows) can also be used to collect field bio-aerosols. This field ready air sampler with a timer can be purchased from Burkard Manufacturing for an approx. costing of £1650 + VAT. Daily air samples are assessed once weekly by an agronomist or grower using on-site field tests (lateral flows). The test is semi-quantitative and measurement of inoculum has been made using a lateral flow test reader (approx. Cost £1000 + VAT) with a standard curve. The standard curve is generated at the time of the weekly test using known spore concentrations of *P. destructor*.

Information on the presence or absence of a critical downy mildew spore threshold in bio-aerosols would provide growers with the capability to identify periods when crops are at risk from the disease. Disease onset and intensity are affected by three factors: disease, host and environment. The timed application and fungicide applied will also be important in control of the disease. In this study, field trials were carried out over two years and across the seasons. This was to reflect the varied climate of the UK and all the year round production of onion crops. Different salad and bulb onion varieties were assessed. Bio-aerosols were evaluated for downy mildew spores using a monoclonal antibody diagnostic probe. The application of fungicides to control downy mildew were applied to crops based on the diagnostic test result and an environmental downy mildew forecast (MILIONCAST). Areas within the crop remained unsprayed to assess disease development in the absence of control

measures. Also, an area was treated for control of onion downy mildew according to the grower schedule.

Based on results in Year 1, a downy mildew lateral flow test reading for predicting disease was proposed. This value was developed in conjunction with the use of an environmental downy mildew forecast model (MILIONCAST). In this way, assessment of the host, disease concentration and environment had been considered. The timed application of downy mildew control regimes according to bio-aerosol concentration reduced crop protection inputs in each of the field trials of 2014 by 50% and provided either the same or improved levels of disease control to that of the grower schedule. In Year 2, three further field trials were assessed in the same way. The disease threshold calculated in Year 1 for the lateral flow test worked well for that season. However, in the following year inoculum potential (capacity of downy mildew spores to initiate infection) appeared greater and so a lower threshold may be required to retain test accuracy. The final trial in 2015 provided environmental conditions very favourable for disease over an extended time period. Identified disease thresholds may not transpose accurately over time i.e. different cultivar type, environment and pathogen. The laboratory test (MTIST ELISA) also failed to identify risk of onion downy mildew spores in the collected bio-aerosols during the final weeks of the trial.

Whilst promising, use of each test should be with caution as downy mildew population structure, spore maturation, viability and the environment will vary. These factors may influence the accuracy of the tests to accurately quantify disease risk. The contamination of air samples with soil may have an inhibitory effect on the immunoassay process. In wet conditions the tubes can fill with water compromising the trapping efficiency of the test.

Financial Benefits

The main financial benefits would be in the use of these tests to reduce unnecessary crop protection inputs to onion cropping systems. Fungicide usage is costly and is one of the major inputs in crop production. Using the lateral flow device the grower/consultant would be able to check for the presence of onion downy mildew in the air and better time the first fungicide application. The cost of these tests must be compared with a typical spend of £260/ha for fungicide treatment. In high risk years it is common to spend in excess of £300/ha on fungicides in a bulb onion crop. However savings will be variable between years and depend on the overall reductions in sprays achieved.

Producers are required under the European Union Sustainable Pesticide Use Directive (2009/128/EC) to apply the general principles of integrated pest and disease management from January 2014.

Action Points

- Disease onset and intensity are affected by three factors: disease, host and environment. The timed application and fungicide applied will be important in control of the disease.
- whilst promising, each test should be used with caution as downy mildew population structure, spore maturation, viability and the environment will vary. These factors may influence the accuracy and robustness of each test especially in salad crops where high crop losses can result from low levels of disease. Reliable disease thresholds for each test cannot be confirmed.
- the use of these tests with environmental models may provide an improved accuracy to identify onion downy mildew infection and sporulation periods. As an integrated disease management approach, information on airborne disease load and environmental data has the potential to assist growers to schedule fungicide applications to crops more effectively and reduce crop protection inputs whilst making cost savings.
- The European Union sustainable pesticide use directive (2009/128/EC) states that professional users will have to apply general principles of Integrated Pest and Disease Management from 1 January 2014.

SCIENCE SECTION

Introduction

In this project the commercial application of an in-field lateral flow test developed in AHDB Horticulture FV 356 (Onions: Further development and calibration of detection tests for conidia of onion downy mildew in combination with MORPH forecast model MILONCAST) was assessed over a two year period. The shelf life of the test was assessed and optimised for extended and improved performance over time. Also, the device was evaluated in commercial onion cropping systems for accuracy in monitoring airborne downy mildew transmission events. Using a digital reader, the results of the 10 minute lateral flow test were compared with a laboratory enzyme-linked immunosorbent assay (ELISA) test (AHDB Horticulture FV 356). MILONCAST, an environmental model that identifies periods when plants are likely to be susceptible to onion downy mildew sporulation and infection was used in conjunction with these diagnostic tests to increase the precision of disease risk assessments. The following treatments at each trial site were followed: downy mildew fungicide applications were made to the onion crop according to a grower fungicide schedule; timed spray applications were made based on the assessment of air samples and the environmental forecast (MILONCAST); no fungicide applications made. Plants at each of the sites were assessed for downy mildew disease.

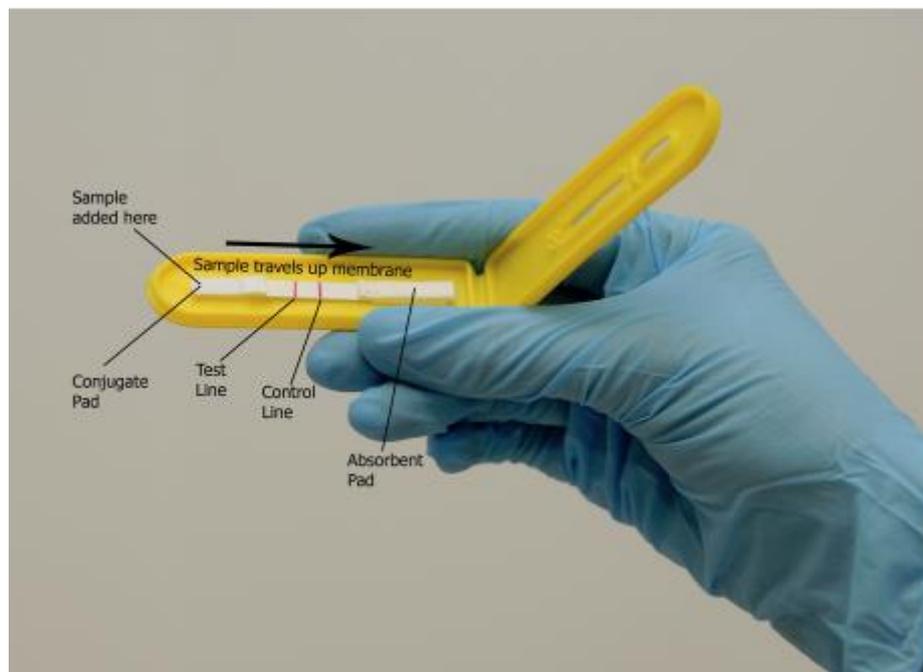
MATERIALS AND METHODS

Onion downy mildew lateral flows: The test framework was constructed using a Millipore 180 HiFlow™ cellulose ester membrane direct cast on to a Mylar backing (Cat No. HF180MC100, Millipore Corp., USA.) attached at either end to an absorbent pad (Cat No. CF6 (Cat No.8116-2250, Whatman), and a sample pad (CO83 Cellulose Fibre pad Cat no. CFSP223000, Millipore). A pre-filter VF2 pad (Cat no. 8124-6621, Schleicher and Schnell, Whatman) was also incorporated between the sample pad and the membrane. A test line of fast prepped *Peronospora destructor* spores (1.85×10^6 spores ml⁻¹ application buffer) was independently applied to the cellulose ester membrane surface using a flatbed air jet dispenser (Biodot Ltd, The Kingley Centre, West Sussex, UK) operating at a line travel speed of 15m s⁻¹. The sprayed membranes were air dried overnight at room temperature (18 - 20°C) and cut in to 5 mm strips. A 30µl volume of UW 242 (1:20 dilution of the antibody made up in conjugate buffer (2% Trehalose, 2% BSA, 2% sucrose in ¼ PBS) was mixed with 5µl of an anti-mouse IgM 40nm gold conjugate (Code BA GAMM 40, British Biocell International, UK). 30ul of the antibody/ gold conjugate solution was then pipetted on to individual clfd sample pads, air dried at 37°C for 35 min and, each pad was attached to the immuno-chromatographic test

strip (Fig. 1). The clfd devices were mounted within a plastic housing device (European Veterinary Laboratory, Netherlands: www.evlonline.nl).

To assess shelf life stability the lateral flows were stored at 4°C and at room temperature (18 - 20°C). At monthly intervals, components of the test were assessed for:

- test and control line stability: The conjugate pad of each of the lateral flows tested was replaced with a freshly prepared desiccated antibody/gold bead pad. Test application buffer was applied drop wise to the lateral flow pad. Using an ESE LFD reader, the test and control lines of each lateral flow were read at 20 minutes (Figure 1a, b)
- conjugate pad stability: Conjugate pads (onion downy mildew monoclonal antibody (UW242) and an anti-mouse probe conjugated to gold spheres) were prepared as described above but with concentrations of sucrose (20%) and mannitol (80mM, 160mM and 320mM) added. Antibody UW242 was used both in tissue culture serum (DMEM, a modification of Basal Medium Eagle (BME) that contains a four-fold higher concentration of amino acids and vitamins, as well as additional supplementary components.) and free of the serum components. Once the conjugate components had been air dried they were stored at room temperature (18 - 20°C) in desiccated pouches. At the point of testing the conjugate pads were removed from storage and inserted into a freshly prepared onion downy mildew lateral flow (Figure 1a).



a

b



Figure 1. The downy mildew lateral flow with test and control development (a) and quantitative measurement using an ESE Quant digital test reader (b).

Batches of lateral flows were made up in Year 1 and Year 2 of the study. The sensitivity of these tests for the measurement of onion downy mildew spores was determined by preparation of a 10 fold serial dilution of *P. destructor* conidia (1000000 to 10). This provides a standard curve over a range of spore concentrations that may occur in field bio-aerosol samples. Aliquots (100µl) of the spore concentrations were applied to the lateral flows. A control of test application buffer alone (i.e. no spores) was included within the study. Using an ESE LFD reader the test and control lines of each lateral flow were read at 5 minutes. This process was repeated for each of the batches. The standard curve is used to evaluate field lateral flow test readings for quantitative measurement of downy mildew spores and as a standard for batch quality control (Figure 2).

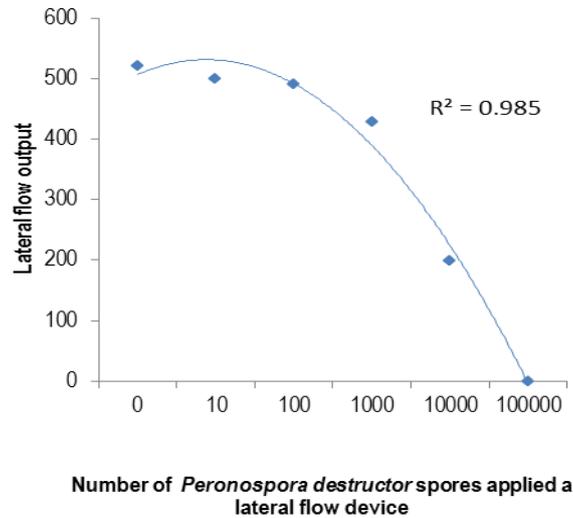


Figure 2. Standard curve used for quality control of lateral flow batches.

