

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

AUTHENTICATION

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

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GROWER SUMMARY

Headline

In this study, the timed application of onion downy mildew control regimes according to bio-aerosol concentration of *Peronospora destructor* reduced crop protection inputs by 50% and provided either the same or improved levels of disease control. An environmental model (MILIONCAST) was used to assess downy mildew infection conditions and the predicted latent period for disease development on infected onion plants to occur. These systems demonstrate an integrated disease management approach towards the reduction of crop inputs to control downy mildew on onion crops. 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.

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. Information from the work will provide data on the optimal use of the system and the potential cost savings.

The pathogen can over winter as mycelium in onion bulbs and sets and, as oospores in debris from diseased foliage. The onion downy mildew has also been shown to be seed borne. When either sets or seeds are planted the mycelium grows within the foliage of the plant. Under favourable conditions the downy mildew pathogen is capable of spore production. This is a diurnal process and both periods of light and darkness are required. Spore production is mainly during the night under high relative humidity of greater than 94 - 95% at temperatures of 6 - 22°C provided there is no rainfall. However high day temperatures exceeding 24°C have been found to inhibit sporulation during subsequent nights. Spore discharge is triggered when relative humidity falls below 59%. The 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, the spores can germinate and infect within hours. Under favourable conditions disease symptoms may be visible within 7 to 10 days.

The control of downy mildew on onions relies mainly on the prophylactic application of fungicides, as frequently as every seven to ten days. Fungicidal control of downy mildew is difficult as fungicides are only effective, if they are applied before or immediately after the disease first appears in the crop. To reduce the impact of fungicides on the environment, integrated disease management (IDM) approaches have been developed. Of these, MILIONCAST (an acronym for 'MILdew on onION foreCAST'), provides improved predictions of onion downy mildew sporulation than those based on DOWNCAST models. However forecasts based on environmental factors are unable to take in to account whether the disease is present or absent within the crop or locality. To overcome this, methods of detecting and quantifying downy mildew spores in crop aerosols have been developed (HDC FV356). Information of disease concentration within the cropping system can be used in conjunction with environmental based forecasting models to provide a precision approach to fungicide application and disease control. The purpose of this study is to validate the combined usage of these two approaches as an integrated disease management system to control downy mildew in commercial cropping systems of the UK onion industry.

Summary

In this study, the timed application of downy mildew control regimes according to bio-aerosol concentration reduced crop protection inputs by 50% and provided either the same or improved levels of disease control.

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 however. Provisional results indicate that a downy mildew bio-aerosol disease risk warning is provided when ELISA results of ≥ 0.8 optical density is recorded.
- 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). A single tube lateral flow reading of OD $\geq 2.5 \times 10^4$ is proposed as the disease threshold for onion downy mildew risk on onion crops. On-site lateral flows for other plant diseases currently retail at circa £7 per test. It is assumed that this costing would apply to a test for downy mildew disease. At present a digital lateral flow test reader would be required for measurement of downy mildew spore concentrations. A reader currently retails at circa £1000 however smart phone readers with downloadable applications are being developed for other diseases.

Information on the presence or absence of a critical downy mildew spore threshold in bio-aerosols provides growers capability to identify periods when crops are at risk from the disease. However, this information is best used in conjunction with an environmental forecast (MILIONCAST) to determine best times to apply control measures. Using this approach should lessen the reliance on mancozeb based fungicides for onion downy mildew control (these are being withdrawn). Also, as part of the sustainable use directive (SUD) http://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides/index_en.htm, this approach will enable producers to demonstrate that they have taken alternative integrated pest management (IPM) measures to prevent disease development before the use of spray applications of fungicides.

Financial Benefits

The main financial benefits will 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 will 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.

Action Points

- air samplers can be used to trap onion downy mildew disease in field bio-aerosols;
- testing of these collected air samples for onion downy mildew disease can be made either by laboratory staff or using lateral flows on-site in the field;
- The use of these tests with environmental models provides an improved accuracy to identify onion downy mildew infection and sporulation periods. This integrated disease management approach provides information on airborne disease load and environmental data. This will 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

A purpose of the project is to assess the commercial application of an in-field lateral flow test developed and reported on in HDC FV 356 (Onions: Further development and calibration of detection tests for conidia of onion downy mildew in combination with MORPH forecast model MILONCAST). The 10 minute field test is for use by growers or agronomists to determine risk of the onion downy mildew pathogen (*Peronospora destructor*) in field air samples. In year one of this project, the following areas were investigated:

