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# ANNUAL REPORT

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To:  
Horticultural Development Council  
Bradbourne House  
Stable Block  
East Malling  
Kent, ME19 6DZ

**BRASSICAS: FURTHER DEVELOPMENT OF  
A SPRAY-TIMING MODEL FOR  
WHITE BLISTER (ALBUGO CANDIDA)  
IN VEGETABLE BRASSICA CROPS  
WITHIN THE BRASSICA<sub>SPOT</sub> SYSTEM**

**HDC PROJECT FV 53e**

**R Kennedy**

Horticulture Research International  
Wellesbourne, Warwick, CV35 9EF

June 2004

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Commercial - In Confidence



# Grower Summary

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**FV 53e**

**BRASSICAS: FURTHER  
DEVELOPMENT OF A  
SPRAY-TIMING MODEL  
FOR WHITE BLISTER  
(*ALBUGO CANDIDA*) IN  
VEGETABLE BRASSICA  
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BRASSICA<sub>SPOT</sub> SYSTEM**

**Annual report 2004**

**Project title:** Brassicas: Further development of a spray-timing model for white blister (*Albugo candida*) in vegetable brassica crops within the Brassica<sub>spot</sub> system

**Project number:** FV 53e

**Project leader:** Dr Roy Kennedy

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**Coordinator:** Richard Mowbray

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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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# 1. GROWER SUMMARY

## 1.1 Headline

A new disease forecasting model for white blister has been successfully tested. It will be used to provide information on the optimal application of sprays for control of white blister in vegetable brassica crops. Growers who use the model will be able to reduce reliance on eradicant fungicides which are costly and at present the main control option for this disease.

## 1.2 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. Like many other leaf spot pathogens white blister has a long latent / incubation period (time between infection and appearance of symptoms). This means that success or failure of control is only apparent in some cases weeks after fungicide applications.

Often this leads to white blister becoming well established in crops before it is really visible. White blister symptoms first appear as yellow spots on leaves and these eventually become white in colour. However often the yellow spots on leaves do not develop further (especially on older leaves) and white lesions are not formed. White blister appears to affect only immature tissue and this is the reason that on some older leaves the white lesions are not readily formed. The maturity of the tissue also affects the time between infection and symptom appearance. This means that mathematical models summarising the effect of environment on the time to symptom appearance must take tissue maturity into account in predictions.

The expected deliverables from this project are:

- An evaluation of the accuracy of a white blister development model for control of white blister in vegetable brassica crops.
- Information of the development time for white blister in vegetable brassica crops and the effect of maturity on time to symptom development.
- The white blister symptom development model programmed within the Brassica<sub>spot</sub> disease forecasting system.

Further development and testing of the system would be required by growers to adapt the information to their own growing systems.

### 1.3 Summary of first year work on FV 53e

#### 1.3.1 Development of the white blister spray timing model

One of the key life cycle stages controlling white blister development in vegetable brassica crops is the time between infection and symptom appearance. By predicting the duration of this time period effective sprays can be predicted which will protect the crop against infection by the white blister pathogen. The effect of temperature on time to symptom appearance by white blister was investigated under controlled environmental conditions within MAFF project HH1713SFV. The results were used to construct models summarizing the effect of temperature on white blister development (Figure 1).

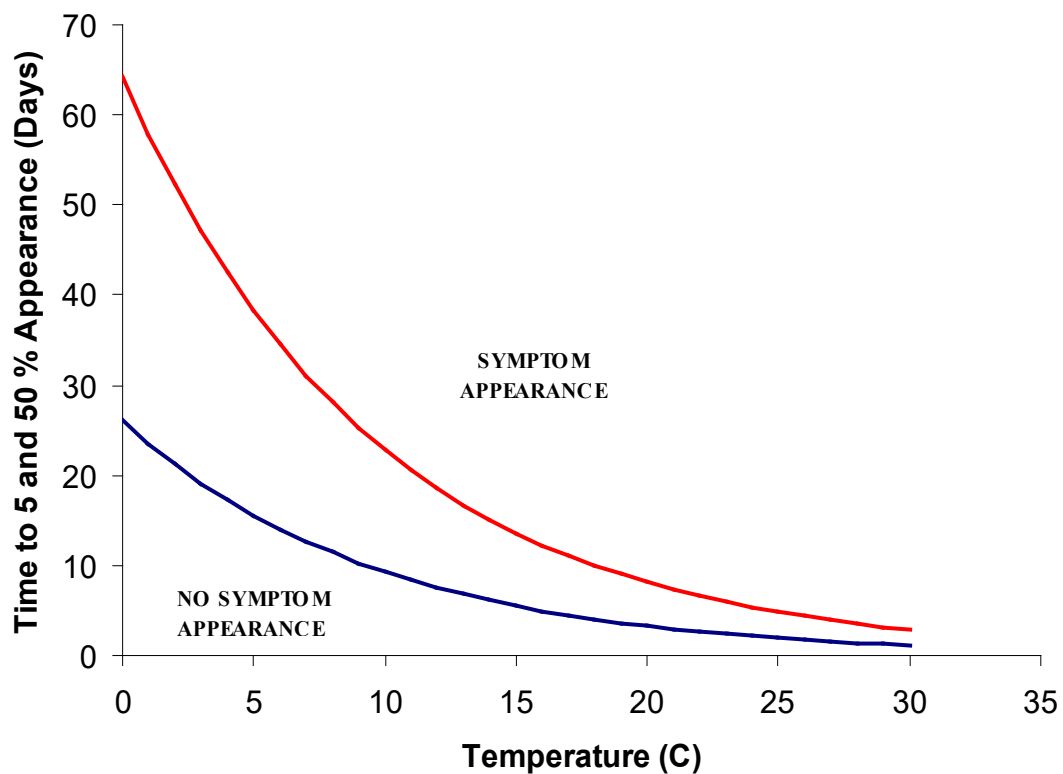


Figure 1. The effect of temperature on number of days to 5 % (■) and 50 % (■) white blister symptom development.



### **1.3.2 Testing the white blister spray timing model in the field**

The white blister symptom development model was programmed within an excel spreadsheet and in computer programme language C (as used in the Brassica<sub>spot</sub> programme) and used in field tests to determine its accuracy in predicting symptom development on inoculated plants. Temperature data was collected from the field and used in conjunction with the model to predict the rate of white blister symptom development under field conditions. This was done to determine which part of the model was most appropriate for practical usage in the field. Tests were only carried out using Brussels sprout plants Cv. Golfer in the first instance but further tests are planned with a range of brassica types. The results show that the predicted time to 50 % symptom development was a good measure of the time to the first observed symptom development in the field. In contrast the time to 5 % symptom appearance was not a good measure of time to symptom development in the field.

### **1.4 Action points for growers**

Using temperature data collected from the field the grower will be able to predict the rate of white blister development in his crop.

- Growers can use the Brassica<sub>spot</sub> system to determine when white blister infection has occurred in the field.
- By linking information on white blister infection with the white blister symptom development model the grower will be able to predict when sprays should be applied.
- Using forecasts of mean temperatures growers can utilize the model to determine well in advance when sprays should be applied.

