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Brassica Leaf Diseases: Development of Disease Forecasting System for Dark Leaf Spot and Light Leaf Spot.

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3 PRACTICAL SECTION FOR GROWERS

In recent years introduction of new cultivars and intensification of cropping has resulted in increasing fungal disease problems within vegetable crops. Many air-borne fungal diseases cause unsightly blemishes which is at odds with the market and consumer demands for high quality produce.

In the past reliance has been placed on routine fungicide usage but, with increasing concern about environmental impact, routine fungicide usage is perceived as costly, wasteful of pesticide and potentially damaging to the environment. Faced with these problems what should the progressive grower do to reduce fungicide usage but maintain quality? If better targeting of fungicide use could be achieved by forecasting disease occurrence quality could be maintained together with reductions in fungicide usage. The increasing efficacy of some newer fungicides might also aid this process.

Several approaches may be made in an attempt to develop practical disease forecasters. The simplest approach is to use conditions which are favourable for the occurrence of key stages in the life cycle (i.e. spore production, infection) to time fungicide application. Where such conditions occur too frequently for practical use, the identification of conditions that favour the completion of a full life cycle of the pathogen may be of value. These types of forecaster may also require modification to include crop growth factors and the effects of geographical location.

The life cycle of pathogenic fungi can be studied under controlled environmental conditions. The effect of critical environmental variables (humidity, temperature and wetness) on disease development can be studied and mathematical models developed to quantify the observed relationships. Use of field studies to validate these relationships are an essential component of the research.

The effect of temperature and surface wetness on infection of brassicas by *Pyrenopeziza brassicae* (light leaf spot) have been investigated under controlled environment conditions. Work describing the observed relationships mathematically is still in

progress. A disease forecaster for *Alternaria brassicae* (dark leaf spot) on Brussels sprouts based on the effect of temperature and surface wetness on infection and of temperature and humidity on sporulation has been developed. The dark leaf spot forecaster predicts when a fungicide spray should be applied for optimal effect or if such a spray is necessary. The number of fungicide applications needed to control the disease without significant reductions in quality can be reduced by using the forecaster to time a spray of iprodione in point **inoculated** plots of Brussels sprouts. However, prediction of episodes of air-borne release of inoculum within the crop may be an important criterion when determining disease occurrence on the buttons. Results of these trials suggest that dark leaf spot infection events on Brussels sprout buttons resulting from splash dispersal of spores require considerably higher levels of foliar disease than similar levels of button infection arising from airborne release of spores. Therefore prediction of airborne release of spores in conjunction with infection criteria may provide an important improvement to the existing system which successfully summarises development of important disease thresholds.

Preliminary trials in 1993 evaluated reductions in the amount of fungicide applied to each crop and the degree of disease control through use of the forecaster in comparison to plots sprayed with routine applications of iprodione and unsprayed control plots. During this season conditions were conducive for high levels of disease development in Brussels sprouts crops by *Alternaria brassicae*. Lesion numbers on leaves throughout the season and on buttons at harvest were measured.

Mean numbers of leaf lesions increased rapidly in unsprayed control plots, after inoculation in early August. High levels of button infection were also observed at harvest in comparison to routine application of three sprays of iprodione. Spraying according to forecast predictions reduced lesion numbers on leaves and buttons to approximately those levels observed in the routine treatment with only one application of iprodione. This represented a saving in the cost of approximately one or two sprays of iprodione.

Although three sprays of iprodione controlled dark leaf spot on the buttons to a higher

degree than on buttons in plots sprayed according to forecaster predictions, the effect was not significant. In the context of a preliminary trial it was impossible to ascertain if this slight reduction in control was economically damaging.

Crop scouting will still be important in identifying the presence of dark leaf spot within the crop. However, when the presence of the disease within the crop is confirmed the forecaster will then be used to determine if and when the disease is likely to build up to a threshold level that, with the onset of airborne spore release, would lead to economic damage on the buttons. The relationship between the amount of disease on the plant and subsequent economic damage could be defined from the criteria known to result in button infection. In this way it may be possible to build into the forecaster some indications of economic impact of the disease on the crop.

In further trials within **uninoculated** commercial crops we are hoping to improve the forecaster predictions of dark leaf spot and ascertain the relationship between these levels of disease and economic impact on Brussels sprout crops. However, some problems still need to be overcome. For example, accurately determining wetness within the crop will be essential. It is still to be determined if wetness within a crop is uniform or can be influenced by topography. These considerations will ultimately determine over what area disease forecaster predictions may extend. The variation in wetness over crop area will be determined in forthcoming trials and it is hoped that this will increase the accuracy of forecast predictions.

In the future, further work may result in a series of predictions for other important air-borne vegetable diseases such as light leaf spot which could be combined with those criteria used for forecasting dark leaf spot. The grower will then have a series of indicators or predictions to aid decisions on crop inputs and assist him in improvement of quality, reduction of costs and minimising of effects on the environment.

4 SCIENCE SECTION

4.1 INTRODUCTION

Dark leaf spot (*Alternaria brassicae* and *Alternaria brassicicola*) and light leaf spot (*Pyrenopeziza brassicae*) are foliar fungal diseases affecting all types of commercial brassica crops. Both diseases are important on Brussels sprouts and form necrotic lesions on leaves, petioles, stems and buttons of all sprout cultivars. If the disease becomes established on the Brussels sprout buttons the blemishes caused can lead to a reduction in grade (Gladders, 1984).

The pathogens are disseminated from infected host tissue by means of spores produced on mature fungal lesions. Spore dispersal can occur by a number of mechanisms including air currents, rain splash or dew droplets. Dispersal in air is potentially over much greater distances than by rain splash. Viable spores landing on healthy plant tissue germinate on and penetrate the host surface (McDonald, 1959; Rawlinson *et al.*, 1978) to form necrotic lesions.

Infection and spore production are important parts of the fungal life cycle (**appendix I**) and depend on environmental conditions at the host surface. Spore production on the lesions of many fungal species is known to require high humidity and similarly the infection process needs the presence of free water on the host surface (leaf wetness) (Rawlinson *et al.*, 1978).

The relationships between important environmental parameters - notably air temperature, humidity and leaf wetness - and the stages in the fungal life cycle, can be accurately determined in controlled environment experiments (Humpherson-Jones, 1991). Relationships derived from these experiments can be used to predict infection or sporulation in the field based on measurements of meteorological parameters within the crop.

Where infection and sporulation occur frequently, and the pathogen completes its life cycle relatively rapidly, prediction of complete cycles of sporulation and infection (**appendix I**) from microclimate measurements can be used as an aid in determining the necessity of control measures (Humpherson-Jones, 1991). This approach has already been used in other crop / disease systems. Some predictive systems are based on just one life cycle stage , for example sporulation of onion leaf blight, *Botrytis squamosa* (Lacy & Pontius, 1983). More complex models have taken account of both sporulation and infection of onion leaf blight (Sutton, James & Rowell, 1986). Other advanced models take into account host factors such as growth or resistance. The VENTEM™ system for apple scab (*Venturia inequalis*) predicts disease on apple cultivars of varying susceptibility (Butt *et al.*, 1992).

An alternative strategy is to draw inferences from field measurements of crop disease and environmental parameters. A regression model can be constructed to give predictions of disease from meteorological data. The onion leaf blight predictor, Inoculum Production Index, developed in New York State, USA uses this methodology (Vincelli & Lorbeer, 1988). However disease predictions derived from this type of model can be subject to inaccuracy when used in different locations. The relationships derived at one site may not apply under different sets of climatic conditions found at other locations.

The approach of constructing a model from an understanding of the pathosystem biology was adopted in this study as this should lead to a more widely applicable, portable disease predictor.

As some laboratory investigations of the dark leaf spot life cycle have already been undertaken (Humpherson-Jones & Phelps, 1989; Humpherson-Jones, *pers. comm.*) the work on dark leaf spot concentrated on constructing and testing models to predict disease thresholds. As *Alternaria brassicae* is the more prevalent of the two fungal species causing dark leaf spot in horticultural ware crops, field experiments have concentrated on this pathogen. Although a difference in optimum temperature for infection has been observed for the two *Alternaria* species causing dark leaf spot

(Humpherson-Jones & Hocart, 1983) this is not expected to affect the field epidemiology.

Little information is available on the epidemiology of the light leaf spot pathogen *Pyrenopeziza brassicae*. Experiments have concentrated on determination of the periods of leaf wetness required for infection of host tissue at different temperatures in controlled environments.

Infected oilseed rape crops have been implicated as a possible source of both diseases in horticultural brassica crops (Gladders, 1984; Humpherson-Jones, 1984). Field experiments took place to determine the role of this potential inoculum source.

Investigations in horticultural crops have been restricted to Brussels sprout (*Brassica oleracea* var. *gemmifera*) crops at this stage. It is expected that some further work will be necessary before extension of the principles to other brassica crops can take place.

4.2 MATERIALS AND METHODS

4.2.1 Model Development

4.2.1.1 Light leaf spot controlled environment experiments

Controlled environment experiments are used to accurately determine the relationship between a single environmental parameter and the completion of important parts of the fungal life cycle (**appendix I**). Previous experiments have determined the temperature and wetness requirements for infection by the dark leaf spot pathogen *Alternaria brassicae* (Humpherson-Jones, *pers. comm.*). In this experiment the effect of varying the period of leaf wetness over a range of temperatures was investigated for the light leaf spot pathogen, *Pyrenopeziza brassicae*. Preliminary glasshouse experiments showed that a period of at least 40 hours of constant leaf wetness was necessary for significant infection at temperatures close to the reported optimum of 15°C (Staunton, 1967).

To investigate the effect of temperature and leaf wetness on infection more fully three replicate experiments were set up. In each experiment one chamber was maintained at each of the following seven temperatures: 4°, 8°, 12°, 16°, 20°, 24° and 28°C under ambient light levels. Brussels sprouts (cv. Aries) were grown in ten-cell modules cut from PG84 trays until the seedlings were at the three to five expanded true leaf stage. Twenty-two modules were placed in each chamber and the seedlings were allowed to equilibrate for 48 hours at each temperature prior to the commencement of leaf wetting.

The seedlings were briefly pre-wetted and were then evenly sprayed to run-off, using a Humbrol paint sprayer, with a suspension of 10^5 conidia ml^{-1} (isolate 1091, obtained from oilseed rape) in sterile distilled water. Conidial suspensions were produced by agitating two week old cultures of this isolate (**appendix IV**), grown on 2% Malt Extract Agar (**appendix V**) at 15°C, in chilled sterile distilled water.

Leaf wetness was maintained by misting the seedlings at regular intervals. Two modules were removed at random, from each chamber, after leaf wetness periods of 0, 1.25, 1.75,

2.25, 2.75, 3.25, 3.75, 4.5, 5.25, 6 and 7 days. After drying with cool air seedlings were transferred to a glasshouse and bottom-watered for one month to allow symptom development. The oldest two leaves on each plant were assessed for number of light leaf spot lesions.

For each replicate experiment there was an uninoculated control chamber at 16°C. On no occasion was any disease observed on these plants. In a further experiment at 16°C infection on Brussels sprouts seedlings cv. Aries by isolate 1111 obtained from cauliflower was compared with that of isolate 1091, obtained from oilseed rape.

4.2.1.2 Light leaf spot *in vitro* experiments

Symptoms of light leaf spot are variable , and *in vitro* experiments with fifteen isolates of various origin (**appendix IV**) compared colony morphology, *in vitro* spore production, spore germination, infectivity and symptom expression.

Three replicate Petri dishes (9cm Sterilin) per isolate were inoculated for each of three media: PDA, PLYSE (**appendix V**) and 2% Malt Extract Agar. A plug of agar from a ten day old culture of each isolate was sub-cultured on to the dish. The dishes were placed in a randomized block design in a 15°C incubator. Colony diameter was measured at 1, 2, 3, 4, 5 and 6 weeks.

Fifteen Petri dishes (2% Malt Extract Agar) of each isolate, with 6 colonies per dish were grown as before with dishes placed in randomized positions in a 15°C incubator. Three dishes of each isolate were sampled destructively at each of 2, 3, 4, 5 and 6 weeks. Each dish was washed with 5ml of sterile distilled water and agitated with a sterile angled glass rod for 30 seconds. The resulting suspension was filtered through a layer of muslin to remove mycelial fragments and the spore concentration determined using a haemocytometer (Improved Neubauer, depth 0.1mm, 1/400mm^2, Weber, England).

Spore germination was assessed by plating 0.25 ml of each spore suspension produced as above from 2, 4 and 6 week old colonies on to a Petri dish of distilled water agar.

Petri dishes were incubated at 15°C (arranged as a randomized block design) for 24 hours before sections of agar (*c.* 4 x 2 cm) were removed from each plate and mounted in cotton blue/lactophenol for microscopic examination. Percentage germination counts were taken with germination considered to have occurred when the germ tube was greater than spore length.

Infectivity of isolates was checked by pooling suspensions from 2 week old colonies for each isolate and spraying on to the pre-wetted leaves of ten Brussels sprout seedlings (cv. Aries) at the three to five expanded true leaf stage. Leaf wetness was maintained by growing the plants inside a sealed plastic bag for 6 days. Disease assessments were taken on plants one month later.

4.2.2 Model Optimisation

Controlled environment experiments have previously been undertaken to investigate the effect of temperature and leaf surface wetness on infection (Humpherson-Jones, *pers. comm.*) and temperature / humidity on spore production (Humpherson-Jones & Phelps, 1989) of the dark leaf spot pathogen *Alternaria brassicae*. Basic models describing the effect of these environmental parameters on infection and sporulation were constructed (see section 4.3.1). The models and assumptions inherent in them were tested using the following measurements taken in crops of Brussels sprouts infected with the dark leaf spot pathogen.

4.2.2.1 Spore trapping

The quantity of dry air disseminated spores present throughout the growing season was measured using a suction spore trap (Burkard Manufacturing Co., Rickmansworth, UK) located in the centre of the infected field crop in 1991, 1992 and 1993. The number of *Alternaria brassicae* spores trapped each hour was counted microscopically.

4.2.2.2 Trap plants

Trap plants, cv. Aries (1991, 1992) and cv. Sheriff (1993), were exposed for periods of either one or three days close to the suction spore trap to assess field infection by the pathogen. Plants were exposed and collected at 1100h GMT as foliage had normally dried at that time. Ten glasshouse raised Brussels sprout plants in FP9 pots, at approximately 8 weeks from sowing in August (or equivalent growth stage), were exposed in the crop each day. Exposed plants were air dried and grown under glasshouse conditions without further wetting of leaf surfaces. At 3 weeks after commencement of incubation the oldest 7 leaves of each plant were assessed for numbers of lesions per leaf. In 1992 each lesion developing on exposed trap plants was isolated on to PLYSE agar medium. The subsequent isolation was examined microscopically for the presence of *Alternaria brassicae* or *Alternaria brassicicola*. Meteorological conditions were monitored within the infected crop as described in section 4.2.3 .

4.2.3 Forecaster Validation

Dark leaf spot models were formulated from controlled environment data and optimised using the methods outlined in section 4.2.2 . Dark leaf spot disease development data collected from field experiments were used to validate models.

4.2.3.1 Disease development

Dark leaf spot disease development was studied in a 15m x 15m plot of Brussels sprouts cv. Aries (1991 and 1992) and cv. Sheriff (1993). Crop spacing was 60cm and agronomic inputs followed standard practise (see **appendix II**).

