Project title:	Onions: Further development and calibration of detection tests for conidia of onion downy mildew in combination with MORPH forecast model MILONCAST.
Project number:	FV 356
Project leader:	Prof. Roy Kennedy
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AUTHENTICATION

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

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CONTENTS

GROWER SUMMARY

Headline

A lateral flow device (LFD) has been developed which can detect spores of onion downy mildew in the air before symptom appearance in the crop. The device has been validated using spore trapping systems.

Background

Onion downy mildew, caused by the fungal plant pathogen *Peronospora destructor*, can cause heavy yield losses in both salad and bulb onion cropping systems. The disease once established in a crop can readily become airborne and inoculum (conidia) is dispersed both locally and over longer distances to infect other salad and bulb onion crops. Once a crop is infected and, if favourable environmental conditions exist, a further period of 7–10 days is required before disease symptoms are then observed. A forecast model (MILIONCAST) is available to describe the effect of environmental conditions on downy mildew conidial production, infection and symptom development. However the model is unable to provide information on whether the crop has actually been exposed to airborne downy mildew inoculum. At present fungicidal control is the only effective means of controlling the disease and avoid crop loss and is applied with no knowledge of disease presence.

HDC FV 356 project reports on the development of a 10 minute in field test detection system to monitor field airborne concentration of onion downy mildew disease inoculum. The results obtained from these tests can, when used in conjunction with an environmental forecast model, assess the likely risk of disease development in an onion cropping system. As part of a risk assessment system producers will be able to make informed decisions on when to apply control treatments. This should enable produces to reduce unnecessary fungicide applications and schedule fungicide applications within areas more effectively.

Summary

The findings of this project indicate that the critical date for applying fungicide applications to the crop can be identified by using a daily ‘infield’ Lateral Flow test in conjunction with a disease forecast system (MILIONCAST). Onion downy mildew disease potential can be identified ahead of visible symptoms. This is a critical point in considerations of disease control. If early applications of fungicide can be targeted when onion downy mildew spores are present at times when the environmental conditions are conducive to infection, the activity of control methods will be enhanced.

The downy mildew lateral flow test has a shelf life of one year at 4^0C and operates over a spore concentration which can elicit disease symptoms in susceptible onion crops.

The downy mildew lateral flow test will be made available commercially in 2014.

Financial Benefits

The main financial benefits will be in the use of the device to reduce unnecessary fungicidal applications to the crop. 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. Lateral flow tests are expected to cost approximately £4-5 per test. This cost must be compared with £40 per hectare for fungicide treatment. In high risk years it is common to spend in excess of £300 per hectare on fungicides in a bulb onion crop. However savings will be variable between years and depend on the overall reductions in sprays achieved.

The amount of spores produced during the night may vary with environmental conditions. Information on the presence or absence of critical spore threshold numbers could help growers to identify periods when disease is likely to spread. However, besides information on sporulation also further information on when conditions are favourable for infection and the latent period are required before growers can decide on the best times to apply control measures.

By using the “in field test” for onion downy mildew in conjunction with the forecast model will enable able better scheduling of fungicide applications to onion crops. There will be less reliance on metalaxyl based fungicides for onion downy mildew control (the authorized use of these are under review).

Action Points

- Use air samplers to trap onion downy mildew disease inoculum ahead of symptom development in the crop.
- Test weekly air samples for onion downy mildew disease inoculum using lateral flow devices.
- Use environmental models to identify onion downy mildew spore release and infection periods.

- Utilisation of the integrated disease management system for control of onion downy mildew. Information on airborne downy mildew inoculum and environmental data will assist growers to schedule fungicide applications to crops more effectively to produce cost savings.

SCIENCE SECTION

Introduction

Downy mildew occurrence in onion crops

Onion downy mildew (*Peronospora destructor*) is geographically widespread and serious disease in bulb and salad onions and in onion seed production. Actual yield losses in bulb onions of 60 to 75% have been recorded (Cook, 1932, Cruickshank, 1958). These losses mainly result from severe infections in bulb onion crops causing early defoliation, reduced bulb sizes and poor storage quality of bulbs (Rondomanski, 1967). 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 (Virányi, 1981). Fungicidal control of downy mildew is difficult and fungicides are only effective, if they are applied before or immediately after disease first appears in the crop (Kennedy, 1998). Fungicidal control is the only effective means of controlling the onion downy mildew and avoiding crop loss.

Biology of Peronospora destructor on onion crops

Large numbers of spores are produced from downy mildew lesions and this is a characteristic of downy mildew pathogens. Sporulation of *P. destructor* is a diurnal process and both periods of light and darkness are required. Sporulation is mainly during the night under high relative humidity of greater than 94 - 95% at temperatures of 6 - 22^{o}C provided there is no rainfall. (Yarwood, 1937, 1943). High day temperatures exceeding 24-25°C or exceeding 27, 28, 29 or 30°C for more than 8, 6, 4 or 2 h, respectively, were found to inhibit sporulation during subsequent nights (Hildebrand & Sutton, 1982). Studies by Hildebrand and Sutton suggested that a combination of night temperature, time of onset of high humidity and duration of high humidity affected the quantity of sporangia produced. Sporangial discharge is triggered when relative humidity falls below 59%. Conidia are thin walled and wind transported over considerable distances. Conidia have been detected at heights of 1500 ft. When conidia are deposited on leaf surfaces, they germinate between 1.5 and 7 h. Progress and spread of downy mildew is dependent on the survival of spore populations until conditions become favourable for germination (Sutton & Hildebrand, 1985). However viability is affected by the prevailing temperature and humidity conditions. At 10^{o}C, spore viability is unaffected by relative humidity. However, at temperatures of 30^{o}C, viability declines rapidly at relative humidifies above 55 %. 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 and when either sets, or seeds are transplanted the mycelium grows within the foliage of the plant. Downy mildew infects all the main onion types grown in the U.K. including common onion (*Allium cepa*) shallots (*A*. *cepa* var. *ascalonicum*) and Welsh onion (*A*. *fistulosum*). Welsh onion is particularly susceptible to downy mildew infection.

Methods for control of onion downy mildew

The control of downy mildew in onions relies mainly on the prophylactic application of fungicides, as frequently as every 10 days. However, to reduce the impact of fungicides on the environment, integrated pest management (IPM) systems have been developed. A reduction in the number of fungicide applications, while maintaining disease control, could be achieved by applying fungicides only at times when conditions are favourable for disease development. The times when large numbers of spores of onion downy mildew are produced are thought to be related to the times when disease spreads. Such direct relationship between airborne spore numbers and disease spread has been proposed for airborne fungal pathogens (Campbell and Madden, 1990), and have been found for *Mycosphaerella brassicicola* on Brussels sprouts (Kennedy *et al*., 2000).

The amount of spores produced during the night may vary with environmental conditions. Information on the presence or absence of critical spore threshold numbers could help growers to identify periods when disease is likely to spread. However, besides information on sporulation also further information on when conditions are favourable for infection and the latent period are required before growers can decide on the best times to apply control measures.

Methods for determining the risk of air-borne diseases in onion crops

Management systems are available for onion diseases (BOTCAST/DOWNCAST /ONION$_{SPOT}$) (Gilles, *et al*., 2004) which can predict the early development of both downy mildew and *Botrytis* in onion crops. This system incorporates the DOWNCAST model (Jesperson & Sutton, 1987), which predicts sporulation and infection events of *P*. *destructor*. DOWNCAST predicts sporulation, but cannot predict the quantity of sporangia produced. Tests of the model in Canada suggested it gave positive predictions of sporulation for 38 out of 45 nights when sporulation was observed. However, in field tests in the Netherlands, DOWNCAST gave positive predictions of sporulation for only 11 out of 24 nights when sporulation was observed (de Visser, 1998). Thus, the model often failed to predict sporulation events in a north-west European maritime climate in which the weather conditions are highly

variable. Battilani and colleagues developed ONIMIL, a forecaster, which is also based on DOWNCAST, and which gives a quantitative prediction for sporulation (Battilani, 1996).