Field Trials: Air sampling equipment were positioned within three commercial onion cropping systems and on a weekly basis provided bio-aerosol concentrations of onion downy mildew (Fig. 3). During these periods air temperature, leaf wetness, relative humidity and rainfall were recorded at 30 min intervals using a Delta T data logger (Delta T Devices LTD., Cambridge, UK.). Environmental data was downloaded daily and used in a mathematical model (MILIONCAST) to determine sporulation risk periods for onion downy mildew. Plants were assessed for onion downy mildew disease. The format for each of the field trials is described below:

Site 1. Worcestershire, OS ref SO8487345194. Salad Onions (cv. Photon) drilled 23/04/14

Three 10m x 9m salad onion beds were identified and marked. Treatments for the control of downy mildew were applied to each of these areas:

- Area 1, received a standard downy mildew spray program at seven day intervals.
- Area 2, received timed spray applications according to the risk of onion downy mildew infection which was based on the assessment of field bio-aerosols and the environmental forecast (MILIONCAST).
- Area 3 received no control treatments for the period.

Detailed information on the air samplers used and, the methods involved in analysis of the field bio-aerosol samples, can be found in AHDB Horticulture FV 356 Annual Report 2011, p 14-17. The three crop trial areas were assessed weekly for downy mildew disease symptoms.



Figure 3. Bio-aerosol equipment operating in Area 3 (no fungicide applications made for onion downy mildew control) of a salad onion crop.

Site 2. Lincolnshire, OS ref. TF238 602, Bulb Onions (cv. Red Tide) and drilled 20/03/2014

Three areas existed as described for site 1, but in the format shown in Figure. 4. The large untreated plot, where no fungicide was applied, provided a dimension of 22 m x 40 m. Areas 1 and 2 (downy mildew fungicide spray trial) consisted of 4 replicate trial plots with each at a dimension of 3.66 m x 8 m.

- Area 1, received a standard downy mildew spray program at seven day intervals.
- Area 2, received timed spray applications according to the risk of onion downy mildew infection which was based on the assessment of inoculum and the environmental risk model (MILIONCAST).
- Area 3 received no control treatments for the period.

2	1	2	1
1	2	1	2

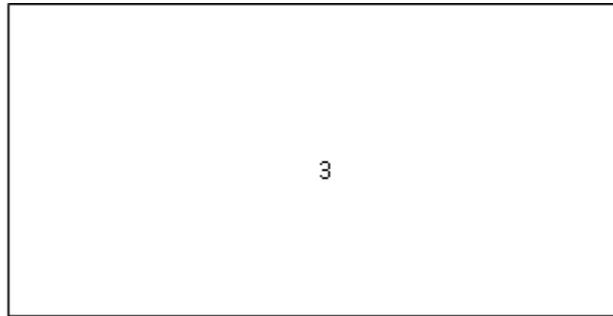


Figure 4. Areas 1,2 and 3 of a bulb onion trial. Air samplers were positioned in Area 3.

Once downy mildew was observed within Area 3 the plot was treated as per Area 1 (weekly spray interval). This was to avoid provision of a high downy mildew pressure within close proximity of the spray fungicide trial. Bio-aerosol equipment was positioned within Area 3 of the trial and included a Microtitre immuno-spore trap (MTIST), a Burkard 7 day volumetric sampler and a Burkard multivial cyclone sampler (Figure 5). A Delta T data logger (Delta T Devices LTD., Cambridge, UK.) was sited at Gedney Drove End (TF454 302) and provided environmental data for the period. The crop trial areas were assessed weekly for symptom expression of downy mildew disease.



Figure 5. Bio-aerosol equipment operating in Area 3 (no fungicide applications made for onion downy mildew control) of a bulb onion crop.

Site 3. Warwickshire, OS ref. SO195 526, Salad Onions (cv. Starlight) drilled 13/08/14.

A bio-aerosol air sampler (MTIST) was sited in an overwintered salad onion crop from October 2014 until April 2015. On a weekly basis field bio-aerosols were assessed for downy mildew disease (Figure 6). Spray timings were made by the grower on the basis of inoculum and environmental risk.



Figure 6. Overwintered salad onion crop at Clifford Chambers, Warwickshire.

In the spring of 2015, an unsprayed trial area was established within the overwintered crop. The remainder of the field continued to be sprayed based on inoculum and environmental risk. Bio-aerosol equipment included a Microtitre immuno-spore trap (MTIST), a Burkard single tube cyclone and a multivial cyclone sampler. Plants were assessed on a weekly basis for signs of downy mildew disease until harvest at the beginning of April 2015.

Site 4. Worcestershire, Bight Farm, Kempsey. OS ref. SO852483, Spring Salad Onions (cv. Totem) drilled 07/03/15, harvested 25/06/2015.

As in 2014, three 10m x 9m salad onion beds were identified and marked in a commercial salad onion crop. Treatments for the control of downy mildew were applied to each of these areas:

- Area 1, received a standard downy mildew spray program at seven day intervals.

- Area 2, received timed spray applications according to the risk of onion downy mildew infection which was based on inoculum and the environmental forecast (MILIONCAST).
- Area 3 received no control treatments for the period.

Bio-aerosol sampling was conducted within the crop from the 1st April 2015 until the 2nd July 2015.

Site 5. Moor Farm, Leasingham Moor, Sleaford. OS ref. TF082496 Bulb Onions (cv. Retano) drilled 23/03/2015 and harvested 14/09/15

Three trial areas existed at the site (Figure 7, Field PM 3).

- Area 1, received a standard downy mildew spray program at seven day intervals.
- Area 2, received timed spray applications according to the risk of onion downy mildew infection which was based on the assessment of field bio-aerosols and the environmental forecast (MILIONCAST).
- Area 3 received no control treatments for the period.

The trial area was situated within proximity of other commercial onion crops. At three of these (Figure 7, Fields PM 17,13,9) downy mildew was identified in patches within the crop (Figure 7, areas marked in red). Approximate distance of these areas to the trial site were between 1700 and 800m distance. The air sampling equipment was not positioned in the crop but beside farm buildings (Figure 7, yellow star; Figure 8). This allowed the air samplers to be run directly from mains voltage instead of 12v batteries. At the time the air samplers were positioned no downy mildew was evident at the trial site.



Figure 7. Location of trial site 4 (OS ref. TF082496) and surrounding area.



Figure 8. Bio-aerosol samplers operating at Sleaford, Lincolnshire.

Site 6. Salford Priors Warwickshire, OS ref. SP051509 Spring Salad Onion (cv. Galactic) drilled 01/07/2015 and harvested 15/10/2015

As at the other described trial sites, three 10m x 9m salad onion beds were identified and marked. During the growing season, treatments for the control of downy mildew were applied to each of these areas:

- Area 1, received a standard downy mildew spray program at seven day intervals.
- Area 2, received timed spray applications according to the risk of onion downy mildew infection. Risk was based on the assessment of field bio-aerosols and the environmental forecast (MILIONCAST).
- Area 3 received no control treatments for the period.

Air samplers were positioned within the trial area and operated off 12 v batteries with solar panels fitted (Figure 9). The trial areas were monitored for downy mildew symptom development as described previously.



Figure 9. Bioaerosols samplers operating at Salford Priors, Worcestershire.

RESULTS

Onion downy mildew lateral flows:

To assess shelf life stability the lateral flows were stored at 4°C and at room temperature (18 - 20°C). At monthly intervals, components of the lateral flow tests were measured for test and control line stability. Biological activity of the downy mildew monoclonal antibody in the conjugate pad was also assessed for a period.

Test line stability. Over a 12 month period, little difference ($r^2=0.9449$) in test line signal was observed between lateral flows stored at 4° C or at room temperature when an application of *P. destructor* spore soluble antigen was applied as a test line (Figure 10). The binding activity at the test line for each did increase at times over the period. This may have been a result of environmental (temperature) or operator variables. For example, the time taken from start to test reading.

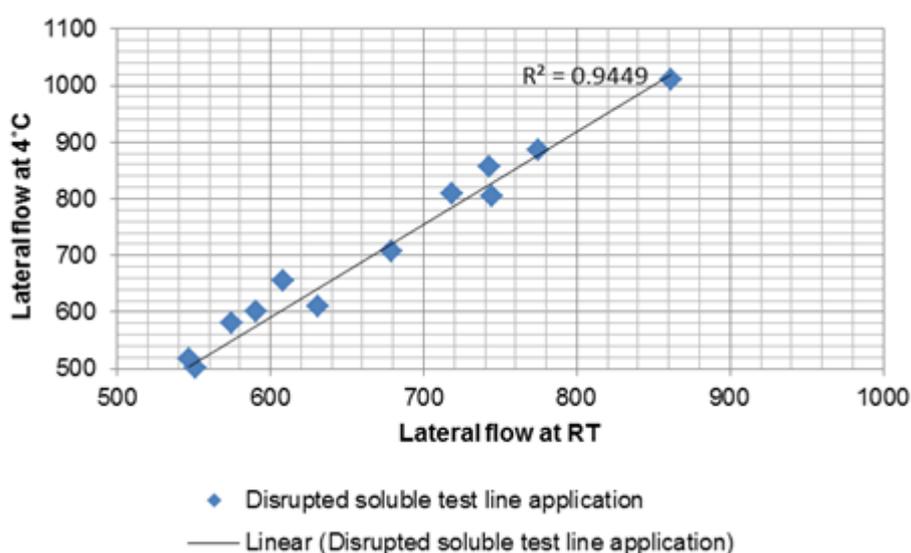


Figure 10. Relationship between the onion downy mildew lateral flows (disrupted *P. destructor* spore soluble test line) stored at 4° and room temperature (18 - 20°C) over a 12 month period.

Antibody conjugate pad stability. Sugars at different molarities were incorporated within the conjugate pad to improve stability of the test format. Testing of these in combination (sucrose (20%) and mannitol (80mM, 160mM and 320mM)) with EMA 242 determined that over time sucrose (20%) and mannitol at 320 mM retained a constant biological activity of the antibody probe to 7 months (Figure 11). By 8 months the control (EMA 242 with no sugar additives) had reduced in activity to its homologous test line antigen (*P. destructor*) by 80%. At 8 months,

the activity of EMA 242 in a stored conjugate buffer of 20% sucrose and 320mM mannitol activity also was compromised. Between month 7 and 8 activity fell by 30 % (data not shown). The onion downy mildew lateral flow in the present format is limited to a 7 month shelf life.

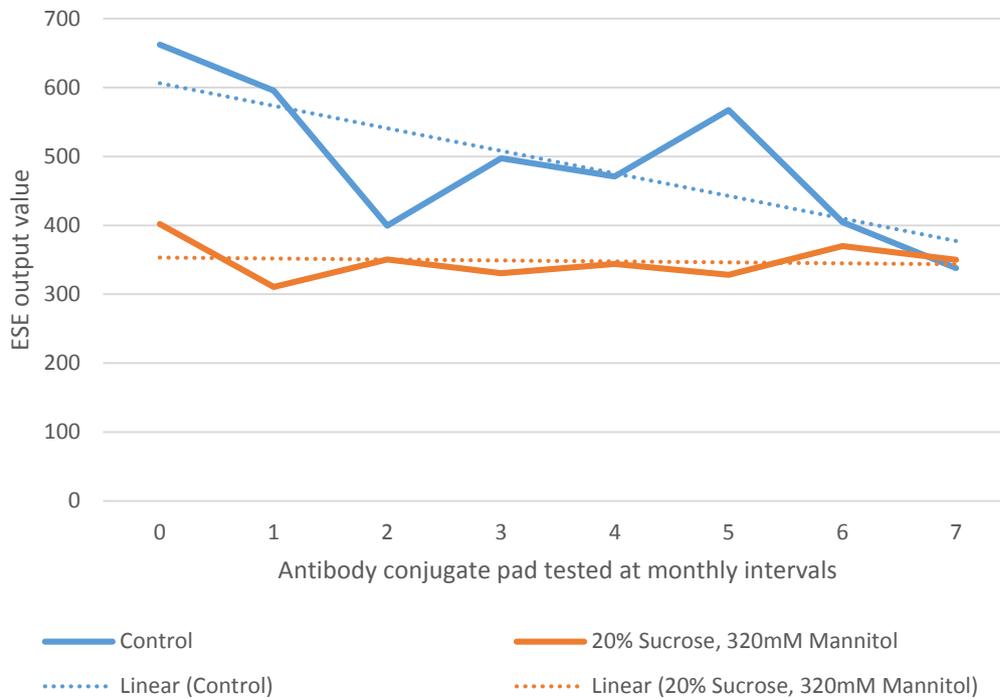


Figure 11. Biological activity of the antibody probe (EMA 242) following desiccation and storage at room temperature

Field Trials

Site 1. Worcestershire, OS ref SO8487345194. Salad Onions (cv. Photon) drilled 23/04/14, harvested week commencing 22/07/14.

