
ANNUAL REPORT

To:
Horticultural Development Council
Bradbourne House
Tithe Barn
East Malling
Kent, ME19 6DZ

HDC PROJECT FV 305

BRASSICAS: DETECTION AND QUANTIFICATION OF VIABILITY OF AIR-BORNE SPORES OF *ALBUGO CANDIDA*

R Kennedy

Warwick HRI
Wellesbourne, Warwick, CV35 9EF

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Signed on behalf of: **Warwick HRI**

Signature: *Simon Bright* **Date:** *21 July 2008*

Name: Professor Simon Bright
Director and Head of Department

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GROWER SUMMARY

Headline

The present study has shown white blister spores (zoosporangia) are commonly found in the air around infected vegetable Brassica crops. They occur in high enough levels to play an important role in transmitting the disease between vegetable Brassica crops in the field and diagnostic kits could be developed to detect them.

Background and expected deliverables

Albugo candida (white blister) occurs frequently in vegetable brassica crops and has a widespread distribution covering most vegetable brassica production areas. White blister like many leaf spot pathogens has specific requirements for its development in vegetable brassica crops. The occurrence of favourable environmental conditions can be used to predict infection but often these over estimate the real risk of disease establishment in crops.

Detection tests for conidia of leaf spots in air samples around vegetable Brassica crops have been developed and are a useful tool in determining the risk of disease development. It is unclear if these tests would be useful for white blister since the main infective white blister propagule is the water borne zoospore. White blister produces zoosporangia but it is unclear if these are transmitted at high enough levels in air samples to enable them to be equated to disease risk. The purpose of this study was to demonstrate if zoosporangia can transmit the pathogen over wider areas under field conditions.

The expected deliverables from this project are:

- Detection and sampling systems for white blister inoculum which will provide information on transmission of white blister in the field.
- Assessment of the variation in level of white blister risk from infected white blister crops.

Summary of the project and main conclusions

The main objective of the work in this report was to determine if zoosporangia of white blister are transmitted over wider cropping areas. Determining the role zoosporangia

play in the transmission of white blister in areas of vegetable Brassica production would need to be assessed if the detection of their presence was to be useful within disease management systems. The present study has confirmed that these relatively large spores are present in detectable quantities in air samples. Numbers of zoosporangia present within spore trap catches varied over the duration of the season. However peaks of over 1000 zoosporangia could be detected in the air during trapping periods in the early part of the growing period. The numbers of zoosporangia present in the air appeared to be related to when pustules emerge on the leaves of the crop. Numbers declined during September and October as temperature conditions became unfavourable for further white blister infection in the crop. The change in the maturity of tissues within the crop also contributed to the decline. There appeared to be a poor relationship between wind speed and numbers of zoosporangia in air samples.

Second year work will investigate the viability of transmitted white blister zoosporangia using molecular techniques in relation to infection of Brassica tissues and production of zoospores (the infective propagule). With information on white blister transmission it is possible that rapid tests could be constructed for use in the field to detect zoosporangia of in the air. These tests could be used to rationalise white blister control sprays.

Financial benefit

Using information on the transmission of white blister in vegetable brassica crops the grower will be able to predict more accurately the risk of white blister at the start of the growing season. The information can be used to determine the potential for using "in field" tests for white blister inoculum detection.

- By linking information on white blister inoculum availability with the white blister spray timing models the grower will be able to predict when symptoms will appear.
- White blister disease control in vegetable brassica crops using this can be integrated with existing tests for dark leaf spot and ringspot inoculum.

Action points for growers

There are no specific action points for growers at this time.

SCIENCE SECTION

INTRODUCTION

Biology of *Albugo candida* (white blister) in Brussels sprouts crops

Albugo candida (white blister) is a member of the *Peronosporales* (oomycota) group of fungal like organisms. It is closely related to the potato blight pathogen and the downy mildews. The life cycle of white blister is quite different from that of true fungi. Recent studies have shown that white blister forms a distinct group within the oomycota. *Albugo candida* the causal agent of white blister attacks at least 29 genera of crucifers (Brassicaceae) including major vegetable brassica types, common weeds and native species (Jacobson et al., 1998). The white blister pustules comprise of zoosporangia which erupt under the epidermis of the plant tissue. Pustules are commonly found on any plant organ (leaves, stems, or flowers) however they do appear more frequently on immature tissues. Zoosporangia produce the infective stage of the organism called a zoospore. The zoospore has a flagella which enables it to be motile in water. It is extremely sensitive to drying and for this reason it is only dispersed by water. Infection of seedlings by white blister occurs over a temperature range of 6-24°C although small amounts of infection also occur at 26°C. At optimal temperatures of 16–24°C only 3–4 h of wetness was required for infection to occur. Temperatures above 24°C restrict infection and spore production but these conditions are rarely found under field conditions. Infection at temperatures below 6°C was not observed after 48 h wetness duration. However the effect of longer wetness durations at these temperatures has not investigated. It is common for white blister to form systemic asymptomatic infections that are not visible for long periods of time. The period between infection and symptom appearance may vary for different vegetable brassica types and for different tissue types (mature or immature). This period can be as long as 3–4 weeks at temperatures of 6–10°C.