In 1991 and 1992 a neighbouring plot of highly susceptible Chinese cabbage (cv. Nagaoka 60) was inoculated with a spore suspension ($5x10^4ml^{-1}$) of *Alternaria brassicae*. Heavily infected trash collected from this plot was spread evenly at the base of the Brussels sprout crop in July in both 1991 and 1992. In 1993 the plot was sited close to the previous year's experiment which had gone through to seed and provided a heavy inoculum source for infection of the new crop at the end of July.

Electronic sensors were sited 30cm from the ground in a small clearing in the crop canopy to measure crop microclimatic parameters. Measurements of air temperature, relative humidity, rainfall and leaf wetness were taken at hourly intervals and stored on an electronic data logger (Delta-T devices, Burwell, Cambs.). Rainfall was measured outside the plot to minimize innaccuracy.

Dark leaf spot lesions were recorded on ten plants which were selected as a stratified sample of five plants along each of two transects in the plot. In an effort to minimise edge effects no sampled plant was closer than 2m from the edge of the plot. At the time of crop inoculation the oldest 60 green leaves of each plant were labelled. The number of dark leaf spot lesions on each leaf (up to a maximum of 25 lesions on each leaf) was recorded regularly.

Disease progress was assessed on the Brussels sprouts buttons from when they first

started to form. Buttons were randomly sampled from the lower, middle and upper thirds of the stem on each of five plants surrounding that assessed for leaf disease. Button diameter and number of lesions were recorded for each of the 150 buttons sampled at each assessment.

4.2.3.2 Fungicide interaction experiment

Four 10m x 10m plots of Brussels sprout cv. Sheriff were isolated from each other to reduce inter-plot interference.

Two plots were inoculated at the beginning of August by using Brussels sprout straw infected with *A. brassicae*. A single application of fungicide was made on 8th October with the timing determined by a prototype *Alternaria* disease forecasting model (section 4.3.2.2). Iprodione was applied to plot 1 (Rovral at 2l ha^{-1} in 200-600l), with chlorothalonil sprayed on plot 2 (Bravo at 3l ha^{-1} in 1000l + Agral at 300ml ha^{-1}).

Dark leaf spot in the two additional plots was controlled with chlorothalonil (Bravo at 3l ha^{-1} in 1000l + Agral at 300ml ha^{-1}) sprays on the 6th and 26th August. Both plots were inoculated as described above during the middle of October.

Dark leaf spot lesions were assessed on twelve tagged plants per plot, three on each of four transects from the centre of the plot (the initial source of inoculum). The oldest fifty leaves per plant were labelled at the end of July. Leaves 1 to 25 were assessed for numbers of lesions in both plots inoculated during August while leaves 21 to 50 were assessed for lesion numbers in both plots inoculated during October.

Button disease was assessed regularly on all plots from commencement of button development. Buttons were randomly sampled from the lower, middle and upper thirds of the stem on each of five plants surrounding that assessed for leaf disease. Button diameter and number of lesions were recorded for each of the 180 buttons sampled at each assessment.

4.2.3.3 Disease transfer from oilseed rape

Field experiments were undertaken to study the potential transfer of both leaf spot diseases from oilseed rape to Brussels sprouts. A 15m x 15m plot of oilseed rape cv. Cobra was sown in the Autumn of 1991. A plot of Brussels sprout cv. Aries was transplanted adjacent to and downwind of the original plot of oilseed rape in May 1992. Fifty oilseed rape plants were inoculated early in February 1992 with a conidial suspension ($10^5 ml^{-1}$) of the light leaf spot pathogen *Pyrenopeziza brassicae* (isolate 1091). Very few disease lesions appeared on these plants, despite a repeated inoculation during March. The experiment was abandoned as disease had failed to reach significant levels by time of harvest and no transfer of light leaf spot to the Brussels sprout plants had been observed.

The experiment described above was repeated in 1993. The result was a similar absence of disease - possibly due to adverse weather conditions for the pathogen in the spring of these two years. A similar design of experiment was set up to study the transfer of dark leaf spot in 1993, using oilseed rape cv. Capricorn. In this experiment a poor stand of rape in the plot prevented build up of disease and any potential transfer into the Brussels sprout crop was precluded.

4.3 RESULTS

4.3.1 Model Development - Dark Leaf Spot

4.3.1.1 Analysis of controlled environment data and model construction

Environmental requirements of dark leaf spot (*Alternaria brassicae*) infection were determined from controlled environment experiments as outlined previously (section 4.2.1), using Brussels sprout cv. Aries. Rate of infection and eventual maximum infection varied around the same optimum temperature. At each experimental temperature most infective penetrations of the host surface occurred over a relatively short time span, indicating that there was little difference between spores in rate of infection. The lowest variation in rate of infection was observed near the optimal temperature.

For each temperature the time for 50% of leaves to become infected, and the maximum percentage of leaves showing infection was evaluated using the probit analysis procedures of the Maximum Likelihood Program (MLP) (Ross, 1987). Since no significant difference was detected between replicates at each temperature the data for three replicates was pooled. Using procedures in MLP (Ross, 1987) and GENSTAT (Payne *et al*, 1987) curves were fitted for the rate of infection (reciprocal of time to 50% infection) against temperature (function I), and maximum potential infection against temperature (function II). Multiplication of the two functions gives a rate function (function III) for an equivalent degree of infection at different temperatures and allows interpolation for temperatures other than those tested.

Hourly integration of function III to a threshold level (from time to 50% infection) indicates the time to an equivalent level of infection across temperatures (**Fig 1**). Function III is a form of thermal time function. It may be integrated for fluctuating hourly temperatures in the presence of leaf wetness, and if the threshold is reached infection is then said to have occurred. If leaf wetness terminates prior to the threshold it is assumed that the spore germ tube will be destroyed and the function is integrated from zero again at the onset of the next leaf wetness period. Environmental conditions

may exist where this assumption is false and the germ tube is not destroyed. Function III forms the basic infection model for the temperature range 6°C to 26°C.

Similar methods had previously been used to formulate a model for spore production on dark leaf spot lesions using controlled environment data (Humpherson-Jones & Phelps, 1989). A function describing the rate of spore production against temperature in the presence of high humidity was determined (Phelps, unpublished). A vapour pressure deficit (VPD) of less than three is required for the function to operate (equivalent to a high relative humidity). Spore production could continue with intervening periods of up to two hours of lower humidity - which is included in the basic spore production model. The time to spore production under constant temperatures and vapour pressure deficit of less than three is shown in **Fig 2.**

Dark Leaf Spot Environmental Requirements

FIG 1

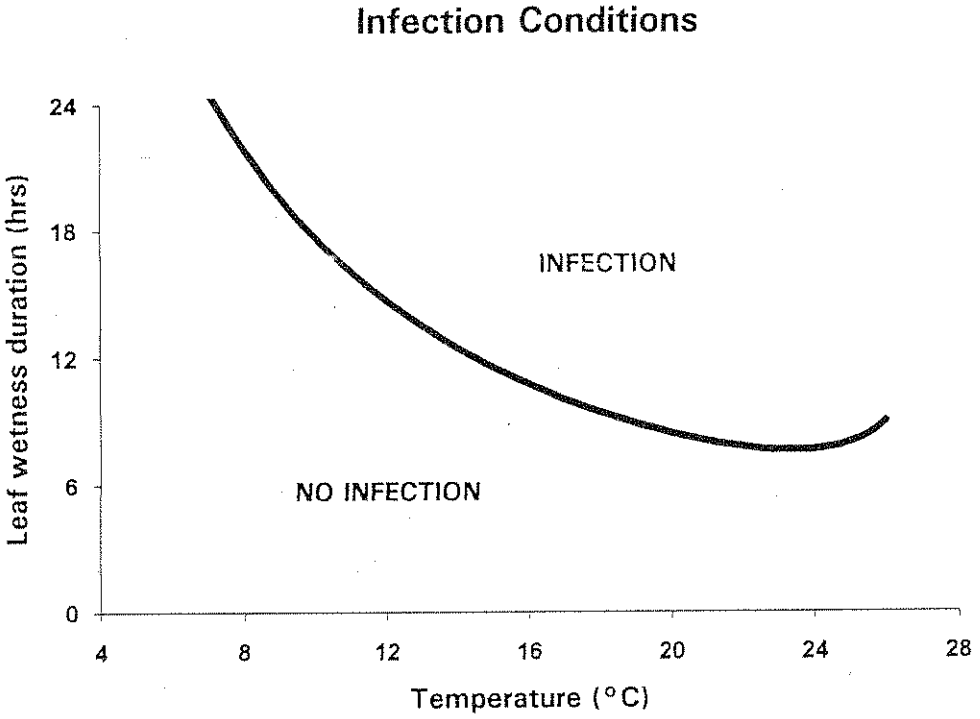
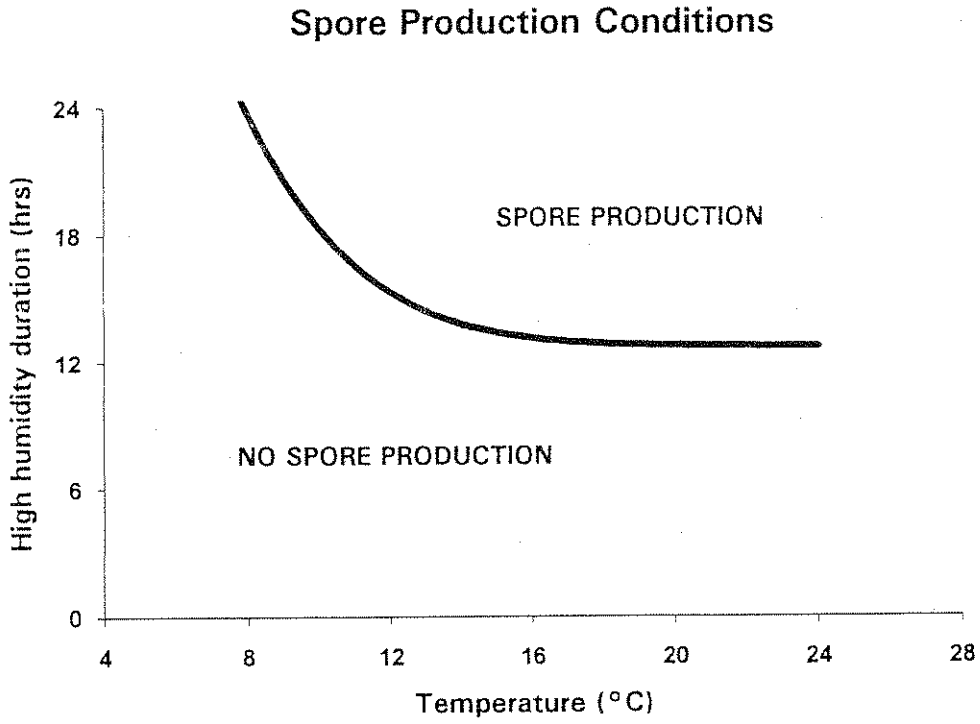


FIG 2



4.3.2 Model Optimisation - Dark Leaf Spot

4.3.2.1 Spore trapping

No conclusions could be drawn from the results of suction spore traps in 1991 as insignificant numbers of spores were trapped. The results from 1992 show in three ways that spore trapping is of limited usefulness.

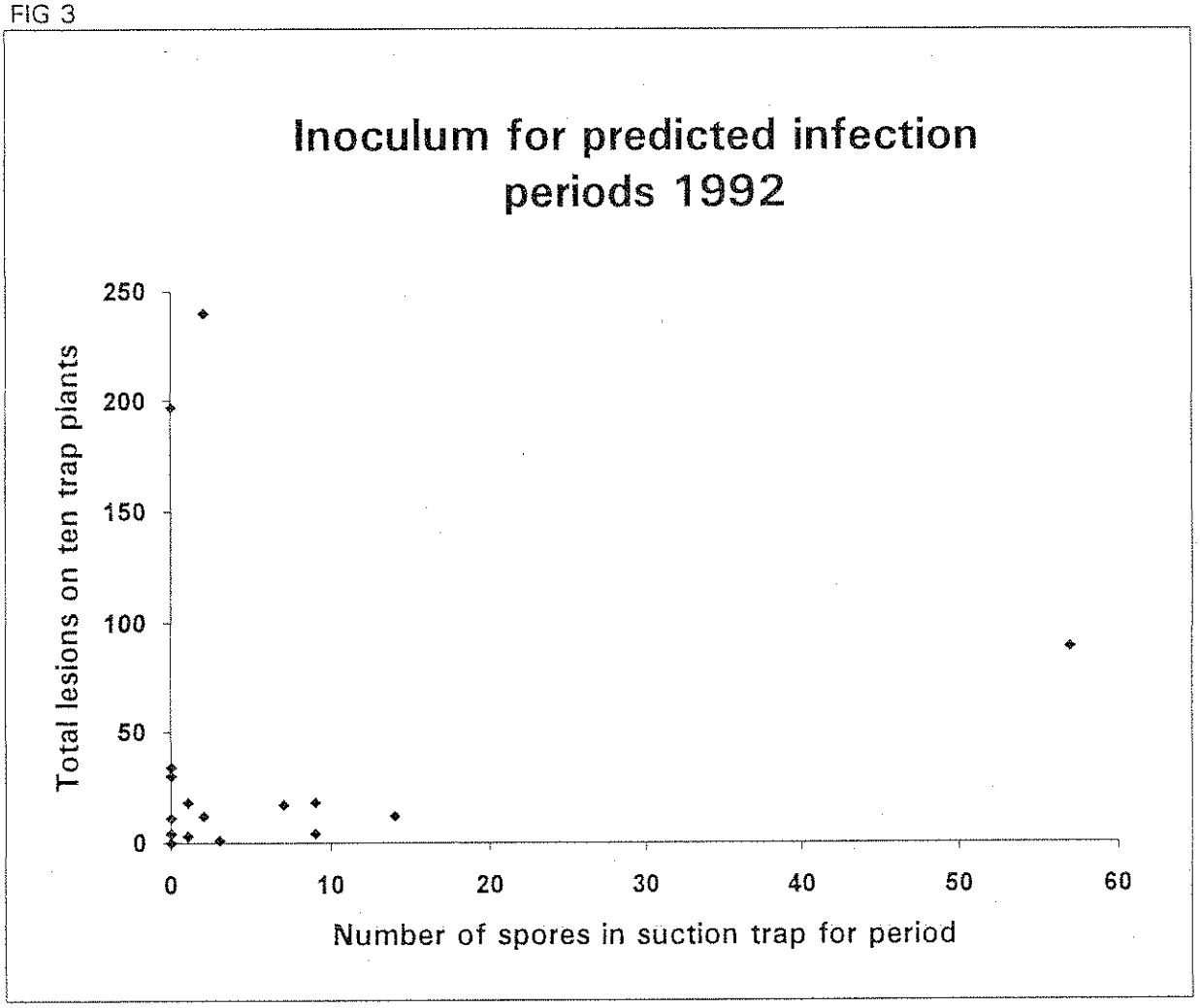
i) There would not be a direct relationship between spore production model predictions and suction trap observations because the latter detect only disseminated airborne spores. Spores may remain on the lesion for a number of days before they are released and dispersed.

ii) Spores can only be detected by suction traps if they are airborne. However, after the onset of winter conditions in mid October no spores were trapped, despite new infections in the crop, suggesting other methods of dissemination.

iii) The number of lesions arising on trap plants after predicted infection periods (**Fig 3**) (a measure of incident inoculum) is unrelated to the number of spores trapped. The suction trap results are not a good measure of total inoculum pressure with many splash dispersal episodes, not recorded by the suction trap, resulting in infection.