Treatment applications made to the crop.

Treatments for the control of downy mildew were applied to two areas of the crop during the trial: Area 1, applications made according to a grower schedule (Table 1); Area 2, timed spray applications to plants based on the assessment of air samples and the environmental forecast (MILIONCAST) (Table 2). No fungicide applications were made to Area 3.

Table 1. Fungicide applications made according to a grower schedule (Area 1)

<i>Date</i>	<i>Product</i>	<i>Active</i>
10/06/14	Invader	Dimethomorph + Mancozeb
	Signum	Boscalid + Pyraclostrobin
20/06/14	Invader	Dimethomorph + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil
26/06/14	Invader	Dimethomorph + Mancozeb
02/07/14	Invader	Dimethomorph + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil

Table 2. Fungicide applications made according to disease risk of onion downy mildew (Area 2)

<i>Date</i>	<i>Product</i>	<i>Active</i>
27/06/14	Invader	Dimethomorph + Mancozeb
11/7/14	Invader	Dimethomorph + Mancozeb

Forecasting risk of onion downy mildew.

A concentration of onion downy mildew spores were predicted daily in the air of the crop using the 'in field' daily lateral flow test (multivial cyclone sampler). These values were derived using the onion downy mildew standard curve (Figure 2) and 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.

Data derived from the weekly MTIST ELISA rose markedly from the 19th June (ELISA output data ≥ 0.8 ; Figure 12a) and triggered a downy mildew warning alert. For this period, the daily lateral flow value recorded values in excess 2.5×10^4 downy mildew spores (Figure 12b). A fungicide spray active towards downy mildew disease was requested and applied to the salad onion plants in Area 2 on the 27th June, 2006. Based on this period providing a source of inoculum (airborne disease) downy mildew symptom expression was forecast (MILIONCAST; Figure 13) visible on susceptible plants from the 10th July (2014).

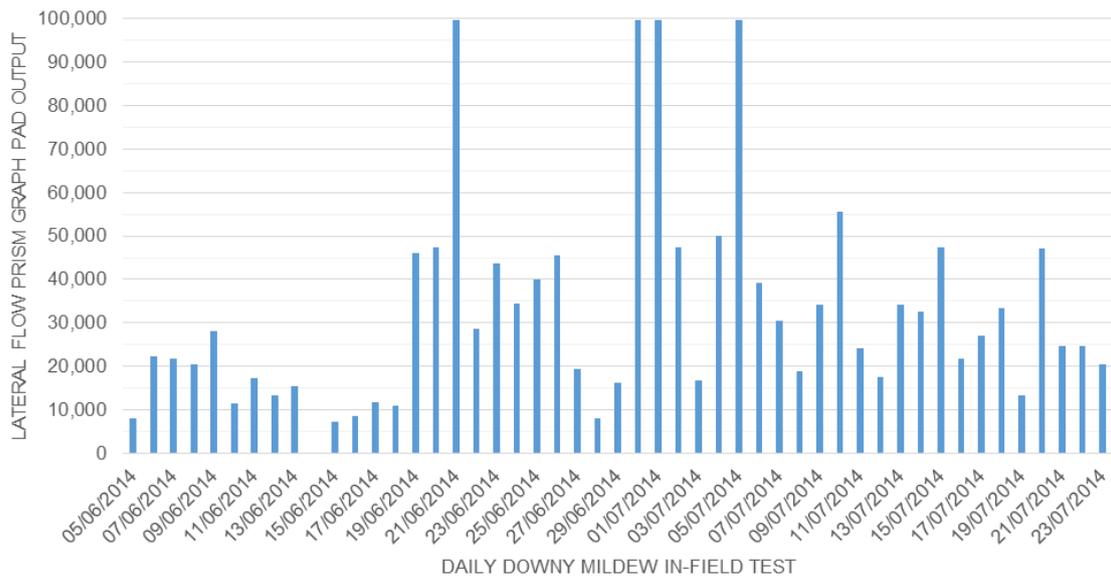
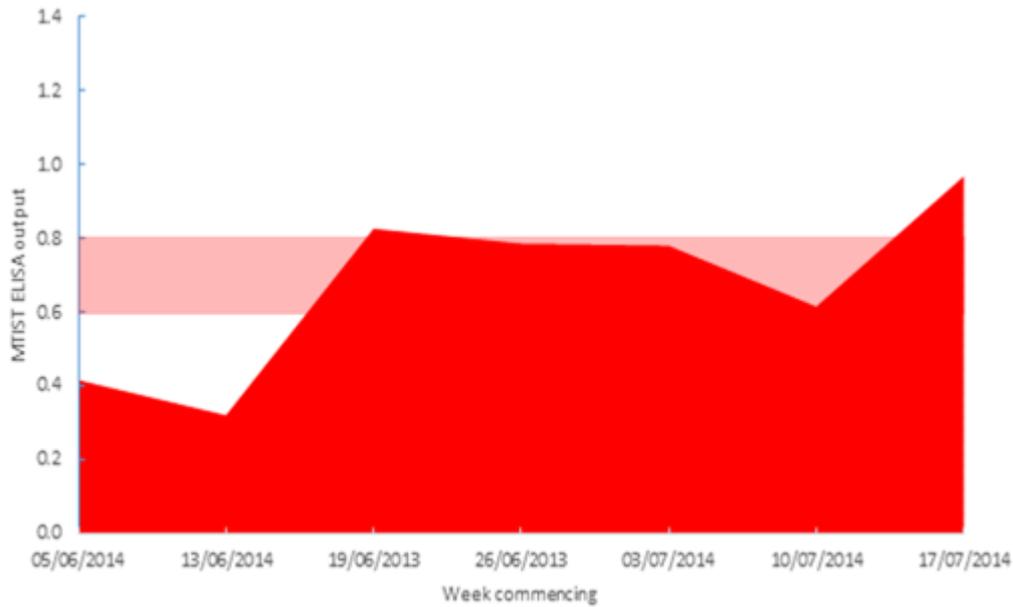
However, according to the disease environmental model, the crop had been at prior risk to disease on the 3rd and 4th June (2014). The bio-aerosol samplers were not sited in the crop until the afternoon of the 5th June.

The MTIST weekly ELISA test determined the crop to be at risk from downy mildew bio-aerosols until the 10th July. MILIONCAST for this period provided warning of disease potential for the 6th, 12 and 13th July. A second fungicide spray active towards downy mildew disease was requested and applied on the 11th July (2014) to the salad onion plants in Area 2. According to MILIONCAST, should downy mildew infection have occurred during this period, a two to three week latent period would be expected for visible symptom expression on untreated infected plants (Figure 13). The MTIST ELISA test indicated downy mildew spores to be in the air at an increased concentration between the 17th to the 23rd of July (2014). However, as the crop was to be harvested the week commencing the 22nd July no treatment for disease control was required. During the trial period the conditions were generally unfavourable for onion downy mildew development (Figure 13).

Assessment of plants for downy mildew disease.

From the beginning of the trial period (5th June) and at weekly intervals, 15 tagged plants in the middle two rows of each 10m treatment block were assessed for downy mildew symptom expression. On the 26th June (2014), the disease was first observed at a very low level in Areas 1 and 3 (1 lesion per 9 plants assessed). No disease was observed on the plants assessed in Area 2 (timed spray applications to plants based on the assessment of air samples and the environmental forecast (MILLIONCAST)). Thereafter, the assessment of the tagged plants was continued on a weekly basis. To increase sample number, salad onion plants across the two middle rows of each 10m treatment block were also included at each assessment. On the 17th July (2014), downy mildew infection was for the first time observed in Area 2 but at a reduced level to Areas 1 and 3 (Figure 14). The crop was harvested the week commencing the 22nd July.

a.



b.

Figure 12 . Measurement of onion downy mildew spores in field bio-aerosols: weekly MTIST laboratory ELISA test (value $\geq 0.6 - 0.8$ estimates risk of downy mildew spores) (a); daily in-field lateral flow test (b).

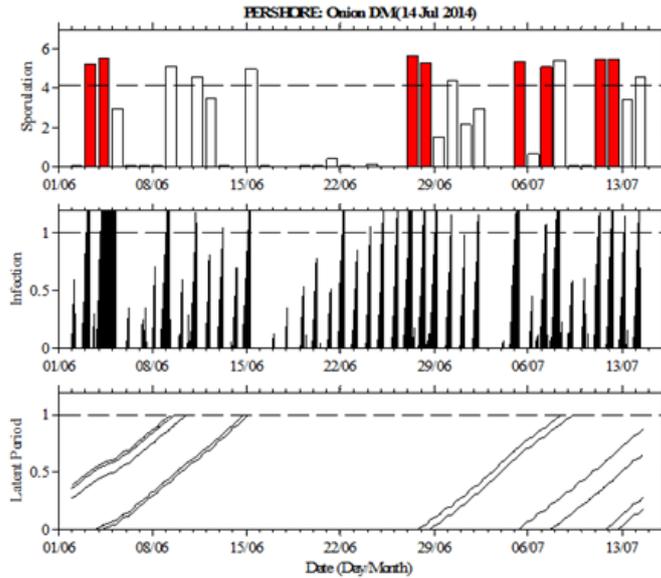


Figure 13. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period (red bar denotes potential for onion downy mildew sporulation and environmental parameters met for infection (threshold of 4 required); unshaded bar denotes potential for onion downy mildew sporulation; latent period shows time for disease expression on plants if downy mildew infection occurs)

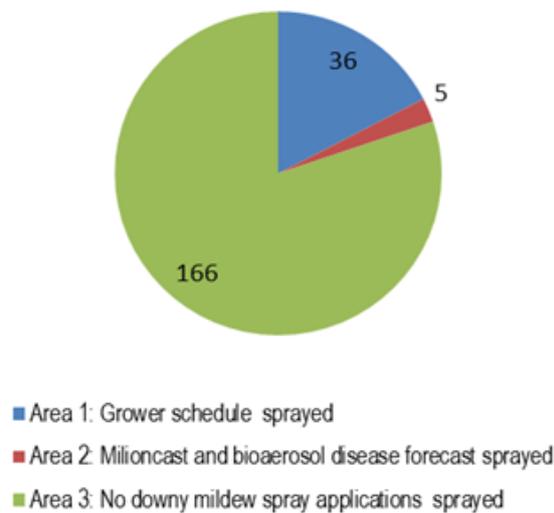


Figure 14. The total number of salad onion plants exhibiting downy mildew symptoms across the two middle rows of each 10m treatment block as recorded on the 17th July, 2014.

Site 2. Lincolnshire, OS ref. TF238 602, Bulb Onions (cv. Red Tide) drilled 20/03/2014, harvested week commencing 01/09/14.

Treatment applications made to the crop.

Treatments for the control of downy mildew were applied to areas of the crop during the trial: Area 1, applications made according to a grower schedule (Table 3); Area 2, timed spray applications to plants based on the assessment of air samples and the environmental forecast (MILIONCAST) (Table 4). No fungicide applications were made to Area 3 until downy mildew disease was observed.

Table 3. Fungicide applications made according to a grower schedule (Area 1)

<i>Date</i>	<i>Product</i>	<i>Active</i>
30/06/14	Valbon	Benthiavdicarb + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil
08/07/14	Unicur	Fluoxystrobin + Prothioconazole
	Dithane NT	Mancozeb
	Valbon	Benthiavdicarb + Mancozeb
14/07/14	Valbon	Benthiavdicarb + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil
23/07/14	Unicur	Fluoxystrobin + Prothioconazole
	Dithane NT	Mancozeb
30/07/14	Invader	Dimethomorph + Mancozeb
06/08/14	Unicur	Fluoxystrobin + Prothioconazole
12/08/14	Invader	Dimethomorph + Mancozeb

Table 4. Fungicide applications made according to disease risk of onion downy mildew (Area 2)

<i>Date</i>	<i>Product</i>	<i>Active</i>
14/07/14	Unicur	Fluoxystrobin + Prothioconazole
	Dithane NT	Mancozeb
23/07/14	Valbon	Benthiavdicarb + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil
30/07/14	Unicur	Fluoxystrobin + Prothioconazole
	Dithane NT	Mancozeb
06/08/14	Invader	Dimethomorph + Mancozeb

Forecasting risk of onion downy mildew.