White blister transmission in vegetable brassica crops

Zoosporangia can germinate directly by producing germ tubes or by releasing 4–12 zoospores. The zoosporangia is relative large (12–18µm in diameter) and its dissemination over larger distances has not been studied. One factor affecting its

dispersal over larger distances is the degree of hypertrophy (distortion) of host tissues. If hypertrophy occurs it forms irregular leaf surfaces creating narrow openings into which air flows can produce considerable wind speeds. This improves the efficiency of dispersal of these spores. However it is not clear what part these spores play in the dissemination of white blister in vegetable brassica crop. The zoosporangia is relatively large and may only be transported over relatively short distances. White blister does not infect oilseed rape so the dissemination of white blister would vary from that of other large spore types. There is no information on the distances that zoosporangia of white blister can be dispersed so the importance of airborne transport by white blister in vegetable brassica crops is unknown.

Water borne dispersal by zoospores of white blister may play a more significant role in epidemic development than zoosporangial dispersal. While zoospores are acknowledged as the main infective propagule infection can also occur directly from zoosporangia. Zoospores are fragile and are killed on drying. However zoospores could be dispersed within wind blown aerosols during periods of rainfall. Zoosporangia release the infective zoospore stage however detecting the poorly airborne zoosporangia is not a guarantee that disease development will occur. The viability of zoosporangia which release zoospores is a critical element in successful disease development within crops. If zoosporangia lose viability they do not produce zoospores and little or no infection by white blister will occur. Detecting the presence of zoospores would be a more accurate estimation of risk. However these are water borne propagules which would be difficult to trap and quantify by growers/end users in the field. Determining the duration of viability by white blister zoosporangia could be one means of determining the potential for zoosporangia to contribute to white blister epidemics. More information on the role played by different spore types in the epidemic development of *Albugo* on vegetable brassicas is therefore required.

Sexual combination by white blister usually occurs in systemically infected tissues. This may also result in distortion of infected host organs. When inflorescences are infected in this way the resulting systems are referred to as a "stagshead". Oogonia and antheridia are formed from the mycelium in the intercellular spaces of the leaf. After the fusion of the antheridium and oogonium the oogonium wall becomes

darker and an oospore is formed. Oospores are larger (30–55µm in diameter) than the zoosporangia however their occurrence and role in epidemics is unclear. White blister infected plant parts can be entirely composed of oospores or there can be both oospores and zoosporangia present within blisters. Weathering and decay of host material release oospores which can germinate directly on new host tissue by a germ tube or by releasing 40–60 zoospores. Each spore type has different criteria for its production and germination. Different germination responses have been reported for different spore types. White blister is very sensitive to temperature change. Spores germinate within the range 10–13°C but require some drying before they can germinate well. Zoosporangia taken from a leaf exposed to sunshine for a few hours are viable but those exposed on a wet leaves fail to produce zoospores. Additionally chilling zoosporangia at 3–5°C improves production of zoospores.

Potential for development of detection tests for white blister

Existing weather based forecasts assume the presence of zoospores is not limiting. Information on environmental factors which affect viability of zoosporangia and release of zoospores is required if successful tests are to be developed for the detection of white blister spores. This information would determine the reliability of airborne spore number as a determinant of disease development. Additionally it would provide more information on when white blister zoosporangia should be trapped and when "in field" tests for white blister should be conducted.

MATERIALS AND METHODS

Plant production for the field and for bait plant usage

Brussels sprouts cv. Golfer were sown in Hassey 308 trays containing a 70:30 Fisons F2 compost (one seedling per cell). Sown trays were placed in a 16/14°C day/night temperature regime. Plants were grown until the 3 true leaf stage at which point they were transplanted into 2 field plots measuring 10m x 10m with a 4 m gap between plots. Transplants had a 50 cm x 50cm planting distance. Plots were treated with 140 Kg N at planting with a 100 Kg N applied as a top dressing after plants had become established.

Collection of white blister field inoculum

At routine intervals plants were inoculated using inoculum collected from a heavily infected seeding field plot of Brussels sprouts cv. Golfer. Infected florets of seedling plants displaying staghead symptoms of white blister were removed and placed in 200 mls of sterile distilled water. After shaking the infected florets were removed from the suspension which contained large numbers of zoosporangia. The suspension was placed at 5°C for approximately 7 hours after which time it was checked for the presence of motile zoospores (the infective stage of white blister). The concentration of inoculum was measured using a haemocytometer.

Plant inoculation

Brussels sprout plants cv. Golfer were inoculated with a white blister zoospore suspension. Plants were sprayed with a 0.05% aqueous suspension of Tween 20 prior to inoculation. At each inoculation time approximately 6–8 plants (at the 6–8 true leaf stage) were inoculated with 70mls of a white blister zoospore suspension. Plants were then placed in a misting chamber for 48 H before being placed outside under field conditions. Plants were routinely monitored and disease symptoms recorded when observed. Infected plants were used to infect field plots of Brussels sprouts. Plots were inoculated by placing 5 infected seedlings in the centre of the field plot.