A new model, named MILIONCAST (an acronym for 'MILdew on onION foreCAST'), was developed based on the data from controlled-environment studies investigating the effect of temperature and humidity on downy mildew sporulation. The rate of sporulation was predicted using controlled environmental data. The accuracy of MILIONCAST was compared to the accuracy of existing models based on DOWNCAST. MILIONCAST gave more correct predictions of sporulation than the DOWNCAST models and a random model. All models based on DOWNCAST were more accurate than the random model when compared on the basis of all predictions (including positive and negative predictions), but gave less correct predictions of sporulation than the random model (Gilles *et al*., 2004). Use of this system, which provides information on the timing of the first fungicide application, may result in better disease management. However inoculum can be imported into disease free crops from other localities/areas but assessments based on environmental risk alone do not take this factor into account. In order to avoid these problems new and rapid methods of detecting and quantifying pathogenic inoculum are required which can be used in conjunction with forecasting models. With this more precision approach there will be reductions in the amounts of fungicide required to control disease. Unnecessary fungicide applications, which are based on weather information alone, will be reduced especially during periods of high risk.

Using air-borne spore numbers within disease forecasting systems

It has been demonstrated that airborne inoculum plays a vital role in the development of epidemics caused by *Botrytis* leaf blight on onion crops (Carisse, 2005). Detection and quantification of airborne spore numbers can be used to predict disease accurately before it is visible in the crop. Peaks of airborne spores are always detected prior to crops becoming infected. This, results from the requirement for a threshold of inoculum to initiate disease establishment in crops and this must coincide with favourable weather conditions. The importance of airborne inoculum has been recognised in the development of many diseases. Its use in practice has been limited because of the difficulties in quantifying it.

Detecting airborne spores of fungal plant pathogens is a useful tool in crop protection if this could be done rapidly and accurately. For example it has been reported that one or two peaks in sporangial concentration in the air of the potato blight pathogen *Phytophthora infestans* preceded the first observed symptoms of the disease in the field (Bugiani *et al*., 1998). These observations were validated in studies conducted by Philion (2003). In these

studies the numbers of sprays applied to control potato blight could be successfully reduced without any impact on crop quality by monitoring the onset of thresholds of potato blight inoculum. Fungicide applications were initiated when the daytime airborne sporangial concentration reached 30 sporangia/m^3 (disease was not yet visible when this threshold was reached). By using these criteria, in combination with disease forecasts based on weather information the number of fungicide applications could be reduced with no impact on disease development. Similar results were obtained for *Botrytis* blight (*Botrytis squamosa*) on onion crops where thresholds of 15 - 20 conidia/m^3 could be used to reduce fungicide application by up to 100% (Carisse *et al*., 2003). Thresholds of inoculum required for disease establishment have also been reported for *M*. *brassicicola* which is the fungal pathogen responsible for ringspot on vegetable brassicas (Kennedy *et al*., 2000). In these studies (with the exception of ringspot) the information on spore number had to be collected manually using a microscope which was slow and time consuming. Tests which, can be conducted in the field are necessary if information on air-borne inoculum concentration is to be of more practical value. The use of air-borne spore numbers, as criteria, within forecasting systems is a new and exciting development in disease forecasting. One “in field test” which could be used in this respect is the lateral flow test.

Immunological tests (Lateral Flow Devices)

Lateral flow assays are only one type of rapid assay which can be employed to quantify target particles or molecules (see Figure.2). However they are now commonly and widely used for detection purposes. They rely upon the specific reaction of sensitised coloured particulates. Antibodies raised to a specific target spore, are bound by passive or covalent means to these coloured particles. These sensitised particles (latex or immunogold particulates are generally used) are then applied using an immersion procedure on to a release pad, to produce a stable particle reservoir for release on to a nitro-cellulose-based membrane. In a standard lateral flow test two lines of reagents are immobilised on to the membrane using a sophisticated reagent dispenser. The constituents of these lines will vary from test to test but commonly only two types of formats are adopted.

The Competitive assay format.

In a competitive assay format the test line comprises of homologous antigen (downy mildew spore components) and the other, the control, is a line of anti-species antibodies. The release pad and membrane are assembled together with an absorbent pad into a plastic housing as illustrated below (Figure 1). The fluid sample is added to the well, releasing the specific antibody bound coloured particles, which then begin to flow across the membrane. If the target antigen (onion downy mildew spore component) is present in the sample

extract, antibody binding will occur to produce a coloured particulate conjugated antibody - antigen complex. Any antibody conjugated coloured particles that fail to bind to target antigen will attach to the immobilised test line as they traverse the membrane. If present at a high enough concentration, a visible line of deposited coloured particulates will form at the test line. The anti-species antibody will capture excess sensitised antibody / coloured particles to produce an internal control line, providing a visible confirmation of antibody / particulate flow.

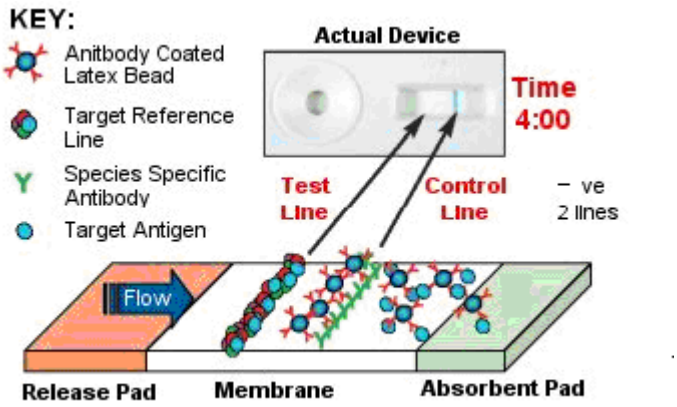


Figure 1. The Competitive lateral flow assay format.

Sufficient antigen target presence (onion downy mildew spores), would induce complete inhibition of antibody attachment to the test line, a result that is indicated by a single line of coloured particle deposition (the control line) denotes a positive results. Two lines of equal colour intensity indicate a negative result.

Developing 'in field' tests for detecting the presence or absence of onion downy mildew inoculum

If accurate 'in field' tests for inoculum are to be constructed they will require specific antibodies that can differentiate between different types of pathogenic spores recognising only onion downy mildew spores. Successful lateral flow test formats have been used to determine their accuracy. Cross-reaction of the test with spores of other pathogenic and non-pathogenic species need to be ascertained. These tests would include other pathogens which are common in onion crops notably *Botrytis squamosa* and *Botrytis cinerea*. Other pathogens found in onion crops include *Cladosporium allii cepae* Many of these pathogens survive on debris in the soil or are found on leaves on onion crops. Consequently tests which detect onion downy mildew conidia should not react with the conidia of other

pathogens common in onion crops. They should also remain non-reactive to other biological and non-biological particles. The level of reactivity of the antibody to onion downy mildew conidia is also important as this will affect the sensitivity of the test and how it can be used to quantify the number of onion downy mildew conidia present in samples.

Project HDC FV 356

Within this project we have sought to develop 'in field tests' which can identify critical transmissible onion downy mildew spore concentrations in collected field aerosols. Years 1 and 2 of the project are previously reported and focus on the optimisation of the air sampling and test format with preliminary field trials data. Within this final report we focus on works carried out in Year three of the project to meet the project deliverables. For this period field trials were carried out in commercial onion cropping systems to assess the 'in field test' and its integrated use within an environmental forecast model (MILIONCAST) to predict the potential for onion downy mildew disease development.

Additional work has focused in Year 3 to assess the suitability of the 'in field' test (downy mildew lateral flow) for commercialisation purposes. Stability of the lateral flow has been examined over time to confirm biological activity and shelf life of the test. The incorporation of a control line to confirm test execution has been investigated.

Materials and Methods

Evaluation of the lateral flow for quantitative detection of transmissive airborne downy mildew inoculum of Peronospora destructor in collected aerosols.