On the basis of this the results for 1993 have not been summarized.

FIG 3


Inoculum for predicted infection periods 1992
Total lesions on ten trap plants
250
200
150
100
50
0
0
10
20
30
40
50
60
Number of spores in suction trap for period

4.3.2.2 Trap plants

Trap plants were exposed for 24 and 72 hour periods to isolate potential infection events from the overall epidemic pattern. The basic infection and spore production models, developed in section 4.3.1, were tested against the observed levels of trap plant infection. The methodology assumes that there is little difference in the environmental requirements for infection on field grown plants in comparison to those used in controlled environment experiments.

Isolations taken from disease lesions produced on trap plants in 1992 were mainly due to infection by *Alternaria brassicae*. There was little infection by *Alternaria brassicicola* in the experimental crop until seed production in the following year. This supports the assumption that *A. brassicae* is the main cause of dark leaf spot in horticultural ware crops. Results from trap plants exposed in the field during 1991 were inconclusive.

The *A. brassicae* basic infection model explained most of the variation shown in levels of infection on trap palnts in 1992. False negative predictions of infection (disease not predicted but observed in the field) were reduced by extrapolating the infection model in the range from 6° to 0°C. Inaccuracies were further reduced by introducing the assumption that germinating spores are not destroyed in the absence of leaf wetness if the air temperature is below 8°C with vapour pressure deficit of under 2. The few remaining false negatives may be explained by prolonged periods of leaf wetness in late autumn, after heavy dew or rain, which were not detected by the current design of electronic wetness sensor.

The basic spore production model is a more accurate predictor of the absence of inoculum in the field (section 4.3.2.1), in prolonged relatively less humid periods, than an accurate guide to its presence. To reduce the false positive predictions (disease predicted but not observed in the field) spore dispersal conditions must be detected. Analysis of the distribution of lesions on trap plants showed that after 16th October 1992 a majority of lesions were clustered and were likely to have arisen from splash dispersed spores. This date corresponds to the onset of reduced temperature conditions in 1992 (**appendix III**), with marked reductions in mean and minimum air temperature. More

pronounced leaf wetness from dew and rain and less evaporative drying were observed after this date. Drying of the sporulating lesions is required for airborne dissemination of inoculum. This rarely occurs under the environmental conditions that were observed after 16th October 1992. It is probable that relatively heavy rainfall is required for spore dispersal at that time of the season. This hypothesis has been confirmed by suction spore trap results (section 4.3.2.1) which show no dry disseminated spores after the marked reduction in air temperatures.

In the early part of the dark leaf spot epidemic (July and August) inoculum production within the crop will be relatively low due to the small lesion size. The potential for inoculum production within the crop will increase as the lower leaves become senescent and the lesions on them expand rapidly. This was observed at the end of August during the 1992 season.

By incorporating the above assumptions into the infection and spore production processes a compound disease forecasting model has been constructed which predicts the observed trap plant infection levels correctly on 86% of occasions in 1992 (**Table 1**). In 1993, when lesion numbers were higher and trap plants were exposed at the time of year when infection was most likely, the model predicted 73% of trap plant infection periods (**Table 2**). An improved leaf wetness sensor would reduce the false negative predictions and new designs of leaf wetness sensor are currently under test.

TABLE 1

Dark Leaf Spot on Trap Plants 1992

(Number of trap plant exposure periods)

Predicted Severity \ Observed Lesions	0	1 - 15	16 - 30	31 - 45	> 45
0	33	23	1	1	2
0.25	3	2	1		
0.5			1		1
1	1	2	2	1	2
2		4	1	1	2

Summary

Predicted Severity \ Observed Lesions	Low	High
Low	61	5
High	7	11

TABLE 2

Dark Leaf Spot on Trap Plants 1993

(Number of trap plant exposure periods)

Predicted Severity	Observed Lesions: 0	1 - 15	16 - 30	31 - 45	> 45
0	6	16	4	2	
0.25					
0.5					
1		3	2	1	3
2		2	1	1	

Summary

Predicted Severity	Observed Lesions: Low	High
Low	22	6
High	5	8

4.3.3 Forecaster Validation - Dark Leaf Spot

4.3.3.1 Disease development

The results of the 1991 field experiments are shown in **Fig 4.** Disease assessments are subdivided into three areas of the plant (strata) which were designated as the lower, middle and upper canopy.

Disease development on the leaves (**Fig 4a**, mean of 200 leaves/stratum), arising from the inoculum introduced into the plot at the end of July, reached the assessment threshold of 25 lesions per leaf by the middle of August in the lower part of the canopy. Leaves of the middle canopy became infected by this time and showed a series of increases, reaching the assessment threshold early in November. The upper canopy showed low levels of disease by mid September which increased sharply during mid October.

Disease on the Brussels sprout buttons (**Fig 4b**, mean of 50 buttons /stratum) increased during early October, soon after the buttons started to expand, with the maximum infection levels reached by late November. Buttons situated on the lower stem showed the greatest increase in disease levels however the upper buttons remained virtually disease free. Disease levels on the buttons were generally relatively low.

The disease forecating model (section 4.3.2.2), which included a time factor of seven days to allow for symptom expression, was run on the crop microclimate data (**Fig 4c**). The increase in predicted disease after 20th September corresponded with an increase in observed disease on middle and upper leaves and on the buttons. The slow increase in disease predicted during August and early September corresponded with the disease levels observed on the middle and upper leaves during this period. The model predicts the timing of disease increase on the lower leaves in early August.

In 1992 fewer leaf disease assessments were made (**Table 3**, mean of 200 leaves/stratum) after the crop was inoculated during July. There was a large increase in disease incidence in the middle and upper canopy between the middle and end of September

1992. The forecasting model predicts the timing of this disease expansion accurately. Predictions outside this time period cannot be validated due to the absence of observations.

A similar pattern of leaf disease development occurred in 1993 as in 1991 (**Fig 5a**). After inoculation in late July disease on lower leaves increased rapidly. However, the middle and upper leaves remained relatively disease free until late September, when disease in both parts of the canopy increased rapidly to the assessment threshold of 25 lesions per leaf. Disease development as predicted by the disease forecasting model (**Fig 5b**) corresponded to the pattern of disease development observed on the middle and upper canopy. Predicted disease development on the lower canopy was less accurate.

Disease development experiments had high levels of inoculum applied which may have explained any differences between the levels of predicted disease increase and that observed within the plot, particularly within the lower canopy. A different pattern of disease development may occur when model predictions are applied to uninoculated field crops (provided a significant initial source of inoculum is not situated nearby).

Disease Development 1991

FIG 4a

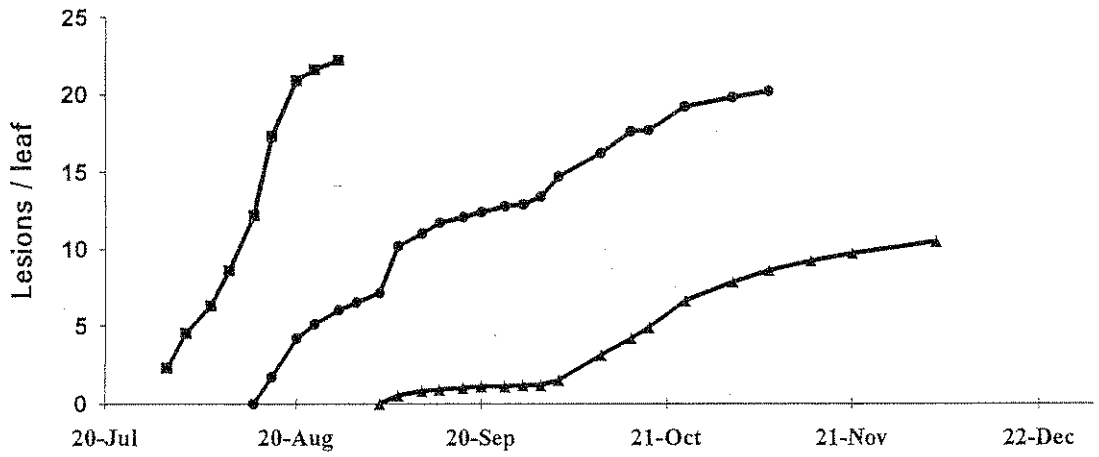


FIG 4b

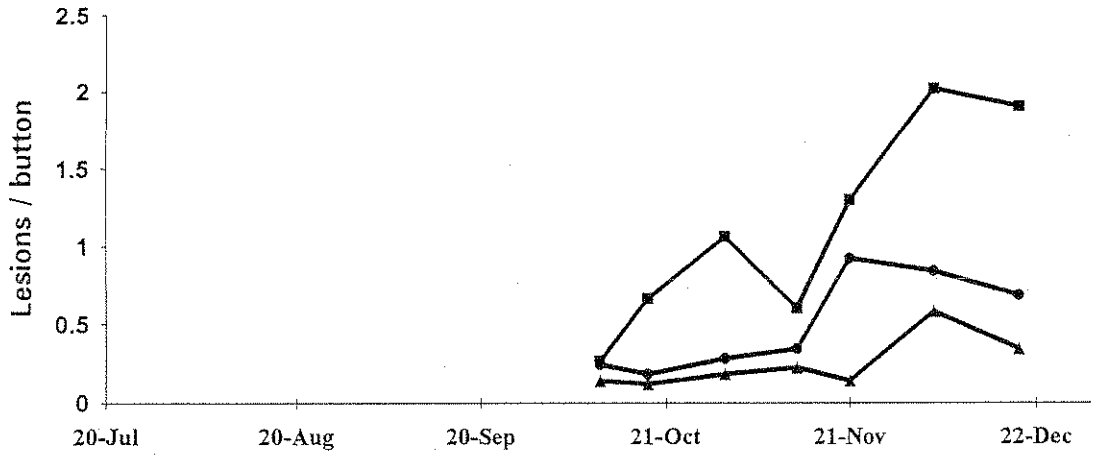
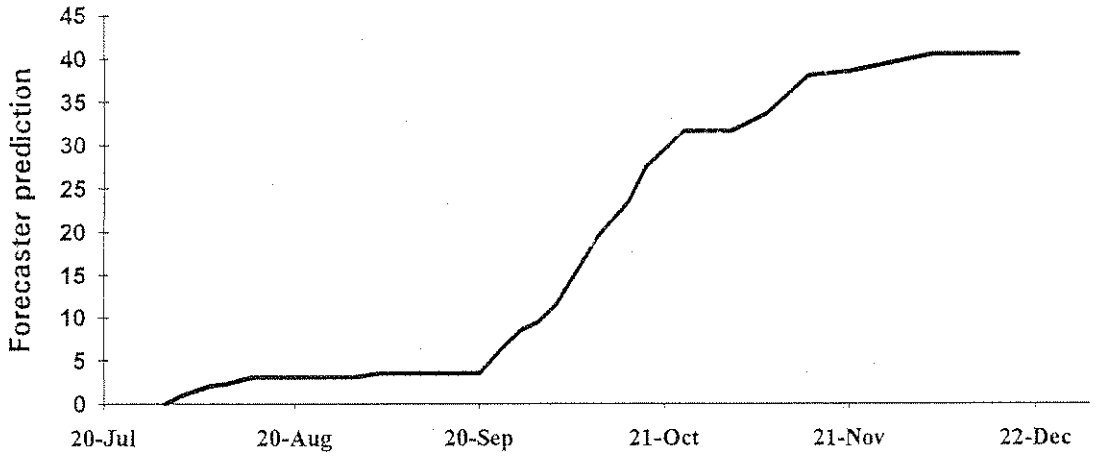
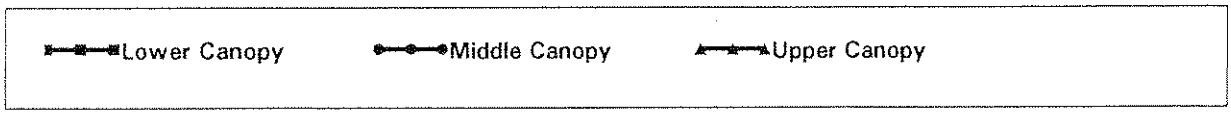


FIG 4c





Disease Development 1992

TABLE 3

				Mean lesions / leaf (200 leaves)		
Date		Forecaster		Lower	Middle	Upper
		Prediction		Canopy	Canopy	Canopy
31/8/92		4.25				
15/9/92		6.75		22.6	10	4.1
29/9/92		23.75		24.4	22.5	22.8
10/10/92		38.75				
15/11/92		39.25				
23/11/92		45.25				

Disease Development 1993

FIG 5a

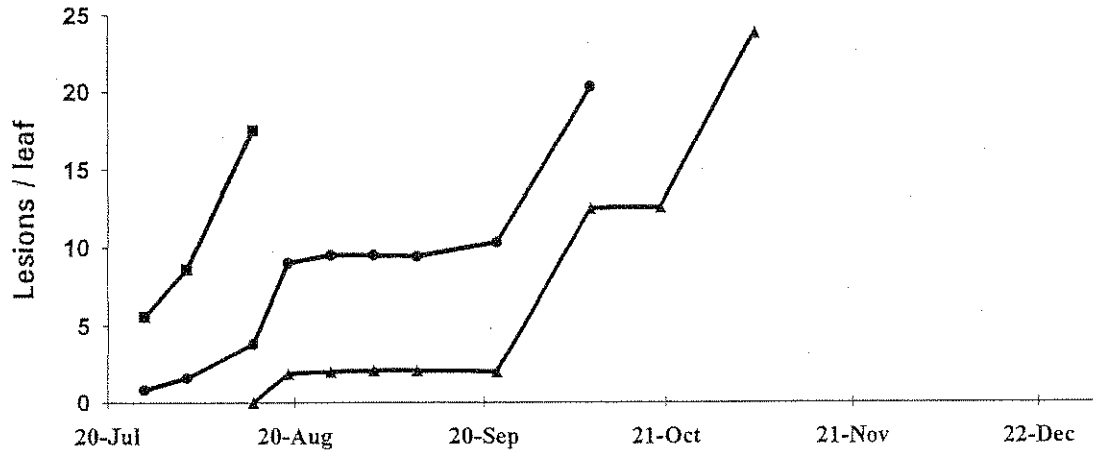
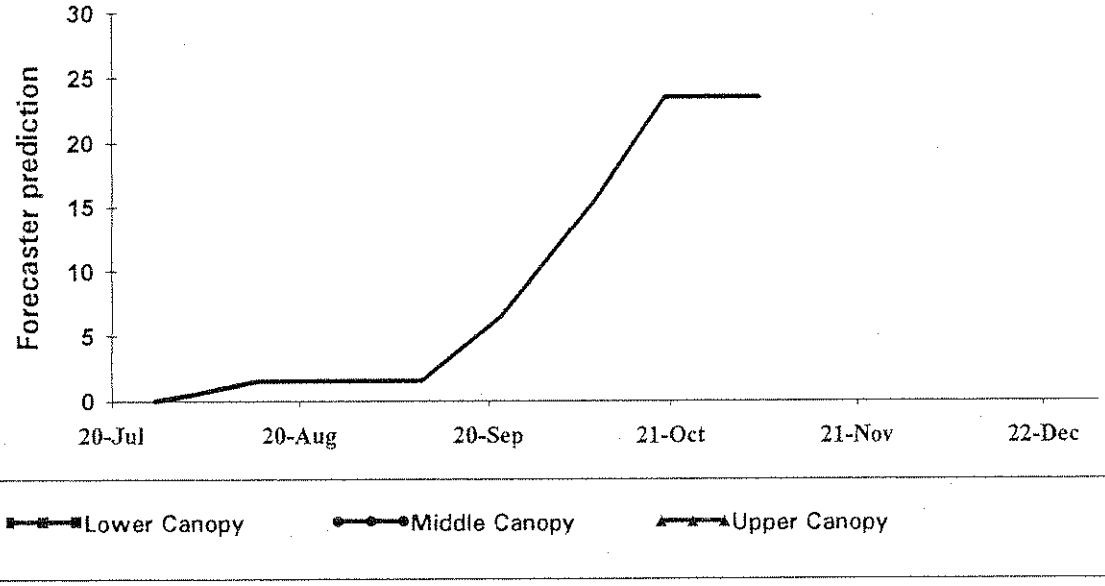


