Project title:	Brassicas: Detection and quantification of viability of air-borne spores of *Albugo candida*
Project number:	FV 305
Project leader:	Dr Roy Kennedy
Year of project:	Final
Date of report:	30 April 2009
Locations:	Warwick HRI, University of Warwick, Wellesbourne
Coordinator:	Fred Tyler
Project commenced:	01 April 2007
Completion:	30 April 2009
Key words:	White blister, transmission, spore viability

Signed on behalf of: **Warwick HRI**

Simon Bright ***8 June 2009***

Signature: …… Date: ……

Name: Professor Simon Bright
Director and Head of Department

CONTENTS **Page**

FV 305: BRASSICAS: DETECTION AND QUANTIFICATION OF VIABILITY OF AIR-BORNE SPORES OF *ALBUGO CANDIDA* (white blister)

GROWER SUMMARY

Headline

- The present study has shown that white blister spores (zoosporangia) are commonly found in the air around infected vegetable Brassica crops. White blister was transmitted over a distance of 0.5 Km. White blister spores survive in the crop under prevailing temperature conditions for at least 28 days.

Background and expected deliverables

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. *Albugo candida* (white blister) occurs frequently in vegetable Brassica crops and has a widespread distribution covering most vegetable Brassica production areas. Detection tests for conidia of dark leaf spot and ringspot 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. Any test would have to differentiate between white blister spores from vegetable Brassicas and white blister which occurs on Shepherd's purse, as these are two distinct species of white blister. 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.
- Detection tests for determining seedborne infection by white blister
- Detection tests for discriminating white blister which occurs on Shepherds purse from that which occurs on vegetable Brassicas.

- 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.

Detecting white blister spores in the field is complicated by the fact that another type of white blister occurs on the weed species Shepherd's purse which is common in Brassica fields and margins. Spores produced by white blister from Shepherd's purse cannot infect vegetable Brassicas and only infect Shepherd's purse. A means of discriminating between these two types of white blister would be essential; if an accurate 'in field' detection system is to be developed. Counting all the spores that look like white blister spores would over-estimate the disease pressure on the crop. Fields with a larger weed problem with white blister infection would lead to potential error and over estimation of the vegetable Brassica white blister risk. However researchers at the Department of Primary Industries in Australia have developed 'genetic fingerprint' methods that can tell the difference between spores from the weed and from the crop. The genetic analysis shows the presence of positive reactions when vegetable Brassica white blister DNA is present. No reactions are recorded when white blister spores from shepherd's purse were tested.

It is unlikely that an antibody-based test that can distinguish between white blister spores from shepherd's purse and those that can infect Brassica crops can be developed. However lateral flow tests can be constructed which will detect the products of molecular reactions. This is a new and rapidly developing field within lateral flow detection systems. In the future lateral-flow

'pregnancy test' type devices could be used to differentiate between pathogenic strains of the same organism. It would be possible to use this technology to develop lateral flow tests which only detect white blister on vegetable Brassicas. The basic molecular tests which can be used to do this have been tested as part of the work reported in this project.

Financial benefit

Using information on detection tests and 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. Detection tests for seedborne infection by white blister could also be used to reduce white blister carryover.

- 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.
- Detection of seedborne infection would also help reduce the costs of production.

Action points for growers

- Transmission of white blister was shown over distances of 0.5 km in the field.
- Seed testing for white blister could be used to reduce initial white blister infection on crops using the methods outlined in the full report.
- Improved control of white blister may be possible by treating propagation plants against white blister infection.

SCIENCE SECTION

INTRODUCTION

Host specialisation of white blister races

Albugo candida (white blister) alternatively called white rust is found worldwide on vegetable Brassica plants. 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. White blister destroys the floral parts of Brassicas and often becomes systemic in the crown region of horseradish roots or in other root parts. 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). Originally Pound and Williams (1963) described six physiological races however other studies report up to 10 races (Liu *et al*., 1996). To date up to 17 races of white blister have been reported however only 5 are reported as pathogenic on vegetable crops. Race 1 infects the leaves and flowering parts of radish but does not affect the radish crop. Race 3 has been reported on horseradish. Races 4 and 5 occur only on cruciferous weed species and do not infect vegetable Brassicas. Race 6 has been reported on watercress. Chinese cabbage, Pak choi and turnip are infected by race 7 while vegetable Brassicas (broccoli, cauliflower, Brussels sprouts, and cabbage) are infected by race 9 only (Hill *et al*., 1988). The occurrence of white blister on cruciferous weed species found commonly in vegetable Brassica crops is potentially problematical given that the spores from this race are morphologically similar to those which infect vegetable Brassicas.

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

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 6-24ºC although small amounts of infection also occur at 26ºC. At optimal temperatures of 16–24ºC only 3–4 h of wetness was required for infection to occur. Temperatures above 24ºC restrict infection and spore production but these conditions are rarely found under field conditions. Infection at temperatures below 6ºC 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 6–10ºC.

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 (30–55um 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 10–13ºC 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 3-5ºC improves production of zoospores.

Development of detection tests for white blister

Existing weather based forecasts assume the presence of zoospores is not limiting. However this is unlikely as the zoosporangia is a large spore form which has limited ability to be spread by wind or other types of transmission. Additionally 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. One problem is the occurrence of white blister on cruciferous weeds. However the race of white blister affecting cruciferous weeds does not infect vegetable Brassicas. Differentiation of zoosporangia and zoospores from these two races would be required if reliable disease risk based information relation to white blister in crops was to be developed. It is possible that both these types of white blister have different requirements for infection and development in on their respective hosts. By gathering information on white blister zoosporangial viability the reliability of airborne spore number as a determinant of disease development could be assessed. 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. The potential accuracy of these tests in the presence or absence of white blister infecting cruciferous weed species would also need to be determined.

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 16/14ºC 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 5ºC 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 in 2007 and 2008. Air samples were taken using a Burkard 24 h volumetric sampler and a microtitre immuno-spore trap (MTIST) in each year. 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 4ºC 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 in 2007. 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 12-14ºC 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 37ºC 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 (30ºC) 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'- tetramethylbenzidene 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 H2S04 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 in 2007 and 2008. 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 16/14ºC 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.

Molecular methods for the detection of white blister zoosporangial viability

DNA extracted leaf and zoosporangial material of white blister using Qiagen Plant Mini Kit. A final volume 50µl was produced using the kit with a 1:100 dilution used in the PCR reaction.