### **1.5 Anticipated practical and financial benefit**

- With optimal timings for white blister spray applications available it is likely that sprays against white blister can be rationalised.
- There will be less need for reliance on metalaxyl based fungicides which should reduce the costs associated with white blister control in vegetable brassica crops.
- By using the white blister spray model in conjunction with others predicting dark leaf spot and ringspot growers will be better able to schedule fungicide applications to crops more effectively to produce cost savings.

## **2. INTRODUCTION**

### **2.1 Control of air borne disease problems in vegetable brassica crops**

The three most important fungal diseases affecting vegetable brassica crops are ringspot (*Mycosphaerella brassicicola*) white blister (*Albugo candida*) and dark leaf spot (*Alternaria brassicae* and *Alternaria brassicicola*). Applications of fungicide to the crop to control these diseases are not straight forward even though the products used are very effective. Long season Brussels sprouts crops can be planted in May and harvested at or after Christmas. This is a considerable period for the crop to remain disease free. With the advent of eradicant fungicides such as tebuconazole (Folicur) and difenoconazole (Plover), which can now be used on both Brussels sprouts and cauliflower crops, the grower has a greater range of options for controlling these diseases. However with increasing restrictions on the amount of each fungicide that can be applied to crops it is important that fungicide usage is properly targeted. White blister belongs to a group of pathogens that are not generally susceptible to azole fungicides (Plover and Folicur). For this reason white blister requires sprays with other types of fungicides which have different modes to action to azole fungicides. Recently BASF has produced a new product which has efficacy against dark leaf spot ringspot and white blister (Signum). However this is an expensive product to use and its over usage would adversely affect the economics of crop production.

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

*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 are comprised of sporangia 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. Sporangia 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.

If symptoms occur on inflorescences they can result in distortion a symptom which is often referred to as staghead. Staghead can result in significant yields loss in seeding crops and on

Brussel sprout buttons. The distortion makes the Brussels sprout button unsaleable but the occurrence on leaf tissues also affects marketability. It is common for white blister to form systemic asymptomatic infections that are not visible for long periods of time. This period between infection and symptom appear 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.

### **2.3 Environmental factors favouring fungal diseases of brassicas**

White blister like other pathogens of vegetable brassicas requires free water for spore germination and infection (Kennedy & Graham, 1995). At optimal temperatures of 20 °C, infection by dark leaf spot spores may occur within 6 h but for substantial disease development at least 10 h of wetness is required (Humpherson-Jones, 1991). Both fungi require at least 12 – 14 h with a relative humidity of greater than 90 % for sporulation to occur (Humpherson-Jones & Phelps, 1989). However ringspot infection requires only short periods of leaf wetness at optimal temperatures, (Kennedy et al., 1999). Ringspot requires prolonged periods of temperature and wetness to complete spore production within fungal structures on the lesion (Cullington, 1995). Once the disease is established relatively short dewfall periods can be very favourable for white blister development. High temperatures result in relatively short periods of time elapsing between infection and symptom appearance on the plant. This is dependant on the temperature under which the plants are grown. Mathematical relationships (models) describing the effect of temperature and wetness on important life-cycle stages have been developed by Warwick HRI. These can be used directly by the grower to provide further information on the occurrence of these critical stages in the crop. These models have been validated for use within commercial crops within HDC project FV53d.

### **2.4 Development of the Brassica<sub>spot</sub> System**

Infection and sporulation models have been programmed within the Brassica<sub>spot</sub> system which comprises of models covering ringspot (*Mycosphaerella brassicicola*) dark leaf spot (*Alternaria brassicae*) and white blister (*Albugo candida*) as follows:

- a) Dark leaf spot/ringspot infection criteria (Crop walking output)
- b) Dark leaf spot disease development criteria (Spray timing output)
- c) Ringspot sporulation criteria (Spray timing output)
- d) White blister infection criteria (Crop walking output)

The system is now widely used to identify periods when there was increased risk of disease development within the crop. Weather information collected from within the crop can be used to determine the likelihood of disease occurrence or fungicide application timings only

if target diseases are present. For example the sporulation models can be used to determine when fungicides must be applied to crops to control disease. These are more useful in long season crops such as Brussels sprouts. Infection models can be used to inform users of the risk of disease occurrence. They can also be used to determine when eradicator fungicides should be applied to crops. Infection models have been found to be more useful for disease control in short season crops such as summer and autumn cauliflowers.

The database within which the forecast models work is called MORPH. MORPH can be used to store weather data which is down loaded on to the same PC using Global Systems for mobile communication (GSM) and radio off-loading. The MORPH system is currently being upgraded within a recently commenced DEFRA project

## 2.5 Current models used within the Brassica<sub>spot</sub> system in commercial crops

The addition of models to the Brassica<sub>spot</sub> system during 1997 – 1999 for use in commercial crops is shown in Table 1. In 1997 the Brassica<sub>spot</sub> system comprised of only the dark leaf spot models which was made available to growers participating in trials using commercial crops of Brussels sprouts. In 1998 the ringspot spray timing model was added to the system which was followed by the white blister disease warning model in 1999. By March 1999 the Brassica<sub>spot</sub> system contained information on the three major disease affecting horticultural brassicas.

**Table 1. Brassica<sub>spot</sub> models used available for use by commercial growers.**

<b>Leaf spot disease</b>	<b>Infection model (disease warning)</b>	<b>Sporulation model (spray timing)</b>
Dark Leaf spot ( <i>Alternaria</i> )	<b>YES</b>	<b>YES</b>
Ringspot ( <i>Mycosphaerella</i> )	<b>YES</b>	<b>YES</b>
White Blister ( <i>Albugo</i> )	<b>YES</b>	<b>NO</b>

## 2.6 Additional models required within the Brassica<sub>spot</sub> system

At present the Brassica<sub>spot</sub> system does not contain any information relating to sporulation by white blister (Table 1). This is an important deficiency in the system as separate applications of fungicide are required to control this pathogen in vegetable brassica crops. Within the Brassica<sub>spot</sub> system models can be used to predict the occurrence of ringspot and dark leaf spot and apply appropriate fungicide sprays at optimal timings. However this is not possible for crops affected by white blister. This may lead to over usage of fungicides for white blister control in the field which could increase crop protection costs unnecessarily. While the disease warning model can be used for white blister control this is not optimal. In many instances, over spraying of crops results from using this information particularly in long season crops where the numbers of spray applications are limited. Over usage of metalaxyl based fungicides has also the potential to cause increased risks of fungicide resistant strains of the white blister pathogen. This has been noted in other crops notably onions where the equivalent pathogen type in onion crops (downy mildew) became more tolerant to fungicide sprays containing metalaxyl.

### **3. MODELS DESCRIBING THE EFFECT OF TEMPERATURE ON WHITE BLISTER DEVELOPMENT**

#### **3.1 Experiments investigating the effect of temperature on white blister development (sporulation)**

The effect of temperature on white blister development and sporulation was investigated under controlled environmental conditions within MAFF project HH1713SFV. The following results describe the data used in modeling studies within project HDC project FV 53e.

