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The results and conclusions in this report are based on an investigation conducted over a one year period. The conditions under which the experiments were carried out and the results obtained have been reported in detail and with 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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GROWER SUMMARY

Headline

Fungicide information helps improve the interpretation of forecasting systems for Brassica leaf diseases.

Background and expected deliverables

Fungicides are vital for controlling leaf spots on vegetable Brassicas and the timing of application is important. If the fungicides are timed right the efficacy of the fungicide could be enhanced and the need for re-application reduced, however optimal timing relies on accurate monitoring or prediction systems that identify when damaging or vulnerable pathogen lifecycle stages occur. Sometimes the conditions can suit a particular pathogen very well and when this occurs fungicides alone cannot treat the problem but fortunately these optimal conditions occur infrequently for many pathogens.

Disease forecasting based on environmental conditions present within the crop is a well established method of improving the effectiveness and reliability of fungicide applications. Forecasting systems for vegetable Brassicas (e.g. Brassica$_{spot}$) comprise of models which describe the effect of environment on key life-cycle stages of each plant pathogen. Leaf surface wetness duration, humidity and temperature are important environmental factors which determine the occurrence of infection and sporulation by air-borne pathogens of vegetable Brassicas. Models which describe the effect of temperature and wetness duration on the infection of vegetable Brassicas by dark leaf spot (*Alternaria brassicae*), ringspot (*Mycosphaerella brassicicola*) and white blister (*Albugo candida*) have been used successfully over many seasons within vegetable Brassica producing areas to improve control of these airborne diseases. The reliability of disease control and potential for reducing fungicide usage has been greatly enhanced. All three of these pathogens require leaf wetness for infection to occur. These requirements have been programmed into computer based models (Brassica $_{spot}$ disease forecasting system).

Models can be used in conjunction with in field weather data collected by data loggers to determine the risk of infection by these pathogens. However information from these models has not been fully integrated with activity of different active ingredients within approved fungicides. There is considerable choice in the number of fungicide products available

however but there is no information on the activity of these fungicides on the leaf surface. . If these systems are to be used more fully information is required on the timescales over which fungicides can successfully control disease when applied after risk periods are identified. For example, how long after a risk period has been identified would applying an eradicant spray be effective?. Additionally, how long do protectants work and remain active on the plant before they are degraded? These are important practical issues because most growers cannot schedule fungicide applications quickly. There is also little information on the impact of fungicide sprays on sporulation (release of spores) by pathogens from mature and developing lesions. This is particularly important for the ringspot pathogen.

This project will provide information on the effect of application time post-infection in combination with dosage and product type on the effectiveness of fungicide applications against dark leaf spot, ringspot and white blister. Addition of this information to disease forecasting systems would reduce the degree of interpretation of results required when using the Brassica$_{spot}$ disease forecasting system. With this new information growers will be able to reach much more informed decisions on fungicidal sprays required under all circumstances in the field. The use of forecasts for white blister infection and time to symptom development will test the feasibility of using protectants to control this disease. If this proved possible it would reduce the costs involved in white blister control in the field. This would have a major impact on control of white blister in long season vegetable Brassica crops.

The expected deliverables from this project are:

- Improved effectiveness of fungicides in relation to forecasted disease risk periods.
- Information on the most effective crop protection inputs for control of white blister infection in vegetable Brassica crops.
- Evaluatation of the effect of chemical sprays on the sporulation of ringspot from established lesions.
- More information on the effect of environment on reduction of fungicide content in leaves after spraying.

Summary of Year one results

Effect of difenconazole (Plover) at recommended doses after ringspot infection

Year one results demonstrated that at recommended dosages difenconazole (Plover) could be applied up to 5 days post infection and still result in complete control of ringspot. If recommended dosages of Plover were applied up to 8 days after infection, ringspot control was observed but only where the mean temperature had been around 5 -10^{o}C after infection. The effect was not observed at mean temperatures after infection of 15^{o}C. Temperatures of 20^{o}C were inhibitory to ringspot post-infection due largely to the degree of leaf fall before symptoms appeared in these experiments.

Effect of Difenconazole and Tebuconazole (Generic Folicur) at increasing time after ringspot infection

There appeared to be differences between fungicides containing Difenconazole and Tebuconazole (Generic Folicur) in their effectiveness when applied at increasing time after infection. Small amounts of disease were recorded if Tebuconazole (as Folicur) was applied only 2 days after infection regardless of incubation temperatures. Conversely Tebuconazole applied at incubation temperatures of 5 and 10^{o}C post application appeared more effective in controlling ringspot over longer time periods in comparison to Plover.

Effect of Chlorothalonil as Bravo post infection for the control of ringspot

Application of Chlorothalonil as Bravo after infection had occurred had little or no effect on the control of ringspot regardless of temperature post-infection. Chlorothalonil has a protectant mode and so this result was not surprising.