Differentiation of white blister zoosporangia from cruciferous weeds and vegetable Brassicas

White blister symptoms on infected leaves of Shepherd's purse were collected from the field and used in DNA extractions as detailed above. DNA extracted from leaves of vegetable Brassicas was used for comparison using the methods described previously. Standardised amounts of diluted DNA (5µl) from both samples were used in tests. Molecular primers

supplied by the Department of Primary Industries were used in the PCR reaction. PCR cycle times were set at 1 min at 95°C before 0.5 min at 95°C followed by a 40 repeat cycle of 0.5 min at 60°C and 1 min at 72°C then a final 10 min at 72°C before the product was held overnight at approximately 18°C. Results were visualised on a 1% Agarose gel containing 8µl gel red. The gel was run at 150 V for 2 h before viewing and photographing under UV light.

Measurement of zoosporangial viability

White blister zoosporangial viability was measured by placing 0.005 g samples of white blister zoosporangia on true leaves of Brussels sprouts seedlings (cv. Revenge) at the 4 true leaf stage. The dry zoosporangia were applied as a coating to the leaves using a camel hair brush. All true leaves were used for each plant and 4 replicated plants were used in the experiment for each environment. Plants were placed under controlled temperature conditions at 5, 10, 15 and 20°C under dry conditions of continuous 90% rh. At each sampling time one replicate leaf from each plant was removed at each temperature and used for DNA extraction as described previously. Samples were analysed using PCR for the presence or absence and amount of white blister DNA present.

Measurement of zoosporangial inoculation concentration on white blister lesion number

Zoosporangial concentrations of white blister were produced by placing collected zoosporangia in a known quantity of sterile distilled water. The concentration of the zoosporangial suspension was checked using a haemocytometer and subsequent dilutions were produced by placing the required amounts of the suspension in sterile distilled water to produce the required concentrations for testing. The test concentrations containing zoosporangia of white blister were sprayed directly on to seedlings (at the third true leaf stage) of Brussels sprouts (cv. Revenge) grown as described previously. Approximately 5 mls of zoosporangial suspension was applied per plant. Plants were transferred to a 100% r.h. environment in a glasshouse at 14°C for approximately 24 h before drying. Plants were maintained moisture free on the foliage in the same glasshouse until symptom appearance. White blister lesions were recorded on two separate occasions on plants with a 14 day interval between assessments.

RESULTS (Year one)

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 2007. 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.

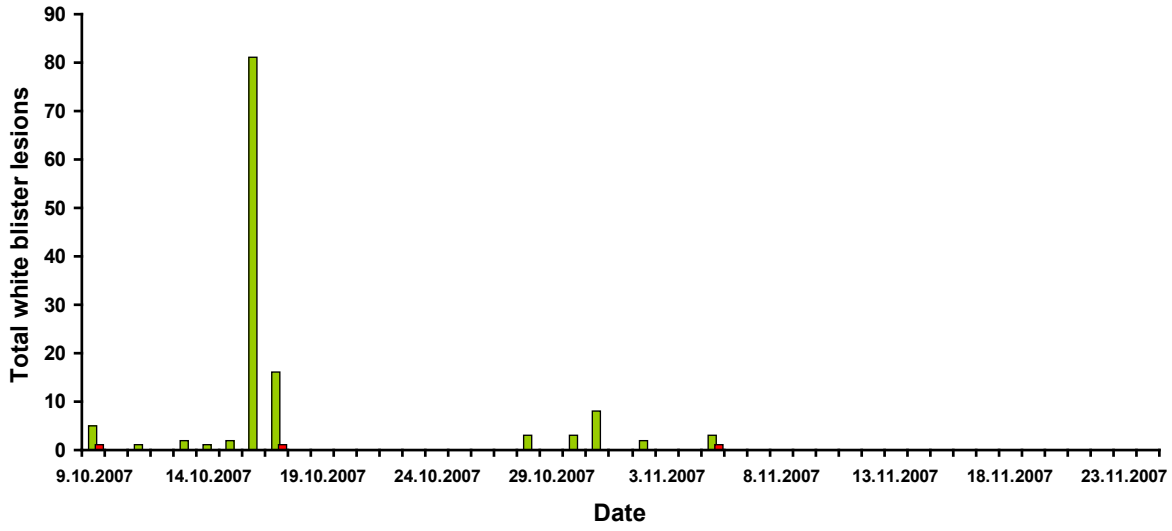

Figure 1. Mean total number of white blister lesions on bait plants exposed 5 m (■) 500 m east (■) and 500m south (■) of a white blister infected field plot of Brussels sprouts (cv.Golfer)

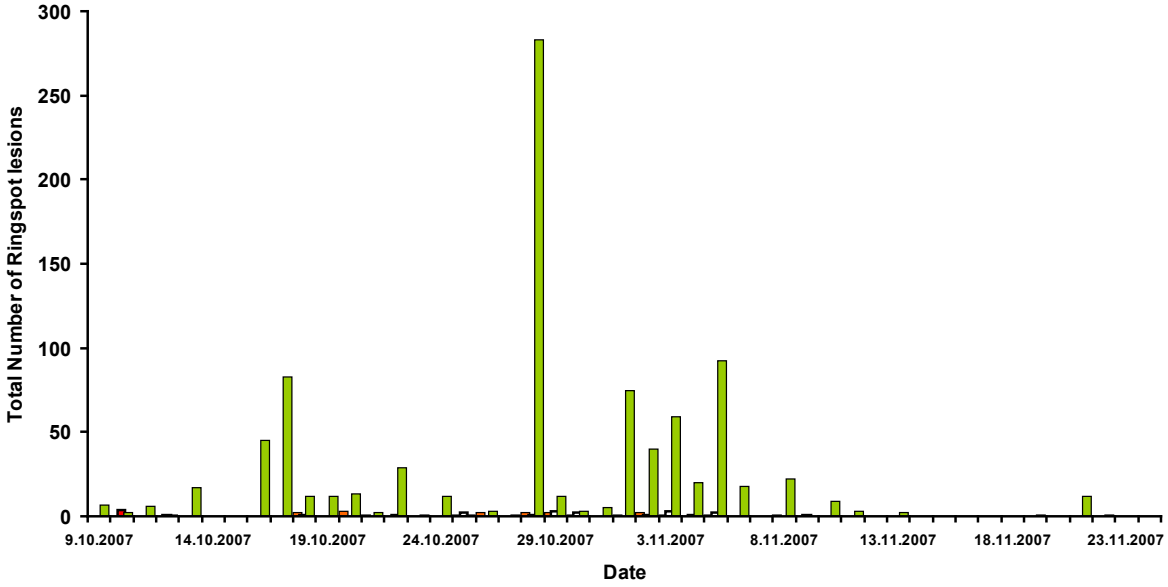

Figure 2. Mean total number of ringspot lesions on bait plants exposed 5 m (■) 500 m west (■) and 500m south (■) of a ringspot infected field plot of Brussels sprouts (cv.Golfer)

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).