##### **3.1.1 Materials and Methods**

###### **3.1.1.1 Plant production**

Seeds of Brussels sprout cv. Golfer were sown (one seed per cell) in a mixture of 70:30 Fisons F2 compost and sand contained in Hassey 307 units. Plants were grown in a 16/14 C day/night temperature regime. Plants were repotted into FP9 pots (one seedling per pot) at the third true leaf stage and grown for a further 2 weeks at 16/14 C day/night temperature regime in the glasshouse. Plants were placed in trays prior to inoculation and unhealthy or atypical plants removed to ensure the uniformity plants used in each experiment.

###### **3.1.1.2 Production of white blister 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). Five week old seedlings of Brussels sprouts in FP 11 pots were inoculated with the zoospore suspension. Inoculum (zoosporangia) for controlled environment studies was prepared by collection from heavily infected seedlings incubated in the glasshouse. Inoculum was collected using a suction pump and hydrated in tap water for 4 h at 5 C before inoculation on to the plant. The concentration of inoculum was adjusted to approximately  $10^4$  zoosporangia per ml using a haemocytometer.

###### **3.1.1.3 Plant inoculation**

Latent period of *Albugo candida* on Brussels sprouts was investigated at constant temperatures of 4, 8, 12, 16, 20, 24 °C and a relative humidity of 75 % during the day with 90 % r.h. at night. Plants were grown in FP9 pots and were inoculated as a single batch in each experiment. Plants were placed at 100% r.h. after inoculation at a temperature of approximately 12 C in a misting tent for a 48 H period. After inoculation plants were removed from the misting tent

and randomly assigned to temperature controlled cabinets held at either 4, 8, 12, 16, 20, or 24C. At each treatment time after the 48 h inoculation period approximately six plants were removed from each cabinet and assessed for disease and plant growth.

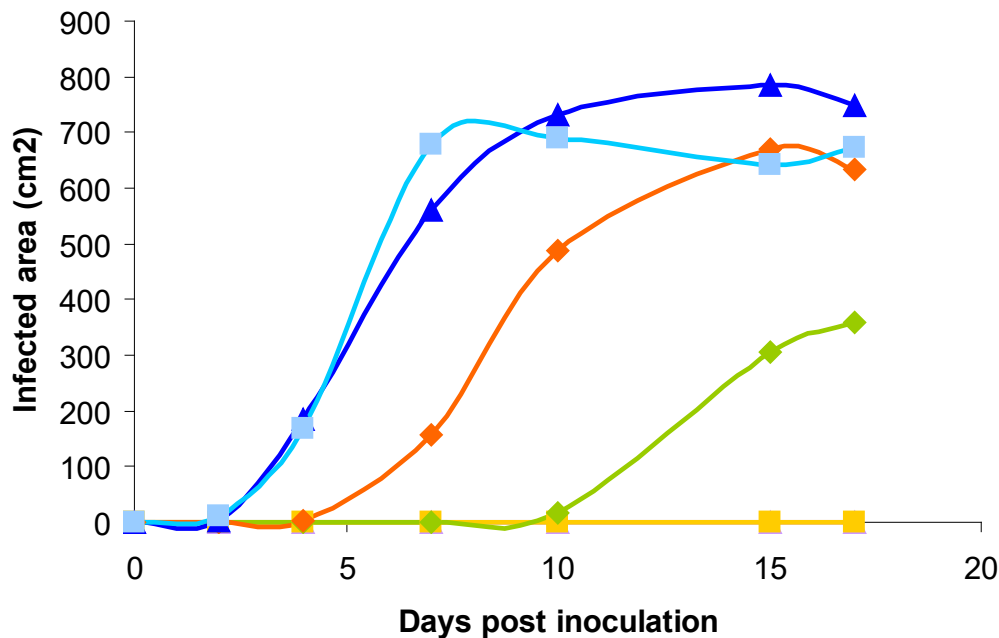
### 3.1.1.4 Disease assessment

The number of lesions and the percentage area of the leaf infected on each leaf of each plant was recorded for each temperature at each assessment time. The leaf area of each leaf was measured (Delta T devices). Leaves were removed from each plant after the number of lesions were counted and the percentage area infected estimated. Detached leaves were then photographed using an image analyser which automatically sensed the leaf area. Disease assessments were taken at 0, 2, 4, 7, 10, 15, 17 and 21 days after inoculation. At each assessment time new plants from each temperature were assessed for disease and leaf area. Experiments were repeated in three separate controlled environment experiments.

## 3.1.2 Results

### 3.1.2.1 Effect of temperature on white blister symptom appearance (infected area)

The results of one controlled environment experiment investigating the effect of temperature on the appearance of white blister infected leaf tissue is shown in Figure 2.

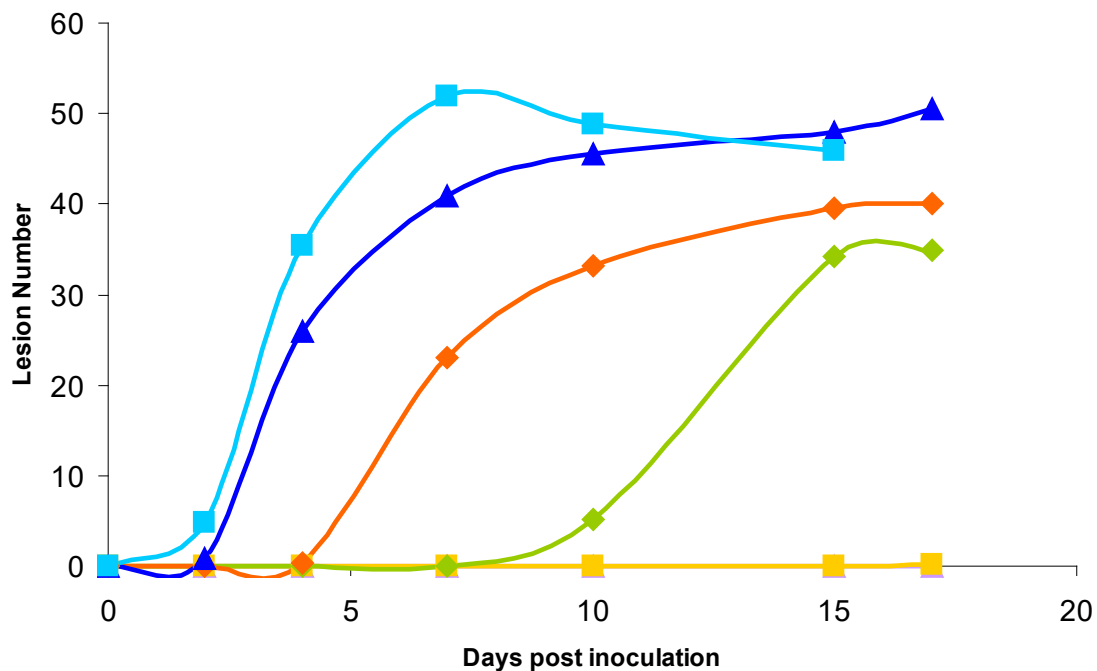


**Figure 2.** The effect of temperature on the appearance of white blister infected leaf tissue at 24 C° (■), 20 C° (▲), 16 C° (◆), 12 C° (◇), 8 C° (■), 4 C° (▲).