FIG 5b



4.3.3.2 Fungicide interaction experiment

Levels of dark leaf spot leaf disease in plot 1, point inoculated during August, (**Fig 6a**, mean of 300 leaves) remained low until the end of September. At this time an abrupt increase was observed on the lower tagged leaves, which was predicted by the disease forecasting model (**Fig 6c**). Application of iprodione on the 8th October was 10 days later than intended from the prototype model prediction. Levels of button disease (**Fig 6b**, mean of 60 buttons/stratum) in the three areas of the crop canopy did not increase after 21st October, despite a relatively high initial incidence due to the late application of the control spray. A further rise in button disease in late November was predicted but the application of iprodione appears to have prevented any further increase in dark leaf spot disease. Lower leaf disease incidence in plot 2, point inoculated during August, (**Fig 7a**, mean of 300 leaves) was almost identical to plot 1 (**Fig 6a**). In the second plot control of disease was by application of chlorothalonil which was similarly delayed. Approximately the same level of initial button disease was observed in plot 2 (**Fig 7b**) as in plot 1 however disease incidence in plot 2 increased as predicted by the disease forecasting model (**Fig 7c**), with the lower buttons heavily infected. A single application of chlorothalonil did not prevent the predicted increase in disease in late November that was observed on the Brussels sprout buttons.

Disease development was followed on two similar plots point inoculated in mid October. The middle canopy leaves of plot 1 (**Fig 8a**, mean of 360 leaves) remained relatively free of dark leaf spot until plot inoculation. Observed disease incidence increased slowly throughout November which was similar to predicted increases in disease (**Fig 8c**). Disease incidence on buttons (**Fig 8b**) increased on those situated on the lower and middle sections of the stem from the end of November onwards, which was also predicted by the disease forecasting model. However, protectant applications of chlorothalonil in August did not prevent plot 2 becoming heavily infected prior to plot inoculation. Middle canopy leaf disease (**Fig 9a**, mean of 360 leaves) increased rapidly during November. The high levels of dark leaf spot in the plot prior to inoculation make comparison of observed disease with predicted disease more liable to inaccuracy. Button disease incidence in plot 2 (**Fig 9b**) increased to high levels, thus any comparisons between plots could not be valid due to the different amounts of inoculum in each plot.

The time of disease increase observed on both leaves and buttons of plot 2 corresponds to predicted disease increase (**Fig 9c**).

Spores of dark leaf spot may be entirely dispersed in splash droplets after the onset of low temperature winter conditions (section 4.3.2). Any increase in disease incidence on buttons on the upper stem would be dependent on the degree of splash dispersal and the location of mature sporulating lesions on the plant.

Plants with higher levels of middle canopy leaf infection should give higher levels of upper stem button infection. Observed infection on upper stem buttons in the October inoculated plot 1 (**Fig 8b**) did not increase from initial levels. Levels of leaf disease were higher in plot 2 and upper stem button disease levels also increased (**Fig 9b**).

The upper stem buttons may however be less susceptible to infection by the pathogen and differences between plots may be explained by differential button development. However initial button disease incidence is relatively high at all three stem heights in both the plots inoculated during August (**Fig 6b** and **Fig 7b**), indicating that there is little difference in susceptibility. In plot 2 (**Fig 7b**) the rate of button disease increase is obviously related to the height of infection in the canopy, faster rate of infection on the lower buttons and the slowest infection rate on the upper buttons which is more evidence supporting the role of splash dispersal at this time of the year. This observation may however be explained by wetness duration being greater on the surfaces of buttons on the lower stem than on higher, more exposed plant parts.

4.3.3.3 Disease transfer from oilseed rape

The difficulties encountered in establishing either disease in oilseed rape have prevented any conclusions being made on this aspect of the epidemiology of the two leaf spot diseases. The climatic conditions encountered in the spring may only intermittently allow disease to build up to potentially damaging levels if transfer to horticultural brassicas occurred. It is probable that oilseed rape would only account for a proportion of the important initial inoculum in horticultural epidemics. Further work is necessary to establish the importance of oilseed rape, and the relationship of disease levels to occurrence and development of both diseases in horticultural brassicas.

August Inoculation : Plot 1

FIG 6a

Iprodione spray 8/10

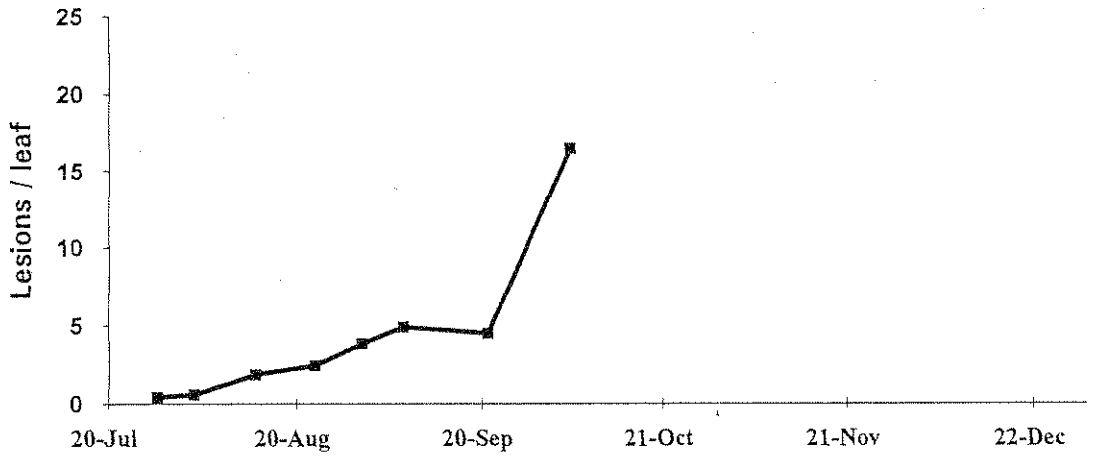


FIG 6b

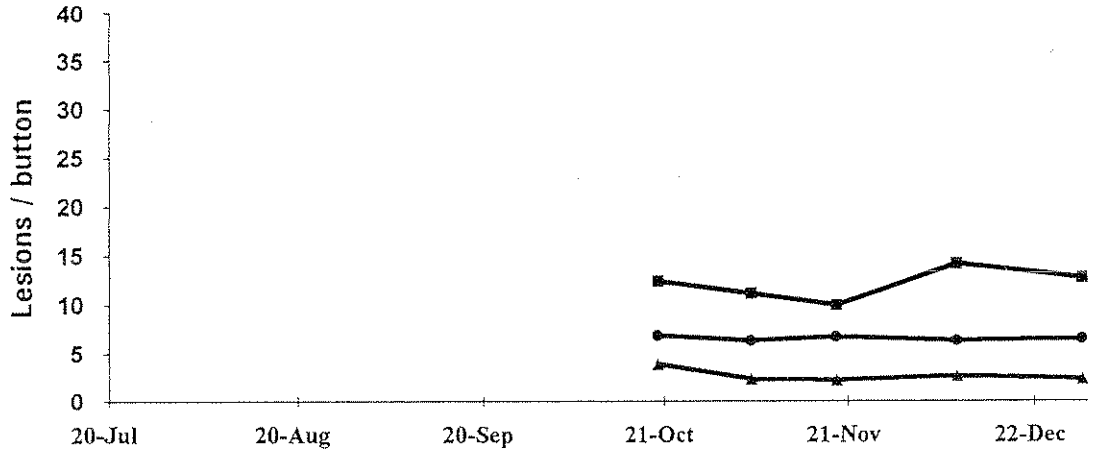
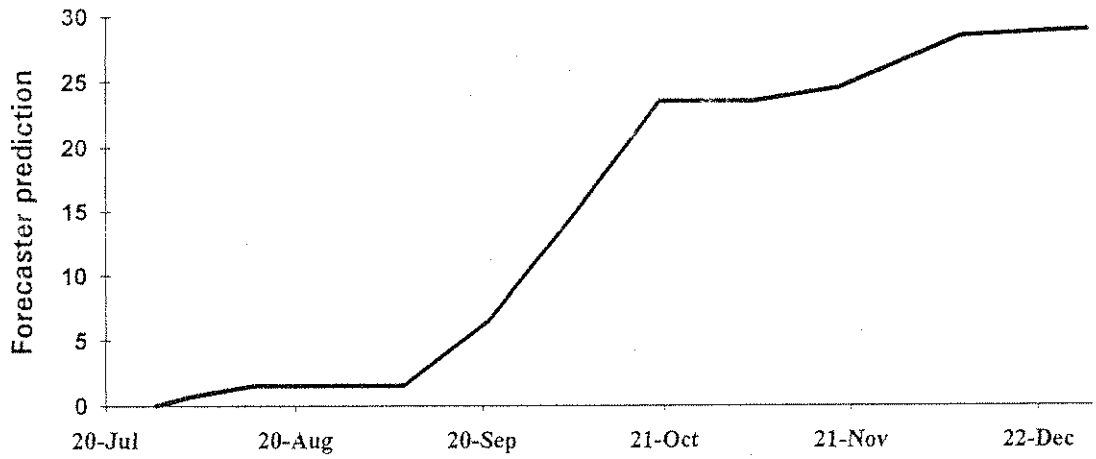
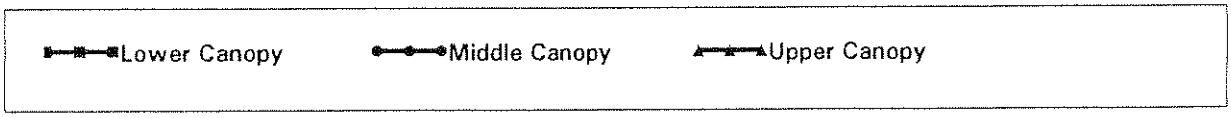


FIG 6c





August Inoculation : Plot 2

FIG 7a

Chlorothalonil spray 8/10

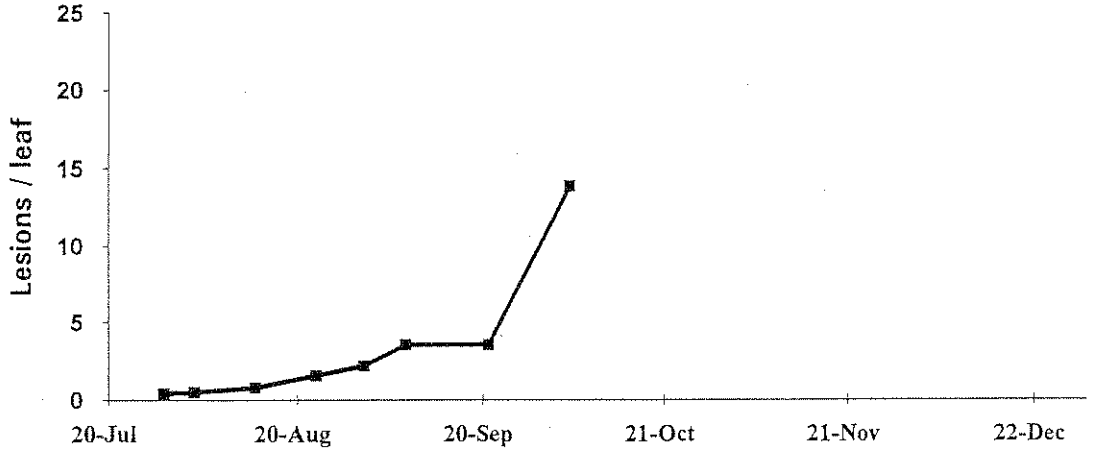


FIG 7b

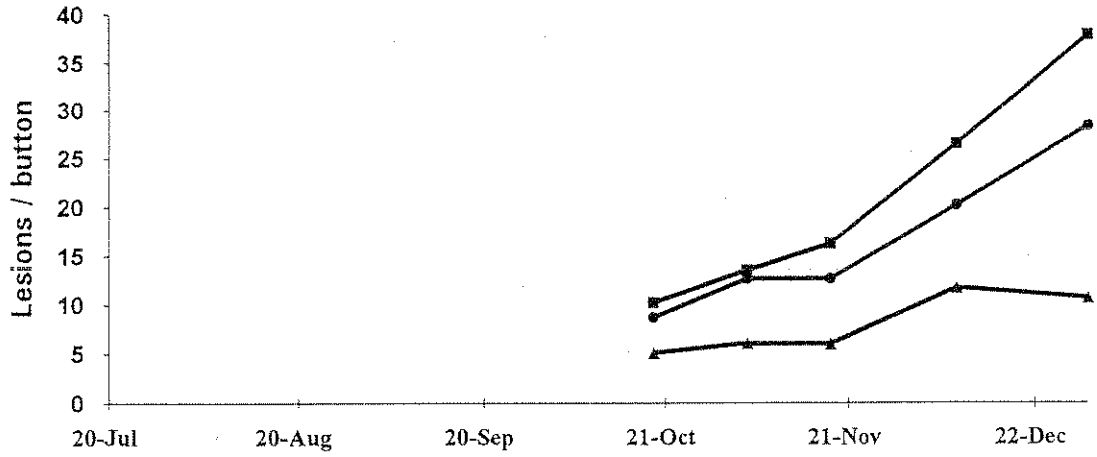
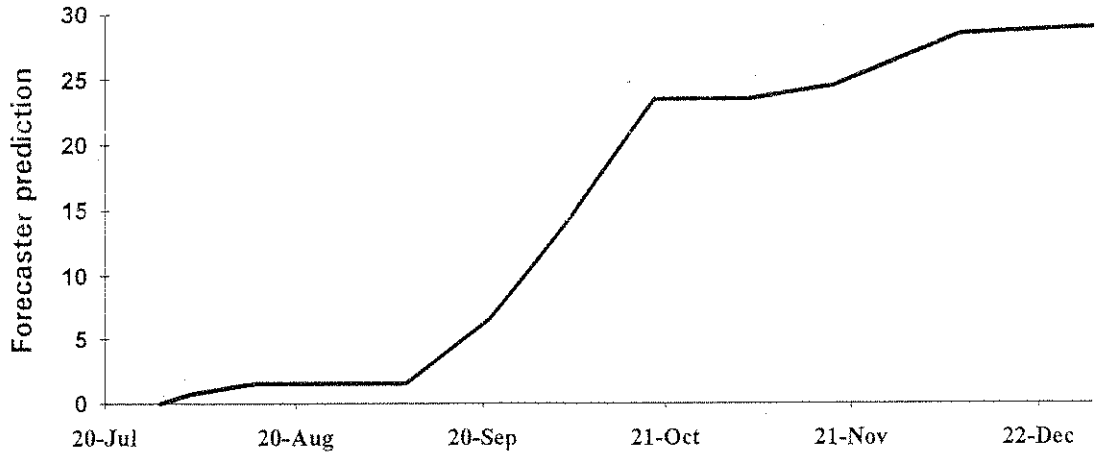
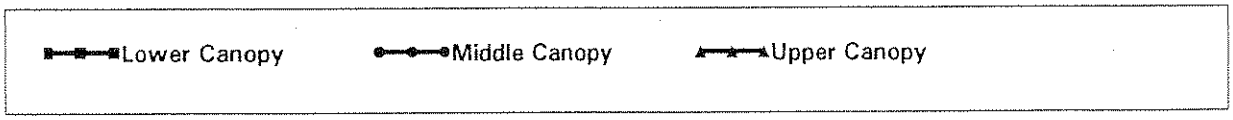