Monitoring airborne inoculum of white blister the in an inoculated overwintered Brassica crop

An over-wintered, heavily infected (dark leaf spot, ringspot and white blister) field plot (20m x 10m) of Brussels sprouts (c.v. Golfer), was monitored continuously over a

period of 3 month for the presence of dark leaf spot and ringspot spores in air samples. Air samples were taken using a Burkard 24 H volumetric sampler and a microtitre immuno-spore trap (MTIST). A daily sample of micro-organisms in the air was collected on glass slides coated with silicone in the volumetric spore trap. The slide was replaced in the trap daily with an unused fresh slide. Prior to field exposure the microstrips for the MTIST trap were stored at 4°C in a sealed container. Air flow through the MTIST sampler was estimated in still air by measuring the air speed at different points across the inlet manifold using a hot film anemometer (air velocity transducer model number 8460, TSI Incorporated, St Paul, MN, USA) and integrating over the area of the inlet. In the tests reported here, the volume flow rate through the device was measured at 57-litre min⁻¹. The MTIST sampler and the volumetric sampler was operated daily for 12 H periods (06:00H – 18:00H) as previous studies had shown that spores of white blister were present in air samples only during daylight hours. For each of the sampling periods twelve *B. oleracea* bait plants (Brussel sprouts cv. Golfer, 10 true leaves), which had been grown in the absence of disease, were positioned adjacent to the spore traps. Further bait plants were positioned 500 m to the south and east of the plot. Plants exposed to the east of the white blister infected plot were positioned adjacent to a Brussels sprout crop which was heavily infected with ringspot. The plot had been given sprays of metalaxyl after transplanting and during its development to prevent infection by the white blister pathogen. After each exposure period, the plants were removed from the field and retained in a glasshouse, at a temperature of 12-14°C for 21 days. Plants were visually examined for expression of white blister.

Detection of ringspot in air samples using ELISA

Field exposed microtitre strips were blocked with 200µl of 1% Casein buffer (1% (w/v) casein PBS) and incubated at 37°C for 45 min. Residual blocking buffer was removed and wells were washed four times for one min each with 200µl PBS, 0.05 % Tween 20 and 0.1% Casein (PBSTw C). Wells 1-4 of each strip then received 100 µl of monoclonal Ab EMA 187 (raised at Warwick HRI to *M. brassicicola*), with the remaining wells of 5-8 each receiving 100µl of PBS, 0.05% Tween 20 and 0.1% Casein. Following incubation in a Wellwarm shaker incubater (30°C) for a period of 45 mins as above, wells were washed three times for one min each with 200µl PBSTincTw. A DAKO duet amplification system was used (DAKO Ltd, Angel Drive, Ely, Cambridge,

UK; Cat no. K0492) to amplify the signal generated by bound tissue culture supernatant antibodies. Wells were washed as described above and 100µl of 3,3',5,5'- tetramethylbenzidine substrate (Sigma, Poole, Dorset, UK; Cat. No. T-3405 and P-4922) was then added to each well. The reaction was stopped by adding 25µl of a 20% 1M H₂SO₄ solution to each well. Absorbance at 450nm was determined with a Biohit BP800 ELISA plate reader (Alpha Laboratories, 40 Parham Drive, Eastleigh, Hampshire, UK).

Micro-climate measurements

Measurements of temperature, humidity, leaf surface wetness and rainfall were collected at 30 min intervals from when the logger was sited in the vegetable brassica crop using a SKYE Datahog II 7 channel logger. Measurements were collected by GSM portable phone Link (Skye Instruments Ltd, Llandrindod Wells, Powys). The logger was powered by a 12 V battery. Environmental data, was collected within MORPH and summarised within BRASSICA_{spot}. Numbers of trapped conidia in the air could be directly compared with corresponding environmental conditions.

Disease assessment on trap plants

Plants were incubated in a glasshouse after field exposure. The number of white blister lesions on each leaf of each trap plant was recorded after 21–28 days incubation in a glasshouse with a 16/14°C day/night temperature regime. The number of lesions on leaves of trap plants were assessed at each trap plant site at the same time. Numbers of ringspot and dark leaf spot lesions on trap plants was also recorded.

Visual microscopic counts of white blister zoosporangia from air samples

Air samples collected using the MTIST air sampler and the volumetric spore trap were checked for the presence or absence of white blister zoosporangia. Microtitre well strips for each sample day were examined visually by using a microscope. The microtitre strip was inverted on the microscope stage and counted directly on the base of each well. Estimates of the numbers of white blister zoosporangia were taken by counting the number of white blister zoosporangia in each well for each

day. The total number of white blister zoosporangia per sample date was expressed, as the volume of air sampled on each day was constant.

RESULTS

White blister and ringspot infection on bait plants exposed within the over-wintered Brussels sprout crop infected with white blister at Warwick HRI in 2007

Due to unusual growing conditions during 2007 crop establishment and infection by ringspot and white blister was delayed. Bait plants could therefore only begin to be exposed within plots where there was an epidemic of either ringspot or white blister at the beginning of October 2008. The number of white blister lesions on bait plants which were exposed daily during the period 9 October 2007 to 8 November 2007 is shown in Figure 1. Only significant numbers of lesions were recorded on bait plants exposed approximately 5 m from the white blister infected plot. Plants were first exposed during October 2007 as at this time sufficient white blister infection had occurred in the plot. The highest numbers of white blister lesions were observed on bait plants exposed on the 16 and 17 October 2008. A smaller peak in numbers of white blister lesions occurred on bait plants exposed on the 31 October 2007. There were very low numbers of white blister lesions recorded on bait plants exposed approximately 500 m to the south and east of the infected plot. Only bait plants exposed to the east of the white blister plot showed infection on the 9 and 17 October and the 5 November 2008. It is likely that weather events during these periods supported longer range transmission events.