There was no symptom development at either 4 or 8 °C over the 17 day period that plants were placed under these constant temperature conditions. At 12 °C symptoms appeared after 10 days however the infected area developed slowly and after 17 days only 300 cm<sup>2</sup> of the total plant area was showing white blister symptoms. At 16 °C white blister symptoms appeared on the first plants after approximately 4 days and area infected increased more rapidly to a maximum of 670 cm<sup>2</sup> of the total plant area inoculated. However symptom development at 20 and 24 °C was very rapid and not significantly different from each other. Symptoms were observed on these treatments after approximately 2 days. Infected area increased rapidly in both treatments to reach a maximum of 670 – 750 cm<sup>2</sup> of the total plant area inoculated after 7 days at each temperature (Figure 2).

### 3.1.2.2 Effect of temperature on white blister symptom appearance (Lesion number)

The results of controlled environment experiment investigating the effect of temperature on the appearance of white blister lesions on leaves is shown in Figure 3 (one experiment shown).



**Figure 3.** The effect of temperature on the appearance of white blister infected leaf tissue at 24 C° (■), 20 C° (▲), 16 C° (◆), 12 C° (◆), 8 C° (■), 4 C° (▲).

The results were similar to that shown for the effect of temperature on the appearance of white blister infected leaf tissue. Lesion numbers increased more rapidly after inoculation at temperatures of 20 and 24 °C after 5 days. Appearance of lesions was much slower at temperatures of 16 and 12 °C with maximum numbers of lesions visible after approximately 15 days. There appeared to be lower numbers of lesions visible at these temperatures in comparison to temperatures of 20 and 24 C° (Figure 3). No lesions were visible at either 8 or 4 C°.



### **3.1.3 Conclusions**

The results show that white blister symptoms do not develop at temperatures below 8 °C. However the optimal temperature for symptom development was at temperatures of 20 °C and above. The results indicate that the disease will not be prevalent in areas where the average daily temperatures occur at 10 °C and below for significant periods of time. This explains why white blister is not prevalent in Scotland and many northerly areas of vegetable brassica production. The results indicate that epidemics will not develop in areas with cooler daily average temperatures because there is not sufficient time for white blister disease development. Where epidemics have occurred they could have resulted from high levels of initial infection on transplants.

## **3.2 Fitting models describing the effect of temperature on white blister development**

### **3.2.1 Materials and Methods**

The best approach to fitting a model to the controlled environment data was to model the spread of primary symptom appearance over specific time intervals (e.g. between 2 and 17 days at 20°C) along with the mean time to symptom appearance. To develop the model a series of candidate frequency distributions were fitted to the times of visible lesion appearance. In addition, times were inverted to provide rates of appearance which are useful when forecasting in field conditions with fluctuating temperatures, and the same distributions were fitted to these. The geometric means of the numbers of lesions per plant were used for model fitting.

These were calculated separately for each of the 3 replicates so that the replication could be used to assess the goodness of fit of the models. Data were collected at 6 temperatures and recorded for up to 21 days. Models were fitted using generalised linear models with a binomial error structure. The binomial totals were defined as the maximum number of lesions attained at each temperature. The candidate distributions were generated from the probit, logit and complementary log-log link functions.

### **3.2.2 Results**

There were very few lesions at 4°C and 8°C and there was evidence of lesion development at 24°C for periods of over 17 days from inoculation. Therefore fitting procedures were based on data from 12°C, 16°C, 20°C and 24°C and 2 to 17 days. Separate fits were made for each temperature. At 16°C, 20°C and 24°C the probit model based on rates of symptom appearance were an extremely good fit. At 12°C a statistical test showed that the fit was

rather poor but visually the fit appeared good and the apparent lack of fit was due to very low lesions until 10 days after inoculation.

To build a forecasting model it was necessary to derive a relationship between the parameters of the frequency distribution and temperature. The probit model implies that the rates followed a normal distribution and so the mean rate was equal to the  $t_{50}$  and could be related to temperature as is standard practice. The relationship was almost linear over the 12-24 °C temperature range but an exponential curve was needed to better describe the lower temperatures. The standard deviation was linearly related to the mean giving rise to a constant coefficient of variation at all temperatures. This is an extremely useful property for building forecasting models.

To produce robust parameter estimates, data from all temperatures between 12-24 °C were combined and the entire model, i.e., the frequency distributions and the temperature data, was fitted.

The final model developed was:

$$R = br^T$$

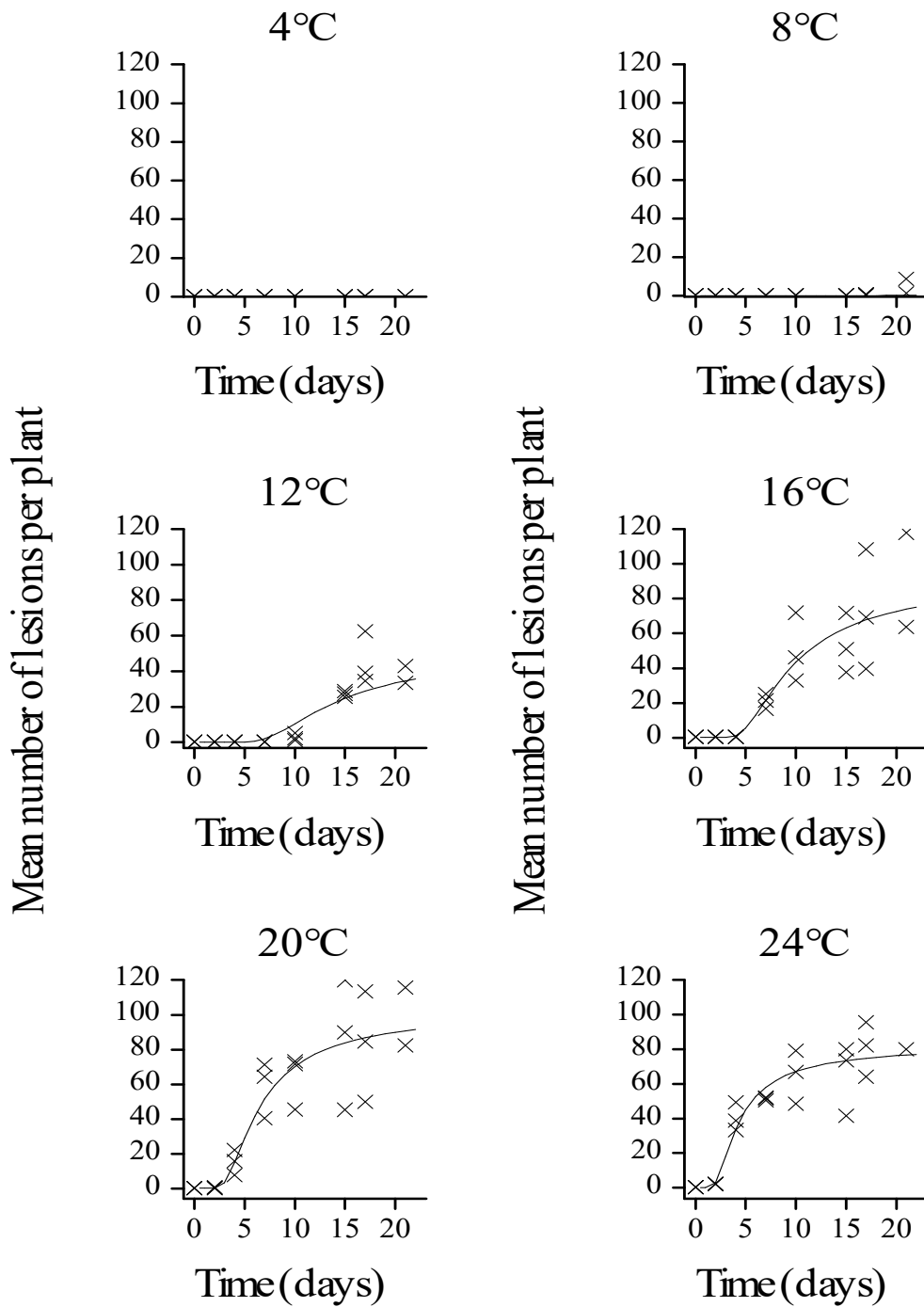
$$s = cR$$

$$f = m\Phi((1/t - R)/s)$$

where  $R$  and  $s$  are the mean and standard deviation of the rates at temperature  $T$ ,  $m$  is the maximum number of lesions,  $\Phi$  is the cumulative normal distribution and  $f$  is the cumulative number of lesions at time  $t$ . The fit of the model is shown in Figure 4.

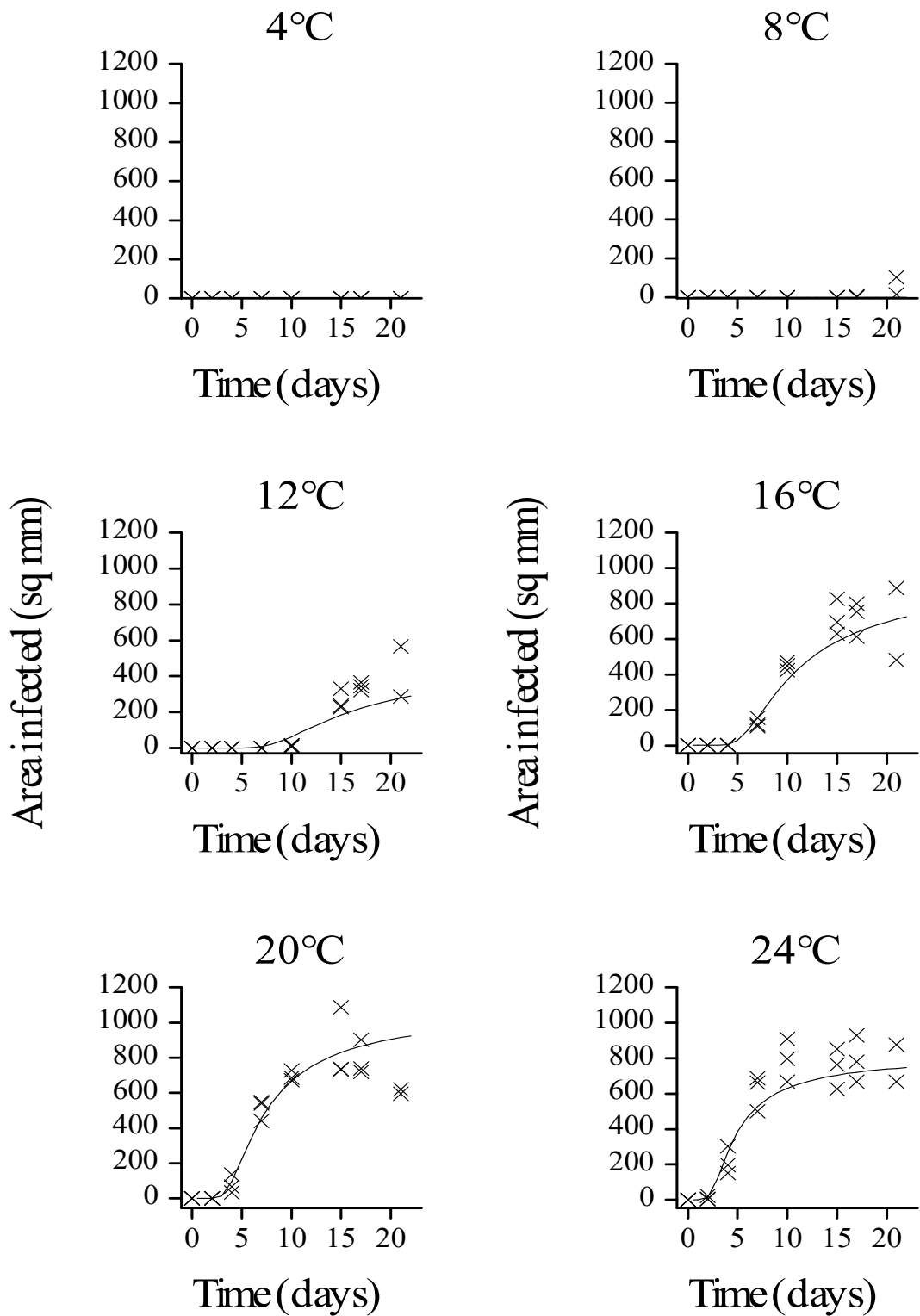
Data were also collected on the infected area ( $A$ ) of each leaf. These data were closely related to lesion numbers ( $L$ ) and the following equation accounted for 98.2% of the variation in the logarithms of the infected areas.

$$\log(A) = 1.2602 + 1.23313 \log(L)$$



**Figure 4. Model fit for the effect of temperature on mean number of white blister lesions per plant.**

Using this relationship the model developed for lesion numbers was applied to infected areas. The fit of the model is shown in Figure 5.



**Figure 5. Model fit for the effect of temperature on white blister infected leaf area.**

### 3.2.3 Conclusions

The results show that there was a good model fit to the controlled environment data over the temperature range 12 – 24 °C. There was little difference between models based on infected area and those based on white blister lesion number. White blister symptom expression is affected by tissue age in that young tissues are more susceptible to infection than older tissues. Leaf area is an expression of tissue age in that the rate of growth of leaves changes over time reflecting their maturity or immaturity. Models describing time to symptom development could be used as a measure which might summarise the effect of tissue age on infection and symptom expression. White blister symptom expression results from two processes. The models developed reflect the tissue age of the plants used in the experiments. Young immature tissues will have a different relationship describing the effect of temperature on time to symptom development in comparison to tissue which is nearing maturity. These differences may be reflected in results when using the model in the field on a range of tissue ages.