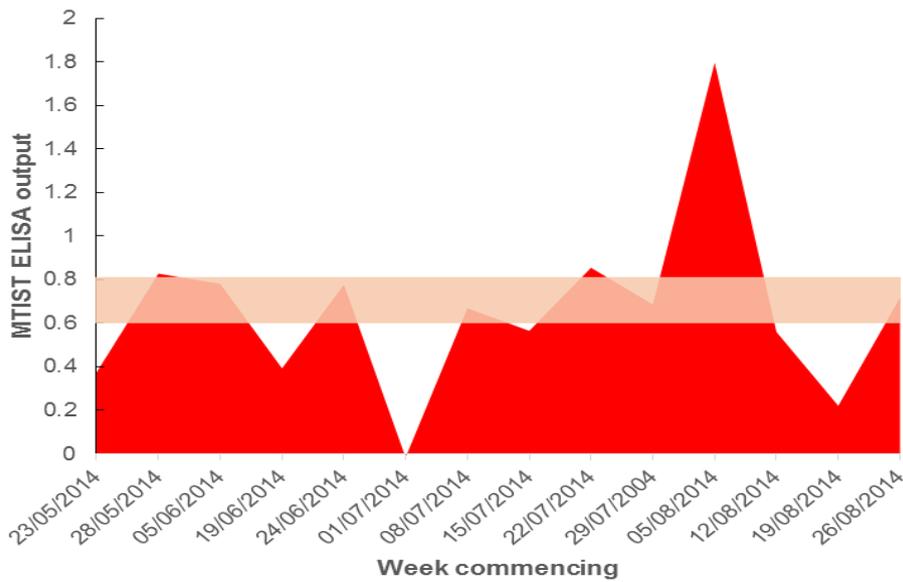
As a result of operational issues, the testing of air samples for downy mildew risk is reported from the 24th June, 2014. No results are available for the weekly collected vials (cyclone air sampler) as a result of excessive soil contamination. The daily collected multi-vial air samples were visually free of soil and recorded an initial spike in downy mildew (lateral flow standard curve field test reading of $\geq 2.5 \times 10^4$ spores) on the 9th July, 2014. For this period, the MTIST air sampler (weekly laboratory test) predicted a moderate to high risk of onion downy mildew spores in the crop bio-aerosol (ELISA value ≥ 0.8 ; Figure 15a). A fungicide spray active for downy mildew was requested on the 11th July and applied on the 14th July (2014). Based on this period as an initial cycle the disease forecast model (MILIONCAST) predicted visible symptoms from the 19th July onwards (Figure 16). The daily infield lateral flow test recorded an increase in downy mildew spores in the crop on this date (lateral flow standard curve field test reading of $\geq 2.5 \times 10^4$ spores; Figure 15b). Thereafter the weekly MTIST ELISA results remained high (≥ 0.8) moderate risk (≥ 0.6) risk of disease until the week commencing 19th August, 2014. The daily lateral flows determined the crop was at a moderate to high risk of downy mildew from the 30th July to the 6th August (daily lateral flow field test $\geq 2.5 \times 10^4$) and thereafter at times until the 20th August. Fungicide sprays active against the disease were applied on the 23rd, 30th July and the 6th August (Table 4). No further protective applications were made until the crop was harvested the week commencing 1st September, 2015.

Assessment of plants for downy mildew disease

Three areas existed at site 2: Area 1, received a standard downy mildew spray program made at seven day intervals (Table 3). Area 2, received timed spray applications (Table 4) according to the risk of onion downy mildew based on the assessment of field inoculum and the environmental forecasts (MILIONCAST). Area 3, remained without fungicide treatment until downy mildew was established within the plot. Thereafter, Area 3 received a weekly spray interval (as per Area 1) and no further assessments for downy mildew were made. This was to avoid provision of a large area of high downy mildew pressure within close proximity to the spray fungicide trials.

Disease was first seen in Area 3 of the crop (no fungicides applied) on the 22nd July. Little or no disease was observed on plants assessed across eight beds of trial Areas 1 and 2 (Figure 17).

a



b

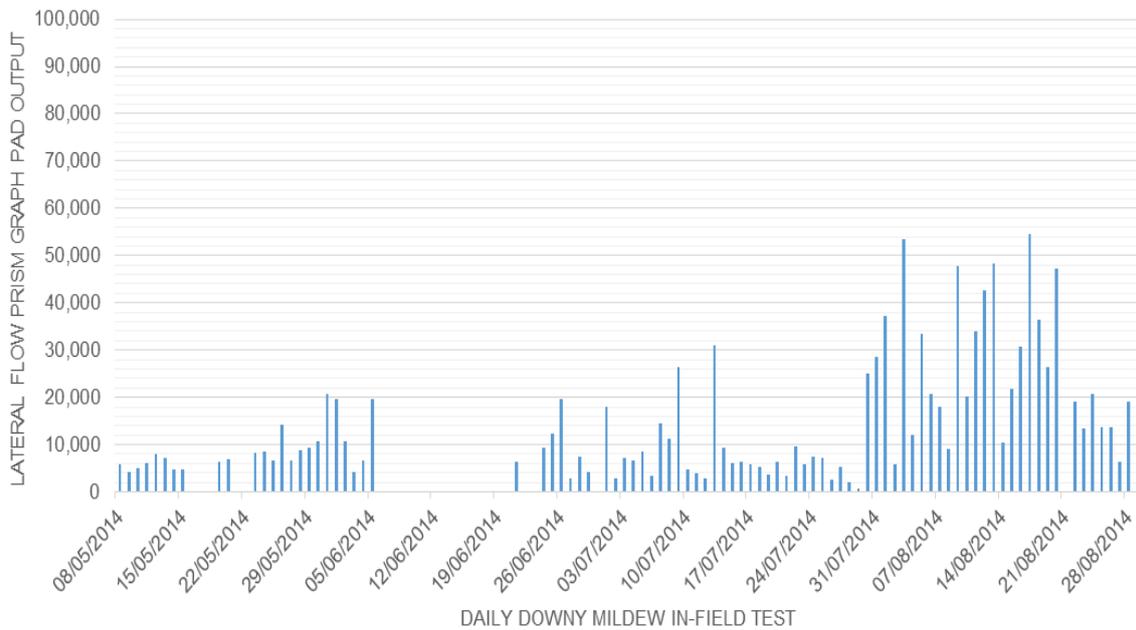


Figure 15. Measurement of onion downy mildew spores in field bio-aerosols: weekly MTIST laboratory ELISA test (value $\geq 0.6 - 0.8$ estimates risk of downy mildew spores) (a); daily in-field lateral flow test (b).

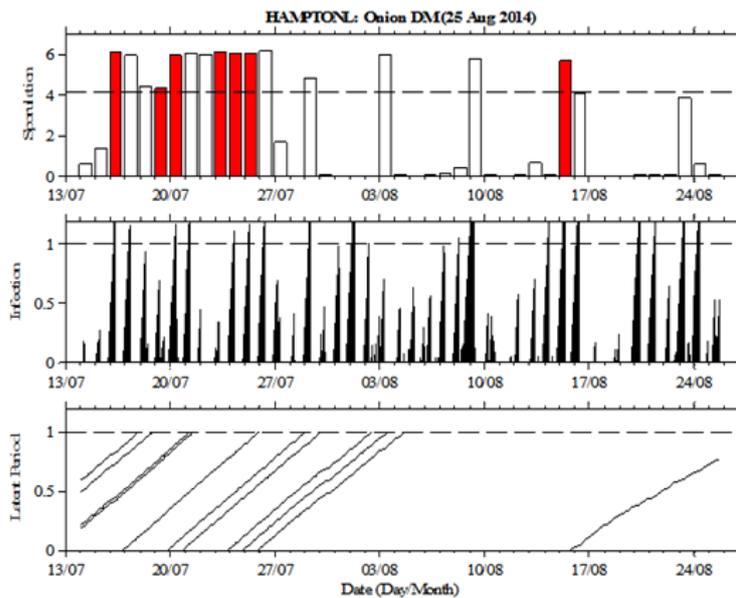
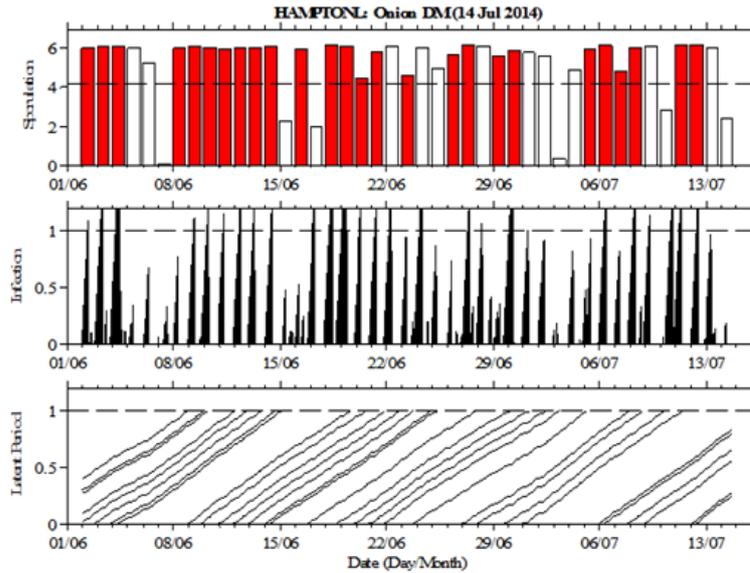


Figure 16. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period (red bar denotes potential for onion downy mildew sporulation and environmental parameters met for infection (threshold of 4 required); unshaded bar denotes potential for onion downy mildew sporulation; latent period shows time for disease expression on plants if downy mildew infection occurs)

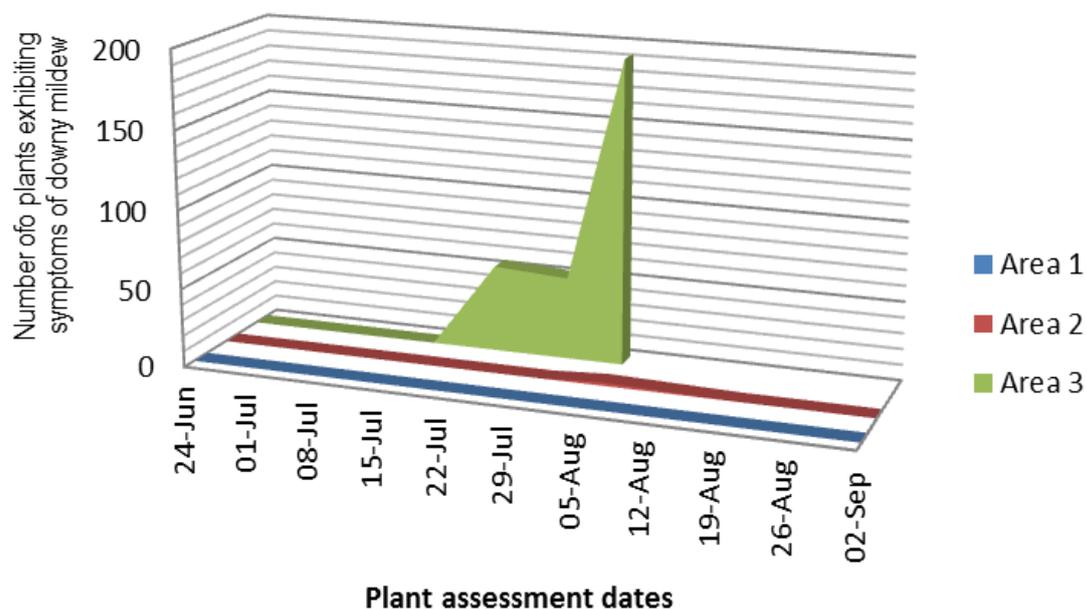


Figure 17. The number of bulb onion plants exhibiting downy mildew symptoms across the three treatment areas as recorded on the 17th July, 2014: Area 1, applications made according to a grower schedule; Area 2, timed spray applications based on the assessment of air samples; Area 3 no fungicide treatment for downy mildew.