A batch of lateral flows were prepared as described in HDC FV 356 Year 2 Annual Report and assessed for quantitative detection of airborne downy mildew inoculum of *P. destructor.* A control as well as a test line were present on each lateral flow (Fig. 2).

Aerosols of *P. destructor* were then produced in a controlled environment chamber and collected using a cyclone air sampler. After which the 1.5ml collection tube was removed from the air sampler and the collected spores suspended in 1ml lateral flow buffer. The *P. destructor* spore concentration was determined by light microscopy and adjusted to a final concentration of $1x10^5$ spore ml^{-1}. A 10 fold dilution series was then prepared in lateral flow buffer to provide a range of $1x10^5$ to 10 spores ml^{-1} buffer. After which 100µl aliquots of each spore concentration were applied to individual onion downy mildew lateral flow devices (Fig. 2). After a 10 minute development the lateral flows were read using an electronic portable reader (ESE reader).

B

Figure 2. A lateral flow device for evaluation of field crop risk to onion downy mildew (A) and a hand held reader ESE lateral flow reader (B)

Assessment of the 2012 lateral flow batch for shelf life stability.

On a monthly basis a 100µl aliquot of lateral flow buffer was applied to each of six stored downy mildew lateral flow devices. The lateral flow devices were prepared as described above, identified as Batch 004 and stored in individually sealed foil pouches at either room temperature (18 - 20ºC) or at 4ºC. Testing was at monthly intervals and the lateral flow results read 10 min. after sample application to the lateral flow device.

Evaluation of the onion downy mildew device to monitor airborne disease transmission events of Peronsopora destructor in commercial cropping systems and in conjunction with the sporulation forecast system 'MILIONCAST'

A commercial salad onion crop which was free of onion downy mildew disease symptoms was identified at Ripple, Worcestershire. Two air samplers were placed within the crop and adjacent to each other. Over a six week period (Nov – Dec, 2011), daily and weekly air samples were collected and analysed for onion downy mildew disease inoculum. The air samplers operated as described below:

- An MTIST air sampler (microtiter immunospore trap) operated for 12 H periods from 06:00 to 18:00 H daily. Held within the base plate of the machine were four eight well microtitre strips. The microtitre well strips were replaced weekly and after each seven day exposure period were sent to the National Pollen and Aerobiology Research Unit (NPARU) for analysis by ELISA for the quantification of onion downy mildew disease inoculum.

- A multi vial cyclone air sampler operated for 12 H periods from 06:00 to 18:00 H daily. At weekly intervals seven 1.5ml microfuge tubes were loaded in to the device and by automation each tube was exposed once to field aerosols for a single 12 H period. The tubes were then collected and each analysed for onion downy mildew disease inoculum using an in 'in field' lateral flow strip (Fig. 1). An electronic reading of the lateral flow test line result was then recorded using a portable hand held device (Fig 1B).

Detailed information on the air samplers used and the methods involved in analysis of the field samples can be found in HDC FV 356, 2011 Annual Report.

Throughout the air sampling period 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.

A commercial bulb onion crop, Worcestershire was then assessed as above over a two month period between March and May, 2012 for incidence of onion downy mildew airborne downy mildew inoculum. A third air sampler (Burkard volumetric) ran continuously over each seven day period to validate the results of the MTIST ELISA and the cyclone air sampler (lateral flow test). Identification and quantification of trapped onion downy mildew disease inoculum was made by conventional bright field microscopic counts at a magnification of 400. Sampling observation times were between 06:00 and 18:00 hrs daily.

Results

Evaluation of the lateral flow for quantitative detection of transmissive airborne downy mildew inoculum of Peronospora destructor in collected aerosols.

The lateral flow which had been developed and optimised for quantitative measurement of airborne disease propagules of *Peronospora destruct*or (onion downy mildew) demonstrated a clear correlation between disease inoculum concentration and test signal depletion when measured using an ESE reader (Fig. 3). The test provided sensitivity over the spore range tested and had the potential to induce symptom development on exposed plants (circa >1000). Test line depletion was observed at 100,000 (visual reading by eye showed no test line). During periods at optimal infection conditions (MILIONCAST) this level of inoculum concentration could give rise to significant disease development on susceptible crops.

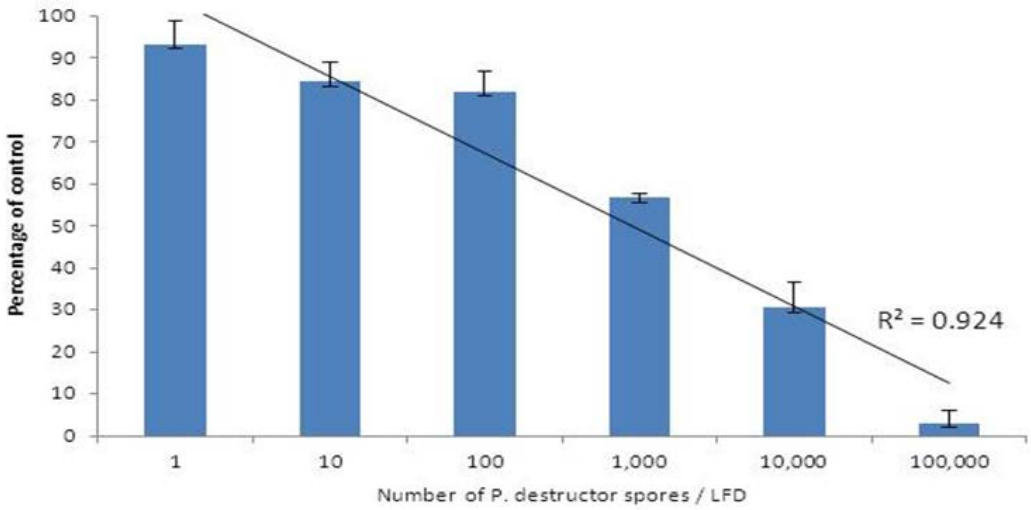


Figure 3. Relationship between the lateral flow ' in field' device and quantification of *Peronospora destructor* spore concentration in collected aerosols.

Assessment of the 2012 lateral flow batch for shelf life stability.

The onion downy mildew lateral flow test shelf life was extended by storage at the lower temperature of 4ºC (Fig. 4). A trend of reduced biological activity was however observed over the 16 month trial period. Nevertheless the activity of the test at 4^0C remained relatively constant within the first six months of use. Thereafter a further reduction of ≈ 5% was observed at 12 months of storage and again at 16 months. When stored at room temperature (18-20ºC) test stability was compromised over time and at 12 months a test signal reduction of >70% was recorded.

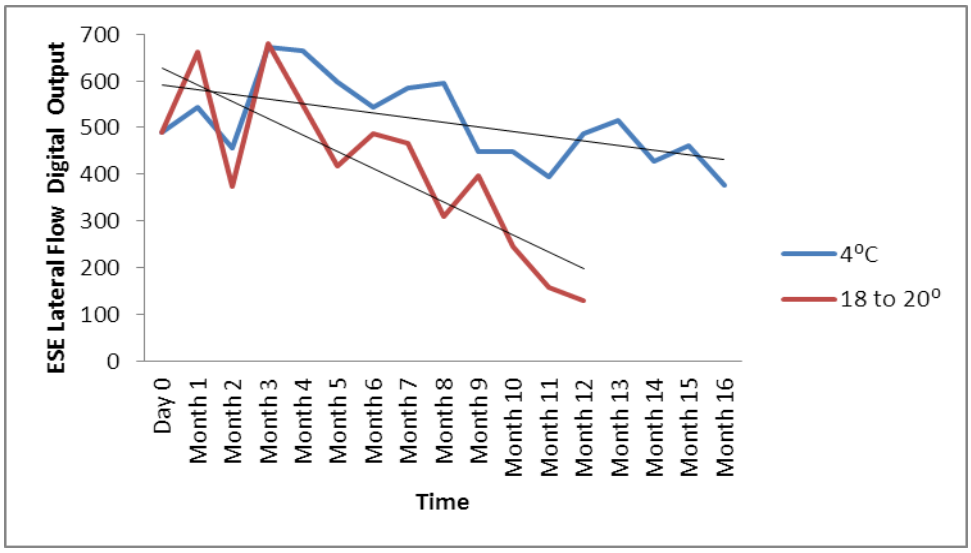


Figure 4. Shelf life of the onion downy mildew 'in field' lateral flow test for monitoring aerosols of *Peronospora destructor*.