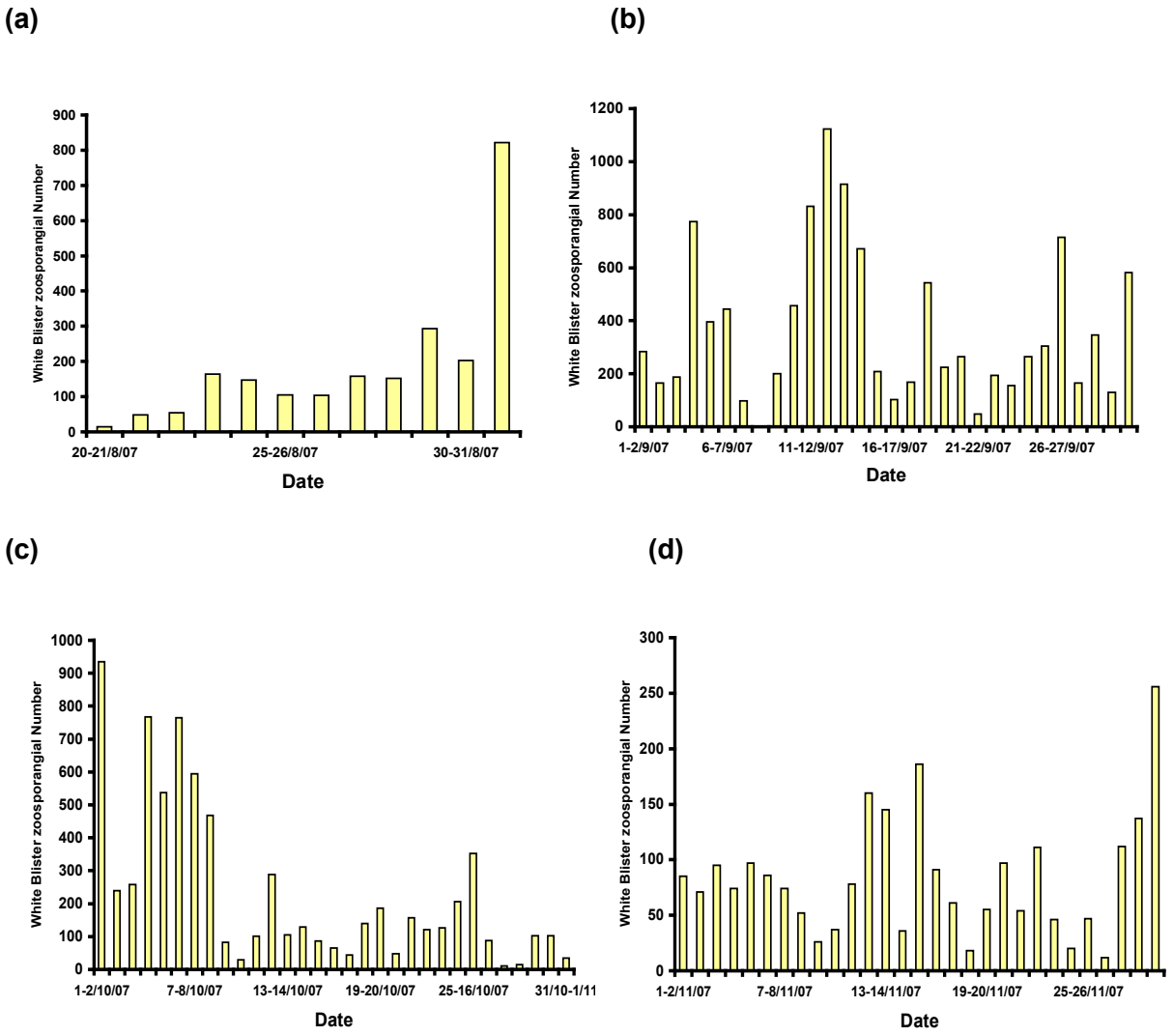

Figure 3. Total daily white blister zoosporangial counts sampled using volumetric air sampler during (a) August (b) September (c) October and (d) November 2007 within a white blister infected field plot at Warwick HRI

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. Zoosporangia of white blister were trapped on to microtitre wells coated with silicone. The total numbers of zoosporangia 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

zoosporangia observed in air samples from the MTIST air sampler and the 24 h volumetric trap.