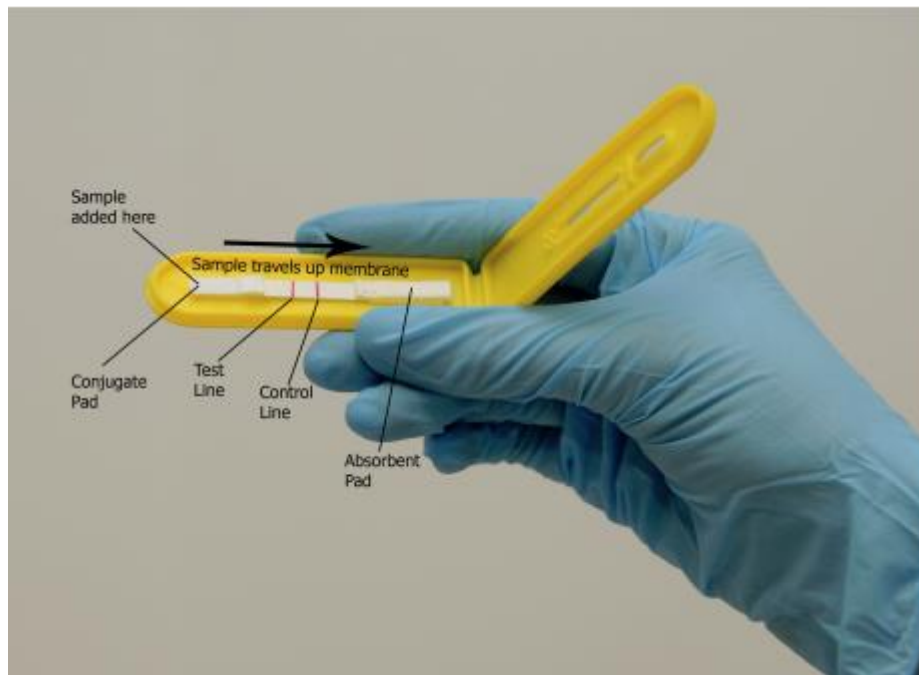
- Lateral flow production: Three batches of 500 downy mildew lateral flow devices were assessed using standardised calibration curves for variability and component stability for the measurement of downy mildew spore concentrations. The process was optimised, quality control standards investigated and a draft protocol developed for routine manufacturing of the tests.
- Lateral flow evaluation: The device was evaluated in commercial onion cropping systems for accuracy to monitor airborne downy mildew disease transmission events. Using a digital reader, the results of the 10 minute lateral flow test were compared with an established laboratory enzyme-linked immunosorbent assay (ELISA) test (HDC FV356). In conjunction with MILIONCAST, an environmental model which identifies periods during which plants would be susceptible to onion downy mildew infection, these diagnostic tests were used to ascribe disease risk of field onion crops.
- Field trials: During 2014, three commercial onion sites were monitored for downy mildew (*Peronospora destructor*) spore transmission events. At two of the sites, the following treatments 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 (MILIONCAST);
 - no fungicide applications made.
- Plants at each of the sites were assessed for downy mildew disease. At a third site an overwintered salad onion crop was monitored for downy mildew disease transmission events.

Materials and methods

Onion downy mildew lateral flows: The lateral flow device comprised of a Millipore HF 240 Hiflow™ cellulose ester membrane direct cast on to 2 mls Mylar backing (Cat no. SHF2400225, Millipore Corp, USA), an absorbent pad (Cat no. GB004, Schleicher and Schnuell, Germany), filtration section (VF2, Millipore Corp, USA) and a sample pad (Cat no. T5NM, Millipore Corp, USA). A flat bed air jet dispenser (BioDot, UK) was used to apply a test line of either a *Peronospora destructor* spore or disrupted soluble spore content to the membrane card. The cards were air dried overnight and cut into 5 mm width strips. A volume of 3 mls of a 1:40 dilution of EMA 242 Monoclonal Antibody (MAb) made up in NPARU conjugation buffer was prepared. To this 3 mls, 600µl of goat anti-mouse IgM 40nm gold conjugate (Code BA GAMM 40, British Biocell International, Cardiff, UK) was added and the tube was incubated on a roller incubator for 10 minutes. Each sample pad of each lateral flow device received 30µl of the antibody gold conjugate solution before air drying in an oven at 37°C for 10 minutes. The lateral flow devices were mounted within a plastic housing device (Schleicher and Schuell, Germany).

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).

Following this study, 1500 lateral flows (three batches of 500 each) were prepared as described above. However, this time the conjugate pads consisted of EMA 242 free of DMEM medium and, with the addition of 20% sucrose and 80mM mannitol to the conjugate pad buffer. 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-aerosols. 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 20 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).

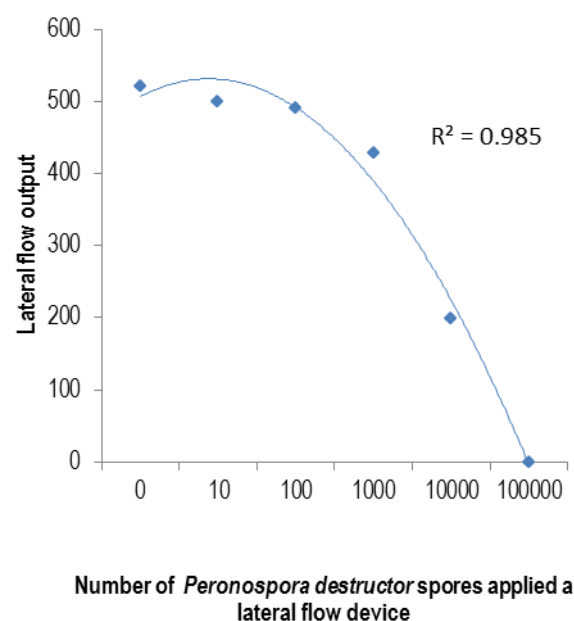


Figure 2. Standard curve used for quality control of lateral flow batches and in the measurement of downy mildew spore concentrations in field bio-aerosols

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. 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 were 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 (variety Photon) drilled 23/04/14

At this site, 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, applications made according to a grower schedule; Area 2, timed spray applications to plants based on the assessment of air samples and the environmental forecast (MILIONCAST); Area 3, no fungicide applications made. Bio-aerosol equipment (Microtitre immunospore trap (MTIST), Burkard 24hr Volumetric glass slide air sampler and a Burkard multivial cyclone sampler) were positioned within Area 3 (Figure 3). Detailed information on the air samplers used and, the methods involved in analysis of the field bio-aerosol samples, can be found in HDC FV 356 Annual Report 2011, p 14-17. The three crop trial areas were assessed weekly for



symptom expression of downy mildew disease.