Evaluation of the onion downy mildew device to monitor airborne disease transmission events of Peronospora destructor in commercial cropping systems and in conjunction with the sporulation forecast system 'MILIONCAST'

Salad Onion Crop – Worcestershire: Daily air samples collected using the multivial cyclone air sampler was tested by lateral flow for downy mildew disease inoculum (*Peronospora destructor* spores). The values generated using the ESE portable field reader were then referenced to a *P. destructor* 'known' spore lateral flow concentration series (Fig. 3) and ascribed a disease risk value (Table 1a). MILIONCAST, an environmental model provided a daily risk forecast of *P. destructor* spore production (sporulation) and infection (Fig. 4 a,b and) in the crop. These two systems (air samples and environmental models ascribing risk of onion downy mildew disease) were assessed together (Table 2). It was observed that the model often forecast daily periods of disease potential but on most occasions no disease was measured in the air samples of the crop canopy. Similarly, there were periods when medium to high spore concentrations were measured in the air samples but the environmental conditions within the crop were not favourable for infection.

During this period only the 13th November was identified as providing the environmental conditions and airborne spore load available for downy mildew infection. The crop however was sprayed routinely throughout the period.

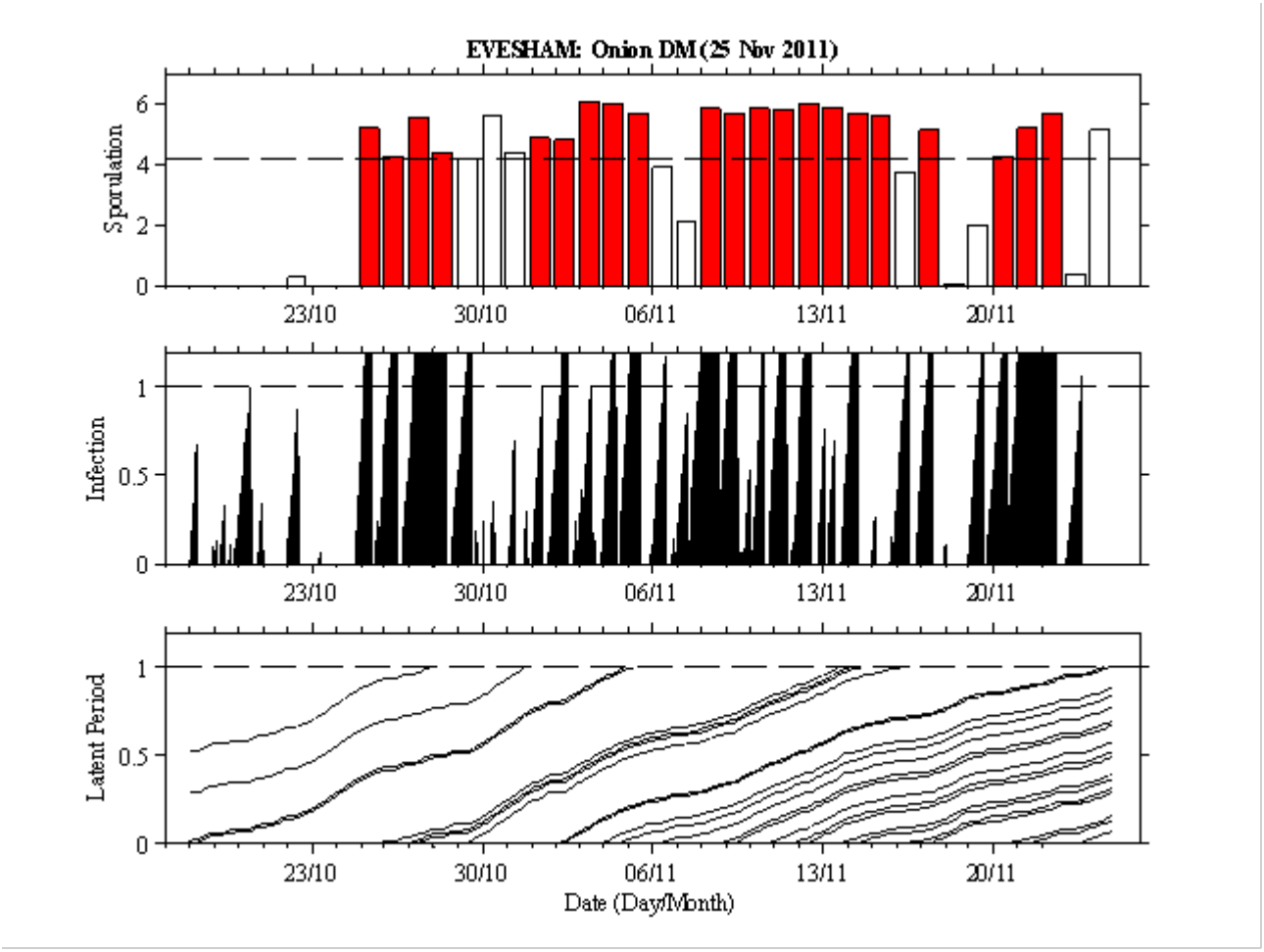


Figure 4a. MILIONCAST environmental model downy mildew disease forecast for the period 17/10/2011 to 26/10/2011 at commercial salad onion site, Worcestershire, UK

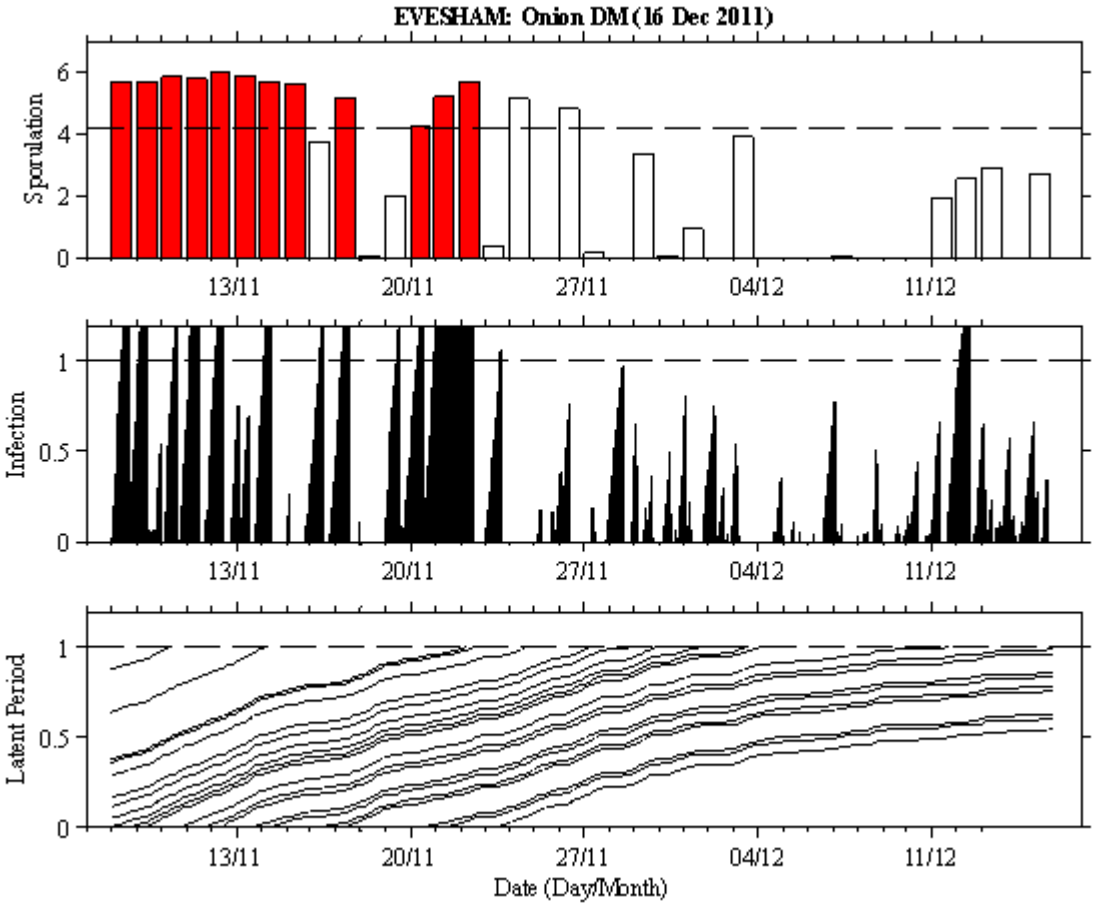


Figure 4b. MILIONCAST environmental model downy mildew disease forecast for the period 09/11/2011 to 17/12/2011 at a commercial salad onion site, Worcestershire, UK