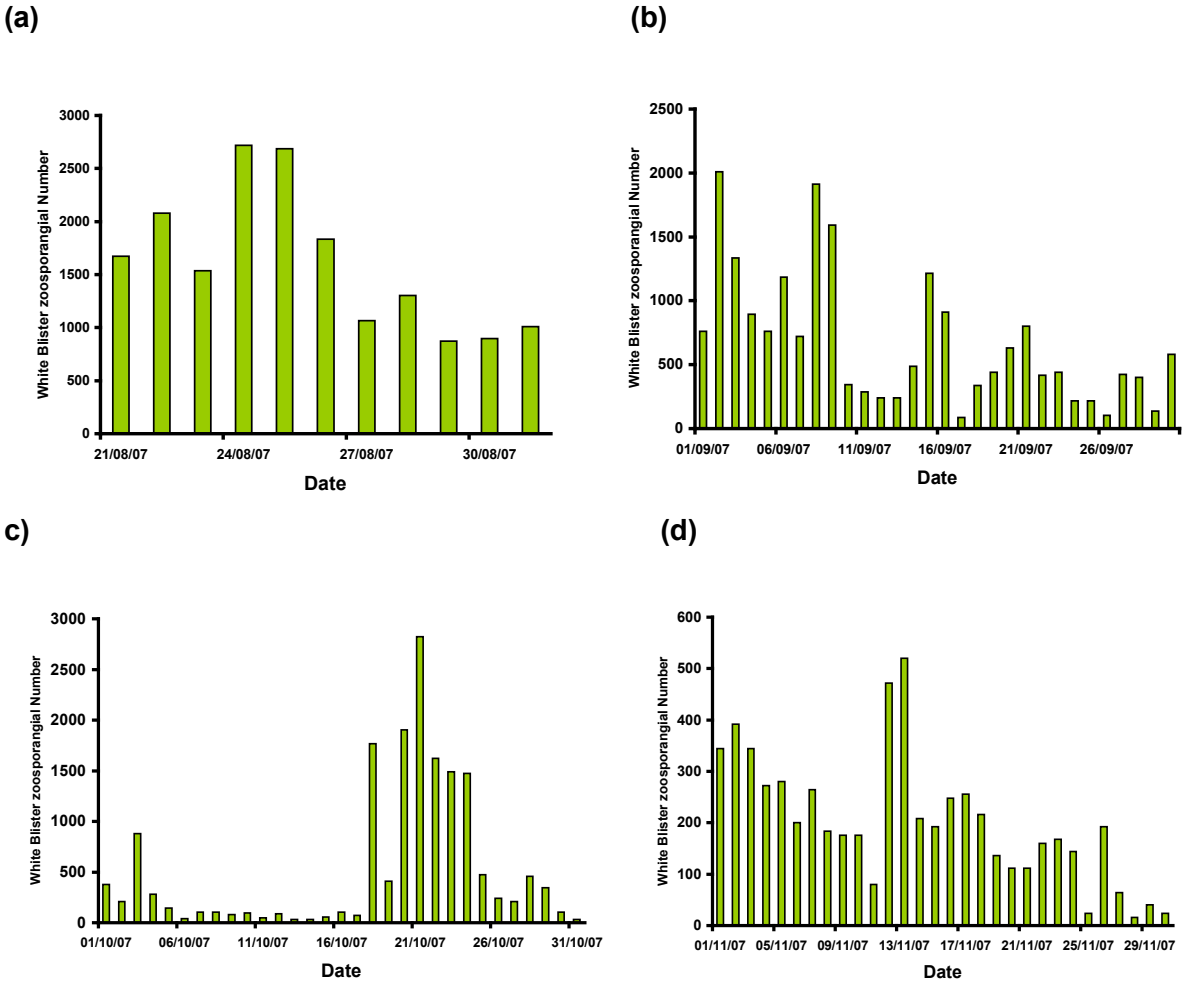

Figure 4. Total daily white blister zoosporangial counts sampled using MTIST high volume air sampler during (a) August (b) September (c) October and (d) November 2007 within a white blister infected field plot at Warwick HRI

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. 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.

Brassica*spot* 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.

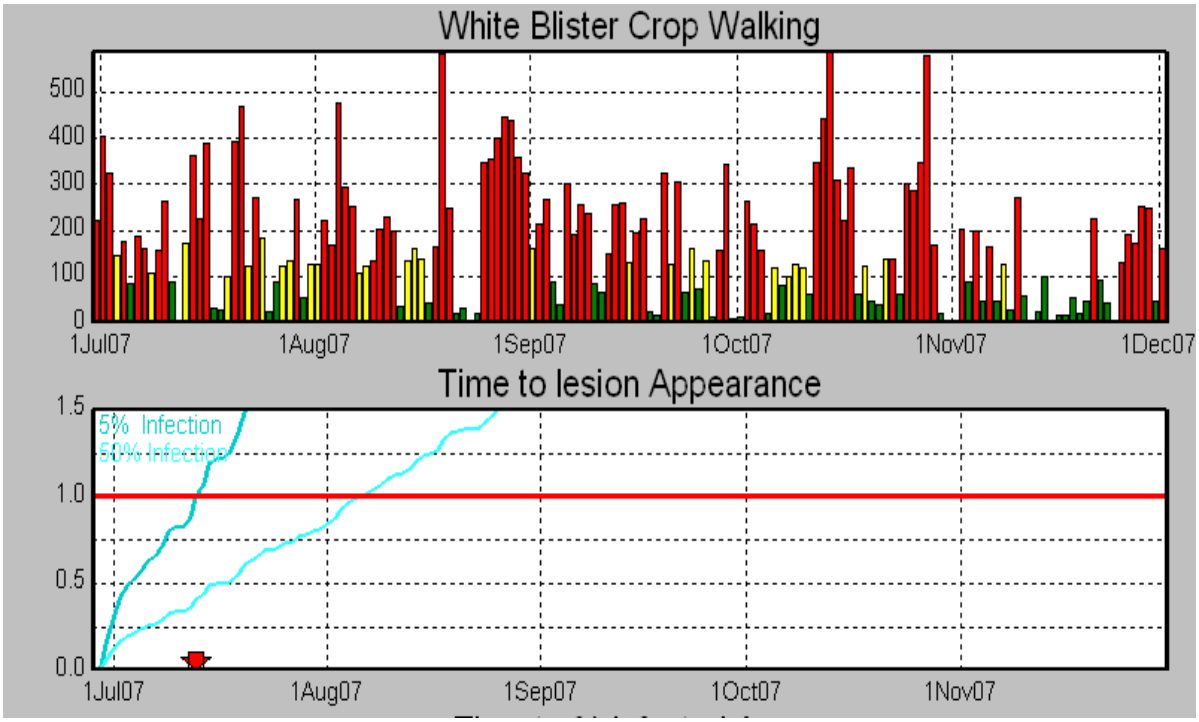

Figure 5. Environmental risk of white blister infection using the white blister II infection model in a white blister infected field plot at Warwick HRI in 2007

Diurnal periodicity of white blister in air samples

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

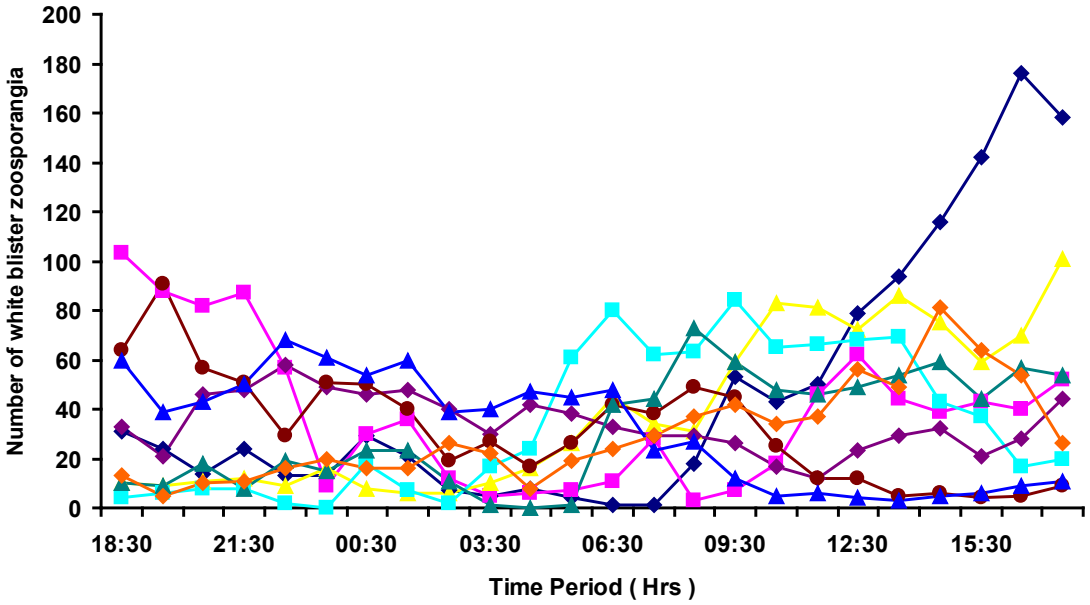

Figure 6. Hourly white blister zoosporangial counts using a 24 h volumetric air sampler during 10 days with zoosporangial counts above 1000 per day at Warwick HRI in 2007

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.