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 (Red Tide) and drilled 20/03/2014

Three areas existed as described at 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 field bio-aerosols and the environmental forecast (MILIONCAST).

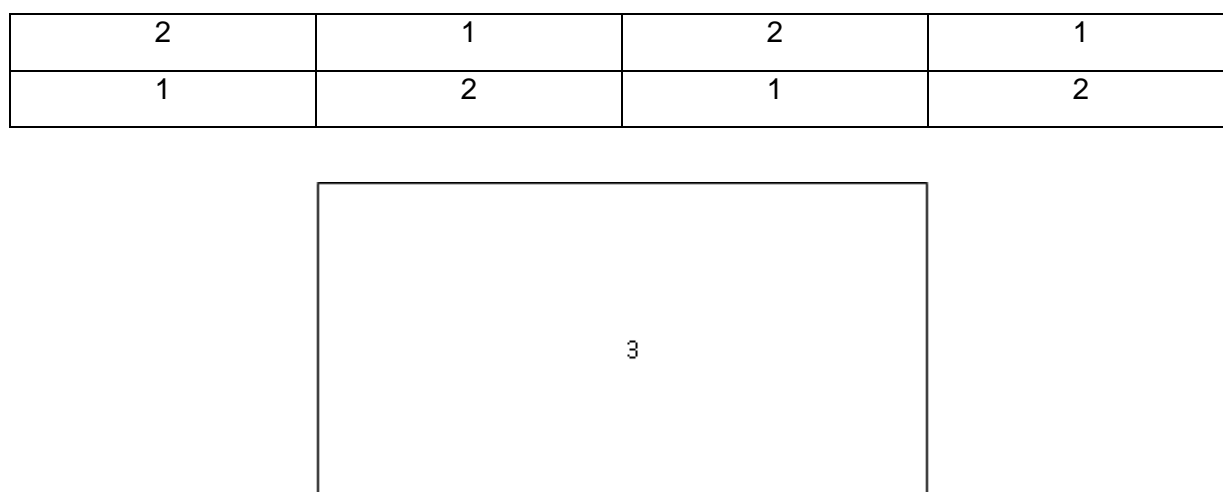


Figure 4. Areas 1,2 and 3 of a bulb onion trial. Air samplers were positioned in Area 3.

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 an area of unusually 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 immunospore 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 (variety Starlight) drilled 13/08/14.

A bio-aerosol air sampler (MTIST) was sited in an overwintered salad onion crop and on a weekly basis assessed for downy mildew disease transmission events. The crop received a comprehensive fungicide spray treatment programme until February 2015. Thereafter an area was left untreated. Plants were assessed on a weekly basis for signs of downy mildew disease.

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.

The lateral flows which had received a downy mildew spore test line application remained relatively constant over time when stored at 4°C. However, when stored at room temperature, the binding activity of the lateral flow test line was seen to reduce by upwards of 50% at 10 months of storage. After which, for this prototype, the testing ceased. When onion downy mildew spores were disrupted and the soluble fraction was retained for test line application, little difference ($r^2=0.9449$) in test line signal was observed between the lateral flows stored at 4°C or at room temperature (Figure 6). 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 more likely operator variables for example, the time taken from start to test reading.

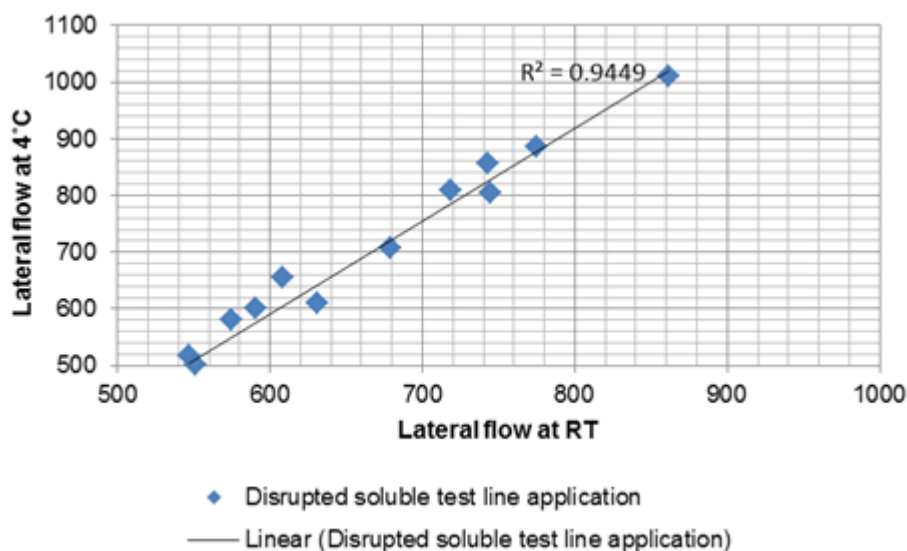


Figure 6. Relationship between the onion downy mildew lateral flows (disrupted soluble test line) stored at 4° and room temperature (18 - 20°C) over a 12 month period.