FIG 7c





October Inoculation : Plot 1

FIG 8a

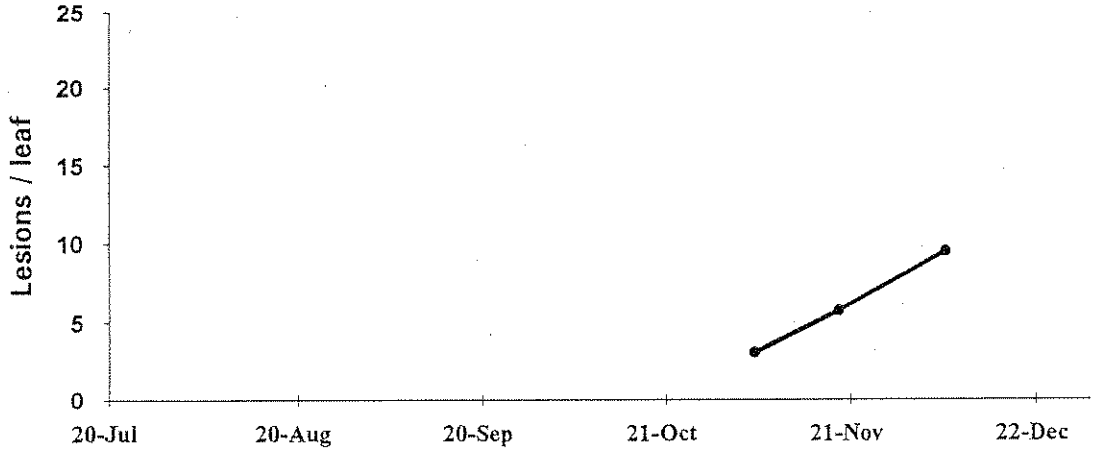


FIG 8b

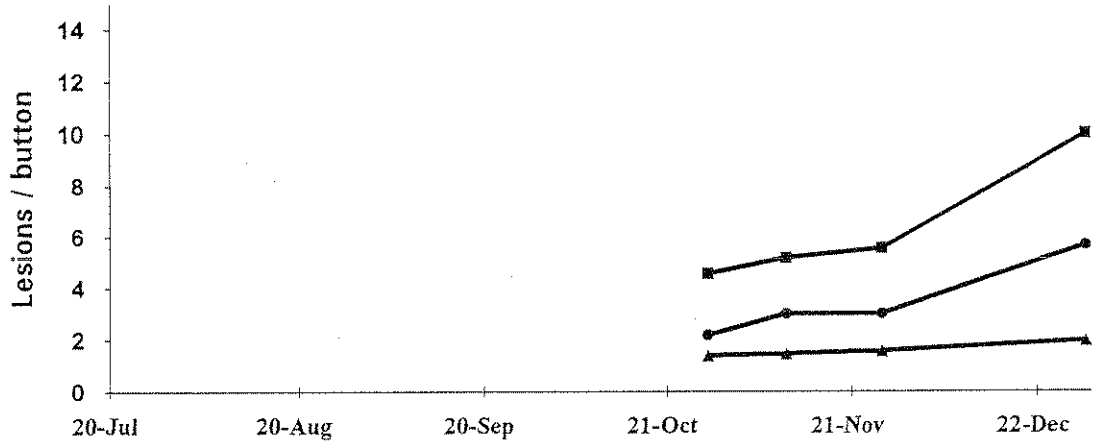
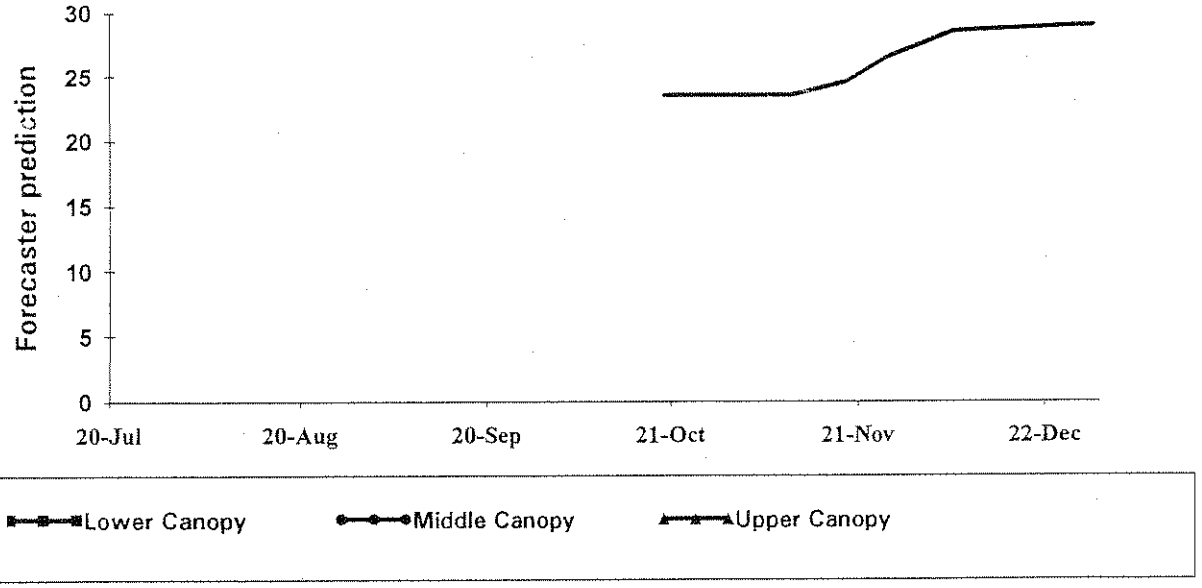


FIG 8c



October Inoculation : Plot 2

FIG 9a

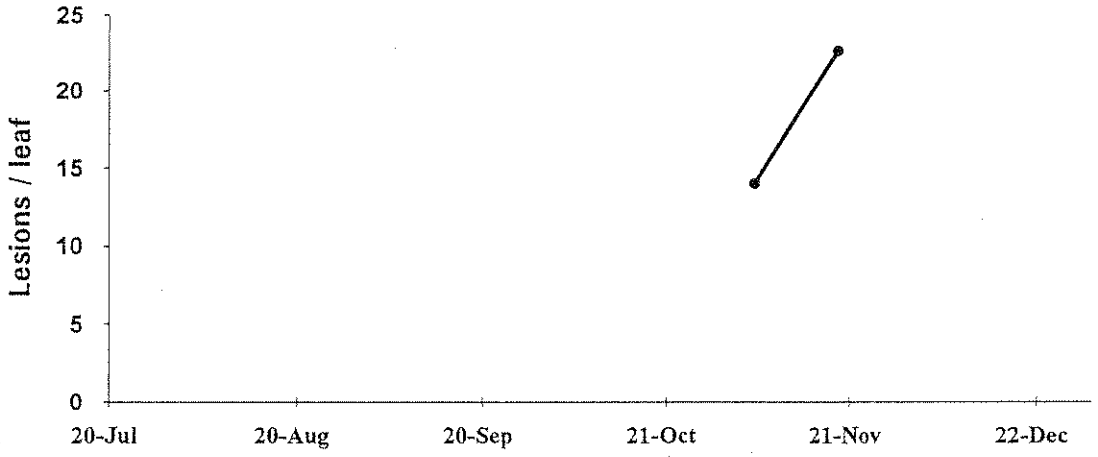


FIG 9b

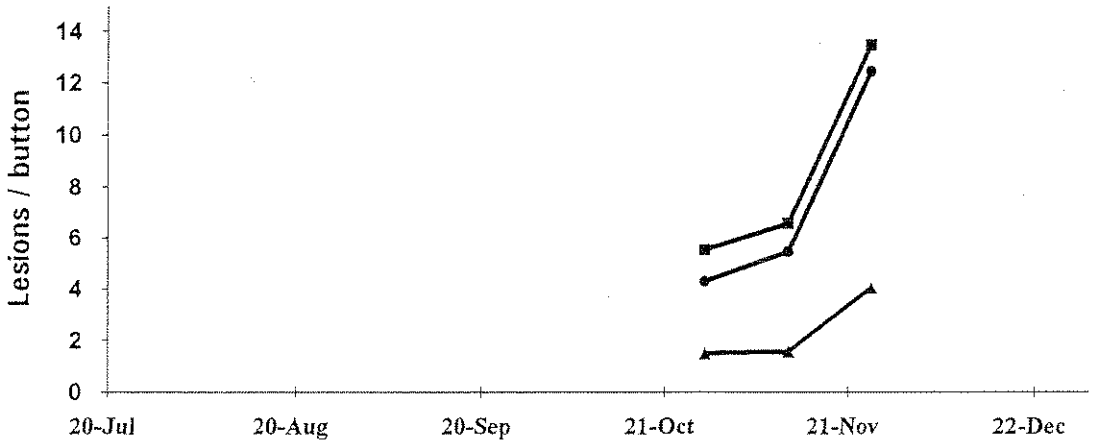
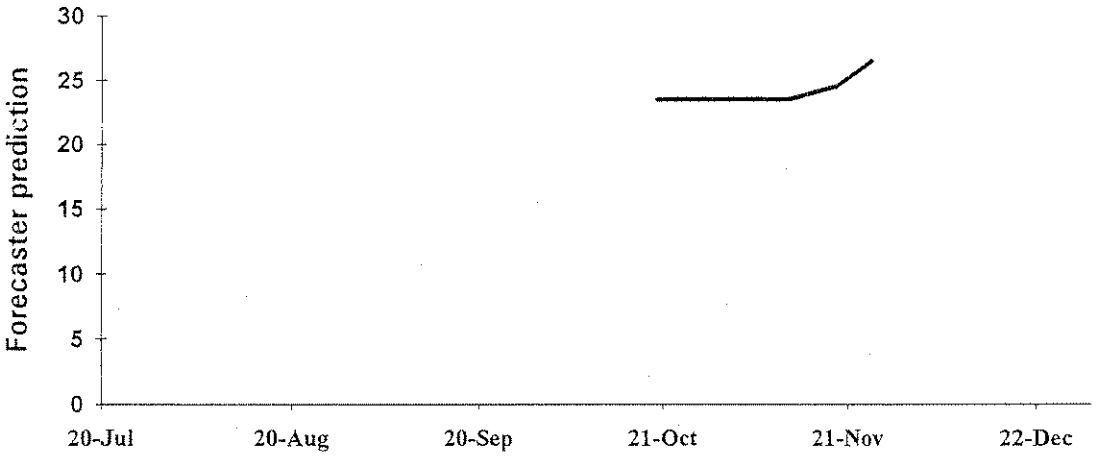
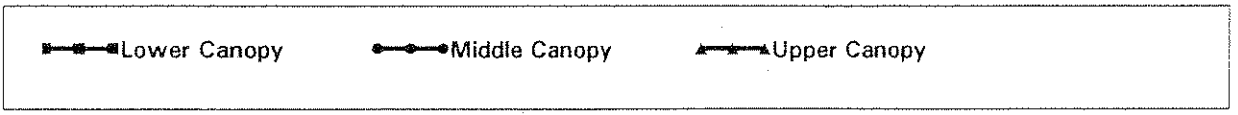


FIG 9c





4.3.4 Model Development - Light Leaf Spot

4.3.4.1 Controlled environment experiments

Experiments to determine the environmental requirements for infection by the light leaf spot pathogen *Pyrenopeziza brassicae* are shown in **Figs 10 - 15.** Probit analysis of the data using GENSTAT (Payne 1993) was applied separately for each replicate at each temperature. An estimate of the potential maximum percentage of leaves infected (the upper asymptote of the sigmoid curve) and the time to 50% of that maximum percentage were derived. Analysis of variance of these variates indicated that there were significant differences between temperatures but not between replicates (**Table 4**).

There was relatively rapid and high levels of infection between 12°C and 20°C , with an optimum around 16°C. At sub-optimal temperatures of 4°C and 24°C, the infection process was slower and asymptotes at 31% of leaves showing infection. Minimal infection was observed at 28°C after 7 days leaf wetness. Curves fitted to this data could be used to predict infection similarly to those formulated for dark leaf spot (section 4.3.1).

Leaf wetness durations required for significant infection by light leaf spot in this experiment were considerably longer than for dark leaf spot (**Fig 1**). Long leaf wetness periods are uncommon during the early part of the growth season. However under winter conditions parts of the crop may be continuously wet. The onset and frequency of these conditions may be an essential requirement for infection and would be easily detected within the crop. However it is not yet known if a germinating spore of *Pyrenopeziza brassicae* can survive dry periods prior to penetration of the host surface which may effect the level of infection within the crop.

An experiment at 16°C with isolate 1111 gave a much faster rate of infection with time to 50% infection of approximately one day in contrast to 1.59 days of leaf wetness required by isolate 1091 (**Table 4**). Significant variation between isolates in their infection requirements may therefore exist. Future work will investigate the potential variation that exists in infection requirements between isolates derived from oilseed rape and those from vegetable brassicas.