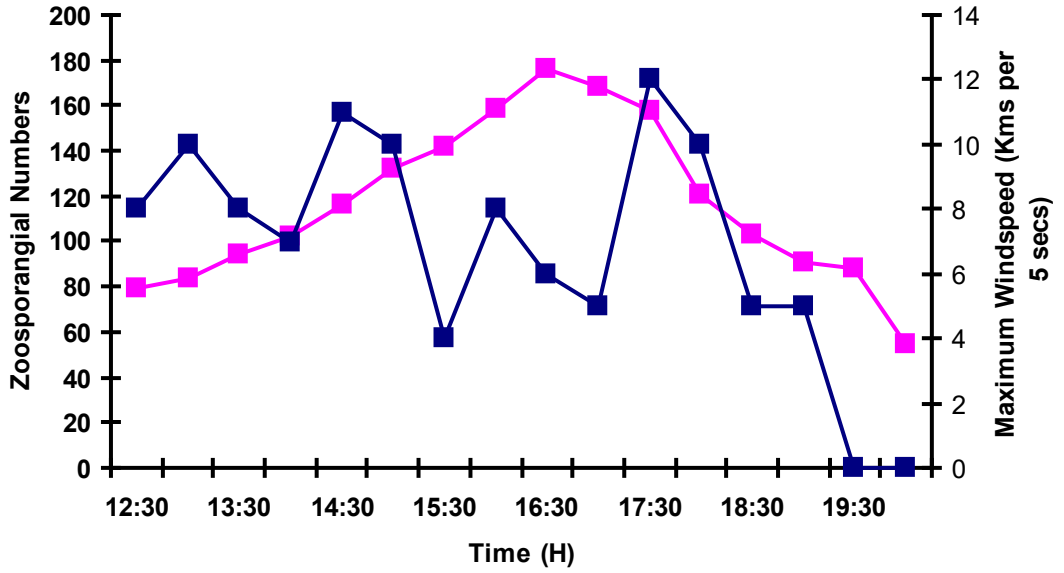

Figure 7. Mean number of zoosporangia in air samples (■) and maximum windspeed (5 secs) (■) in air samples from an white blister infected plot of Brussels sprouts (cv. Golfer) at Warwick HRI

RESULTS (Year two)

White blister infection on bait plants exposed within the over-wintered Brussels sprout crop infected with white blister at Warwick HRI in 2008

Due to unusual growing conditions during 2008 white blister establishment and infection was at low levels. Bait plants were exposed within plots during September and October at varying distances from the plot. The number of white blister lesions on bait plants which were exposed daily during the period 1 September 2008 to 17 October 2008 is shown in Figure 8. There were very low numbers of white blister lesions recorded on bait plants exposed within the plot. No bait plant infection was observed at exposure sites at varying distances north (the prevailing wind direction) from the infected plot.

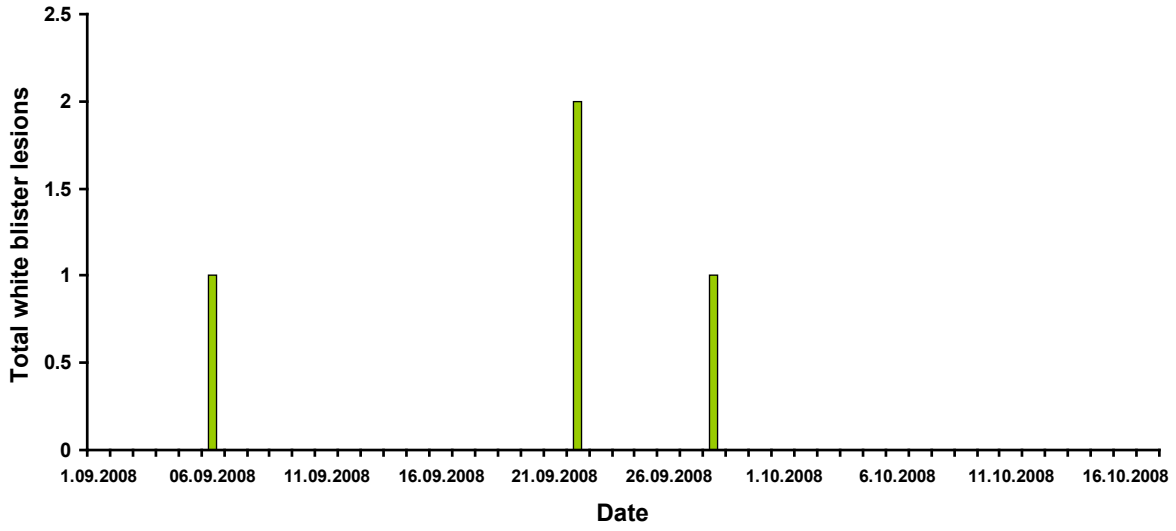

Figure 8. Mean total number of white blister lesions on bait plants exposed in the plot (■) 240 m north (■) and 420 m north (■) of a white blister infected field plot of Brussels sprouts (cv. Golfer)

White blister counts taken from air sampled using an MTIST sampler

White blister inoculum availability from a heavily infected Brussels sprout crop at Warwick HRI was measured using an MTIST high volume sampler in 2008. Zoosporangia of white blister were trapped on to microtitre wells coated with silicone. The total numbers of zoosporangia were counted using a microscope. Daily zoosporangial counts in wells in the trap are shown in Figure 9 during September 2008. The pattern of white blister zoosporangia availability matched the pattern of white blister lesions produced on trap plants however the available inoculum was low throughout the trapping period. No inoculum was observed in the trap vessels after the 30 September 2009.