Table 1. Assigned Crop risk value in a salad onion crop to onion downy mildew disease by lateral flow 'in field test' (a) and an environmental forecast (MILIONCAST) (b)

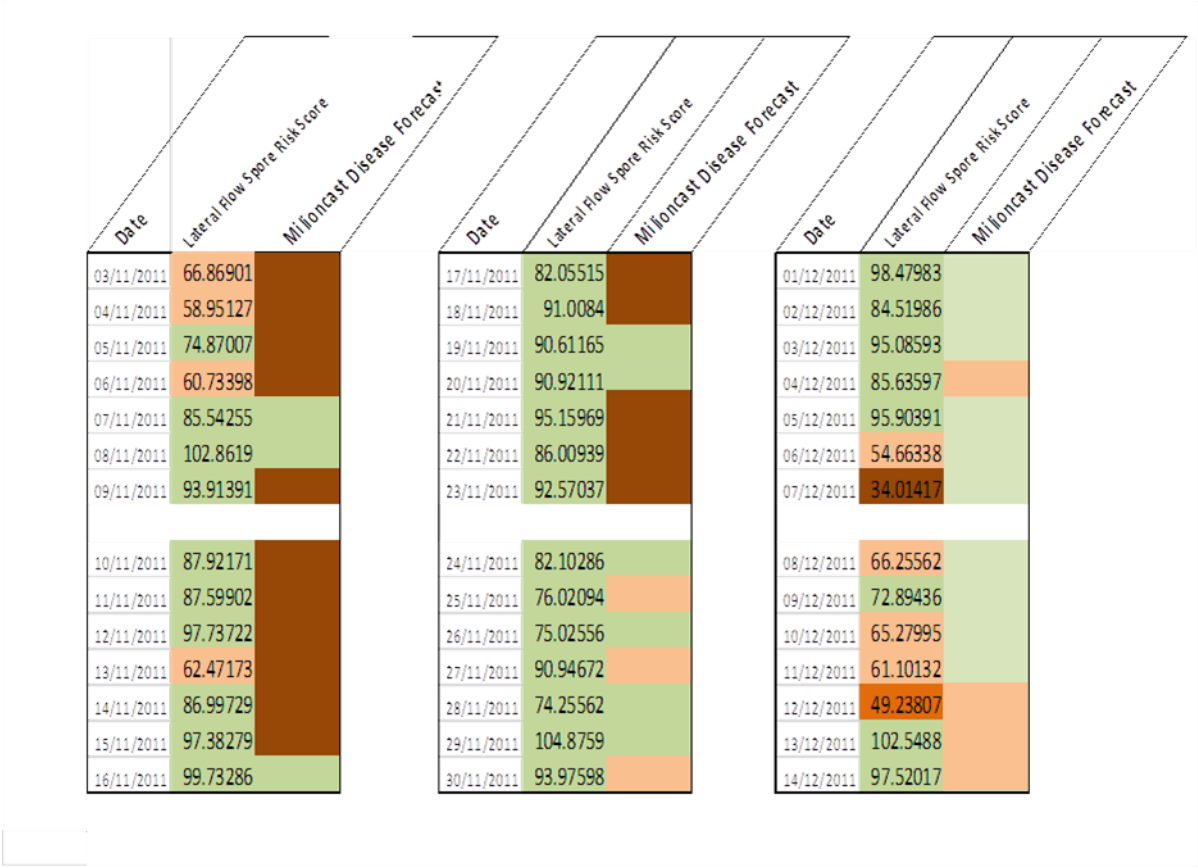
1a

ESE % lateral flow conversion	Downy mildew spore risk ascribed to crop
> 70	None/Low
<70	Low /Moderate spore
<50	Moderate spore concentration
<30	High spore concentration

1b

Milioncast downy mildew disease risk forecast	
None / Low	
Spore production	
Near spore / infections conditions	
Spore production and infection conditions	

Table 2. Monitoring daily onion downy mildew disease risk in a salad onion crop using lateral flow devices and an environmental weather based disease forecast (MILIONCAST)



Date	Lateral Flow Spore Risk Score	Milioncast Disease Forecast
03/11/2011	66.86901	
04/11/2011	58.95127	
05/11/2011	74.87007	
06/11/2011	60.73398	
07/11/2011	85.54255	
08/11/2011	102.8619	
09/11/2011	93.91391	
10/11/2011	87.92171	
11/11/2011	87.59902	
12/11/2011	97.73722	
13/11/2011	62.47173	
14/11/2011	86.99729	
15/11/2011	97.38279	
16/11/2011	99.73286	

Date	Lateral Flow Spore Risk Score	Milioncast Disease Forecast
17/11/2011	82.05515	
18/11/2011	91.0084	
19/11/2011	90.61165	
20/11/2011	90.92111	
21/11/2011	95.15969	
22/11/2011	86.00939	
23/11/2011	92.57037	
24/11/2011	82.10286	
25/11/2011	76.02094	
26/11/2011	75.02556	
27/11/2011	90.94672	
28/11/2011	74.25562	
29/11/2011	104.8759	
30/11/2011	93.97598	

Date	Lateral Flow Spore Risk Score	Milioncast Disease Forecast
01/12/2011	98.47983	
02/12/2011	84.51986	
03/12/2011	95.08593	
04/12/2011	85.63597	
05/12/2011	95.90391	
06/12/2011	54.66338	
07/12/2011	34.01417	
08/12/2011	66.25562	
09/12/2011	72.89436	
10/12/2011	65.27995	
11/12/2011	61.10132	
12/12/2011	49.23807	
13/12/2011	102.5488	
14/12/2011	97.52017	

For this period airborne downy mildew inoculum of *P. destructor* (onion downy mildew) was also assessed on a weekly basis using an MTIST air sampler and the results processed at the National Pollen and Aerobiology Research Unit (Fig 5.) With the exception of the fourth and fifth weeks of field sampling the crop was considered at risk from airborne *Peronospora destructor* disease inoculum (ELISA >0.25). However it was not possible to evaluate the crop for disease development as routine fungicide sprays were applied throughout the monitoring period.