Integral also to the onion downy mildew lateral flow is the stability of the antibody probe during the processes of dehydration, storage and rehydration. For this purpose sugars at different molarities were incorporated within the conjugate pad. Testing of these in combination (sucrose (20%) and mannitol (80mM, 160mM and 320mM)) with EMA 242 (either in or free of tissue culture medium) determined that sucrose (20%) and mannitol at 80mM retained biological activity of the antibody probe over the time period assessed (Figure 7). In this study, the removal of the tissue culture supernatant (DMEM medium) was beneficial.

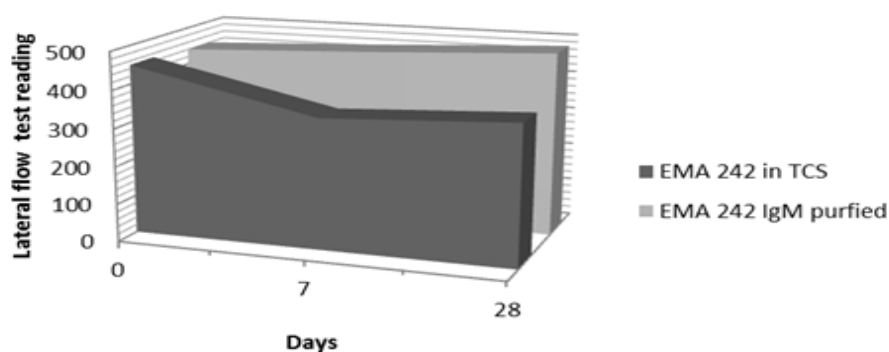


Figure 7. Biological activity of the antibody probe (EMA 242) following desiccation and storage at room temperature to the onion downy mildew pathogen.

Field Trials

Site 1. Worcestershire, OS ref SO8487345194. Salad Onions (variety 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.

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 8a) and triggered a downy mildew warning alert. For this period, the daily lateral flow value recorded values in excess of 2.5×10^4 to 1×10^5 downy mildew spores (Figure 8b). A fungicide spray active towards downy mildew disease was requested and applied to the salad onion plants in Area 2 on the 27th June, 2014. 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; Figure 9), be 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.

Thereafter, 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 9). 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.

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 thereafter 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 10). The crop was harvested the week commencing the 22nd July.

Table 1

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

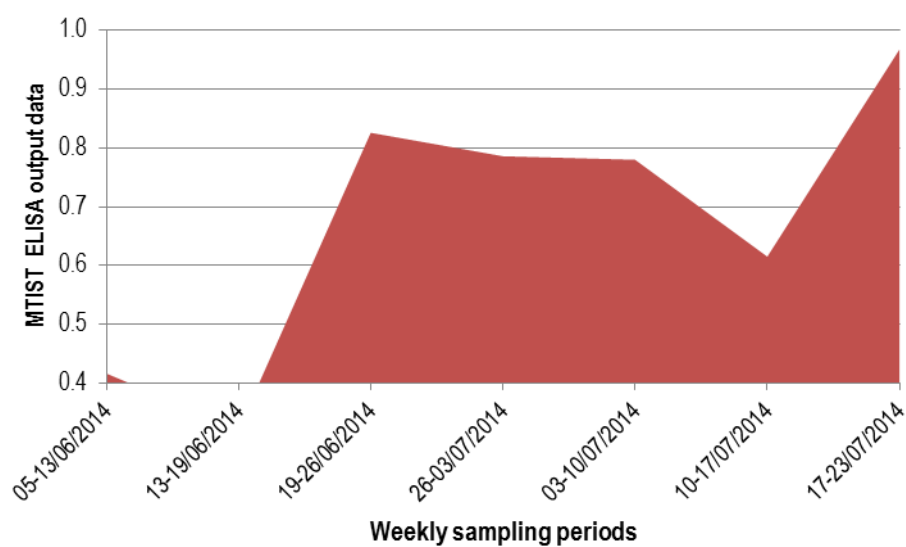
<i>Date</i>	<i>Product</i>	<i>Active</i>
10/06/14	Invader	Dimethomorph + Mancozeb
	Signum	Boscalid + Pyraclostrobin
20/06/14	Invader	Dimethomorph + Mancozeb
	Olympus	Azoxystrobin + Chlorothalonil
26/06/14	Invader	Dimethomorph + Mancozeb
02/07/14	Invader	Dimethomorph + Mancozeb
	Olympus	Azoxystrobin +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

a.



b.