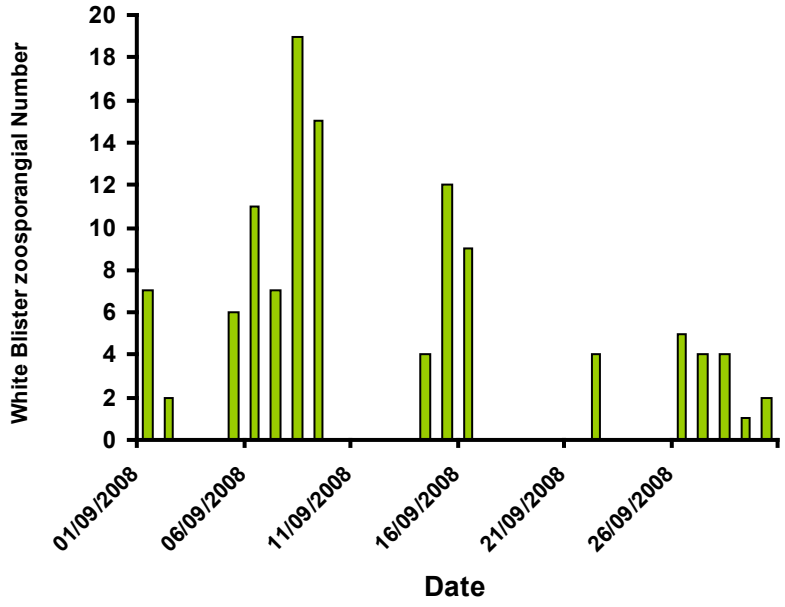

Figure 9. Total daily white blister zoosporangial counts sampled using MTIST high volume air sampler during September 2008 within a white blister infected field plot at Warwick HRI

Brassica*spot* output for white blister at Warwick HRI 2008

The Brassica*spot* output at the Warwick HRI field site is shown in Figure 10. There was missing data due to data logger errors from the 29 July 2008 until the 25 August 2008. Relatively low numbers of days with infection conditions were recorded during the whole season. However infection within field plots was not observed until the beginning of September 2008. The growth of the sprouts was restricted due to waterlogging and low temperatures. High risk infection periods were recorded continuously at the end of August 2008 and during September 2008. Relatively few high risk infection periods were recorded during October 2008. Four days during November 2008 had conditions which could give rise to white blister infection according to the model. Very low levels of white blister infection were observed within infected field plots. High risk infection conditions were recorded during mid December 2008.

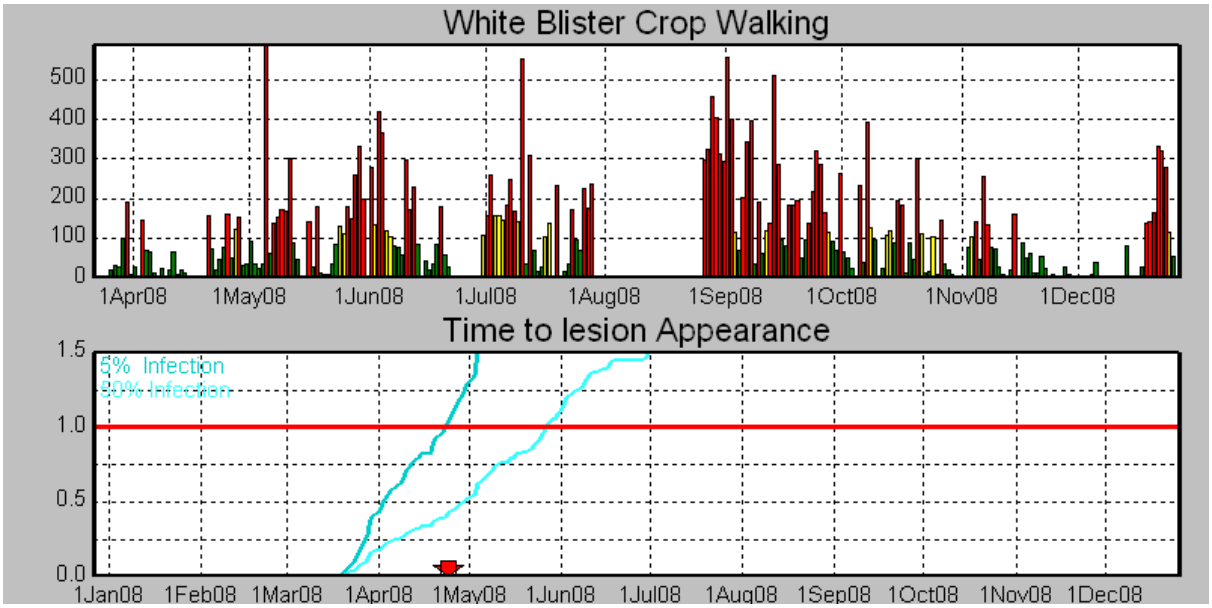

Figure 10. Environmental risk of white blister infection using the white blister II infection model in a white blister infected field plot at Warwick HRI in 2008

Differentiation of white blister species found in the field within commercially produced Brassica crops

Molecular probes supplied by the Department of Primary Industries, Victoria, Australia were used to detect variation between white blister found on vegetable Brassicas and that found on Shepherd's purse or Charlock. The results of using molecular probes F09, H05, D03, A08, C02 and F01 are shown on Table 1. The results show that probe D09 can differentiate between white blister on Shepherds Purse and white blister found on vegetable Brassicas in the UK (Plate 1). A PCR product was obtained when DNA from white blister infected leaf material from vegetable Brassicas was used with the D09 probe. In comparison to white blister infected leaf material from Shepherd's purse did not give any PCR product on gels. Probe A08 was also able to differentiate between the two types of *Albugo*. However reliable detection using this probe under all conditions was compromised in comparison to D09. Other probes tested were unable to determine differences between the two *Albugo* types.

Table 1: Molecular detection of white blister zoosporangial type

Molecular Primers/White blister type	F09	H05	D03	A08	C02	F01
Vegetable brassica leaf	+	+	+	+	+	+
Shepherds Purse leaf	+	+	-	-	+	+
RELIABILITY	***	**	***	* / **	*	*

*** = EXCELLENT

** = FAIR

* = POOR

Reliability determined on frequency of false positives for negative control and consistancy of results

Plate 1: Differentiation of Vegetable Brassica white blister DNA and Shepard's Purse white blister DNA

Measurement of white blister zoosporangial viability

Table 2. Identification of viable DNA samples of white blister zoosporangia after incubation at 28 days incubation at 5, 10, 15 and 20°C

Track Identifier	Sample	DAY	TEMP °C	DILUTION	PRIMER
1	leaf	28	5	1:100	DO3
2	leaf	28	10	1:100	DO3
3	leaf	28	15	1:100	DO3
4	leaf	28	20	1:100	DO3
5	leaf	12	5	1:100	DO3
6	leaf	12	5	1:100	DO3
7	MQ water				DO3
8	leaf	28	5	1:100	FO1
9	leaf	28	10	1:100	FO1
10	leaf	28	15	1:100	FO1
11	leaf	28	20	1:100	FO1
12	leaf	12	5	1:100	FO1
13	leaf	12	5	1:100	FO1
14	MQ water				FO1