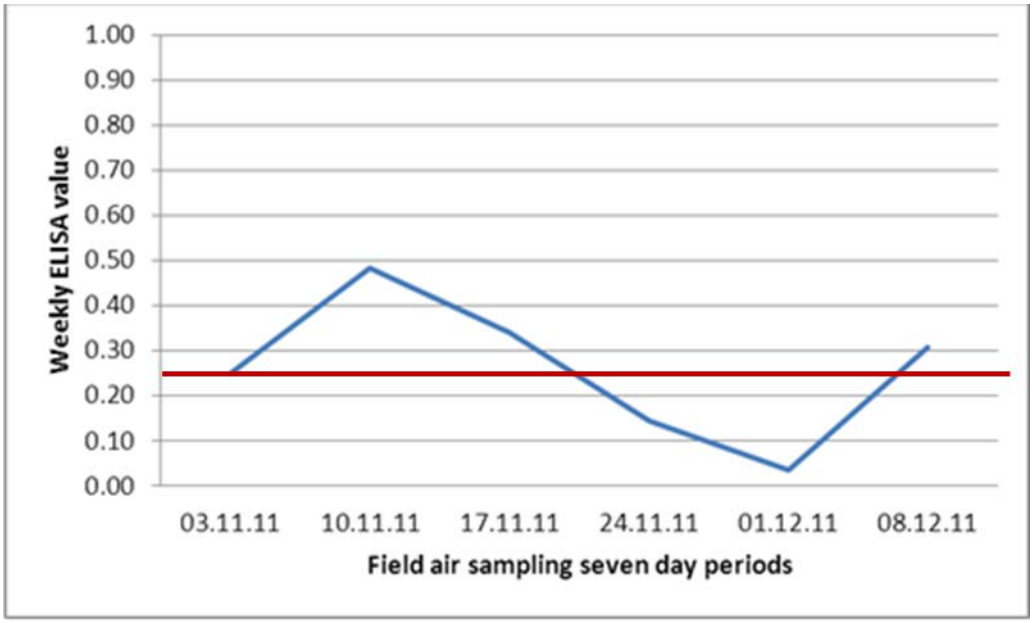
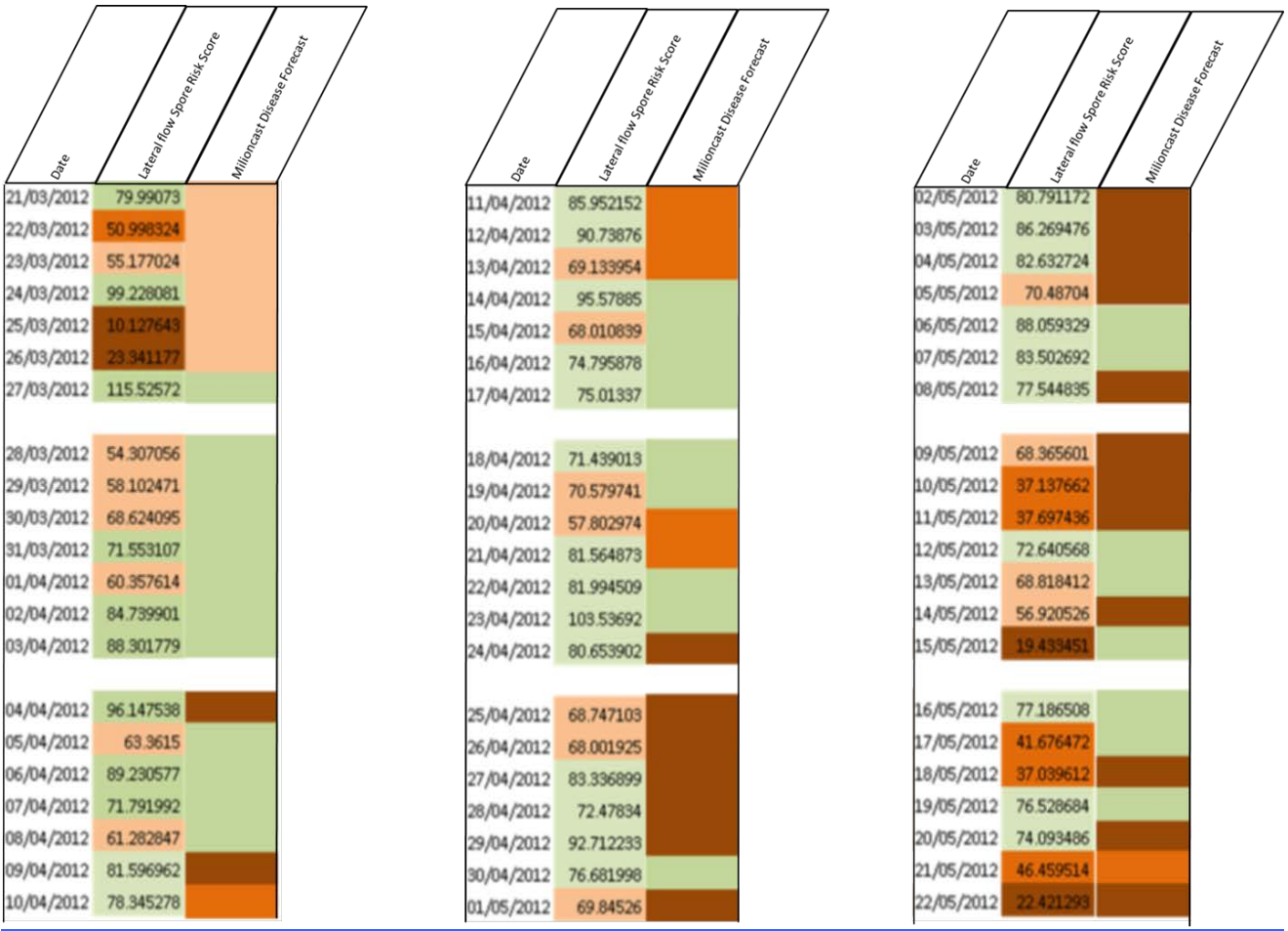


Figure 5. Monitoring daily onion downy mildew disease risk in a salad onion crop from weekly MTIST collected air samples and processed by PTA-ELISA.

Bulb Onion Crop – Worcestershire: Daily air samples collected using the multivial cyclone air sampler were tested 'in field' by lateral flow for downy mildew disease inoculum. The values generated using the ESE portable field reader were then referenced to a *P. destructor* 'known' spore lateral flow concentration series (Fig. 3) and ascribed a downy mildew disease risk value (Table 1a). All weeks gave rise to periods when the crop was exposed to 'low level' downy mildew airborne transmitted disease pressure (Table 3). High risk periods were observed in weeks 1, 8 and 9 (Table 3). There was a correlation (r^2=0.9625) between the *P. destructor* spore concentrations collected by the ' in field' lateral flow daily air sampler and the weekly absorbance values of the MTIST ELISA (laboratory test) when cumulative weekly values of the 'in field' system were compared (Fig. 6).

Significant onion downy mildew sporulation periods within the crop (conditions favourable for *P. destructor* spore production) were predicted by the environmental model (MILIONCAST) in weeks 1, 3 to 9 inclusive. High risk events for downy mildew infection were predicted in each of the weeks 3 to 9 inclusive (Table 3, Figs. 7a,b). Routine fungicide sprays were applied throughout the trial period and no disease development was observed.

Table 3. Monitoring crop risk in a bulb onion crop to onion downy mildew disease by lateral flow 'in field test'



Date	Lateral flow Spore Risk Score	Milioncast Disease Forecast
21/03/2012	79.99073	
22/03/2012	50.998324	
23/03/2012	55.177024	
24/03/2012	99.228081	
25/03/2012	10.127643	
26/03/2012	23.341177	
27/03/2012	115.52572	
28/03/2012	54.307056	
29/03/2012	58.102471	
30/03/2012	68.624095	
31/03/2012	71.553107	
01/04/2012	60.357614	
02/04/2012	84.739901	
03/04/2012	88.301779	
04/04/2012	96.147538	
05/04/2012	63.3615	
06/04/2012	89.230577	
07/04/2012	71.791992	
08/04/2012	61.282847	
09/04/2012	81.596962	
10/04/2012	78.345278	

Date	Lateral flow Spore Risk Score	Milioncast Disease Forecast
11/04/2012	85.952152	
12/04/2012	90.73876	
13/04/2012	69.133954	
14/04/2012	95.57885	
15/04/2012	68.010839	
16/04/2012	74.795878	
17/04/2012	75.01337	
18/04/2012	71.439013	
19/04/2012	70.579741	
20/04/2012	57.802974	
21/04/2012	81.564873	
22/04/2012	81.994509	
23/04/2012	103.53692	
24/04/2012	80.653902	
25/04/2012	68.747103	
26/04/2012	68.001925	
27/04/2012	83.336899	
28/04/2012	72.47834	
29/04/2012	92.712233	
30/04/2012	76.681998	
01/05/2012	69.84526	