The area affected by the disease is related to the number of white blister infections which appear over time and their growth on the leaf. However the results suggest that changes in leaf area cannot be used in this way and that symptom expression in response to temperature is related to lesion appearance and not lesion expansion.

### 3.3 Models describing the effect of temperature on rate of white blister symptom development

#### 3.3.1 Results

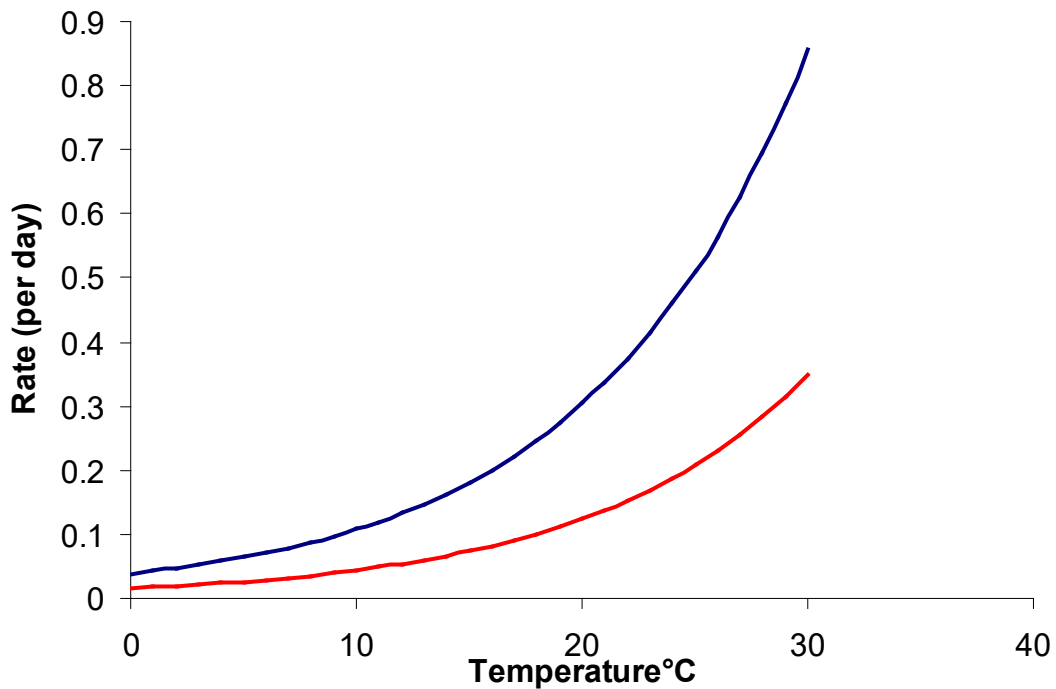
Using the models developed (see section 3.2) the effect of temperature on rate of symptom expression (time to 5 and 50 % symptom expression) could be calculated. Rate functions for 5 % and 50 % symptom development were derived from the model describing the effect of temperature on symptom development at each temperature (Figure 6).

$$(\text{time to 5 \% symptoms}) = (0.01557 * 1.1091^{\text{Temp}}) * (1 + 0.884 * 1.65)$$

$$(\text{time to 50 \% symptoms}) = (0.01557 * 1.1091^{\text{Temp}})$$

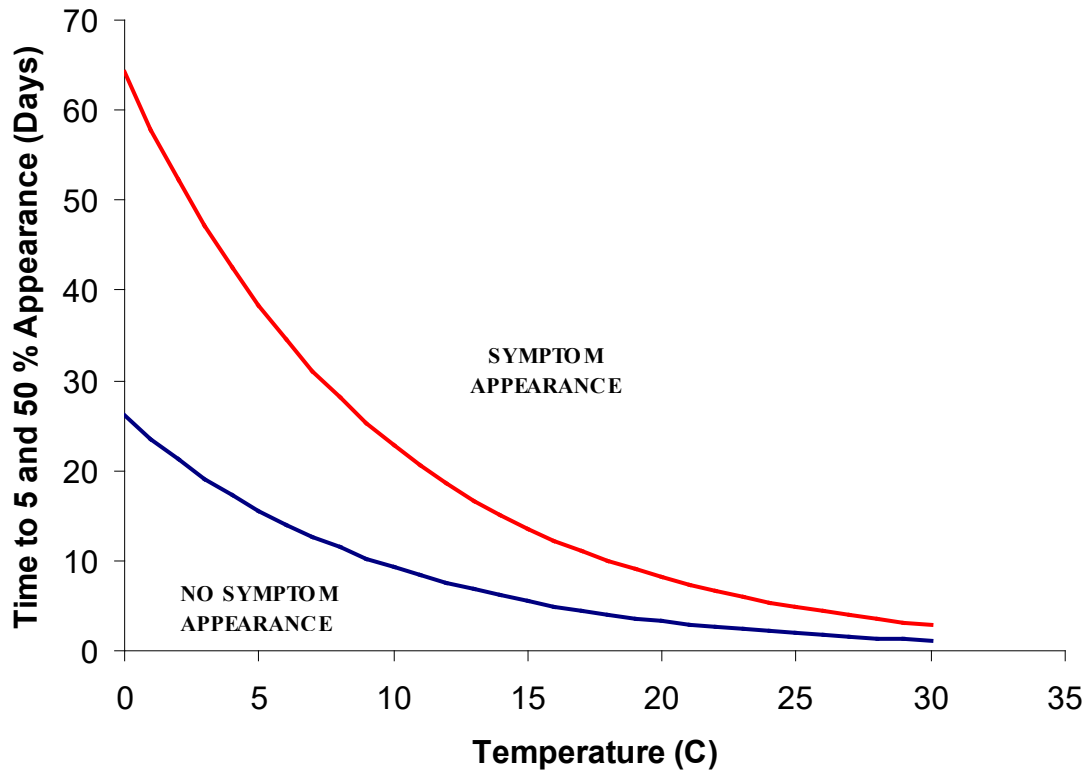
These rate functions can be used to calculate the time to symptom development over the temperature range. However these rate functions have been calculated on the basis of a 48 h infection period at an average hourly temperature of 14 °C. This will result in the estimates of time to symptom development being less accurate. Some adjustment maybe required to

compensate for this initial infection period as white blister infection may have mostly occurred after a few hours at these temperatures.



**Figure 6. The effect of temperature on rate of 5 % (■) and 50 % (■) white blister symptom development.**

The effect of temperature on time to 5 % white blister symptom development is shown in Figure 6. This analysis shows that at 4 °C the predicted time to 5 % symptom development was 18 days. However in controlled environment studies no symptom development occurred at 4 °C indicating that some adjustments may be required to the model at these temperatures. Time to 5 % white blister symptom development was approximately 12 and 9 days at 8 and 12 °C respectively. Relatively rapid symptom development was predicted at 16, 20 and 24 °C of 6, 4 and 3 days respectively (Figure 7).