Light Leaf Spot Infection

FIG 10

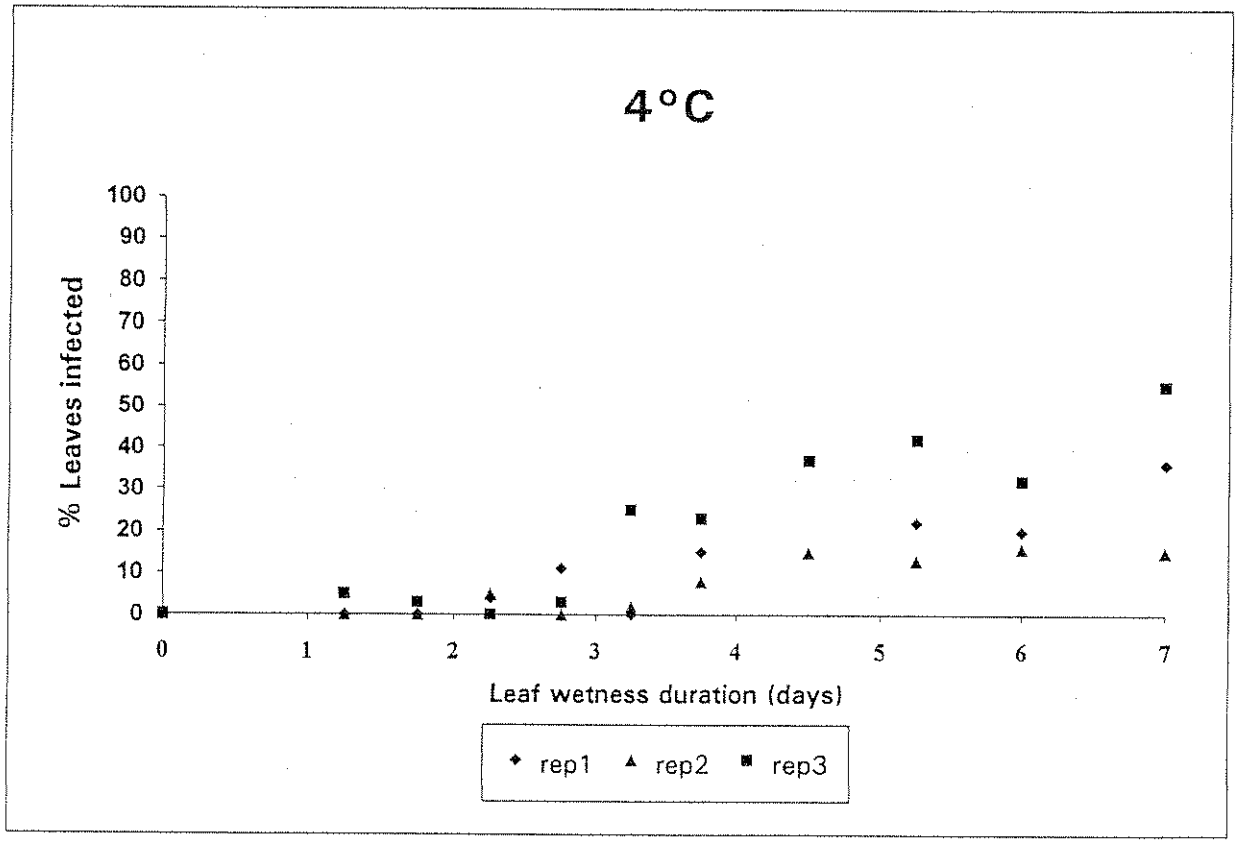
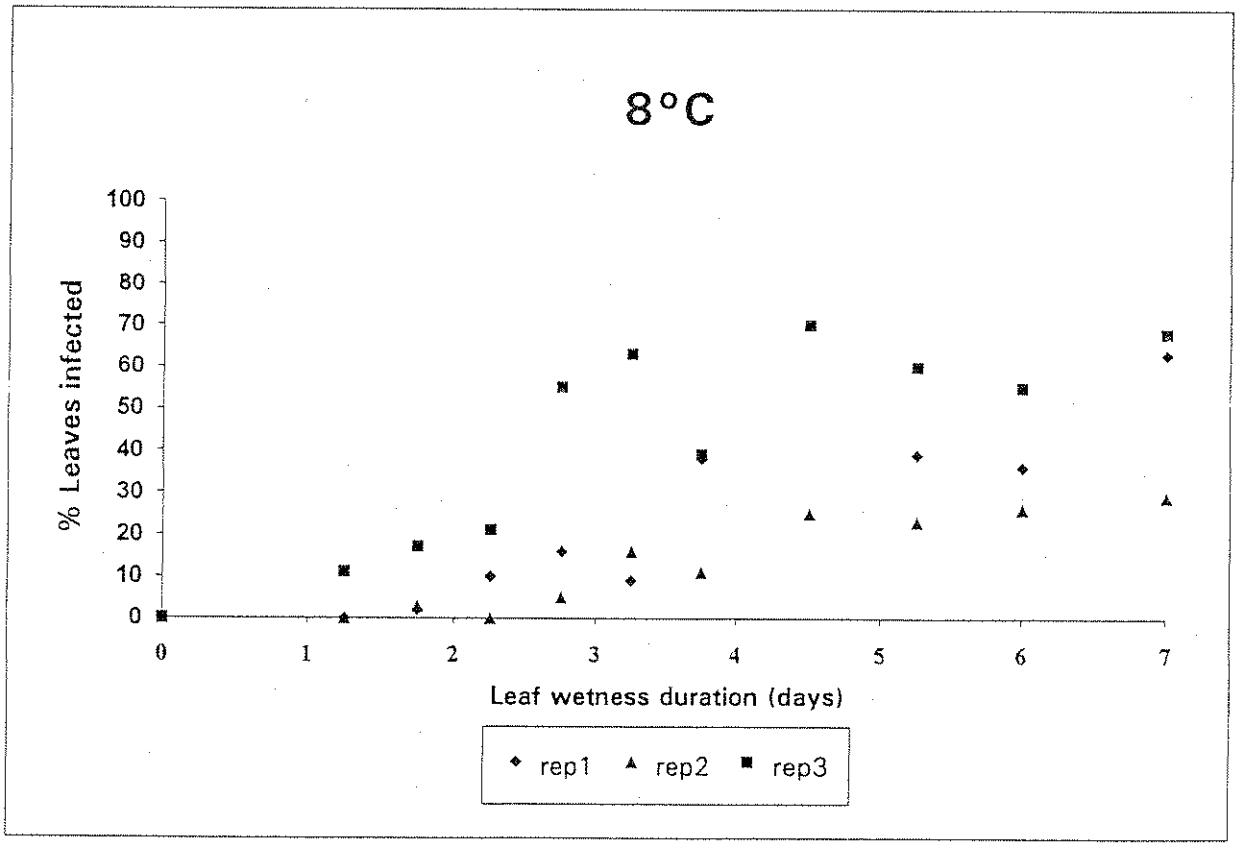


FIG 11



Light Leaf Spot Infection

FIG 12

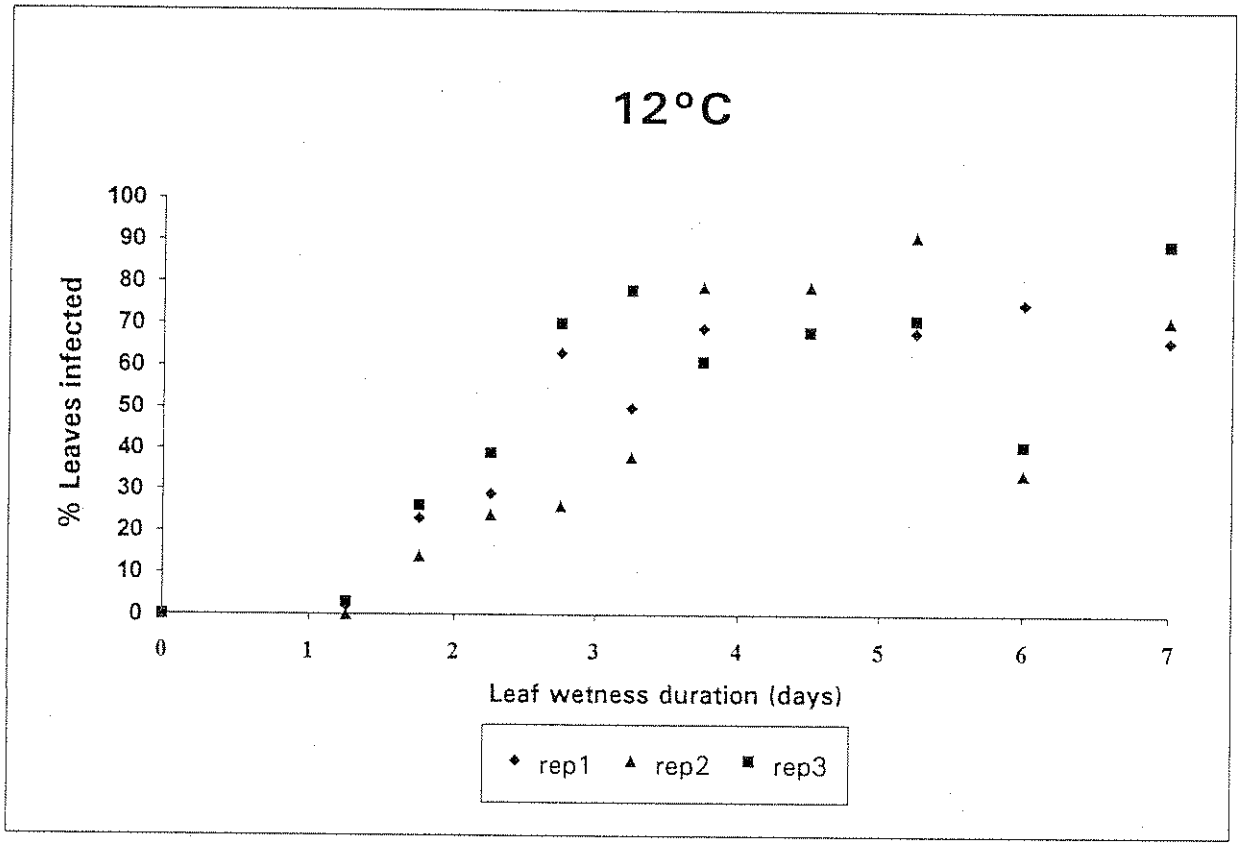
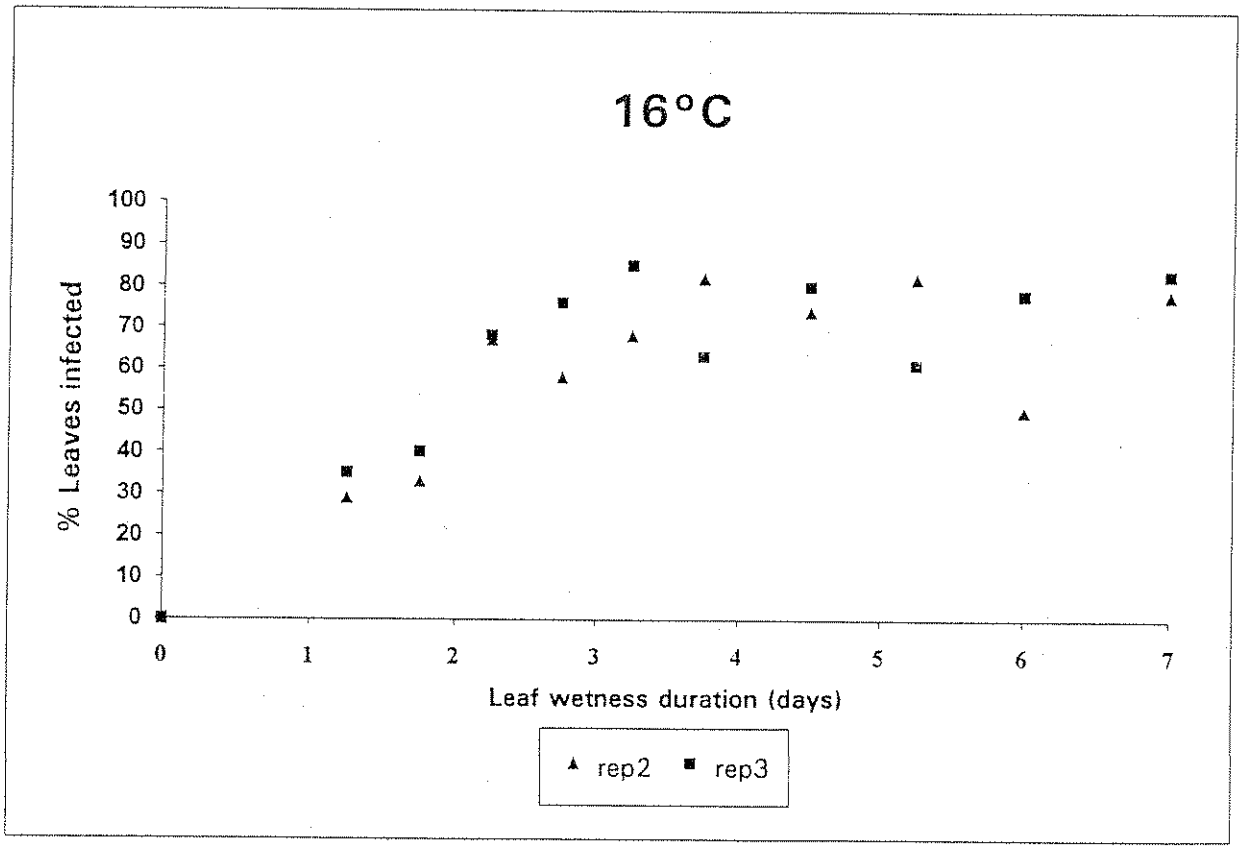