Site 3. Warwickshire, OS ref. SO195 526, overwintered salad onions (cv. Starlight) drilled 13/08/14, harvest date April 2015.

Based on environmental data (MILIONCAST) and MTIST downy mildew spore risk periods fungicide sprays were applied to the crop (Table 5). No fungicides were applied between January and April 2015 and the crop remained free of downy mildew disease. Daily lateral flow readings were low (<1000).

Table 5. Fungicide applications made to the crop according to grower schedule (September – December 2014)

<i>Date</i>	<i>Product</i>	<i>Active</i>
25/09/14	Invader	Dimethomorph + Mancozeb
15/10/14	Invader	Dimethomorph + Mancozeb
30/10/14	Invader	Dimethomorph + Mancozeb
24/11/14	Fubol Gold	Metalaxyl – M + Mancozeb

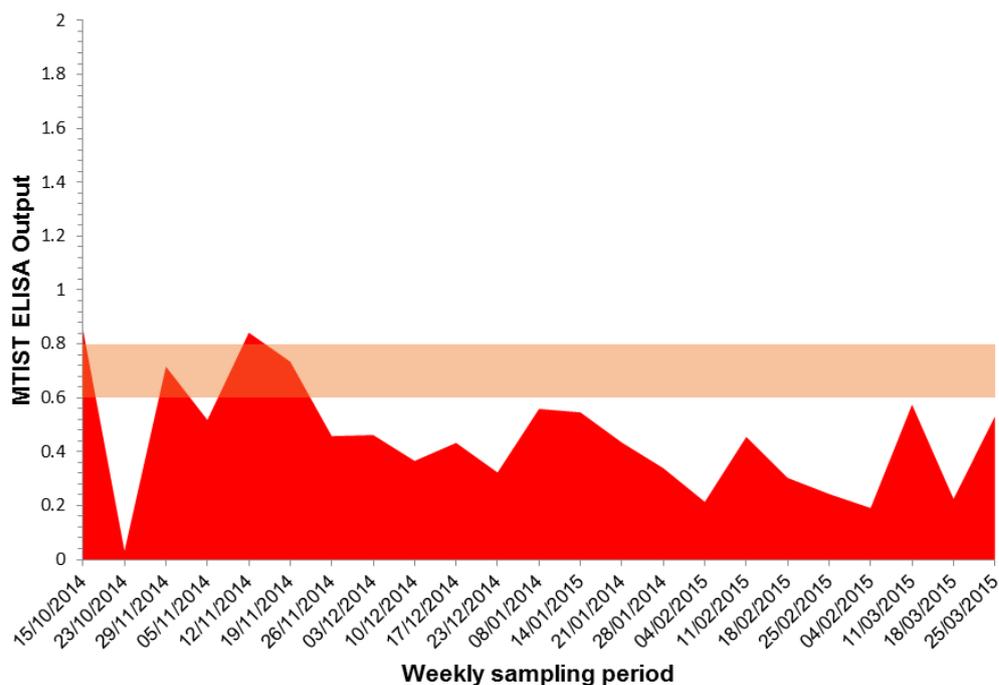


Figure 18. Measurement of *Peronospora destructor* spores in field bio-aerosols: weekly MTIST laboratory ELISA test (value $\geq 0.6 - 0.8$ estimates risk of downy mildew spores)

Site 4. Worcestershire, Bight Farm Kempsey. OS ref. SO852483, Spring salad onion (cv. Totem) drilled 07/03/15, Harvested w/c 25/06/2015.

For the week commencing 06th May 2015 the MTIST ELISA value recorded a value of ≥ 0.8 (Figure 19a). Downy mildew infection periods were predicted by MILIONCAST. Disease symptoms were predicted visible by the end of May (Figure 20). The lateral flow results for the sampling period however remained low ($< 10,000$; Figure 19b) indicating no risk of onion downy mildew development. Although on the 6th May a daily lateral flow recorded a value of > 5000 (Prism Graph Pad Output). A lateral flow was deemed void on 13th May (test line incomplete) and a tube was recorded full of water on the 5th May. This would have compromised the trapping efficiency of the cyclone ‘multivial’ air sampler and the reliability of the test. A fungicide spray for control of downy mildew was applied to Area 2 of the crop on the 19th May 2015 based on the MTIST ELISA value and the small corresponding peak (lateral flow 6th May).

Until the final assessment (25/06/2015) no disease was observed on the plants in the three trial areas (Area 1, applications made according to a grower schedule; Area 2, timed spray applications based on the assessment of air samples; Area 3 no fungicide treatment for downy mildew). On this date the mean number of plants (1008) assessed in each treatment

area recorded sporulating downy mildew development on 0.03 % plants (Area 1), 0 (Area 2) and 0 (Area 3). Five spray applications had been made in Area 1, while Area 2 had received one downy mildew control application. For the week commencing 25/06/2015 the MTIST ELISA recorded a value of ≥ 0.8 (Figure 19a). On this date the daily lateral flow test recorded a value of $>10,000$ (Prism Graph Pad Output, Figure 19b). A downy mildew control spray application was not applied as the crop was within the harvest interval.

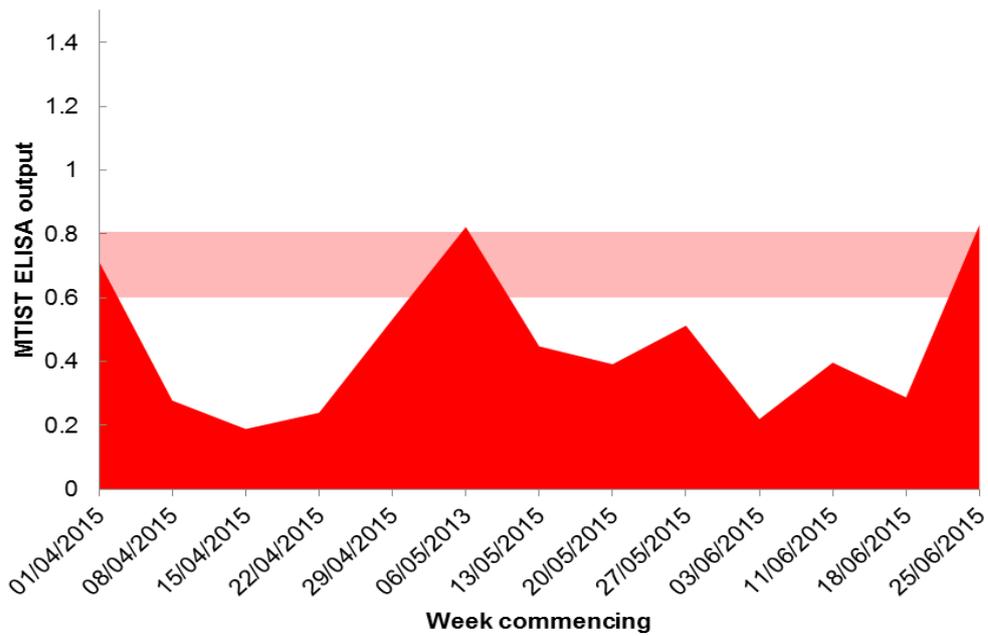


Figure 19a. Measurement of *Peronospora destructor* spores in field bio-aerosols: weekly MTIST laboratory ELISA test (≥ 0.6 - 0.8 estimates risk of onion downy mildew spores)

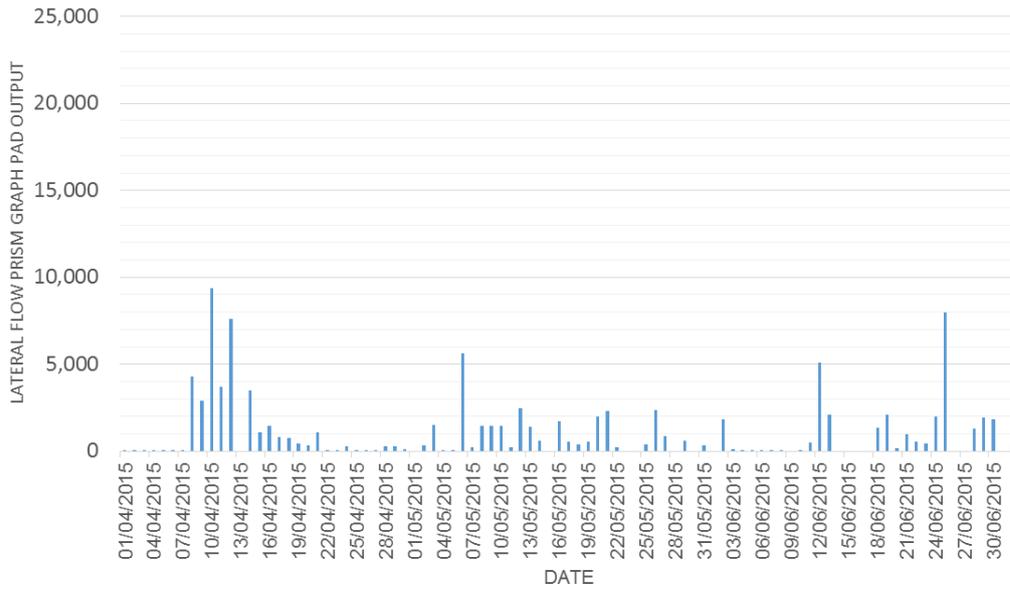


Figure 19b. Daily measurement of onion downy mildew spores in field bio-aerosols using in-field lateral flow test.

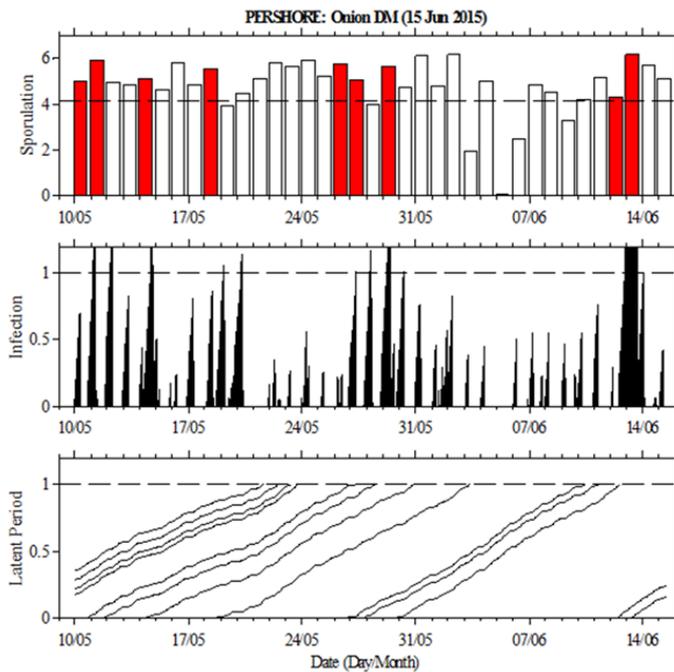


Figure 20. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period (red bar denotes potential for onion downy mildew sporulation and environmental parameters met for infection (threshold of 4 required); unshaded bar denotes potential for onion downy mildew sporulation; latent period shows time for disease expression on plants if downy mildew infection occurs)

Table 6. Fungicide applications made according to grower schedule (Area 1)

<i>Date</i>	<i>Product</i>	<i>Active</i>
13/05/15	Invader	Dimethomorph + Mancozeb
21/05/15	Invader	Dimethomorph + Mancozeb
30/05/15	Invader	Dimethomorph + Mancozeb
03/06/15	Invader	Dimethomorph + Mancozeb
11/06/15	Invader	Dimethomorph+ Mancozeb

Table 7. Fungicide applications made according to disease risk of onion downy mildew (Area 2)

<i>Date</i>	<i>Product</i>	<i>Active</i>
19/ 05/15	Invader	Dimethomorph + Mancozeb

Site 5. Sleaford, Moor Farm, Leasingham Moor. OS ref. TF082496 Bulb Onions (cv. Retano) drilled 23/03/2015 and harvested 14/09/ 2015.