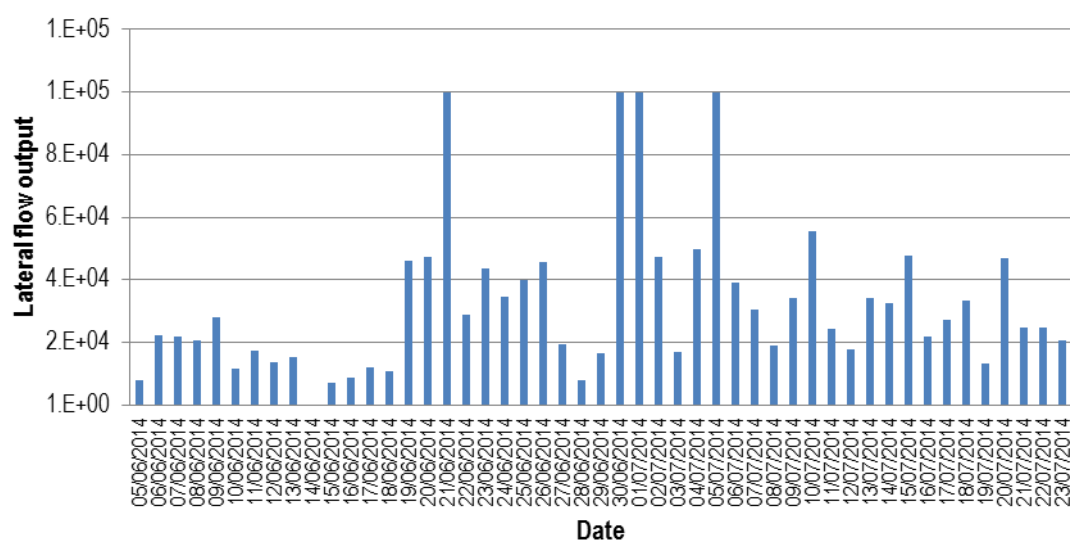


Figure 8 . Measurement of onion downy mildew spores in field bio-aerosols: weekly MTIST laboratory ELISA test (a); daily in-field lateral flow test (b).

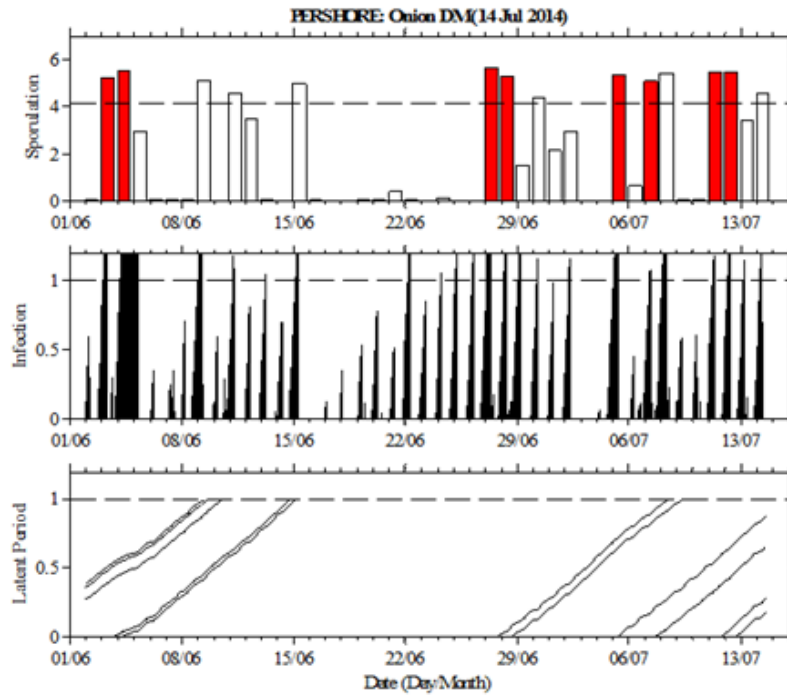


Figure 9. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period

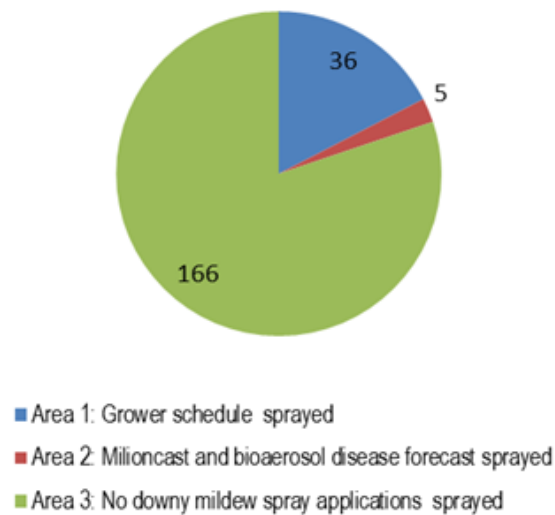


Figure 10. The total number of salad onion plants exhibiting downy mildew symptoms across the two middle rows of each 10m treatment block as recorded on the 17th July, 2014.

Site 2. Lincolnshire, OS ref. TF238 602, Bulb Onions (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.

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 multivial air samples however appeared visually free of soil and recorded an initial spike in downy mildew bio-aerosol concentration on the 9th July (lateral flow standard curve field test reading of $\geq 2.5 \times 10^4$ spores). 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 11a). A fungicide spray active towards downy mildew disease was requested on the 11th July and applied on the 14th July (2014). Based on this period reaching the disease threshold, the development of downy mildew symptom expression on plants would, according to the disease forecast model (MILIONCAST), be visible from the 19th July onwards (Figure 12). 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 11b). Thereafter the weekly MTIST ELISA results remained at high (≥ 0.8) or moderate risk (≥ 0.6) risk of onion downy mildew disease until the week commencing 19th August, 2014. The daily lateral flows determined the crop was at 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 spray actives 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 infection based on the assessment of field bio-aerosols and the environmental forecast (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 13).