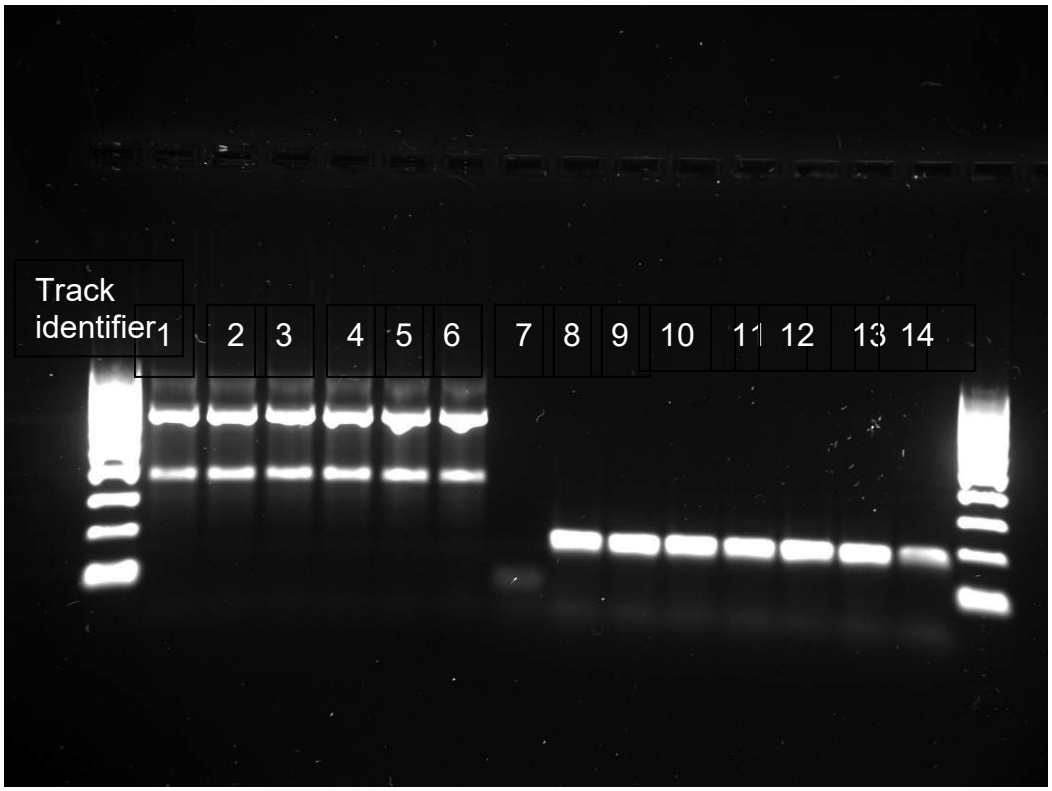

Plate 2. Identification of viable white blister DNA in leaf samples after 28 days incubation at 5, 10, 15 and 20°C

The results show that after 28 days regardless of temperature viable DNA of white blister could be detected on inoculated leaf surfaces. Tracks 1-4 (Plate 2) gave positive reactions with white blister primer DO3. Tracks 1- 4 contained DNA extracted from leaf surfaces inoculated with white blister zoosporangia. The leaves were attached to plants which had been incubated for 28 days at either 5, 10, 15 or 20^{o}C. This primer did not react with the water sample. Similar results were obtained with molecular primer FO1 however this primer did react with the control water sample. The results show that regardless of temperature white blister DNA was detectable on the leaf after 28 days storage.

DISCUSSION

Aspects of the life cycle of white blister in vegetable Brassica crops which affect optimal detection

Symptoms of white blister, within crops are difficult to detect when there are low levels of disease present. The extended incubation period at lower temperatures also contributes to the difficulty in detection. For example at continuous temperatures of 5ºC symptoms of white blister will not become visible.