At the trial at Sleaford, Lincs, the air samplers were not situated within the crop. Risk of downy mildew transmission was recorded by the MTIST ELISA (Figure 21a) in July and August (week commencing 08/07/2015 and 12/08/2015). The lateral flow 'provisional risk threshold' ($\geq 2.5 \times 10^4$ spores 2014 field trials) gave only a single daily risk period on the 8th July, 2015 (Figure 21b). The forecast model (MILIONCAST) for this period only predicted sporulation periods for onion downy mildew. Infection conditions were not identified by the MILIONCAST until the 14th July (Figure 22). Nevertheless, an assessment of each trial area (3 beds per treatment) identified disease in Area 3 (no fungicide application) and Area 2 (forecast sprayed) on the 15th July (Figure 23). Disease remained low with <1.5% plants affected in each of the trial areas for the remainder of the trial period (Figure 23).

Seven spray applications targeted for downy mildew control were made to Area 1 (grower schedule). Two applications were made to Area 2 (forecast). On each occasion these were applied late (as a result of the summer holiday period and staff). The first spray application was made according to the ELISA and lateral flow risk reading week commencing 8th July, 2015. The second fungicide application was made as a result of the MTIST value recorded on the 12th August 2015. No lateral flow readings were available for this period (12-18th August) as the air sampler was non-operational at this time.

During the trial period the wells of the MTIST and the cyclone air sampler microfuge tubes were noticeably contaminated with soil. In particular the following dates were identified 18/7, 19/7, 22/7, 29/7 to the 4/8/2015.

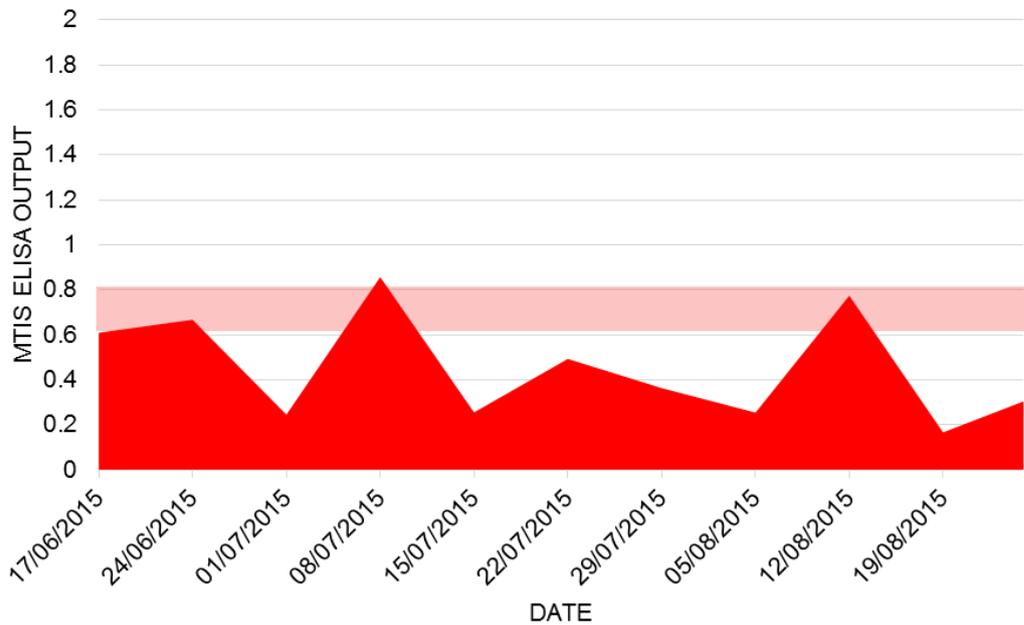


Figure 21a. Measurement of *Peronospora destructor* spores in field samples: weekly MTIST laboratory ELISA test ($\geq 0.6 - 0.8$ estimates risk of onion downy mildew spores).

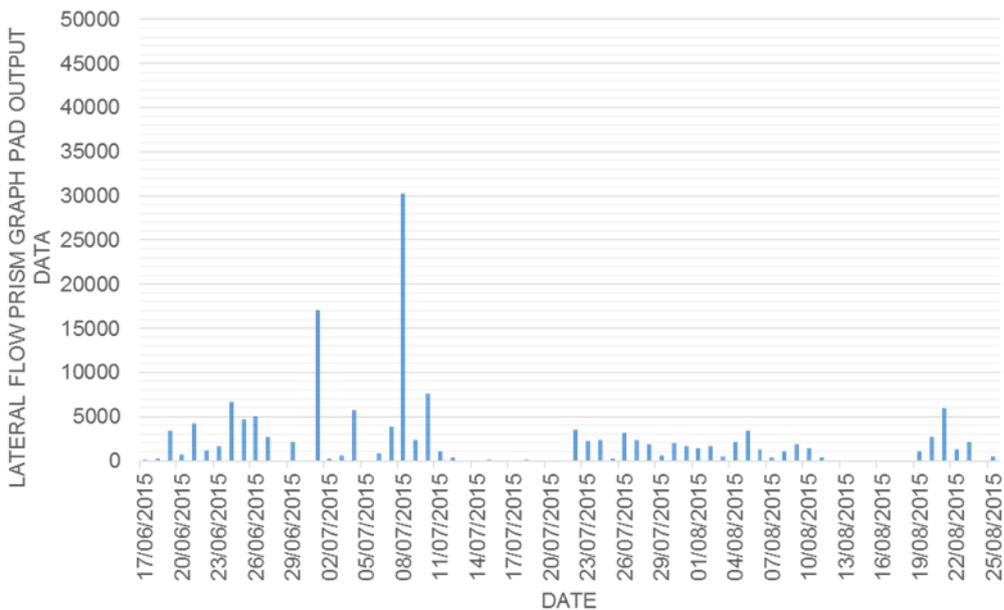


Figure 21b. Daily measurement of onion downy mildew spores in field bio-aerosols using in-field lateral flow test.

Table 8. Fungicide applications made according to a grower schedule (Area 1)

<i>Date</i>	<i>Product</i>	<i>Active</i>
25/06/15	Unicur	Fluoxystrobin + Prothioconazole
	Dithane NT	Mancozeb
08/07/15	Valbon	Benthiavdicarb + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil
21/07/15	Invader	Dimethomorph + Mancozeb
30/07/15	Unicur	Fluoxystrobin + Prothioconazole
06/08/15	Optimo Tech	
13/08/15	Invader	Dimethomorph + Mancozeb
20/08/15	Unicur	Fluoxystrobin + Prothioconazole

Table 9. Fungicide applications made according to risk of onion downy mildew disease (Area 2)

<i>Date</i>	<i>Product</i>	<i>Active</i>
20/07/15 *	Dithane NT	Mancozeb
26/08/15	Optimo Tech	Pyraclostrobin + Dimethomorph

*Applied late due to delay in processing results (summer holidays).

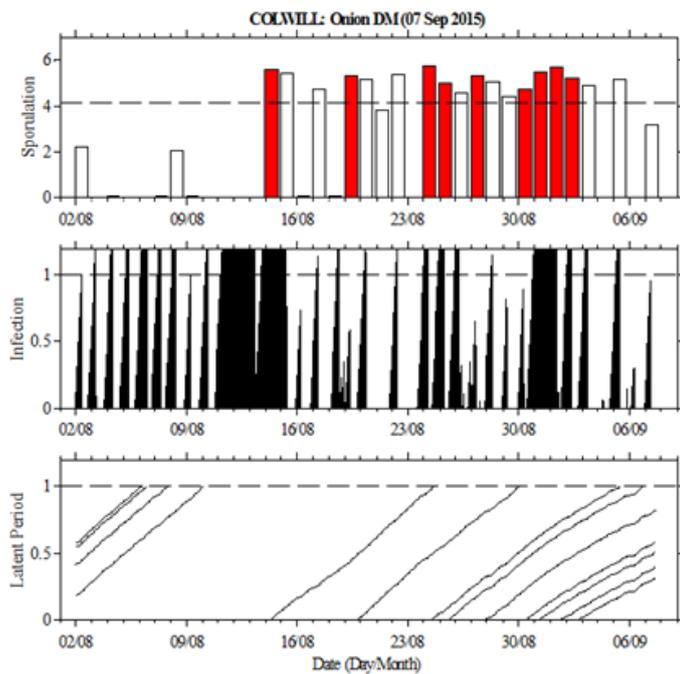
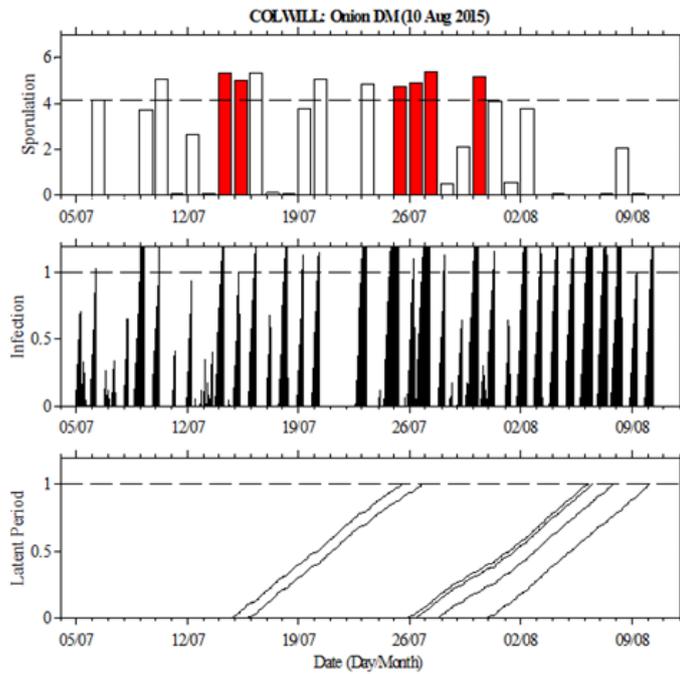


Figure 22. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period (red bar denotes potential for onion downy mildew sporulation and environmental parameters met for infection (threshold of 4 required); unshaded bar denotes potential for onion downy mildew sporulation; latent period shows time for disease expression on plants if downy mildew infection occurs)

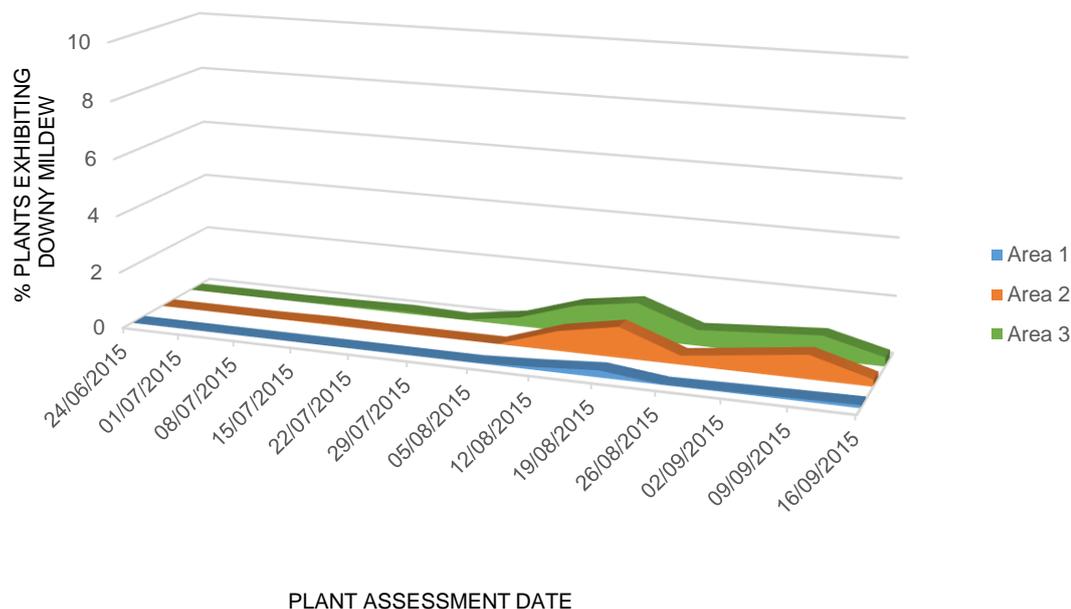


Figure 23. The number of bulb onion plants exhibiting downy mildew symptoms across the three treatment areas: Area 1, applications made according to a grower schedule; Area 2, timed spray applications based on the assessment of air samples; Area 3 no fungicide treatment for downy mildew.