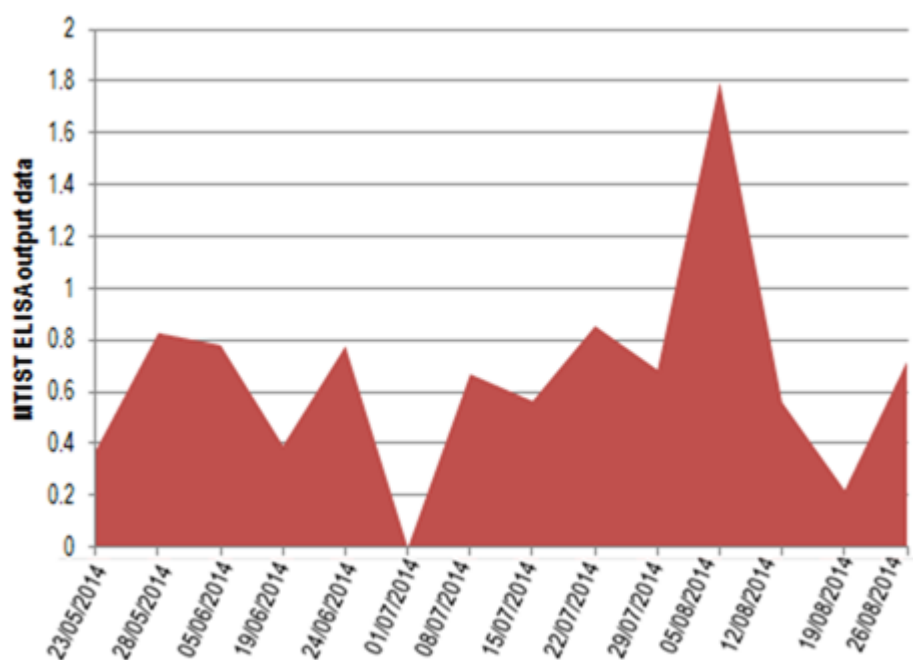
Table 3. Fungicide applications made according to a grower schedule (Area 1)

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

a



b

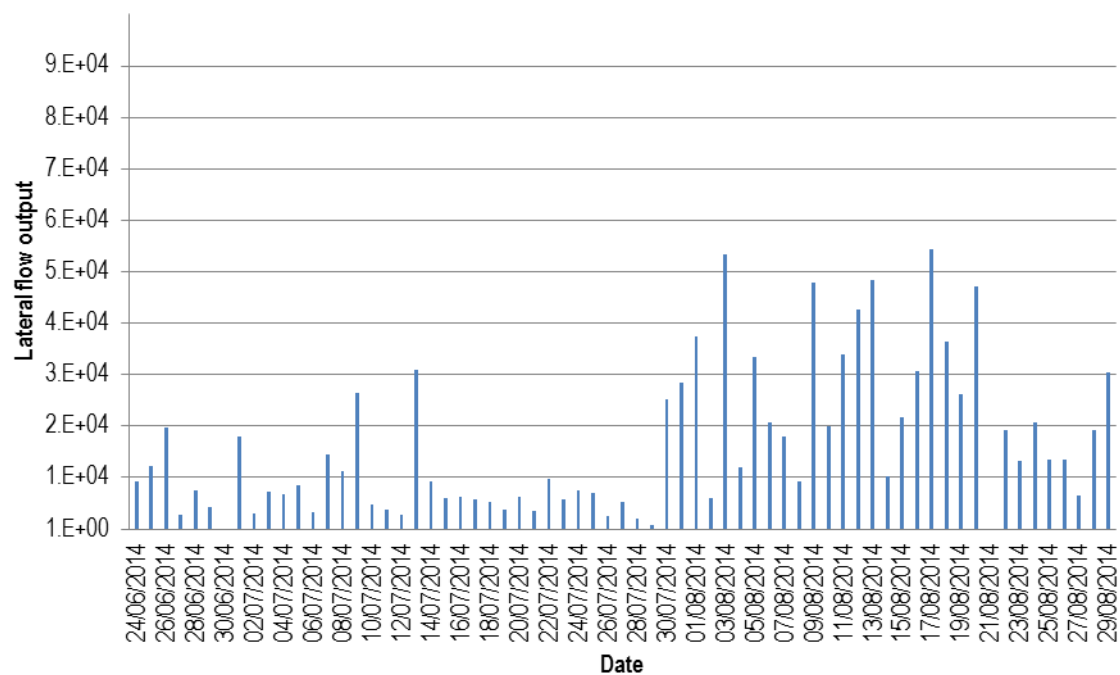
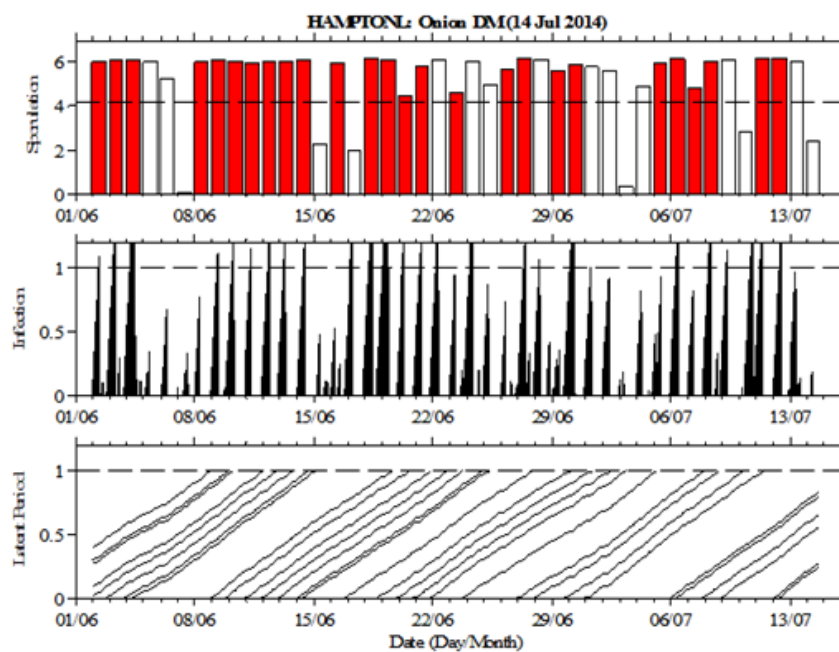