Date	Lateral flow Spore Risk Score	Milioncast Disease Forecast
02/05/2012	80.791172	
03/05/2012	86.269476	
04/05/2012	82.632724	
05/05/2012	70.48704	
06/05/2012	88.059329	
07/05/2012	83.502692	
08/05/2012	77.544835	
09/05/2012	68.365601	
10/05/2012	37.137662	
11/05/2012	37.697436	
12/05/2012	72.640568	
13/05/2012	68.818412	
14/05/2012	56.920526	
15/05/2012	19.433451	
16/05/2012	77.186508	
17/05/2012	41.676472	
18/05/2012	37.039612	
19/05/2012	76.528684	
20/05/2012	74.093486	
21/05/2012	46.459514	
22/05/2012	22.421293	

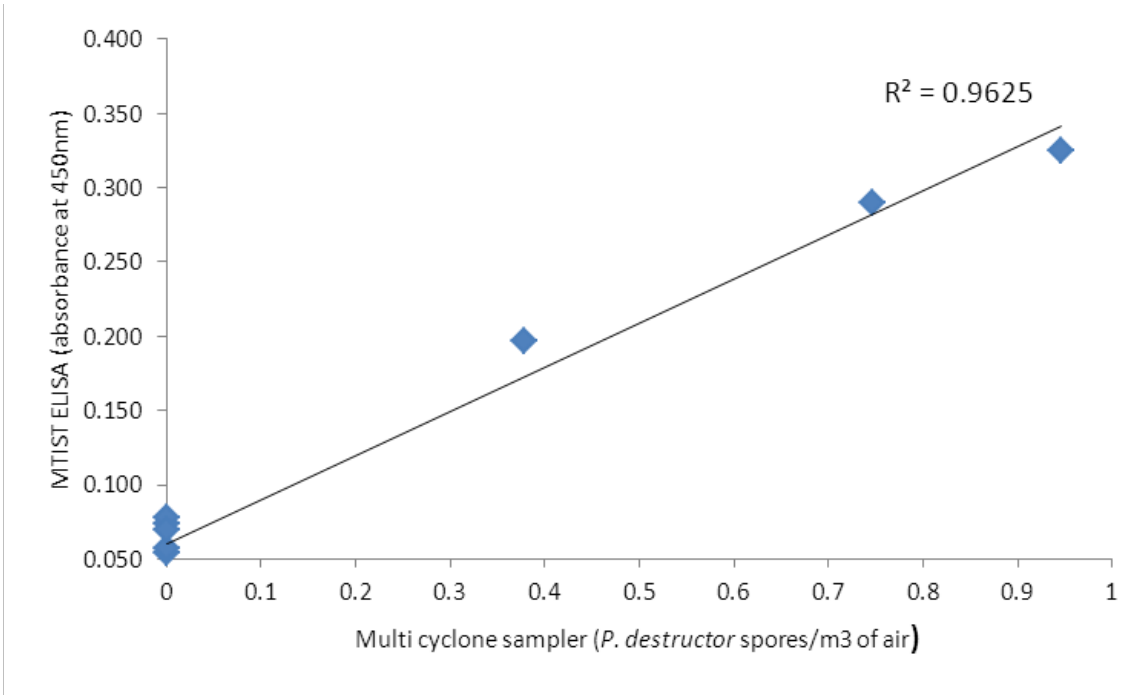


Figure 6. Monitoring weekly field air samples for presence of *P. destructor* disease inoculum using a laboratory assay (MTIST ELISA) and a field based (lateral flow) test.

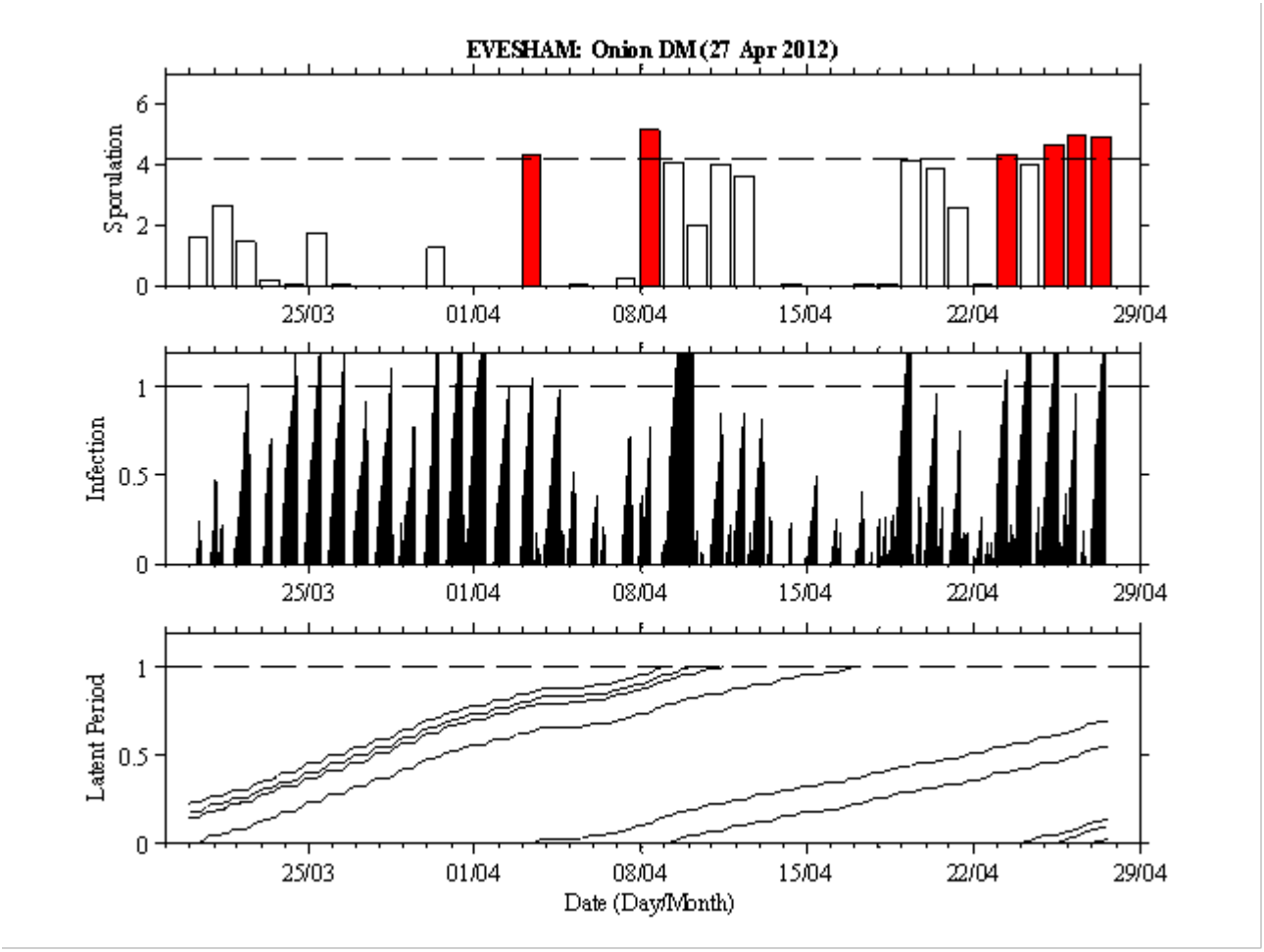


Figure 7a. MILIONCAST environmental model downy mildew disease forecast for the period 20/03/12 to 29/4/2012 at a commercial bulb onion site, Worcestershire, UK