**Figure 7.** The effect of temperature on the time (days) to 5 % (■) and 50 % (■) white blister symptom.

### 3.3.2 Conclusions

Use of the models to predict time to symptom appearance showed that the model was accurate on data derived from controlled environments. However the model did over estimate the time to symptom appearance at 4 and 8 C. In controlled environment studies no symptom appearance was observed over a 21 day period at these temperatures. However, predicted time to 5 % symptom appearance was 17 and 11 days approximately at 4 and 8 °C respectively using the model. Time to 50 % symptom appearance at 4 and 8 °C was 42 and 28 days respectively. This part of the model may need further refinement using additional data sets collected from the field.

## **4. VALIDATION STUDIES USING THE WHITE BLISTER SYMPTOM DEVELOPMENT MODEL**

### **4.1 Validation studies using inoculated plants placed under fluctuating temperatures under field conditions**

#### **4.1.1 Materials and Methods**

##### **4.1.1.1 Plant production**

Brussels sprouts cv. Golfer were grown in FP11 pots in 70:30 Fisons F2 compost (one seedling per pot). Plants were grown in a 16/14 C day/night temperature regime until the 6-8th true leaf stage at which point they were used within the experimental regime. Sequential sowings of plants ensured that all plants used in the experiment were at approximately the same growth stage.

##### **4.1.1.2 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 zoospores was measured using a haemocytometer.

##### **4.1.1.3 Plant inoculation**

Brussels sprout plants cv. Golfer were inoculated with the 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 70 mls 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.

##### **4.1.1.4 Disease assessment**

The number of lesions on each leaf of each plant was recorded for each inoculation date at each assessment time. Disease assessments were taken at regular intervals after each inoculation period. The number of lesions on leaves of each plant was assessed after first symptoms had appeared.



#### **4.1.1.5 Programming the model**

The model was programmed in C and also within an excel spreadsheet so that weather data for testing the model tests could be used to determine the time to symptom appearance. The relationships for time to 5 and 50 % symptom development were programmed in this way. Temperature data recorded at 30 min intervals were utilised in these tests. The model programmed with the excel spreadsheet was used as the reference for comparison of other programmed systems and for checking the validation of the model results. Further adjustments were made to the programmed model on the basis of results from the excel spreadsheet.

#### **4.1.1.6 Collection of environmental data in the field**

The air temperature was recorded at 30 min intervals at the point of exposure of inoculated plants in the field. The temperature was recorded using a Smartlog meteorological monitoring station (Aardware Design, Walton on Thames). The temperature data was downloaded daily using a GSM phone link and used for comparisons of observed and predicted (using the model) symptom development.

#### **4.1.1.7 Testing the model**

The programmed model was used to determine the time to symptom development under field conditions. Test plants which had been inoculated and placed in the field were used in these tests (CV. Golfer) only. The temperature data from the field was used in conjunction with the rate (Figure 6) which was accumulated to reach a value of 1 at which point symptoms were predicted as having occurred in the field (expected symptom appearance). The time to observed symptom appearance was plotted against expected symptom appearance (in days) and a regression function ( $r^2$  value) calculated for the fit of the relationship between the two. The closer the  $r$  value to 1 the better the fit between observed and predicted symptom development. It was assumed that plants became infected immediately after inoculation.

### **4.1.2 Results**

#### **4.1.2.1 Effect of temperature on white blister symptom appearance**

The relationship between time to first observed and 50 % expected symptom development is shown in Figure 8. The relationship between time to 5 % observed and 5 % expected symptom development is shown in Figure 9. There was a very good relationship ( $r^2 = 0.9478$ ) between time to first observed symptom development and expected time to 50 % symptom development according to the white blister symptom development model. There was a poorer relationship ( $r^2 = 0.3729$ ) between time to first observed symptom development and expected time to 5 % symptom development according to the white blister symptom development model. Tests were conducted on plants which were at the same stage in development.

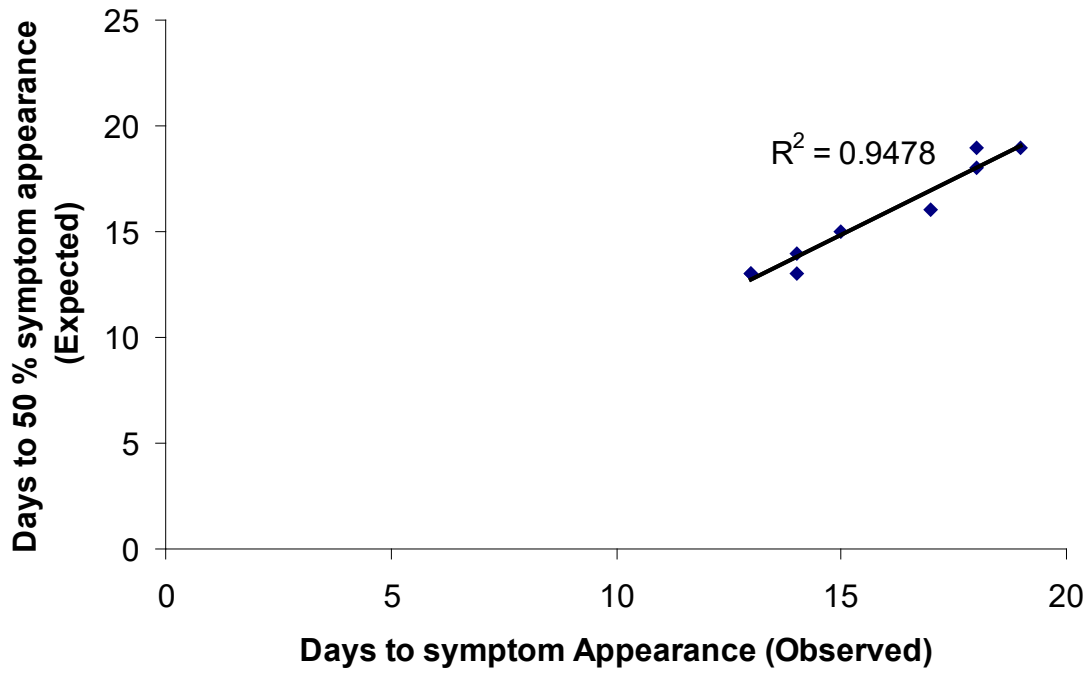


Figure 8. Days to first observed and 50% expected symptom development.