Site 6. Warwickshire, OS ref. SP051509 Salford Priors, Salad Onion (variety Galactic) drilled 01/07/2015 and harvested 15/10 2015.

For the period of assessment the weekly MTIST bioaerosols processed by ELISA did not meet the ≥ 0.8 high risk threshold. Although, between the 13th August and 3rd September, 2015 values > 0.6 values were recorded (Figure 24a). The daily lateral flow test remained low throughout. The highest value was recorded on the 25th September (>5000 prism graph pad, Fig 24b). The efficiency of the multivial 'daily' cyclone air sampler was compromised on one occasion (17th September) recording a full tube of water. Also, the tubes on a number of occasions were recorded as heavily contaminated with soil. In particular the following dates: 10-12/09/15, 19-20/09/15 and 23-24/09/15. Battery charge remained above 12 volts throughout and the air samplers were checked weekly for sampling efficiency.

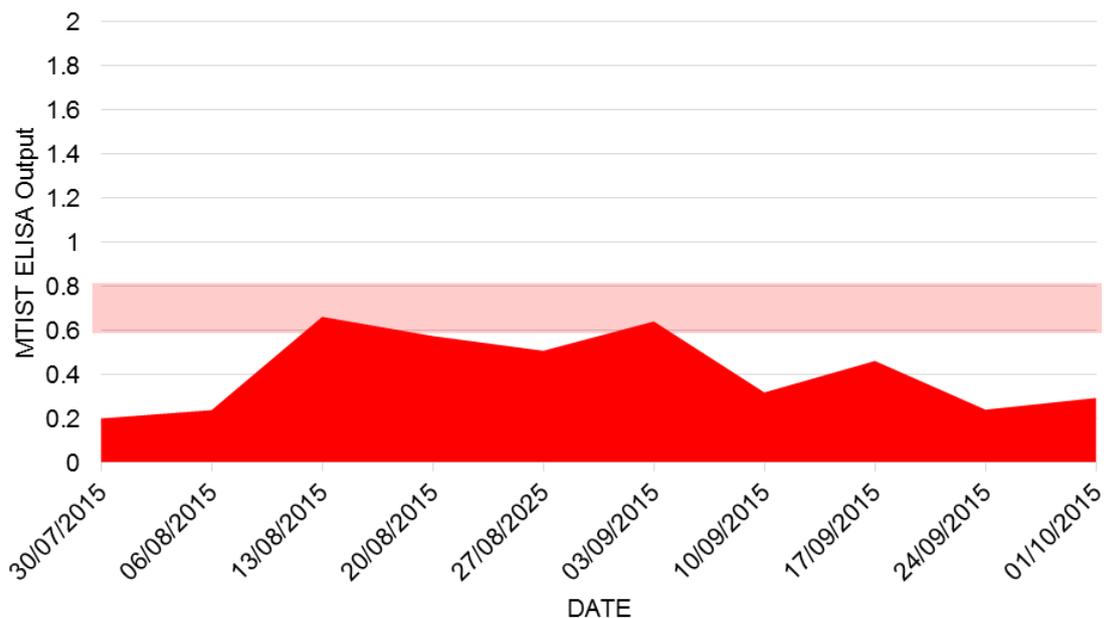


Figure 24a. Measurement of *Peronospora destructor* spores in field bio-aerosols: weekly MTIST laboratory ELISA test ($\geq 0.6 - 0.8$ estimates risk of onion downy mildew spores).

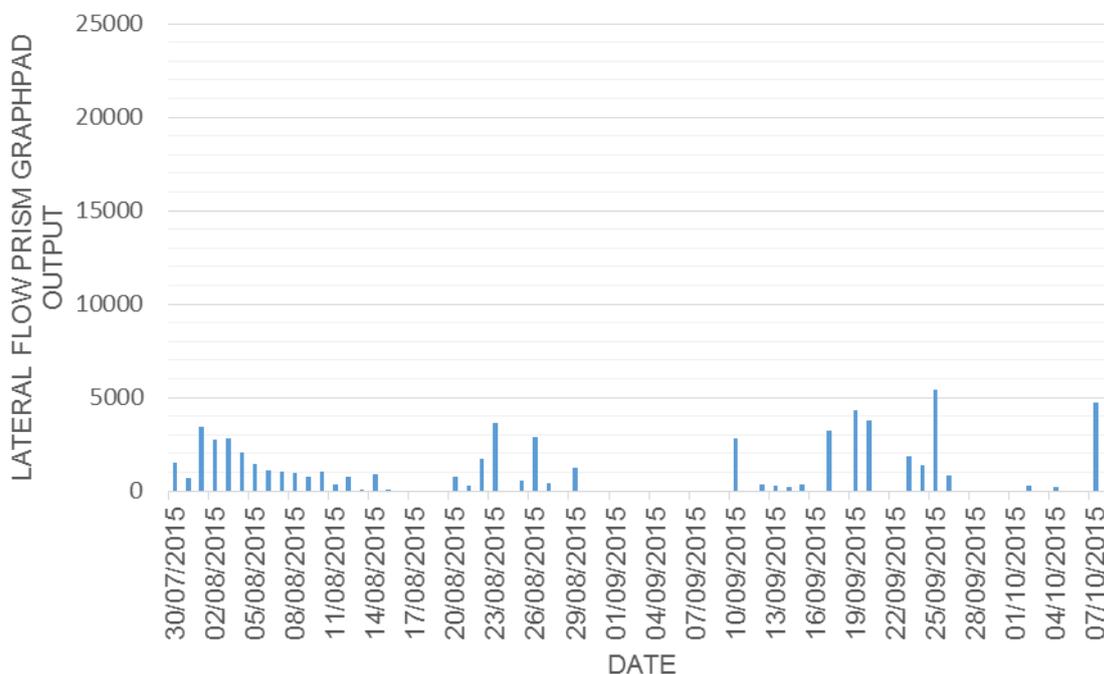


Figure 24b. Daily measurement of onion downy mildew spores in field bio-aerosols using in-field lateral flow test.

From July to the end of the trial conditions were non-limiting for downy mildew infection (Figure 25). Low level downy mildew disease was first recorded in the onion crop on the 17th September 2015 (Area 2, 0.12% plants and Area 3, 0.45% plants). According to the onion downy mildew model forecast (MILIONCAST) infection would have occurred around the 1st September 2015 (Figure 25). A fungicide spray application was made on the 3rd September (Invader, Table 11) to Area 2 (forecast spray) and applied based on the MTIST ELISA risk (three weeks at or near 0.6 (moderate risk). No further disease was observed in Area 2 (forecast spray schedule) until 1st October 2015 (1.3% plants infected). At this time 15% of plants were infected in the unsprayed area of the crop. By the 8th October 100% of plants in the unsprayed crop (Area 3) showed onion downy mildew disease and 46% in Area 2 (forecast spray). The grower fungicide schedule of weekly downy mildew control measures showed no sign of disease until the 8th October (0.33% plants affected) (Figure 26).

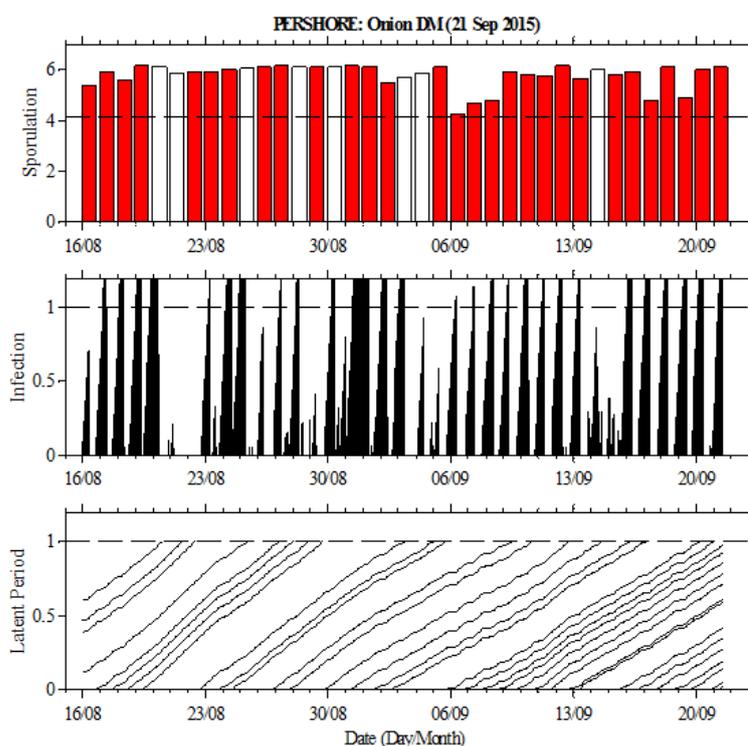


Figure 25. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period

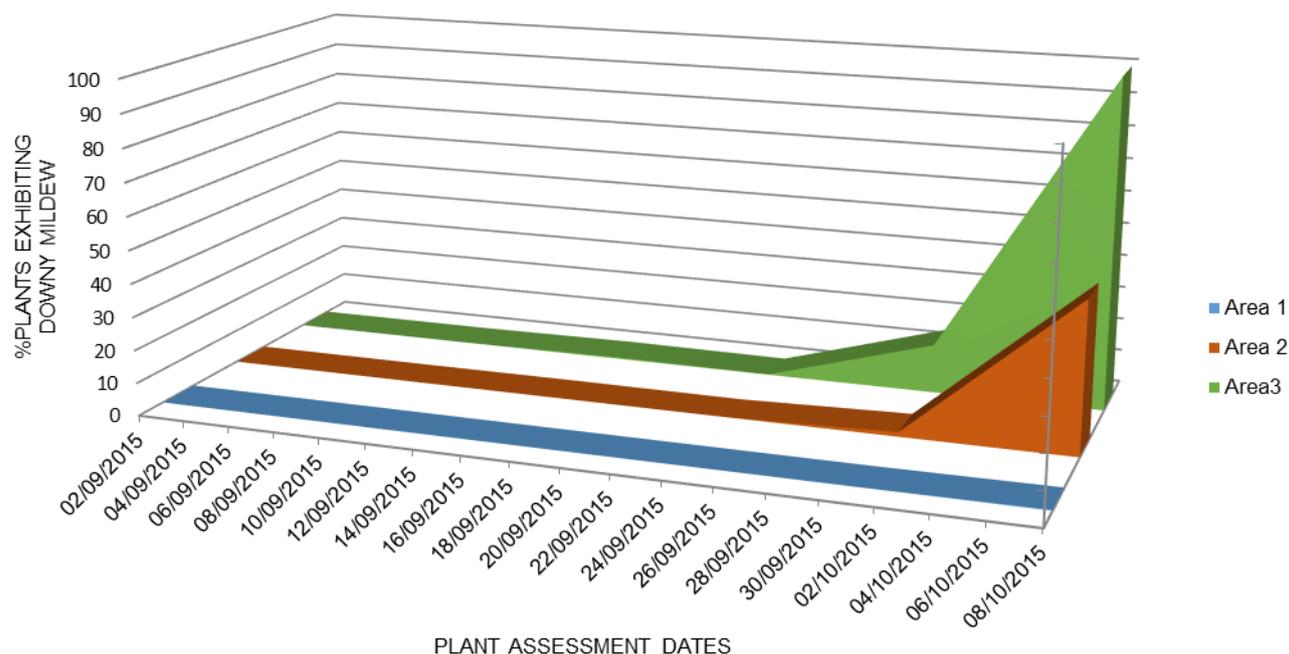


Figure 26. The number of bulb onion plants exhibiting downy mildew symptoms across the three treatment areas: Area 1, applications made according to a grower schedule; Area 2, timed spray applications based on the assessment of air samples; Area 3 no fungicide treatment for downy mildew.