Figure 11. Measurement of onion downy mildew spores in field bio-aerosols: weekly MTIST laboratory ELISA test (a); daily in-field lateral flow test (b).



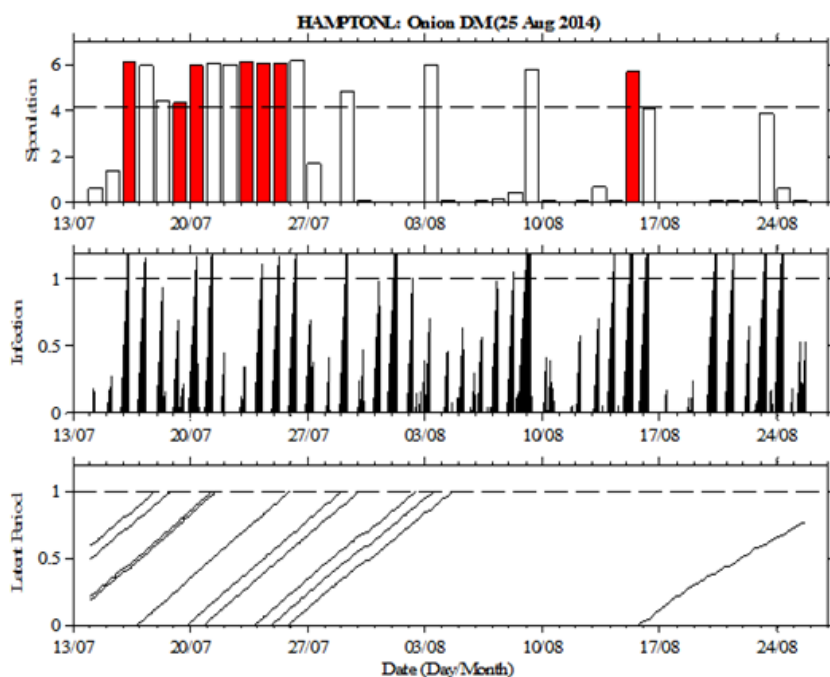


Figure 12. MILIONCAST – An environmental forecast for risk of onion downy mildew sporulation, infection and disease latent period

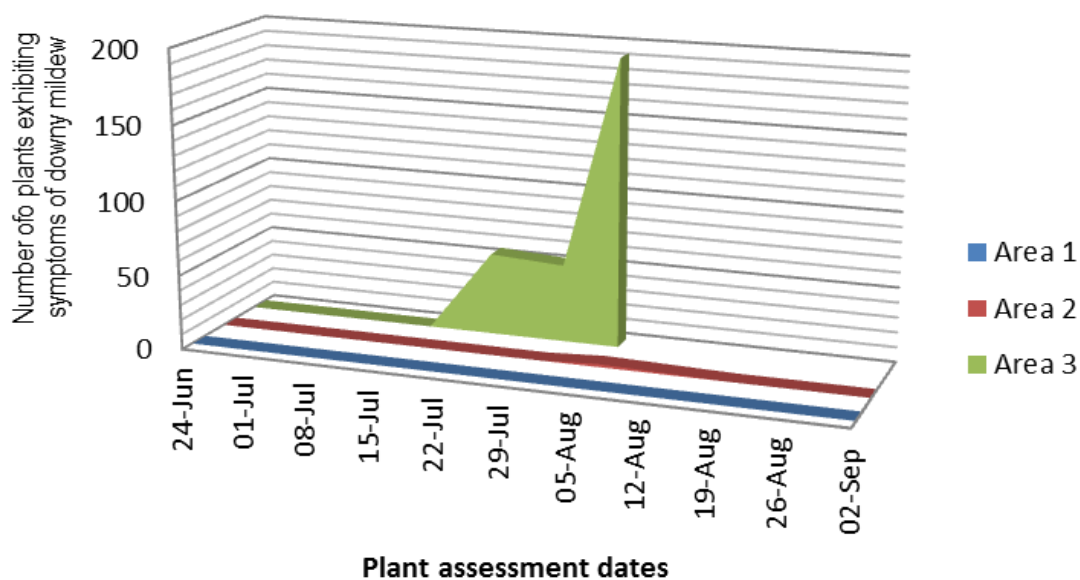


Figure 13. The number of salad onion plants exhibiting downy mildew symptoms across the three treatment areas as recorded on the 17th July, 2014: Area 1, applications made according to a grower schedule; Area 2, timed spray applications based on the assessment of air samples; Area 3 no fungicide treatment for downy mildew.