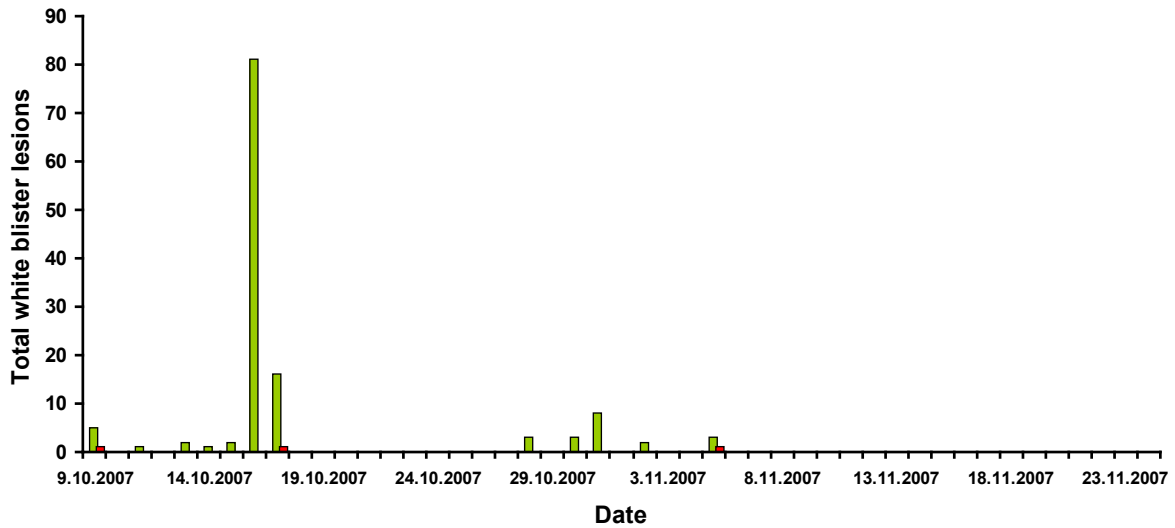


Figure 1. Mean total number of white blister lesions on bait plants exposed 5 m (■) 500 m east (■) and 500m south (■) of a white blister infected field plot of Brussels sprouts (cv.Golfer).

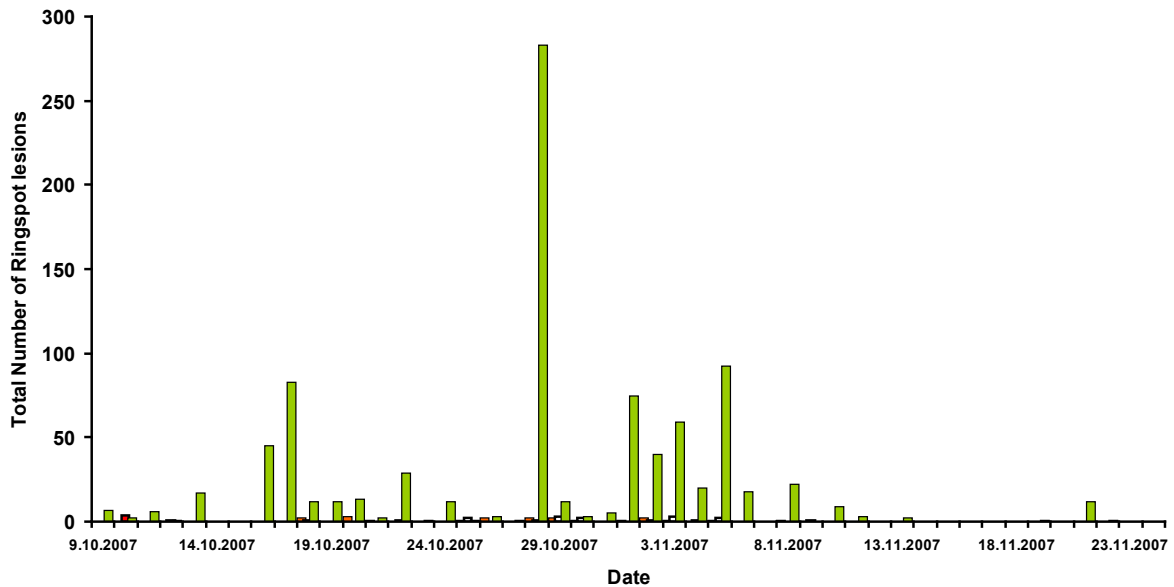


Figure 2. Mean total number of ringspot lesions on bait plants exposed 5 m (■) 500 m west (■) and 500m south (■) of a ringspot infected field plot of Brussels sprouts (cv.Golfer).

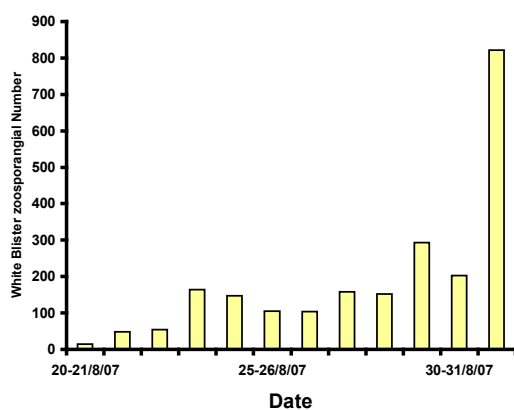
The pattern of ringspot infection on bait plants differed to that observed for white blister (Figure 2). Peaks of ringspot infection were observed on bait plants on the 16, 17 and 28 October 2008 and on the 1, 2, 3 and 5 November 2008. High numbers of

ringspot lesions were observed on bait plants in comparison to white blister throughout the trial. Negligible numbers of ringspot lesions were observed on bait plants positioned close to the white blister infected field plot.