Using the white blister MORPH model to time fungicide sprays

Spray timing trials using the combined white blister infection and symptom development model in MORPH demonstrated that the model can be used effectively to control white blister in crops of Brussels sprouts using reduced numbers of control sprays. White blister disease development was lower than expected during 2008. Two routine sprays with Nativo at recommended dosages did not appear to control white blister in comparison to one spray of the industry standard Folio Gold applied according to the white blister disease development model. Additionally applying Bravo at the recommended dosage according to the white blister disease development model also reduced the level of white blister in the crop. White blister was assessed only on the leaves of the plants and no infection on Brussels sprouts buttons was observed in the trial.

Summary of Year two results

Effect of Boscalid and Pyraclostrobin (Signum) application at recommended dosages after ringspot infection

The effect of incubation temperature after infection on the efficacy of recommended dosage of Boscalid and Pyraclostrobin (both in Signum) on ringspot infection at different incubation temperatures was investigated. The results show that 5, 10 and 15^{o}C reduced the development of ringspot lesions when applied after infection in comparison to 20^{o}C. Temperatures of 5^{o}C post inoculation improved the efficiency of Signum in comparison to other incubation temperatures. Signum applied up to 3 days post inoculation at 5^{o}C reduced ringspot infection significantly. Boscalid and pyraclostrobin applied as Signum were effective in reducing new ringspot lesion development up to 7 days after infection at 5, 10 and 15^{o}C in comparison to 20^{o}C. However application times over 7 days post infection were less effective but still controlled ringspot lesion number in comparison to plants incubated at 20^{o}C. The full interaction between applications of Signum at recommended dosages and incubation temperatures of 5, 10, 15, and 20^{o}C post inoculation could not be ascertained within this experiment because the control plants used lost their leaves and therefore did not express any ringspot symptoms which could be counted. The mean numbers of ringspot lesions per plant were low at approximately 3 by the end of the experiment.

Effect of temperature on fungicide content in leaves

Difenconazole (Plover) content in leaves declined more rapidly in comparison to Tebuconazole (Folicur) at temperatures of 5^{o}C. After approximately 21 days, levels of Plover in leaves of sprayed plants had declined to 0% of that found in the control plants. However 30% of the initial level of Folicur was present after 21 days at 5^{o}C. At higher temperatures (10, 15, and 20^{o}C) the levels of Plover were much higher as a percentage of the initial concentration. Levels of Plover were similar to those of Folicur found at each of the temperatures tested. The levels of Boscalid (applied in Signum), in sprayed leaf samples, dropped by 50 % regardless of incubation temperature over the experimental period. However Pyraclostrobin (also in Signum) in leaf samples were reduced to approximately 30 – 40% of that found in controls after seven days at 10, 15 and 20^{o}C. At 5^{o}C Pyraclostrobin was detected in higher amounts 7 days after spraying but dropped to low levels 14 days after application.

Measuring the effect of fungicides on inoculum production by the ringspot pathogen

The effect of spraying recommended dosages of Difenconazole (Plover) on inoculum production by established lesions of the ringspot pathogen was investigated. The results of

initial experiments show that sprays of Difenconazole initially depressed the level of inoculum production. However 7 days after fungicide application inoculum production by lesions began to rise to the levels observed on control plants.

Summary of Year three results

Effect of temperature on fungicide content in leaves under field conditions

Brussels sprouts cv. Millennium were raised under glasshouse conditions and transplanted into large pots of 20 – 30 cm diameter. Plants were grown to the fifteen to twenty leaf stage in under 12 – 14 ^{o}C day temperatures. Plants were divided into four equal groups and either left unsprayed or sprayed with either Difenconazole (as Plover), Tebuconazole (as Makhteshim AGAN tebuconazole generic product) or Boscalid/ Pyraclostrobin (as Signum). Each set of harvested leaves from each plant were separated and analysed (by hplc-uv methodology) separately. There were ten replicated sprayed plants per fungicide treatment and ten unsprayed control plants. The results show a rapid decline in fungicide levels in leaves during the first 7 days post application of Plover, Generic Folicur and Signum (Boscalid). There was some reduction in the amount of Signum (Pyraclostrobin) in leaf samples 7 days post application however levels were only marginally lower. There was a further reduction in levels of all fungicides in leaf samples taken between day 7 and day 42 post application. After 42 days post fungicide application levels of Plover, and Signum were reduced to virtually zero. Generic Folicur however remained relatively high in sprayed leaf samples after 42 days. The results confirmed controlled environment experiments using these fungicides carried out in year two of the project.

Effect of fungicides on ringspot inoculum production

The effect of fungicides on ringspot inoculum production was investigated in controlled environment experiments. The effect of application of Difenconazole (Plover), Tebuconazole (Nativo), Boscalid. and Pyraclostrobin (Signum) could not be measured as too few ringspot ascospores were produced in the unsprayed control treatments included in this experiment.