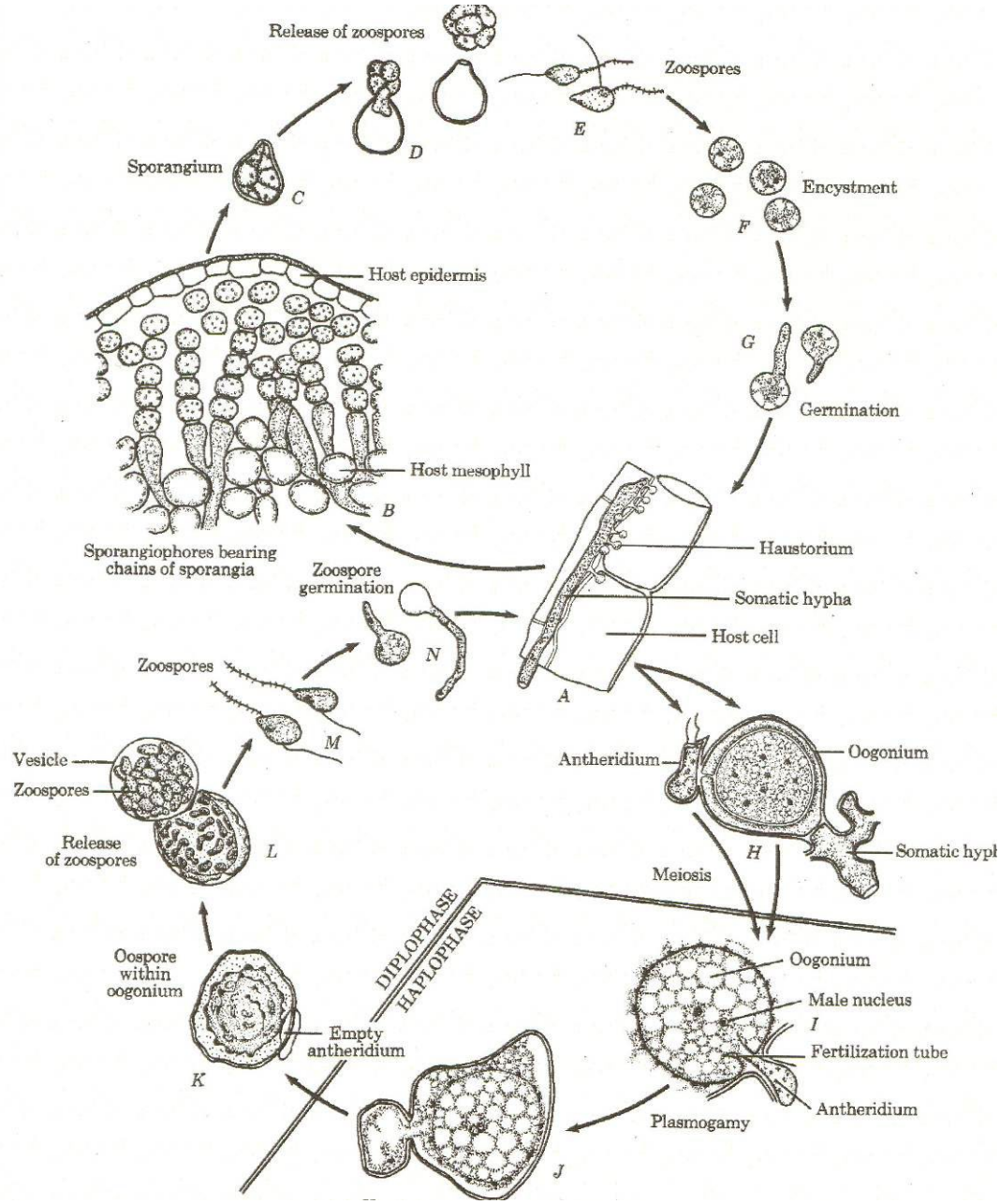

Figure 10. Life Cycle of *Albugo candida* (white blister) on vegetable Brassicas

Symptomology of white blister on young plants is poorly understood and lesions can become enlarged and can lead to distorted tissue while the leaf tissue remains immature. Mature tissue will show white blister symptoms much more rapidly. There is potential for a large difference in symptom expression on mature and immature tissues. 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. 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. Leaf age is a major factor in determining the number of white blister pustules that develop on the leaves. The more mature leaves do not give rise to the numbers of pustules found on immature leaves (Verma et al., 1983). The effect of leaf age may indirectly affect the effect of temperature on time between infection and symptom appearance. For example at continuous temperatures of 5ºC symptoms of white blister will not become visible. Only vegetable Brassicas are infected by white blister as oilseed rape (*Brassica napus*) appears to be highly resistant to this pathogen. The detection of spores of white blister in vegetable Brassica crops is dependent on which spore form is more important in determining disease risk. The zoospore is water borne but there is evidence that it can be liberated and dispersed during periods of rainfall. The zoosporangia which gives rise to zoospores is a larger spore form which can be dispersed by wind. Both these sources of dispersal are not optimal and its unclear how important between crop wind dispersal of zoosporangia is. In this study dispersal of zoosporangia was relatively low. Successful transmission of white blister in Brassica plants over distances of less than 500 m was rarely observed. The DNA tests could be used to differentiate zoosporangia which infect vegetable Brassicas and those which infect Shepherd's Purse. These two types of zoosporangia appear similar but those produced on Shepherd's purse cannot infect vegetable Brassicas. Zoospores within water samples were extremely difficult to detect due to the low numbers and their sporadic production. Samples tested for DNA could not detect zoospores within water samples taken from infected Brussels sprouts crops. There was also difficulty in ascertaining if zoospores were present in the absence of zoosporangia from which they are produced.

However there is substantial evidence for seed borne infection by white blister (Faggian, unpublished) which gives rise to seedling infection within propagation systems. Successful infection under propagation conditions can cause infection on seedlings at the time of transplanting. The environmental conditions during propagation are optimal for white blister infection. Temperature conditions are within the range necessary for infection (e.g. not higher that 22–24ºC or lower that 6–8ºC). Frequent watering of seedlings gives rise to optimal wetting

conditions on cotyledons where seed borne infections will be expressed. Consequently it might be useful to spray seedlings against white blister before they are planted in the field.

Viability of white blister zoosporangia in vegetable Brassica crops

The spore form of white blister which is airborne is the sporangia (also called the zoosporangia). 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 results from this project show that the zoosporangia can remain viable for long periods of time (at least one month) regardless of temperature. This means that the crop is unlikely to become disease free after it has become infected despite the narrow window of temperature requirements for infection (no infection is possible at high and low temperatures). The role of zoosporangia as survival structures in the life cycle of white blister maybe more significant, than at first thought. Experiments could not determine the numbers of zoosporangia which would give rise to infective zoospores. However, only small numbers of zoosporangia have the ability to produce zoospores. Freshly produced zoosporangia do not produce 100% germination rates and germination through the production of zoospores declines quickly with zoosporangial age. However zoosporangia can germinate directly by the production of germ tubes. Zoosporangia could retain the ability to germinate directly but this has not been investigated within the current project.

Rapid tests for white blister inoculum

Detecting white blister spores in the field, in order to improve the accuracy of forecasts, is complicated by the fact that the fungus also occurs on the weed species shepherd's purse which is common in farm fields and margins. Spores produced by strains of white blister that grow on shepherd's purse cannot infect vegetable Brassicas and only infect Shepherd's purse. A means of discriminating between these two types of white blister is required if we are to develop an accurate 'in field' detection system – if we just counted all the spores that look like white blister spores it would result in an over estimation of the disease pressure on the crop. Fields with a larger weed problem with white blister infection would lead to potential error and over estimation of white blister risk.

The Department of Primary Industries in Australia have developed genetic methods that can tell the difference between spores from the weed and from the white blister which infects the Brassica crop. The genetic analysis shows the presence of light coloured bands when vegetable Brassica white blister DNA is present. No bands or reactions are recorded when white blister spores from shepherd's purse were tested. This test means that we can

differentiate between the two types of white blister. This would be important if accurate methods of “in field” detection were to be developed.

With information on white blister detection and transmission rapid tests could be constructed for use in the field to potentially detect zoosporangia 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 zoosporangia. Trapping formats for zoosporangia would also need to be integrated with numbers of zoosporangia 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 5ºC 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 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.

REFERENCES

Hill, C.B., Crute, I.R., Sherriff, C. & Williams, P.H. (1988). Specificity of *Albugo candida* and *Peronospora parasitica* pathotypes towards rapid cycling crucifers. Cruciferae Newsletter **13**:112 -113.

Jacobson, D. J., Lefebvre, S. M., Ojerio, R. S., Berwald, N. and Heikkinen, E. (1998). Presistent, systemic, asymptomatic infections of *Albugo candida,* an oomycete parasite, detected in three wild crucifer species. *Canadian Journal of Botany* **76**, 739-750.

Kennedy. R., & Wakeham, A.J. (2006) Impact of fungicide resistance on light leaf spot control on vegetable Brassicas in Scotland. *Aspects of Applied Biology* **78**, 51 – 58.

Liu, J.Q. Parks, P. & Rimmer, S.R. (1996). Development of monogenic lines for resistance to *Albugo candida* from a Canadian *Brassica napus* Cultivar. Phytopathology **86**, 1000 – 1004.

Pound, G.S., Williams, P.H. (1963) Biological races of *Albugo candida*. *Phytopathology* **53**, 1146-1149.

Verma., P.R., Spurr, D.T. & Petrie, G.A. (1983). Influence of age and time of detachment on development of white rust in detached *Brassica campestris* leaves at different temperatures. *Canadian Journal of Plant Pathology* **5**, 154 – 157.