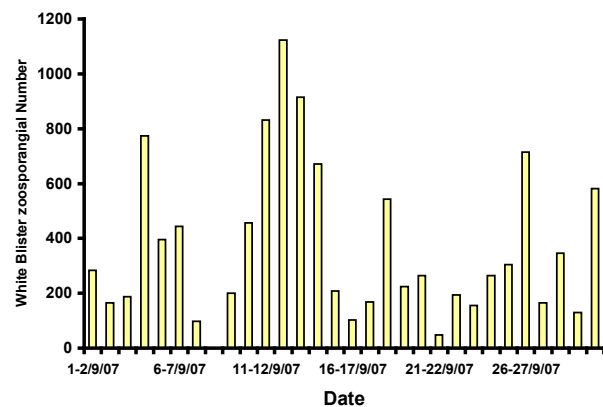
White blister microscope counts (24 H volumetric trap)

Inoculum availability from a heavily infected Brussels sprout crop at Warwick HRI was measured using a volumetric spore trap. Zoosporangia of white blister were trapped on to glass slides coated with silicone. The total numbers of zoosporangia were counted using a microscope. Daily zoosporangial counts are shown in Figure 3 over the period August 2007 to November 2007. Due to late crop establishment and growth white blister infection within the field plot did not occur until August 2007 at which point trapping studies commenced. Small numbers of zoosporangia were observed on slides during early August 2007 (Figure 3a). High numbers (approximately 800) of zoosporangia were observed at the end of August 2007. There was an increase in zoosporangial numbers trapped in air samples during September 2007 (Figure 3b). The highest numbers of white blister zoosporangia were recorded in air samples from the 12 to the 13 September 2007. Peaks in zoosporangial numbers were also observed during the 1-2 and the 4-9 October 2007 (Figure 3c). During much of October 2007 lower levels of zoosporangia were trapped in daily air samples collected from the 24 H volumetric trap. There were lower numbers of zoosporangia in air samples collected during November 2007 although peak numbers of approximately 200 were observed on the 15-16 and the 29-30 November 2007 (Figure 3d).

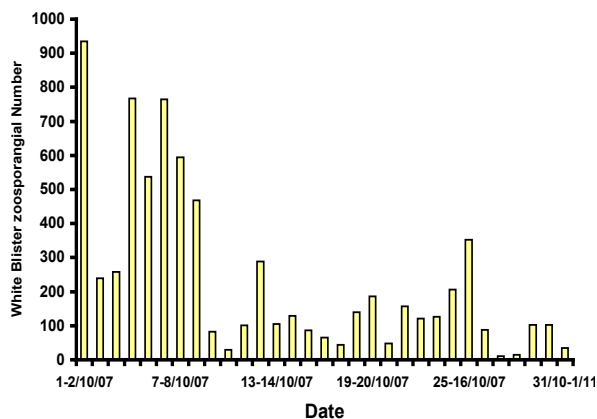
(a)



(b)



(c)



(d)

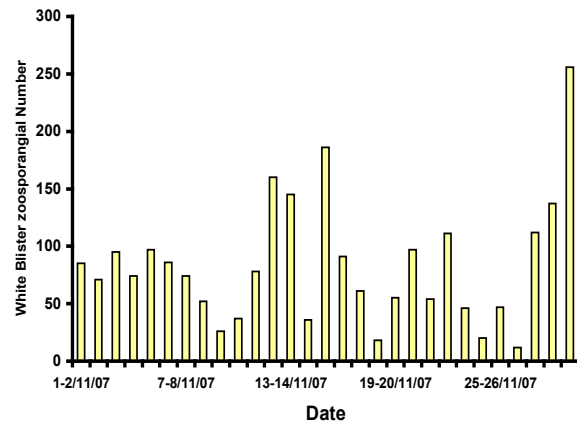
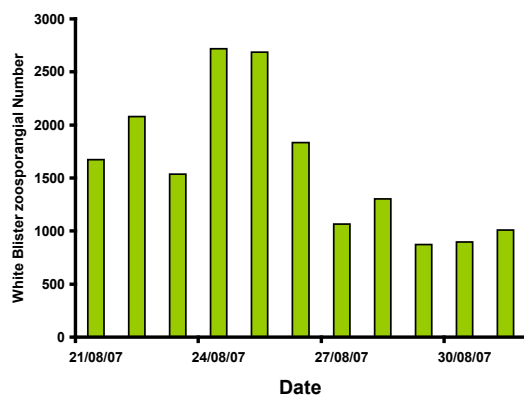


Figure 3. Total daily white blister zoosporangial counts sampled using volumetric air sampler during (a) August (b) September (c) October and (d) November 2007 within a white blister infected field plot at Warwick HRI.