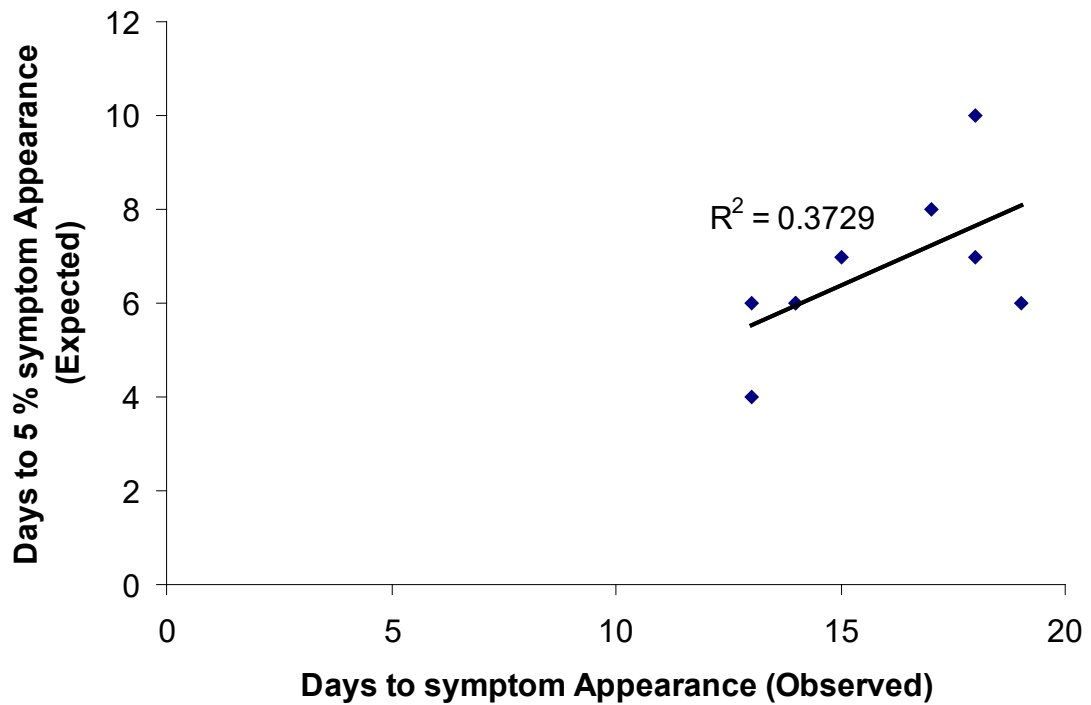


Figure 9. Days to first observed and 5% expected symptom development.

### **4.1.3 Conclusions**

The results show that the predicted time to 50 % symptom development is a good measure of the time to first observed symptom development in the field. In contrast the time to 5 % symptom appearance was not a good measure of time to symptom development in the field. One potential reason for this result arises from the assumption that plants were infected immediately after inoculation. This assumption may result in a more inaccurate relationship between time to 5 % symptom development and expected symptom development. By assuming infection occurs quickly expected symptom development will always occur before observed symptom development. Conversely this may favour time to 50 % symptom development, as a criteria, for white blister symptom development and the results support this finding. Further tests are required where the white blister infection model is used to determine the point of plant infection. This can then be used as the starting point for use of the white blister symptom development model. This approach will be used in further tests with the model on additional data sets. The white blister symptom development model also needs to be tested on plants of differing maturity as this may also be another source of variation that needs to be accounted for.

## **5.0 DISCUSSION**

### **5.1 Development of the white blister symptom development model**

Fungal diseases of vegetable brassica crops occur as a complex. Each has its own specific requirements for development which means that one or other disease can exploit the variety of conditions which occur during the vegetable brassica growing season. Therefore if all diseases are to be predicted information on the environmental criteria necessary for development of each disease will be required. Forecasts, of more than one disease in the crop can result in increasing opportunities for synergy in fungicide sprays. The addition of spray timing forecasts for white blister to the Brassica<sub>spot</sub> system will increase the potential for fewer applications of fungicide. White blister control depends 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 spray timing model will enable more reliance on protectant fungicides such as Amistar. The protectant fungicide can 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 hoped that white blister can be reduced to low levels in the crop removing the need for applications of metalaxyl. The model can also be used to determine when the crop can be checked for signs of disease. When used in conjunction with the white blister infection model the symptom development model can determine when symptoms resulting from infection will appear in the crop. This will reduce the need for crop walking and should mean that growers and producers can determine more accurately when to walk the crop and what diseases to look for.

### **5.2 Testing the white blister symptom development model**

The white blister symptom development model was developed using controlled environment data. For it to be fully reliable it must be extensively tested using information derived under field conditions. This report shows how the model was produced, programmed and tested (under field conditions). A limited number of data sets were available for these tests however these show when the model is used to predict the appearance of symptoms on plants with immature leaves the results are accurate to within one day. Further work is required using data from field plants which is more mature as it appears that this is one source of inaccuracy not accounted for in the model. It is possible that some manipulation of the function might make it more accurate on mature tissue. The model also requires further testing on other vegetable brassica varieties. Both these aspects will be investigated in the year two work. One variable which requires urgent investigation is if vegetable brassica types or cultivars vary in the length

of time required for symptom development. This would be useful information which could be incorporated into the model if significant. Some varieties already appear to vary in the environmental criteria necessary for rapid symptom development.

### **5.3 Practical usage of the white blister prediction system within *Brassica spot***

Combining the white blister symptom development model with those for ringspot and dark leaf spot which are already present within *Brassica spot* should increase the reliability and effectiveness of disease prediction in vegetable brassica crops. New and rapid methods of detecting and quantifying pathogenic inoculum are becoming available. These tests will still require mathematical models such as the white blister symptom development model to be effective. The models will effectively determine when tests for inoculum should be carried out. White blister is not wind borne over great distances because this phase of its life-cycle has large spores (called zoosporegia). Additionally, the infective stage, “the zoospore”, is only produced in the presence of free water and these are splash borne within the crop. It would therefore be less effective to use measures of windborne inoculum for this pathogen in comparison to ringspot or dark leaf spot of brassicas. The long period required at low temperatures for white blister symptom development means that this disease should be easy to control in the early stage of crop development after transplanting. It is possible that much of the infection arises from infected transplant production as only immature tissues are capable of producing symptoms. Additionally oilseed rape is not susceptible to infection by white blister which means that there should be fewer reservoirs of inoculum from which crops can become infected. As a result it is possible that by using the white blister symptom development model there may be a high chance of reducing the incidence of white blister to very low levels reversing the trend with this disease seen in recent years. This will depend on uptake and usage of the model by growers.

### **5.4 Further disease forecasting criteria for foliar diseases of vegetable brassica crops**

A survey of growers conducted in 1995 highlighted their requirement for forecasting systems to predict the occurrence and severity of white blister (*Albugo candida*), dark leaf spot (*Alternaria spp.*) and ringspot (*Mycosphaerella brassicicola*) the three major leaf spots of Brussels sprouts. Fungicides application is the only option for controlling these diseases at present. With information now available on optimal timing of white blister sprays there is now a system available which covers all the major vegetable brassica diseases which occur in the U.K. With the changes in fungicide products available for vegetable brassica crops other problems which were minor in the past have become more prevalent. A good example of this is the powdery mildew pathogen *Erysiphe cruciferarum*. This pathogen has become more prevalent since triadimenol (Bayfidan) lost approval for use in vegetable brassica crops. This has placed more

reliance on other chemicals with activity against powdery mildew notably tebuconazole and sulphur. However the advantage of forecasting systems is that they have the capability for information on new problems to be added in the future while retaining information on existing pathogens. This means that regardless of changes in fungicide regulations information exists which can be used to enhance the activity of those fungicides still available to the grower.

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