Site 3. Warwickshire, OS ref. SO195 526, over wintered salad onions (variety Starlight) drilled 13/08/14, harvest date scheduled April 2015.

A bio-aerosol air sampler (MTIST) was sited in an overwintered crop of salad onions and assessed on a weekly basis for onion downy mildew disease transmission events. During October and early November a number of disease risk periods were identified (ELISA value ≥ 0.8 ; Figure 14). Thereafter, risk of *P. destructor* spores in the air declined below the proposed threshold diseases risk value (ELISA value ≥ 0.8). The crop to date has received a comprehensive fungicide spray treatment programme for downy mildew. However, from February 2015 an area will remain unsprayed and thereafter assessed weekly for downy mildew symptom development. The trial remains in place until the harvest date schedule of April, 2015.

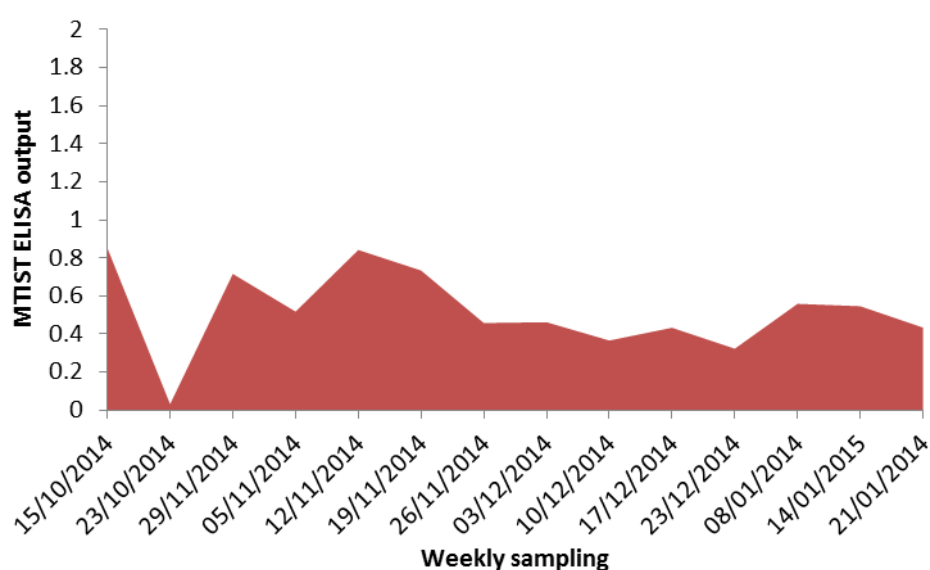


Figure 14. Measurement of *Peronospora destructor* spores in field bio-aerosols: weekly MTIST laboratory ELISA test (value ≥ 0.8 = onion downy mildew disease threshold)

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 shelf life stability of the onion downy mildew lateral flow test was markedly improved when a disrupted soluble spore concentration was applied as the test line. Also for the period of study, the biological activity of the downy mildew antibody probe was stabilised under desiccation by the addition of sucrose (20%) and mannitol (80mM) to the conjugate pad. This process was improved when the antibody probe was free of tissue culture serum (DMEM). 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.

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 to that 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 application treatments for prevention of downy mildew disease and, compared to the unsprayed Area 3, reduced the number of infected plants by 82.5%. In comparison, Area 2 (disease forecast timed applications) had received only two application treatments but reduced the number of infected plants by 95.7% compared to the control. 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 identified an airborne peak in downy mildew disease in the first weeks of July 2014. The first timed fungicide application was made shortly thereafter on the 14th July, 2014. According to MILIONCAST (environmental disease forecast) this infection period would lead 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 application treatments 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 application treatments had been applied to this area for control of downy mildew disease.

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. However, during the colder months of the year (late November to February 2015) the risk of onion downy mildew has subsided. This study remains in place until the harvest date schedule for April, 2015.

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 the addition of sucrose (20%) and mannitol (80mM).
- Downy mildew disease was observed in untreated onion field crops following an MTIST ELISA weekly bio-aerosol disease warning of OD reading ≥ 0.8 .
- An onion downy mildew disease warning was triggered using a weekly multivial air sampler test (daily air samples provided in seven tubes for testing on-site by lateral flow) when a single tube reading is $\geq 2.5 \times 10^4$.
- In this study the timed application of downy mildew control regimes according to bio-aerosol concentration reduced crop protection inputs by 50% and provided either the same or improved levels of disease control.
- The efficiency of the cyclone air sampler was affected at times by rain (water collection in the tubes). In Lincolnshire, the collection of soil was observed in tubes which were exposed for a full seven day period. The collection of soil within the tube will lead to a reduction in air sampling efficiency and may have led to inhibition of the lateral flow test. By using a multivial cyclone air sampler (weekly air sampler across seven tubes (24h collection periods)) this effect was markedly reduced.

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