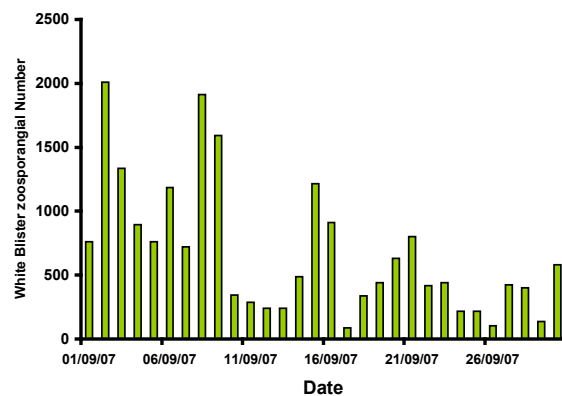
White blister counts taken from air sampled using an MTIST sampler

Inoculum availability from a heavily infected Brussels sprout crop at Warwick HRI was measured using an MTIST high volume sampler. Zoospores of white blister were trapped on to microtitre wells coated with silicone. The total numbers of zoospores were counted using a microscope. Daily zoosporangial counts are shown in Figure 4 over the period August 2007 to November 2007. There were some differences in the numbers of white blister zoospores observed in air samples from the MTIST air sampler and the 24 H volumetric trap.

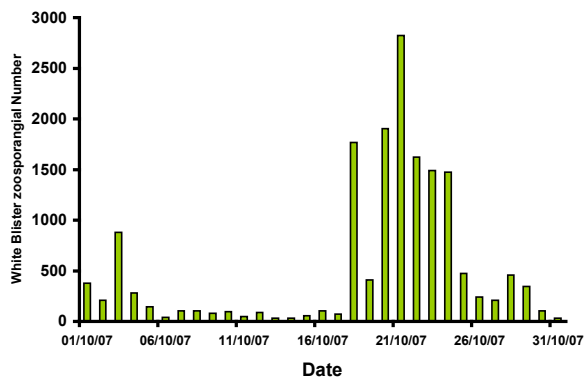
(a)



(b)



c)



(d)

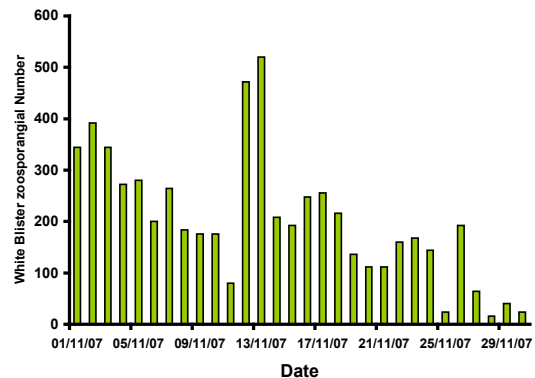


Figure 4. Total daily white blister zoosporangial counts sampled using MTIST high volume air sampler during (a) August (b) September (c) October and (d) November 2007 within a white blister infected field plot at Warwick HRI.

The MTIST air sampler was operating at a higher air sampling volume however the peaks in white blister zoosporangial numbers did not correspond between the two types of traps. Peaks in zoosporangia numbers in air samples from the MTIST trap occurred after 24 August 2007. 2368 Peaks in zoosporangial numbers occurred in samples from the MTIST trap on the 2, 3, 6 and 8 September 2008. There were lower numbers trapped after the 10 September 2007 (with the exception of the 15 September 2007). Low numbers of white blister zoosporangia were observed in air samples from the MTIST trap from the 1 – 17 October 2007. However peak numbers of zoosporangia occurred in air samples in the MTIST trap from the 18–24 October 2007. A similar pattern in zoosporangial numbers was observed in air samples from the volumetric and MTIST trap during November 2007. Peak numbers of approximately 500 zoosporangia were trapped in the MTIST sampler during the 12 and 13 November 2007.

Brassicaspot output for white blister at Warwick HRI 2007

The Brassica_{spot} output at the Warwick HRI field site is shown in Figure 5. Infection conditions conducive to plant infection were recorded during the whole season. However infection within field plots was not observed until August 2007. High risk infection periods were recorded continuously at the beginning and end of August 2007 and during mid October and at the end of October 2007. High levels of white

blister infection were observed within infected field plots. High risk infection conditions were also continuously recorded at the end of December 2007.