Table 10. Fungicide applications made according to risk of onion downy mildew disease (Area 1)

<i>Date</i>	<i>Product</i>	<i>Active</i>
28/08/15	Invader	Dimethomorph + Mancozeb
04/09/15	Invader	Dimethomorph + Mancozeb
11/09/15	Invader	Dimethomorph + Mancozeb
18/09/15	Fubol Gold	Metalaxyl – M + Mancozeb
25/09/15	Invader	Dimethomorph + Mancozeb

Table 11. Fungicide applications made according to risk of onion downy mildew disease (Area 2)

<i>Date</i>	<i>Product</i>	<i>Active</i>
28/08/15	Invader	Dimethomorph + Mancozeb

DISCUSSION

Onion downy mildew lateral flows

Over a 12 month period and at two storage temperatures (4°C or room temperature (18-20°C)), the test line stability of the onion downy mildew lateral flow test was markedly improved when a disrupted soluble spore concentration was applied. Also, the biological activity of the downy mildew antibody probe was improved when free of tissue culture serum (DMEM) and applied to the conjugate pad in sucrose (20%) and mannitol (320mM) prior to storage under desiccation. The ratio of sugar to protein has previously been reported as influential in retaining protein in its native-like state and against aggregation and deamination (protein degradation) (Cleland *et al.*, 2001; Meyer *et al.*, 2009). The structural preservation of the antibody (diagnostic probe) is critical for its capability to bind successfully to homologous target antigen (downy mildew spore) on hydration. Combining the test line antigen stability with the biological activity of the conjugate pad a shelf life of 7 months is reported for the downy mildew lateral flow test.

Field Trials

Site1: Data derived from the weekly MTIST ELISA did not trigger an airborne downy mildew warning alert at site 1 until the period 19 to the 26th June (ELISA ≥ 0.8). In accordance to this test result, a fungicide spray active towards downy mildew disease was applied to Area 2 of the salad onion crop. The lateral flow values (multivial spore trap) for this period recorded onion downy spore concentrations in excess of 2.5×10^4 . Thereafter these test values (ELISA ≥ 0.8 ; lateral flow 2.5×10^4) were used to trigger downy mildew spray treatments. Based on this period providing a source of inoculum (airborne disease) the development of downy mildew symptom expression would, according to the downy mildew environmental disease forecast (MILIONCAST), be visible on susceptible plants from the 10th July 2014. When the crop was assessed on the 17th July, onion downy mildew disease was for the first time observed in Area 2 of the crop. The infection level observed was at a much lower level than observed in Areas 1 (grower spray regime) and 3 (no fungicide treatment). For these areas (1:2:3) a plant disease ratio of 7:1:33 was recorded. At the final plant assessment, Area 1 had received four downy mildew sprays and, compared to the unsprayed Area 3,

reduced the number of infected plants by 82.5%. By contrast, Area 2 (disease forecast timed applications) received only two sprays and the number of infected plants was reduced by 95.7% compared to the untreated Area 3. It should be noted however that the second application was made within the two week spray interval of harvest.

Site 2: At the second trial site, the weekly MTIST ELISA and the in-field lateral flow test results of the multivial cyclone air sampler, both identified a peak in test values for the first weeks of July 2014. The first timed fungicide application was therefore made shortly after on the 14th July 2014. According to MILIONCAST (environmental disease forecast) if untreated this infection period would have led to visual symptom and sporulation of onion downy mildew disease on infected plants from the 19th July. When the treatment areas were assessed on July 15th, no disease was observed. However on the 22nd July, disease was for the first time observed in Area 3 (untreated area of the crop) and on 55 of the plants assessed. No disease at this time was observed in Areas 1 and 2. To prevent the control area becoming a local source of unnaturally high downy mildew disease pressure the area thereafter received downy mildew control treatments as per Area 1 (grower schedule).

At the final plant assessment (2nd September), Area 1 had received seven sprays for prevention of downy mildew disease and no infection was observed on the plants. Similarly at this time, no downy mildew infection was recorded in Area 2 (disease forecast timed applications). However, only four sprays had been applied to this area for control of downy mildew disease consisting a 43% reduction in control inputs.

Site 3: At the overwintered salad onion site, a bio-aerosol air sampler (MTIST) recorded a risk of onion downy mildew disease during October and the early weeks of November 2014. Routine fungicide spray applications were made to the crop by the grower and no disease was observed. From January 2015, weekly MTIST air samples and daily multivial cyclone lateral flow tests recorded low downy mildew disease risk. No fungicide sprays were applied and no disease was observed in the crop.

Site 4: Potential for downy mildew disease was identified by both types of air samplers (MTIST and multi-vial daily air sampler) at this site on the 6th May, 2015, although, the lateral flow test remained well below the 'estimated' downy mildew risk threshold as identified in 2014 (year 1 of the project). Throughout the trial no disease was observed in Area 2 (forecast downy mildew control) or Area 3 (no control measures applied) of the crop. Five spray applications

for control of the disease were made to Area 1 (grower schedule) compared to just one control measure in Area 2. Consisting an 80% reduction in disease control inputs.

Site 5: Downy mildew disease pressure was recorded on crops in the locality between 1700 and 800m distance of the trial site. MTIST bio-aerosol tests identified downy mildew risks in the first two weeks sampling. Only low level readings were observed for the daily lateral flow devices (5000 Prism Graph Pad). The environmental model (MILIONCAST) determined no risk of downy mildew infection for this period. As a result, no fungicide applications were made. Only on one date (8th July, 2015) during the trial period did the lateral flow test record a value in excess of the proposed disease threshold value. For this period the MTIST also predicted a risk of onion downy mildew. Disease was observed at a low level in the crop seven days later (0.03% plants affected in unsprayed crop). In total seven downy mildew sprays were applied to Area 1 and two were applied to Area 2 (forecast spray) consisting of a 70% reduction in sprays. At the end of the trial period downy mildew disease remained low (<3%) and was observed in each of the trial areas. Although least apparent in the grower area (Area 1).

Site 6: The trial carried out during July to October 2015 provided mainly non-limiting conditions for development of onion downy mildew disease on plants. Disease on volunteers was noted prior to disease establishment on the crop of salad onions in the trial area. The MTIST ELISA recorded downy mildew risk periods (or close to estimated threshold) from the 13th August through to the 10th September. Low level downy mildew disease was recorded in the onion crop on the 17th September 2015 (Area 2, 0.12% plants and Area 3, 0.45% plants). According to MILIONCAST, infection of plants would have occurred towards the end of August into September. The timed spray application (identified by MTIST only) and applied 3rd September to Area 2 provided some control with 1.3% plants infected in Area 2 on the 1st October compared to 15% plants in the unsprayed area.

Onion downy mildew sporulation was readily observed on infected plants in Areas 2 and 3 from the end of September. By the 8th October, 100% of plants in the unsprayed crop were infected and 46% in Area 2. The routine sprayed crop remained free of downy mildew disease. On the 8th October sporulation was observed on infected plants in Areas 2 and 3,. Daily lateral flow results remained low throughout the trial period. However, the highest values were however recorded on the 25th September and 7th October 2015 (5000). This increase in values may reflect the downy mildew sporulation periods observed in the crop. Nevertheless, compared to the downy mildew risk value proposed in Year 1, these values would not have

activated the application of a downy mildew control measure. This would suggest that the identified disease thresholds may not transpose accurately over time i.e. different cultivar type, environment and pathogen. For this period, the MTIST ELISA failed to identify downy mildew spores in the collected bio-aerosols. Both air samplers operated efficiently for the period, so it is unlikely that there was equipment failure or a problem with the positions of the samplers.

It is not clear why risk of downy mildew spores was not identified during this period. It is possible that the target epitope (antigenic determinant that the diagnostic antibody binds to) was limited on this occasion. Population genetic analysis confirm that natural populations of many species (includes oomycetes) are highly variable. Also, the spore age (maturation) and the effect of the environmental conditions on the spore could affect antigen availability and retention during the ELISA process. For the sampling dates of 10-12/09/15, 19-20/09/15 and 23-24/09/15 the cyclone multivial tubes were heavily contaminated with soil. Diagnosis with soil has its challenges and problems can arise with inhibition of the assay by contamination of pesticide residues and organic matter (Otten et al., 1997; Hong et al., 2003; Stewart-Wade, 2011). In this study, the air samplers were placed within close proximity to Area 1 of the crop (grower spray schedule). There is potential that in combination or alone the accumulation of pesticide drift or soil within the sample collection vessels could have led to an inhibition of the immunoassay. The environmental conditions (wet, high humidity and long dew periods) may also have exacerbated the impact of this. Both the MTIST and cyclone sampler will under wet conditions retain water within the sample collection vessel. Although, to help combat this the MTIST was fitted with a cowl (Kennedy & Wakeham, 2000).

Disease onset and intensity will be affected by three factors: host, pathogen and environment (Figure 27). These factors may influence the accuracy of the tests to accurately quantify disease risk. The proposed 'disease threshold' identified in Year 1 of the study may not transpose well between the different trials i.e. cultivar type, environment and pathogen. Sensitivity of the tests may therefore be limited by variation in pathogen inoculum potential i.e. fewer spores capable of causing the same amount of disease. The results from this trial raise doubts to the robustness of the tests to accurately monitor risk of onion downy mildew disease.

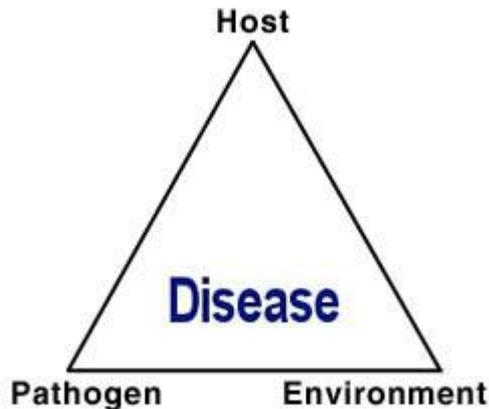


Figure 27. Disease onset model

Conclusions

- Application of a disrupted soluble downy mildew spore concentration to the lateral flow as a test line, provided a binding stability over the 12 month testing period, whether stored at 4°C or room temperature (18-20°C).
- To retain biological activity of the desiccated IgM downy mildew monoclonal antibody probe it is best applied to the lateral flow conjugate pad free of tissue culture supernatant and with sucrose (20%) and mannitol (320mM). Stability of the conjugate pad is restricted to a 7 month period.
- The lateral flow diagnostic test relies on cultivation of downy mildew spores on host material for preparation of the test line and standard curve. Repeatability and reproducibility of the test could be affected by spore maturation, expression of antigen, concentration accuracy. Variation in downy mildew spore antigen between lateral flow batches could affect the accuracy of the test threshold. In Year 2 of the study, low lateral flow values were generated for onion downy mildew risk compared to Year 1. An inaccuracy within the standard curve could markedly affect the test output.
- Disease onset and intensity will be affected by three factors: host, pathogen and environment. These factors may influence the accuracy of the tests to accurately quantify disease risk. Thresholds may require adjustment based on environmental criteria. Salad onions are grown at times when conditions can vary greatly. The existing threshold may however be appropriate for bulb onions as yield loss is different between bulb and salad onions. Also, bulb onions are grown over the same period each year and during the summer when conditions are less likely to be continuously non-limiting for onion downy mildew.

- The efficiency of the cyclone air sampler was affected at times by rain (water collection in the tubes) and soil. This will affect air sampling efficiency and soil contaminants may provide inhibition of the immunoassay test. By using a multivial cyclone air sampler (weekly air sampler across seven tubes (24h collection periods)) this effect was reduced but not eliminated.

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