Financial benefit

Further information on the efficacy of protectant and eradicant fungicides on dark leaf spot, ringspot and white blister would be useful when selecting control options for controlling disease in crops after infection has been predicted. Often spraying fungicides in relation to risk

periods can be delayed because of adverse weather or the availability of spray equipment. This information once obtained within this project could be added to the Brassica$_{spot}$ forecasting system. This information could then be referred to by users when crop protection inputs were required. Appropriate control dosages could be used which would enable growers and producers to assess the economics of effective crop protection regimes.

Action points for growers

- Growers using the Brassica$_{spot}$ infection models have up to 5 -7 days to apply either Plover or Folicur and still maintain effective control.

- The application interval of Generic Folicur is much longer (approximately 14 days) in comparison to Plover especially at mean temperatures of 5 – 10^{o}C. Mean daily temperatures can be ascertained from the Brassica$_{spot}$ weather model.

- Significant levels of Generic Folicur could be measured up to 42 days post application of this fungicide.

- Sprays of Plover applied to established ringspot lesions can reduce inoculum production and slow epidemic development.

SCIENCE SECTION

Introduction

Fungicide usage is a key component within crop protection regimes for controlling leaf spots on vegetable Brassicas. Timing of fungicide application can enhance the efficacy of a treatment and reduce the need for reapplication. Optimal timing of pesticide applications often relies on accurate monitoring or prediction systems which identify when damaging or vulnerable lifecycle stages are occurring. Fungicide efficiency can be improved when these are applied when conditions are most suitable for the pathogen. Fortunately optimal conditions occur infrequently for many pathogens. However it is difficult to be accurate in designating favourable conditions for pathogen infection as night temperatures and local conditions where the crop is grown often become important. Approaches based on monitoring environmental conditions locally and using these collected variables with mathematical models has overcome these issues for many diseases. Vegetable Brassicas forecasting systems have been developed such as the Dutch Plant Plus system or Brassica $_{spot}$ (using the MORPH platform) for running disease forecasting models on PC.

Most of these systems comprise of models which describe the effect of environment on key life-cycle stages of plant pathogens. Leaf surface wetness duration, humidity and temperature are important environmental factors which determine the occurrence of infection and sporulation by air-borne pathogens of vegetable Brassicas. Models which describe the effect of temperature and wetness duration on infection of vegetable Brassicas by dark leaf spot (*Alternaria brassicae*) , ringspot (*Mycosphaerella brassicicola*) and white blister (*Albugo candida*) have been used successfully over many seasons within vegetable Brassica producing areas to improve control of these airborne diseases. The reliability of disease control and potential for reducing fungicide usage has been greatly enhanced. All three of these pathogens require leaf wetness for infection to occur. Dark leaf spot for example requires free water for spore germination and infection. At optimal temperatures of 20^{o}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. Dark leaf spot requires at least 12–14 h with a relative humidity of greater than 90% for sporulation to occur. One of the most difficult air-borne pathogens to control in vegetable Brassicas is ringspot caus*ed by Mycosphaerella brassicicola.* Due to the cosmetic nature of damage by *M. brassicicola* in horticultural production many opportunities exist for crop loss by this pathogen. Small amounts of disease can lead to downgrading in the value of production or total crop loss. However, ringspot

infection requires shorter periods (10 h) of leaf wetness at optimal temperatures for infection to occur. Ringspot requires prolonged periods of temperature and wetness to complete spore production within fungal structures on the lesion (Cullington, 1995).
Models describing these requirements form the basis of the Brassica *spot* disease forecasting system. Models can be used in conjunction with in field weather data collected by data loggers to determine the risk of infection by these pathogens. However this information has not been fully integrated with the activity of different approved fungicides. There is considerable choice in the number of fungicide products available however the activity of these fungicides on the leaf surface has not been characterised. If these systems are to be used more fully information is required on the timescales over which fungicides can successfully control disease when applied after risk periods are identified.

The effect of weather conditions on the efficacy of many fungicides has not been studied. An important consideration is the diurnal variation in temperatures which can be over 15^{o}C between night and day and it is unknown if all fungicides have equal performance under varying temperature conditions. Similarly the occurrence of fungicide residues could also be linked to mean temperatures. Many fungicides used in crop protection regimes in vegetable Brassica crops have activity against more than one pathogen. For example difenconazole (Plover) controls both ringspot and dark leaf spot. Fungicidal products may vary in their control of different pathogens when applied at reduced dosages. While this information is important experimentally it is not possible for insurance purposes to apply reduced dosages of fungicides. The recommended rate of application appears on the label for each fungicidal product. With greater importance attached to fungicide residues, information on the efficacy of reduced fungicidal dosages and their concurrent residual measurements in leaf tissues would help inform growers and producers. Residues would also be affected to some extent by variation in environmental conditions. Information on these aspects is limited due to the recent approval of some fungicidal products and the commercial confidentiality that fungicide manufacturers attach to specific data. It would be important to ascertain any divergence in the response of pathogens to reduced dosages of fungicides. Use of reduced fungicide dosages would be at the growers own risk. It would allow growers to determine the likely impact of fungicide residues on crops given spray applications close to harvest intervals or prevailing temperature conditions which were likely to impact on crop residues.