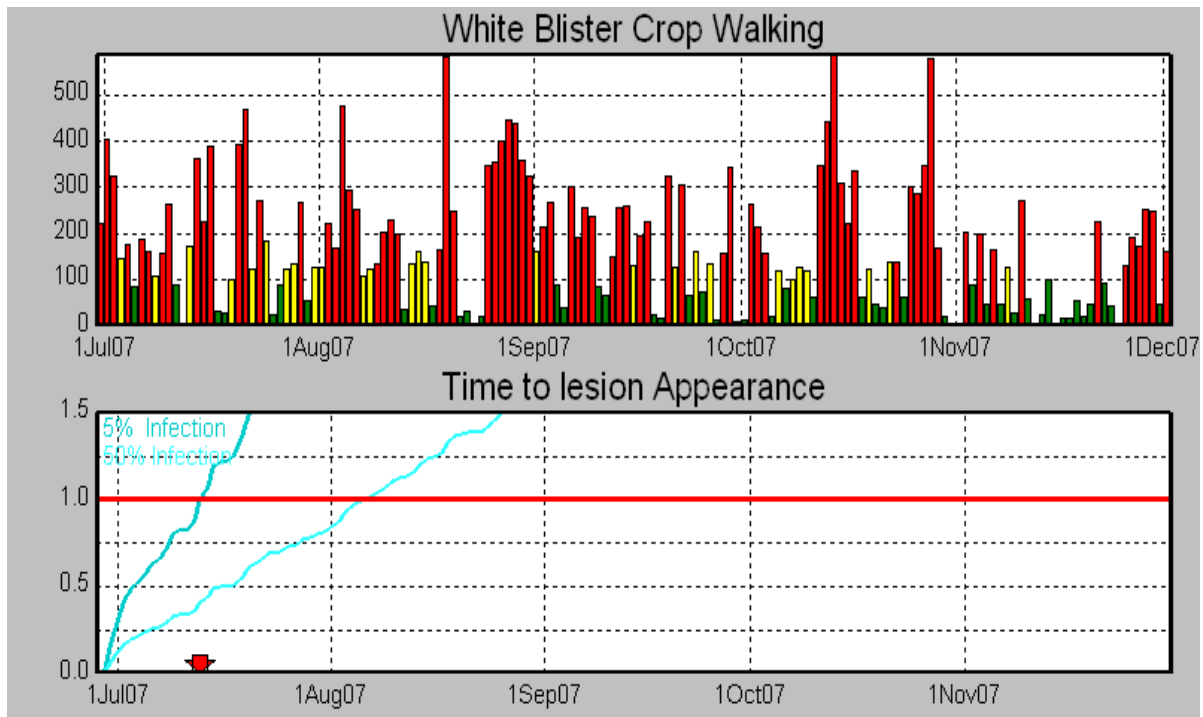


Figure 5. Environmental risk of white blister infection using the white blister II infection model in a white blister infected field plot at Warwick HRI.

Diurnal periodicity of white blister in air samples

The diurnal periodicity of white blister zoospores in air samples is shown in Figure 6. The ten days with the highest numbers of zoospores in air samples were plotted on an hourly scale to determine if zoospore numbers in the air occurred at specific times during the day. The results indicated that there was no pattern in the occurrence zoospores over 24 H periods.

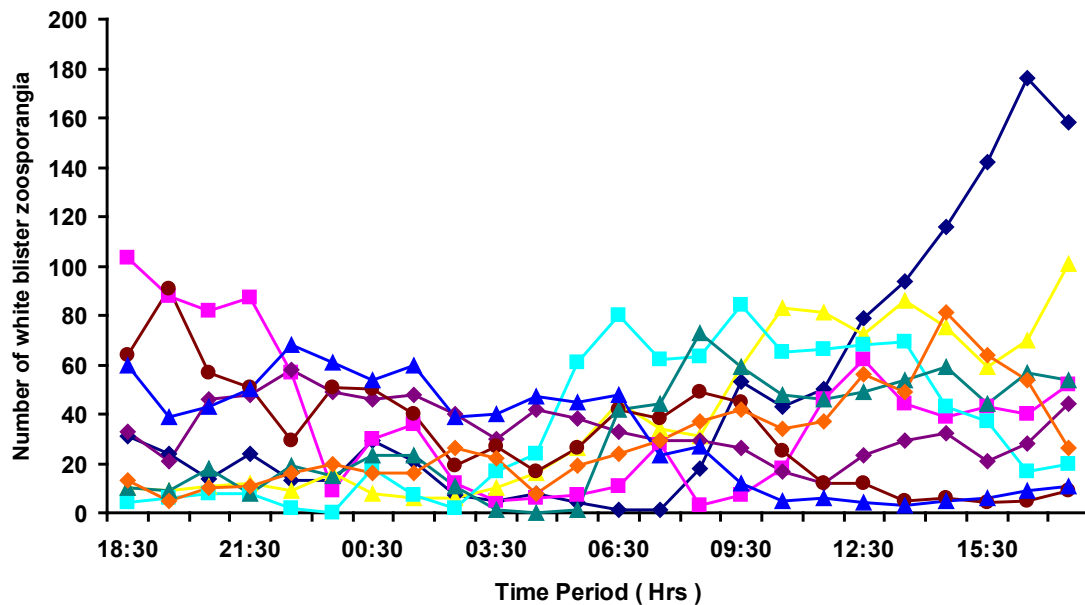


Figure 6. Hourly white blister zoosporangial counts using a 24 H volumetric air sampler during 10 days with zoosporangial counts above 1000 per day at Warwick HRI in 2007.

There appeared to be a relationship between maximum windspeed recorded and the numbers of white blister zoosporangia in air samples. However the numbers of data sets available was not enough to facilitate a full analysis. Windspeeds of above 5 Km for 5 secs gave elevated levels of white blister zoosporangia in air samples (Figure 7). However there was zoosporangia in air samples at low windspeeds with some zoosporangia in the air when there was no wind measurements. Most zoosporangia occurred in the air during the day time.