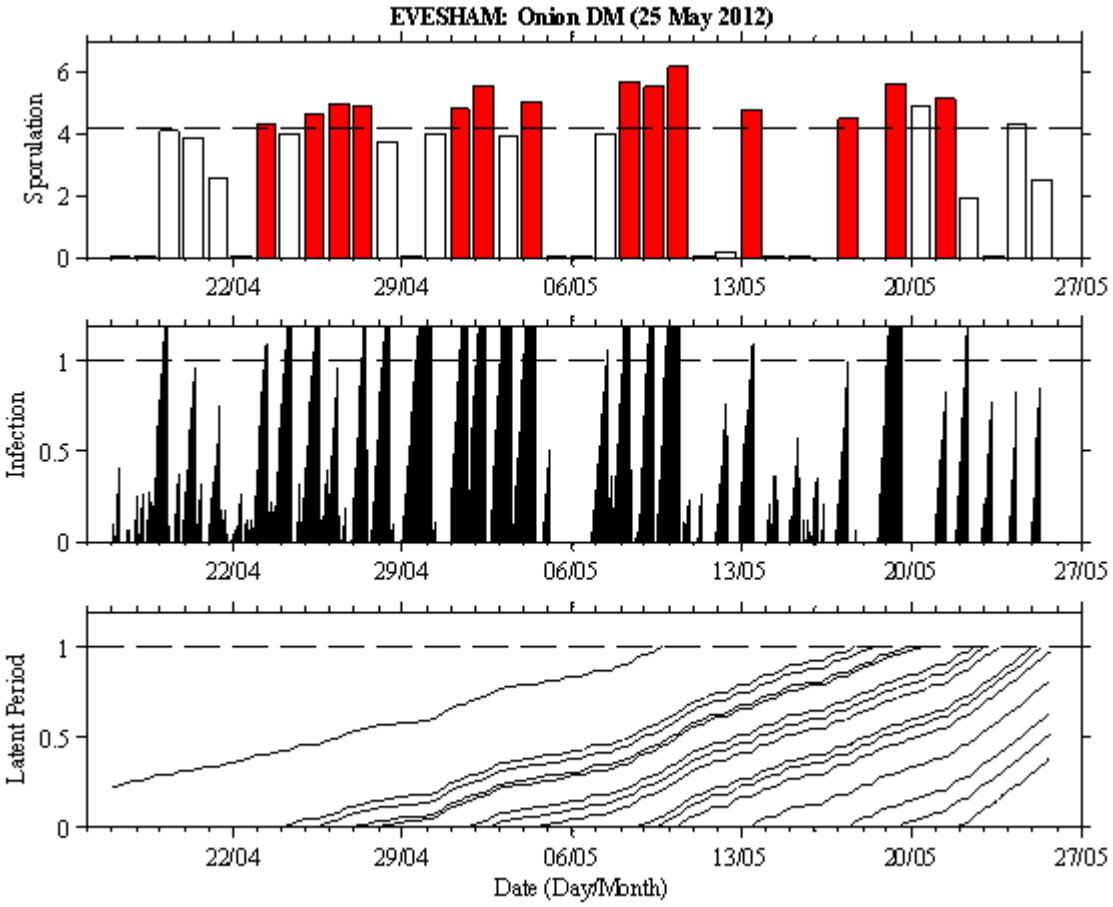


Figure 7b. MILIONCAST environmental model downy mildew disease forecast for the period 17/04/12 to 27/5/2012 at a commercial bulb onion site, Worcestershire, UK

Discussion

The onion downy mildew lateral flow prototype used in Year 3 of the study was observed to operate over a proposed onion downy mildew disease threshold concentration of ≥1000 *Peronospora destructor* spores per daily sampling period. Using a digital reader the test provides a quantitative spore reading of between 100 spores to 100,000 spores.

The shelf life of the lateral flow test was increased to one year when the tests were stored at 4ºC. Storage at room temperature (18 – 20º C) is not recommended for periods of more than one month.

Over winter field trials show that during November 2011 a daily risk forecast (MILIONCAST environmental model) was issued on most days. This determined that fungicide sprays were applied routinely throughout the trial period. No disease was subsequently observed on the crop. However when the air samples for this period were examined using the lateral flow test the potential for disease was identified only in the first two weeks of sampling (3-6th, 13th November). Until the 6th of December no further periods of available inoculum at a concentration required to initiate disease were observed in the crop. Similarly, the results of the MTIST ELISA (weekly laboratory analysis) showed airborne downy mildew inoculum concentration to build over the first two weeks of sampling (3-16th November) and then drop to very low levels until the final week (week commencing 8th December 2011). From these results it would suggest that the number of spray applications could have been reduced to a single treatment to target the period when spore concentrations were identified within the crop canopy and conditions were ascribed for infection by the environmental model (3-16 November, 2011).

Similarly in the first week of monitoring in the commercial Spring salad onion crop (Worcestershire) heavy disease pressure was identified in the air by the two air sampling devices but infection conditions were not forecast by the environmental model until the third week (4/4/2012). Thereafter and, within weekly intervals, onion downy mildew airborne downy mildew inoculum was forecast at medium and high risk by the 'in field' lateral flow test in some of the daily air samples. For each of these weekly periods the environmental model ascribed a level of risk for downy mildew sporulation and infection events. The MTIST ELISA (laboratory test) correlated with the numbers of *P. destructor* spores collected by the infield test air sampler (r^2=0.9625). Utilising information from both the environmental model and the air sampling systems would ascribe risk of downy mildew infection from the third week. Pesticide applications were however made for the whole period.

Conclusions

The findings of this report indicate that the critical date for applying fungicide applications to the crop can be identified by using a daily 'infield' Lateral Flow test in conjunction with an environmental disease forecast system (MILIONCAST). Onion downy mildew disease potential can be identified ahead of visible symptoms. This is a critical point in considerations of disease control since if early applications of fungicide can be targeted to when onion downy mildew spores are present and, at times when the environmental conditions are conducive to infection, the activity of control methods will be enhanced and efficiency savings over the growing season made.

The downy mildew lateral flow test has a shelf life of one year at 4ºC and operates over a spore concentration which can elicit disease symptoms in susceptible crops.

In Year 2 of the study it was reported that the use of weekly inoculum estimates were not useful. However, following studies of the diurnal periodicity of *Peronospora destructor* spore release, it was found in Year 3 that by altering the daily sampling hours of each air sampler, the MTIST weekly tests then correlated with the daily air samples of the 'in field test'. Depending on the situation and / or requirements of the cultivator, weekly or daily tests could be used as an indicator of airborne *P. destructor* spore concentrations. Weekly air sampling would require the positioning of an MTIST air sampler in the crop. Air samples would be collected on a weekly basis and the sample sent to the laboratory by post for processing. Results can be returned to the end user within 24 hours receipt of the sample. Alternatively if daily 'in field' assessment is favoured an eight day multi vial air sampler can be positioned within a crop and operated remotely over the period to provide eight daily air samples. Depending on the daily MILIONCAST environmental disease forecast outputs, the samples can either be tested or discarded. The advantage of the daily test is that the air sample can be processed by the end user i.e. grower, agronomist and informed disease control measures taken immediately. At present a portable ESE digital reader is encouraged for use with the lateral flow test to provide a quantitative result.

Establishing a network of traps (3-4 traps) which could be applied to localities could be used as an early warning system for onion downy mildew. With high sampling rates these traps if positioned to reflect prevailing wind patterns could be used to designate onset of disease risk in different areas and pinpoint specific disease transmission events affecting different crops. When used in conjunction with the environmental model (MILIONCAST), protective applications of fungicide can be precisely timed at different locations. This will help the

onion industry meet any shortfall in fungicide types in the future by improving efficiency of existing approved products.

Knowledge and Technology Transfer

Prof Roy Kennedy gave a presentation titled 'Detection, forecasting and control of vegetable diseases' to the Salad & Herb Growers Technical Update meeting on the 7th Feb-2012 at Pershore College. UK.

Prof Roy Kennedy gave a presentation titled 'Effect of environment on disease of protected crops' at Getting to the Heart of Horticulture on the 13th January 2012. The event was sponsored by the NFU and Wychavon Council

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