FIG 13



Light Leaf Spot Infection

FIG 14

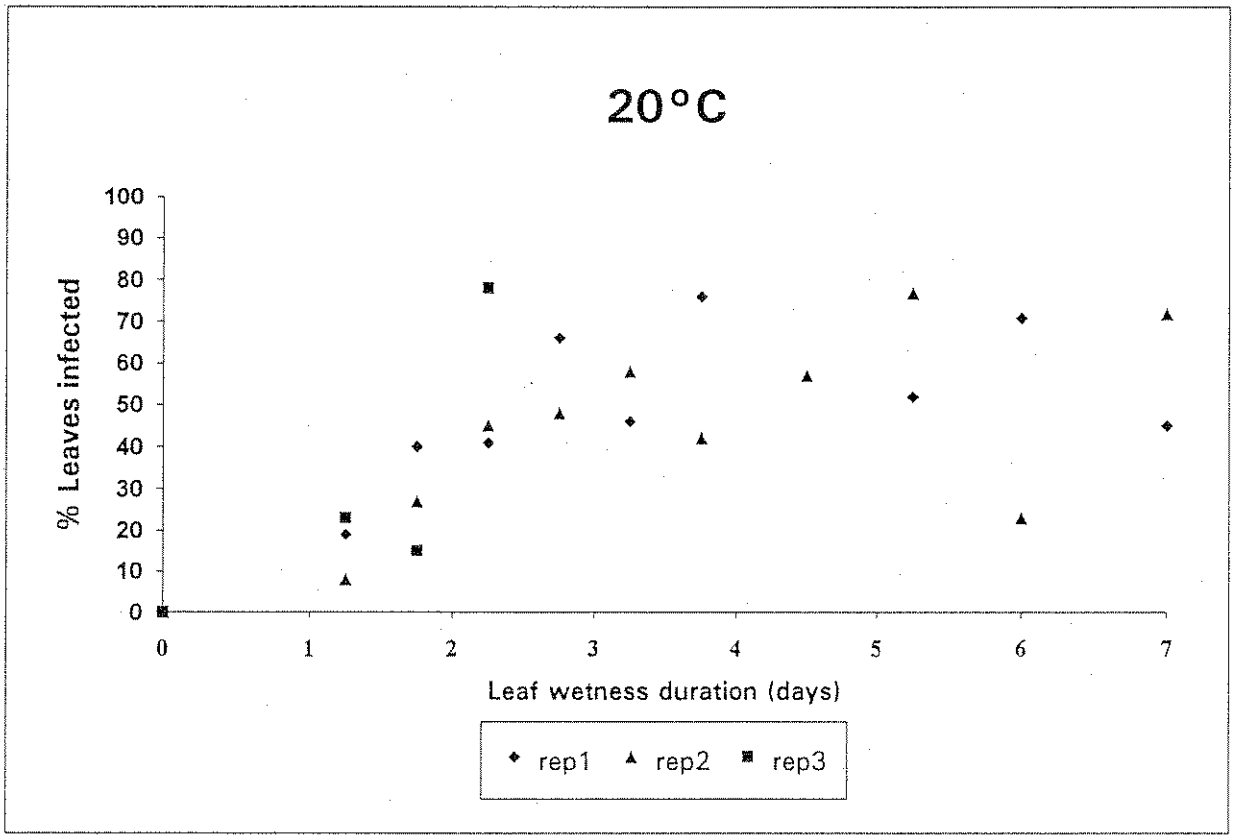


FIG 15

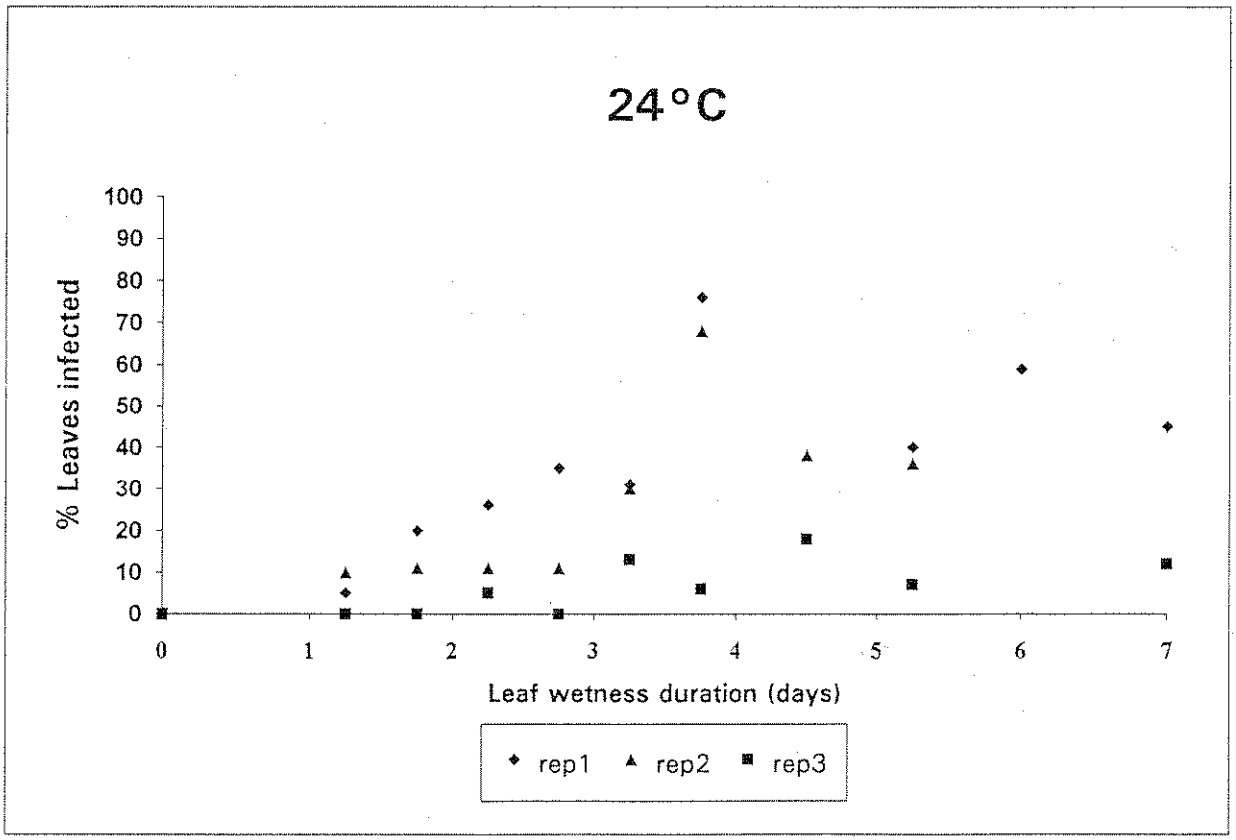


TABLE 4

Light Leaf Spot Infection Experiment

(mean of three replicates)

Days of leaf wetness to 50% infection

Temp (C)	4	8	12	16	20	24	28
	4.07	3.13	2.32	1.59	1.76	2.38	Minimal Infection

sed = 0.364

Maximum % of leaves showing infection

Temp (C)	4	8	12	16	20	24	28
	31.5	45.8	68.8	74.6	70.4	31.4	Minimal Infection

sed = 14.06

4.3.4.2 *In vitro* experiments

Experiments outlined in section 4.2.1.2 were undertaken to investigate the potential variation between isolates of light leaf spot. Mean radial growth rates of colonies at four weeks in Petri dishes varied from 1.67 to 4.67 mm per week (**Table 5**). Analysis of variance showed highly significant ($p<0.001$) variation between isolates and between media. Further analysis, with isolates grouped on the basis of geographical and host origin (**appendix IV**), showed significant variation ($p<0.05$ to $p<0.001$) within groups of isolates. Similar variation was found at all sampling times.

In vitro spore production on 2% Malt Extract Agar is most prolific on two week old colonies (**Table 6**). Highly significant differences between isolates ($p<0.001$) were observed at all sampling times. Isolates were grouped, as above, and highly significant variation ($p<0.001$) was found within all groups at all times except isolates 1094, 1095 and 1096 (**appendix IV**). No significant differences were observed between these isolates for *in vitro* spore production.

Percentage germination of spores produced *in vitro* was measured at 2, 4 and 6 weeks (**Table 7**) and was highest after two weeks incubation at 15°C but declined with increasing incubation time. Analysis of variance showed highly significant differences between isolates and within most groups of isolates at all sampling times. There was no significant difference in percentage germination at four weeks among isolates 1094, 1095 and 1096 - similar to the observation for *in vitro* spore production.

All isolates fulfilled Koch's postulate but some distinct differences between isolates were observed. On some plants with particular isolate combinations the only symptom expression observed after one month incubation was the extrusion of white spore masses on the leaf surface (conidiamata).

While these results should be interpreted with caution, there is reason to believe that there are differences between isolates of the light leaf spot pathogen occurring on vegetable brassicas which could complicate any future attempt to forecast the occurrence of this disease.

Light leaf spot in vitro experiments

TABLE 5 **Colony radial growth rate at four weeks**
(mm per week, mean of 3 replicates)

			Media	
Isolate		2% MA	PLYSE	PDA
1091		3.5	4	4.67
1092		3	4	4
1093		3.17	4.17	4.33
1094		2.5	3.83	3.33
1095		2.67	3.17	3
1096		2.33	2.5	2.83
1097		2	3.33	3.67
1098		2.33	3.33	2.67
1099		1.83	3.83	3.5
1100		2.5	3.67	3.67
1101		2.83	3.67	3.67
1108		2.67	4	4.33
1111		1.67	3.67	3.67
1112		1.67	3.83	3.67
1113		2	3.67	3.5

Light leaf spot in vitro experiments

TABLE 6 **Colony spore production after incubation at 15°C.**
(x10000 per ml, 5ml per dish, mean of 3 replicates)

		Incubation Time		
Isolate		2 Weeks	4 Weeks	5 Weeks
1091		70	14	3
1092		437	360	54
1093		12	2	1
1094		380	91	7
1095		233	7	6
1096		550	18	13
1097		287	22	5
1098		3233	1083	340
1099		230	163	43
1100		500	377	110
1101		520	300	70
1108		1633	790	500
1111		1153	1097	760
1112		193	123	88
1113		610	337	82

TABLE 7 **Spore germination after colony incubation at 15°C.**
(% germination after 24 hrs, mean of 3 replicates)

		Incubation Time		
Isolate		2 Weeks	4 Weeks	6 Weeks
1091		89.7	74	56
1092		91.3	59.3	24
1093		73.3	71.7	46.6
1094		89	82.3	32
1095		98	83.7	32.7
1096		98.3	85	84
1097		97.3	82.3	66.3
1098		88.3	80.3	71
1099		96.7	94	94.7
1100		95.3	93.3	94
1101		90.7	93	86.7
1108		93.7	93.3	90.1
1111		96.3	93.3	95.7
1112		91.7	66.7	16.2
1113		69	71.3	13.7

4.4 CONCLUSIONS

4.4.1 Dark Leaf Spot

1. Controlled environment experiments have been used to formulate models for fungal life cycle stages which can be successfully applied in the field. Hourly integration of a thermal time function under certain microclimatic conditions provides the basis of the model.

2. Trap plant data allows biological inferences to be made and permits optimization of models for use in the field. The following conclusions have been derived from their use:
   - germinating spores survive absence of leaf wetness when air temperature < 8°C and vapour pressure deficit < 2.
   - the basic infection model can be extrapolated in the range 6°C to 0°C.
   - spore dispersal is largely by means of splash after the onset of reduced temperature conditions in winter.
   - inoculum potential of the crop is low prior to lower leaf senescence.
   - electronic leaf wetness sensors underestimate duration late in the year , improved sensors have been designed and built.

3. Button disease spread is dependent on major dry spore release (affecting the whole stem) prior to onset of reduced temperature conditions in winter. The vertical splash dispersal of spores after that date depends on the height of major leaf infection.

4. Observed increases in disease levels correspond closely to predictions from the optimised epidemic model.

5. The model has been shown to be applicable for two Brussels sprout cultivars, and further work could lead to use on other horticultural brassica types.

6. The optimised epidemic model has the potential to be developed as a forecasting system for use as an aid to determination of the timing of fungicide application.

4.4.2 Light Leaf Spot

1. *In vitro* studies with isolates of *Pyrenopeziza brassicae* of different host and regional origin showed differences in colony growth, spore production, spore germination and *in planta* symptom expression and infectivity.

2. The sporadic absence of necrosis observed with some isolates could explain the reported influx of of disease on Brussels sprout buttons in the apparent absence of leaf disease.

3. The optimum temperature for infection of Brussels sprouts was 16°C and infection occurred in the range from 4°C to 24°C. Leaf wetness duration for 50% infection was approximately 36 hours at optimal temperatures and more than double this at the extremes of the range.

4. The long leaf wetness durations required for infection by the light leaf spot pathogen could be used as a basis for prediction of disease in the field.

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4.6 ACKNOWLEDGEMENTS

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4.7 APPENDICES

I Leaf Spot Life Cycle

II Typical Crop Diary

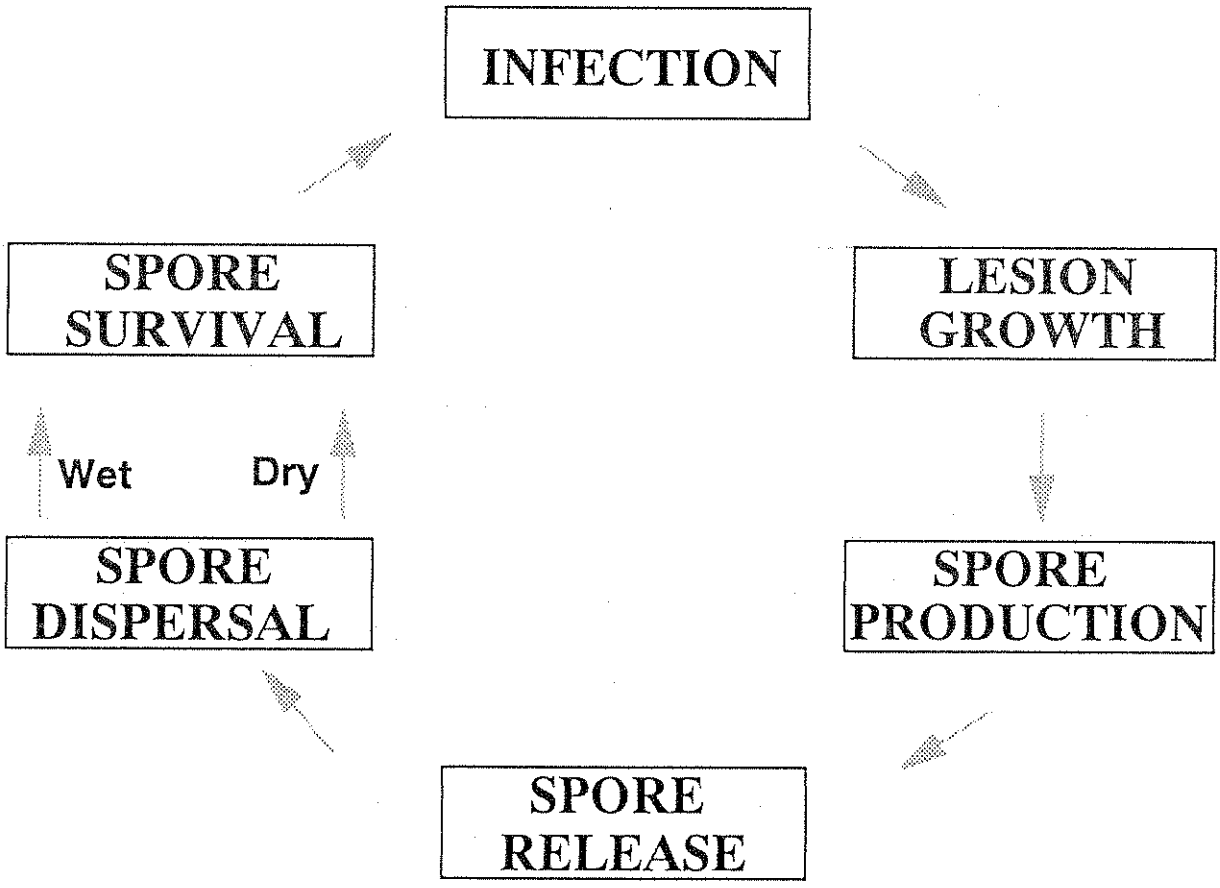
III Crop Meteorological Data 1991, 1992, 1993

IV Light Leaf Spot Isolate List

V Agar Media

APPENDIX I

LEAF SPOT LIFE CYCLE



APPENDIX II

Typical Crop Diary

20/04 Brussels Sprout seed sown in Hassy 308 modules.

Seedlings grown in glasshouse.

17/05 Nitram fertiliser applied at 190Kg/ha.

17/05 Plot area powerharrowed.

18/05 Plot marked for planting.

20/05 Seedlings manually transplanted at 60cm spacing.

20/05 Application of Yaltox at 0.4g/plant for cabbage root fly control.

22/05 Spray of Albrass at 9.0L/ha and Dacthal at 6.0 Kg/ha for weed control.

16/06 Plot irrigated.