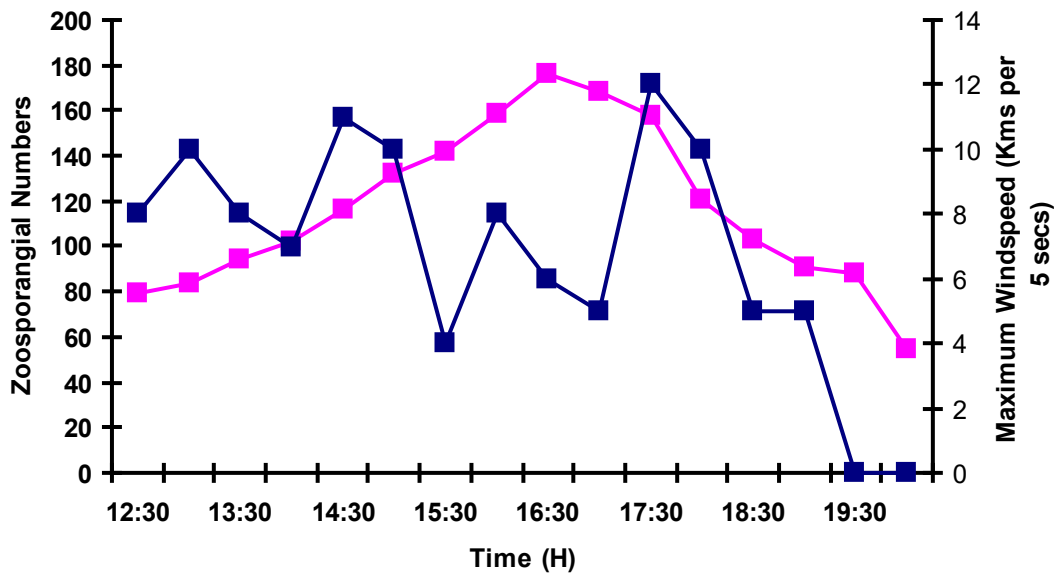


Figure 7. Mean number of zoosporangia in air samples (■) and maximum windspeed (5 secs) (■) in air samples from an white blister infected plot of Brussels sprouts (cv.Golfer) at Warwick HRI.

DISCUSSION

Disease forecasting criteria for white blister in vegetable brassica crops

Fungicides application is the only option for controlling diseases in vegetable Brassica crops at present. Information on the optimal timing of fungicide applications is therefore important if good control of fungal pathogens is to be achieved. The addition of disease development forecasts for white blister to the disease forecasting system (Brassicapot) within MORPH for vegetable Brassica crops has improved the forecasting of white blister within vegetable Brassica crops. However further development to include the effect of spatial aspects of disease transmission are required. This would help reduce the time required to manage and control disease in the field.

White blister control in the past has depended on the application of fungicides containing metalaxyl. This chemical is combined with chlorothalonil (Folio Gold). However other chemicals are available which are more protective in action such as azoxystrobin (Amistar). Use of the white blister forecasting models has enabled more reliance on protectant fungicides such as Amistar. The white blister disease

development model enables protectant fungicides to be applied just before white blister symptoms appear in the crop reducing any further spread of disease in the crop. Fungicides containing metalaxyl are the most expensive fungicidal product applied to vegetable brassica crops. Reducing the usage of this chemical will help reduce the costs of vegetable brassica production. Metalaxyl can be applied only when disease pressure is high however by using protectants effectively it is clear that white blister can be reduced to low levels in the crop removing the need for applications of metalaxyl. The white blister infection model can also be used to determine when the crop can be checked for signs of disease.

Both models (white blister infection and white blister disease development) have been programmed within the Brassicaspot (MORPH based) system which means that both pieces of information are displayed concurrently. However one of the objectives of the work in this report was to determine if zoospores of white blister are transmitted over wide areas. If zoospores are observed to be transmitted over larger distances their presence could be used as a means of determining when white blister initially occurs in vegetable Brassica crops. The information can also be used as an effective management tool for technical managers who have big areas of crop to manage and limited time constraints.

Rapid tests for white blister inoculum

With information on white blister detection and transmission rapid tests could be constructed for use in the field to potentially detect zoospores of white blister in the air. By using techniques outlined in FV233 and FV233a, early detection of white blister in air samples could be made possible. The lateral flow device would however need to be tested with portable air samplers in the field to determine the optimal trapping format for white blister zoospores. Trapping formats for zoospores would also need to be integrated with numbers of zoospores found above infected crops in the absence of white blister on plants.

Symptoms of white blister, within crops are difficult to detect at low levels especially in large cropping areas. Additionally the symptomology of the disease on young plants is poorly understood and the latent period varies under different temperatures. For example at continuous temperatures of 5°C symptoms of white blister will not become

visible. By detecting the presence of white blister zoosporangia, it would be possible to determine action thresholds for vegetable Brassica crops at different stages in their development. The lateral flow device if used to detect zoosporangia of white blister in the field would require construction and validation.

Detecting white blister zoosporangia would be particularly useful early in the season as a method of preventing disease transfer between vegetable Brassica crops grown at different stages in the season. The use of weekly estimates of inoculum in air samples has also been reported (Kennedy et al., 2006) for other diseases, notably *Pyrenopeziza brassicae* (light leaf spot of horticultural and arable brassicas). Tests which can be conducted in the field are necessary if information on air-borne inoculum concentration is to be of more practical value. Results of the trials reported in this report demonstrate that zoosporangia of white blister could be used as a means of determining when the first application of fungicides is required against this disease. Potential exists for linking these estimates of white blister inoculum to mathematical models describing the environmental factors which affect white blister development. Use of this approach might improve the efficiency of inoculum detection systems, disease forecasts and ultimately improve disease control. However this would require investigation in future work.

There are also criteria within the white blister II model which have not been tested which could be linked to estimates of white blister zoosporangia in the air. This refers primarily to the expected time to 50% plant infection. It is possible that, this criteria, if accurate, might have some usage for differentiating white blister infection events or the likelihood of epidemics becoming established in crops at different levels of available white blister inoculum.

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