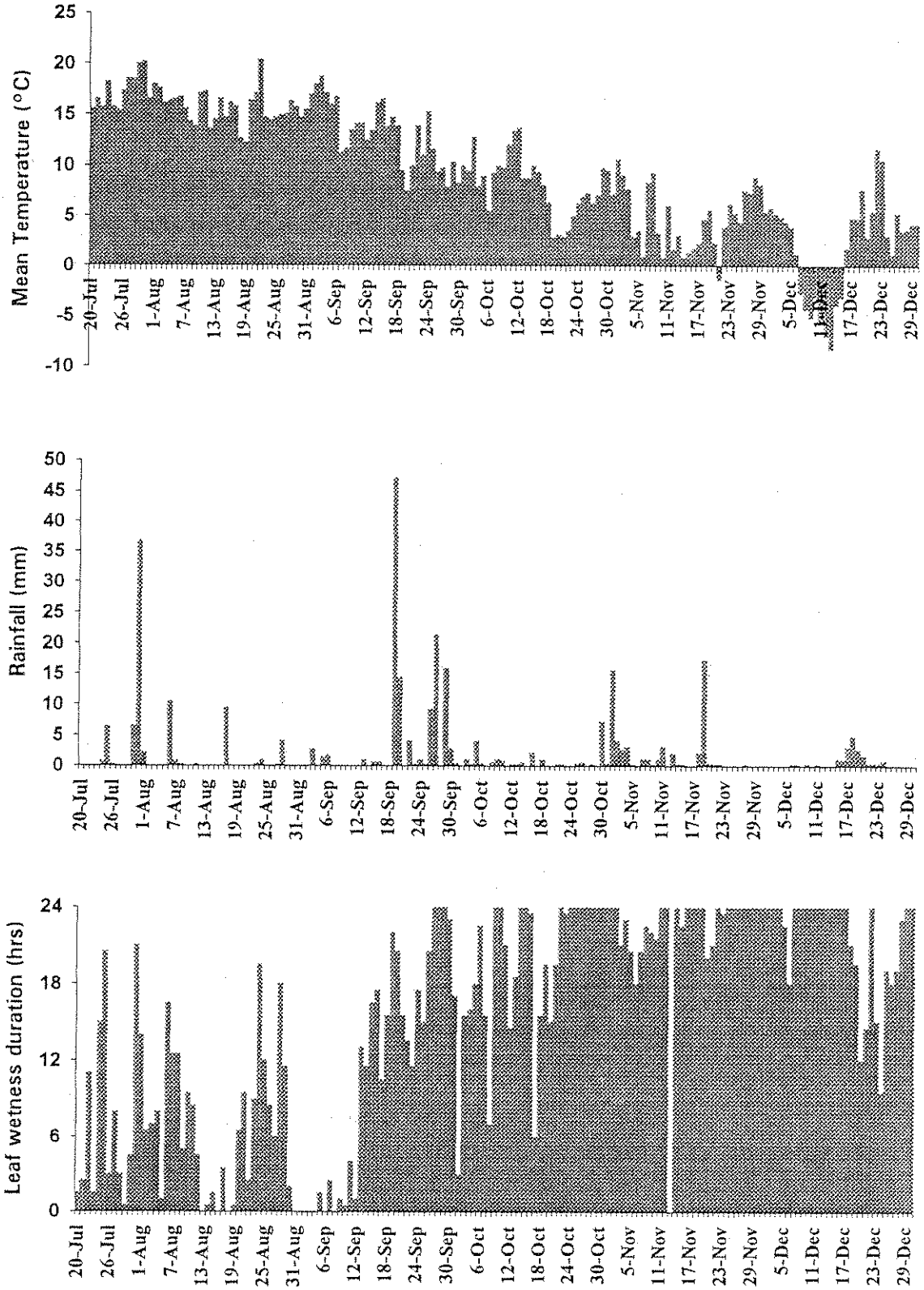
01/07 Nitram fertiliser applied at 120 Kg/ha as top dressing.

01/07 Plot irrigated.

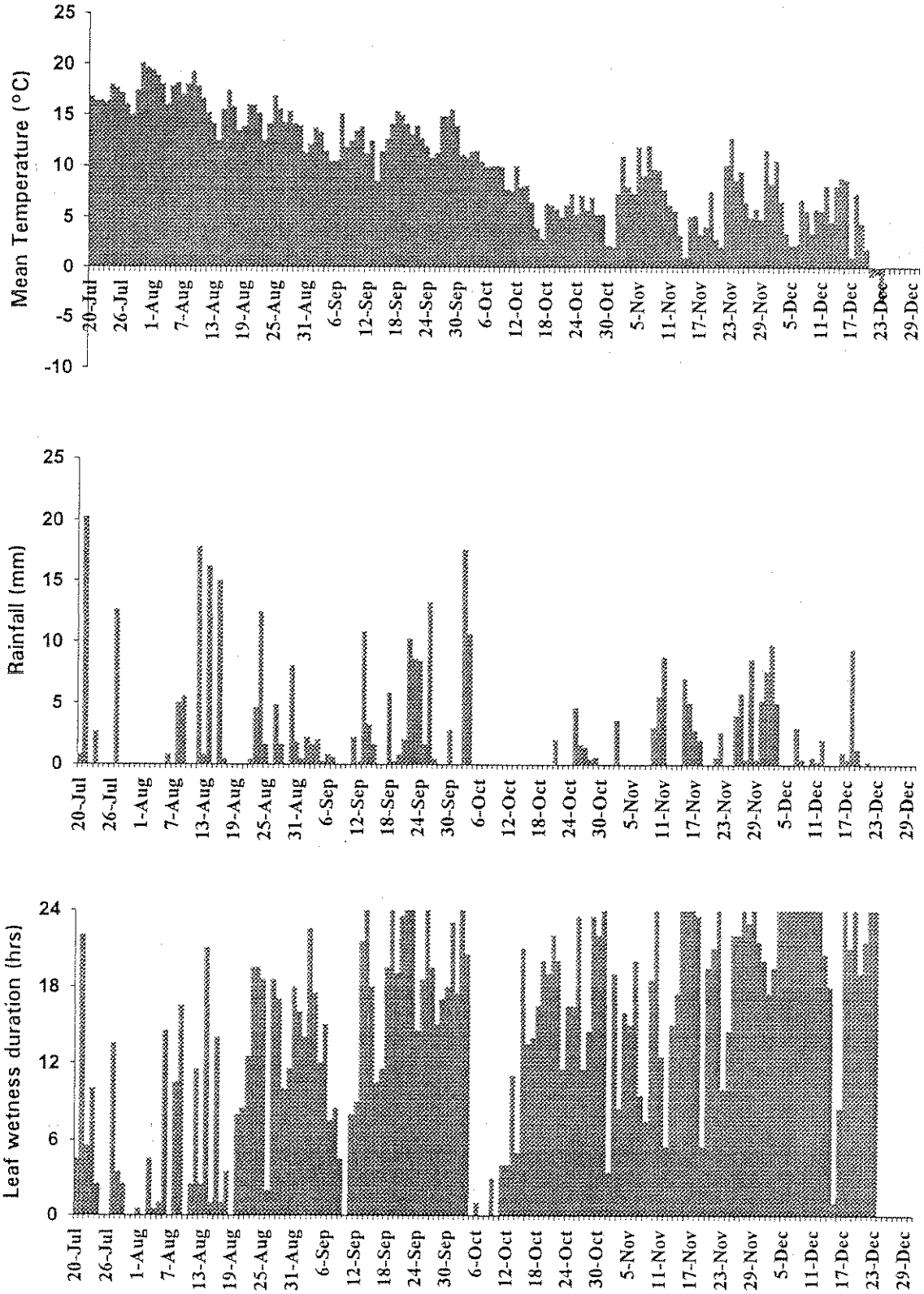
26/07 Metasystox at 560ml/ha applied to control aphids.

APPENDIX III

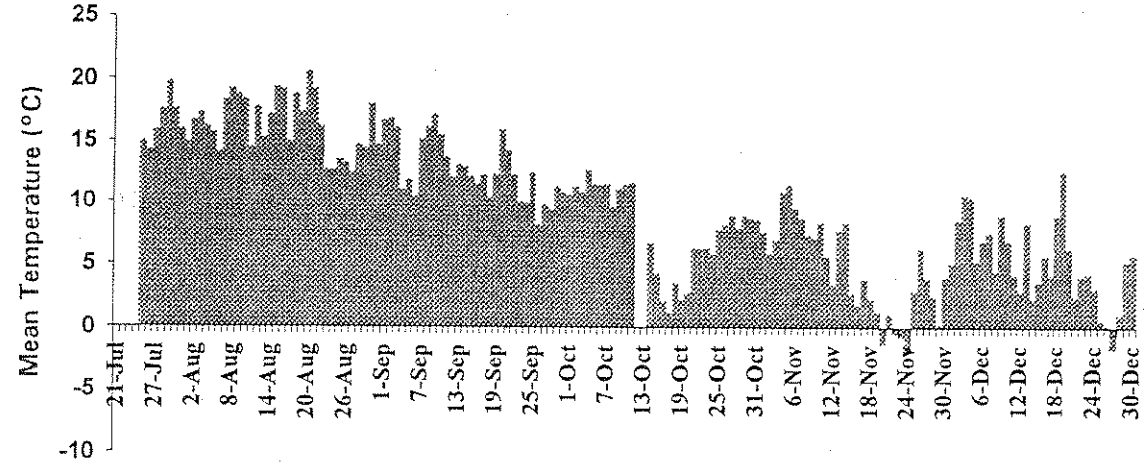
Met Data 1991

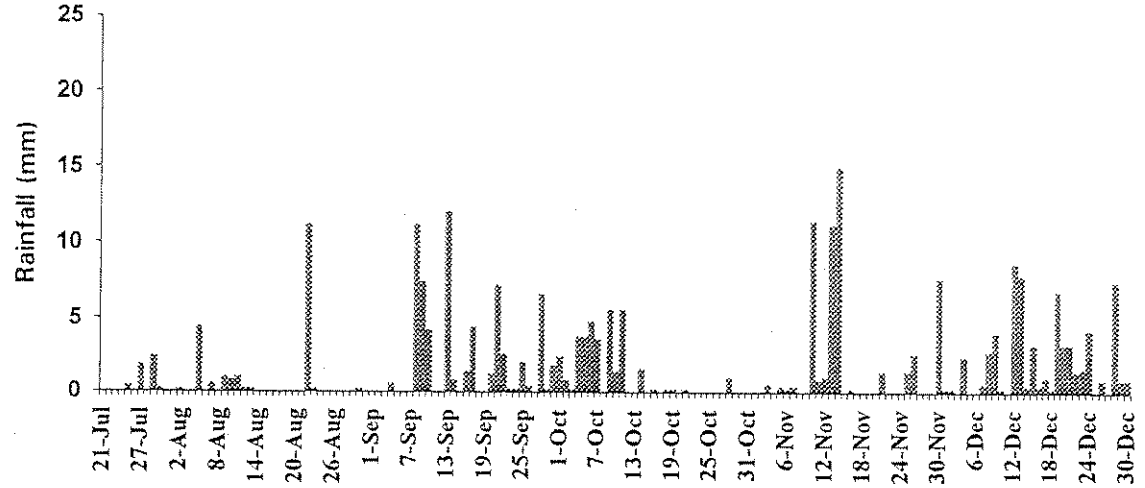


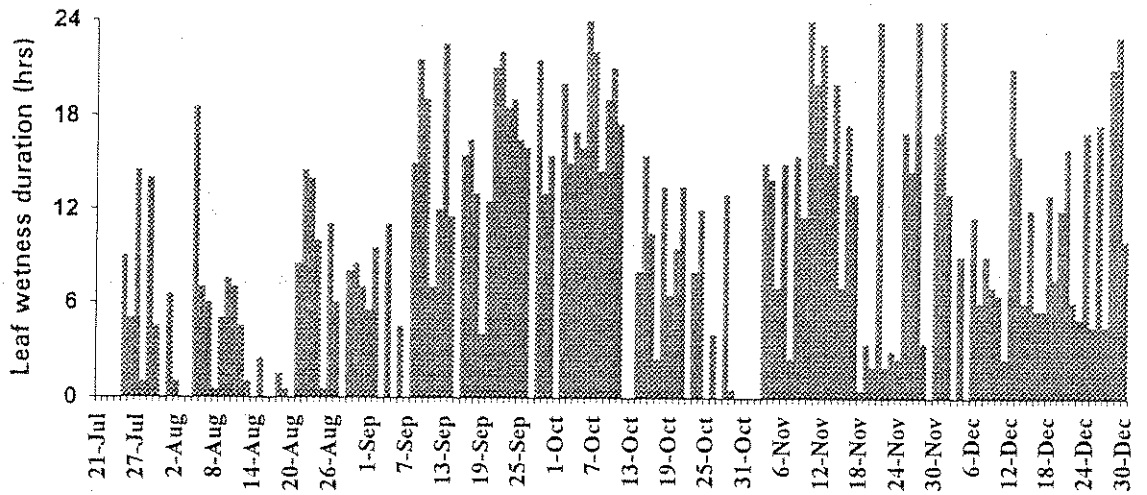
Met Data 1992



Met Data 1993







APPENDIX IV

Source of Light Leaf Spot isolates used in experiments

Isolate Code	Crop	Geographical Location
1091	Oilseed Rape	Hertfordshire
1092	Cabbage	Wellesbourne , Warks.
1093	Oilseed Rape	Hertfordshire
1094	Brussels Sprout	N Hereford. , Field A
1095	Brussels Sprout	N Hereford. , Field B
1096	Brussels Sprout	N Hereford. , Field C
1097	Brussels Sprout	S Hereford. , Field A
1098	Brussels Sprout	S Hereford. , Field A
1099	Brussels Sprout	S Hereford. , Field B
1100	Brussels Sprout	S Hereford. , Field C
1101	Brussels Sprout	S Hereford. , Field D
1108	Brussels Sprout	Wellesbourne , Warks.
1111	Cauliflower	Cornwall , Field A
1112	Cauliflower	Cornwall , Field B
1113	Cauliflower	Cornwall , Field C

nb. Field A-D refer to different fields on the same farm.

APPENDIX V

Preparation of 1l Agar Media

2% Malt Extract Agar (2% MA)

Malt Extract	20g
Agar	20g
Distilled Water	*c.* 1l

1. Boil Malt extract in water until dissolved.
2. Add agar and boil until dissolved.
3. Make up to 1l with more distilled water.
4. Sterilize at 15 p.s.i. for 20 minutes.
5. pH is *c.* 3-4 and may be adjusted with NaOH solution.

Potato Dextrose Agar (PDA)

Raw potato	*c.* 300g
Dextrose	20g
Agar	20g
Distilled Water	*c.* 1l

1. Scrub and peel potatoes, cut as 12mm cubes.
2. Rinse and weigh 200g of these.
3. Boil cubes in the water until soft (*c.* one hour).
4. Mash and strain through two layers of muslin cloth.
5. Add agar and boil until dissolved.
6. Add dextrose and stir until dissolved.
7. Make up to 1l with distilled water.
8. Sterilize at 15 p.s.i. for 20 minutes.

Prune Lactose Yeast Agar (PLYSE)

Dried prunes	400g
Yeast Extract	1g
Lactose	5g
Agar	20g
Distilled Water	*c.* 2l
Streptomycin antibiotic	0.1g dissolved in 10ml sterile water
Erythromycin antibiotic	0.1g dissolved in 10ml sterile water
Sodium Hydroxide (NaOH) solution	0.8g in 100ml distilled water

1. Simmer prunes in 1l of water for one hour.
2. Strain through two layers of muslin.
3. Sterilize this stock solution at 15 p.s.i. for 20 minutes.
4. Mix 100ml stock solution and 850ml distilled water on a hot plate.
5. Add yeast extract and lactose and dissolve.
6. Add agar and dissolve.
7. Make solution up to *c.* 965ml with distilled water.
8. Adjust pH to 5.5 with *c.* 15ml NaOH solution.
9. Sterilize at 15 p.s.i. for 20 minutes.
10. Add antibiotics when solution has cooled, just prior to pouring plates.