Additionally there is little information on the impact of fungicide sprays on sporulation by pathogens from mature and developing lesions. This is particularly important for the ringspot pathogen. This project will provide information on the effect of fungicide application post-

infection in combination with dosage and product type on dark leaf spot, ringspot and white blister. Addition of this information would reduce the degree of interpretation of results required when using the Brassica$_{spot}$ disease forecasting systems. With this new information growers will be able to reach much more informed decisions about fungicidal sprays required under all circumstances in the field. With the changes in the determination and accuracy of fungicide residues and its cost it will be increasingly important for growers to determine where fungicide residues are likely to occur. Controlling plant pathogens with little or no measurable fungicide residue will be an important consideration. Harvesting crops to avoid residue problems is necessary and if information on the environmental factors affecting residues could be obtained it would have a major impact on disease control in long season vegetable Brassica crops.

Materials and Methods

Controlled Environment Experiments

Plant production

Seeds of Brussels sprout cv. Revenge 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^{o}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^{o}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 of plants used in each experiment.

Inoculation of plants with ringspot ascospores

The effect of temperature on fungicide dosage and application timing after infection in Brussels sprouts was investigated at constant temperatures of 5, 10, 15, 20^{o}C and a relative humidity of 98 % day and night. Plants were grown in FP9 pots and were inoculated as a single batch in each experiment. Plants were placed at 100% rh after inoculation at a temperature of approximately 12^{o}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 5, 10, 15, 20^{o}C. 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.

Disease assessment

The numbers of lesions of different pathogens were counted at each assessment time before and after application of fungicides. Plants were assessed within each cabinet at 3, 7, 14 and 21 days after removal and fungicide application. Lesions of ringspot were counted at two time periods 14 days after fungicide application.

Application of fungicide dosages in controlled environment experiments

Sprays were applied to plants used in CE experiments using a Berthoud Vermorel 2000 HP knapsack fitted with a hollow cone nozzle. All fungicides were mixed into 1 litre of water, with the standard commercially recommended rates of fungicides as follows: Plover - 0.75ml/litre, Folicur - 1ml/litre, Bravo 500 - 2.2ml/litre and Signum - 1gm/litre. Reduced dosages were produced at 33 and 66% of active ingredient/product. Reduced dosages were used in experiments investigating their effect on disease control except where Signum was applied. Signum was applied in controlled environment experiments at recommended dosages only. Reduced dosages were not used in fungicide residue experiments. Unsprayed controls were included in each experiment. No other sprays (insecticides) were applied to the experimental plants during the experiment.

Application of fungicide dosages in Field experiments

Brussels sprout plants cv. Petrus were grown in 15 cm diameter pots. Approximately **Sprays** were applied to plants used in field experiments using a Berthoud Vermorel 2000 HP knapsack fitted with a hollow cone nozzle. All fungicides were mixed into 1 litre of water as in experiments in controlled environments as follows: Plover - 0.75ml/litre, Folicur - 1ml/litre, Bravo 500 - 2.2ml/litre and Signum - 1gm/litre. Signum was applied in field experiments at recommended dosages only. Reduced dosages were not used in fungicide residue experiments conducted under field conditions. Sprays were applied on day 0 and the leaves from five replicate plants per treatment were harvested at 3, 7, 14, 21 and 42 days after fungicide application and analysed for fungicide residues. Unsprayed controls were included in each experiment. No other sprays (insecticides) were applied to the experimental plants during the experiment.

Trapping ringspot ascospores after application of fungicides

Ascospore production from ringspot infected leaves sprayed with recommended and reduced dosages of Plover (difenconazole) was measured under CE conditions using methods described previously (Kennedy *et al*., 2000). Plants were grown in FP9 pots and were inoculated as a single batch in each experiment. Plants were placed at 100% rh after

inoculation at a temperature of approximately 12^{o}C in a misting tent for a 48 h period. After inoculation plants were removed from the misting tent and placed in a glasshouse with bottom watering. After the onset of symptom appearance (14 days) infected plants were divided into two groups with one group unsprayed and the other sprayed with 0.3 litres of Plover in equivalent amounts of water. Sprayed plants were placed in CE cabinets (one concentration per cabinet) at constant temperatures of 15^{o}C and a relative humidity of 98 % day and night. A microtiter immuno-spore trap (MTIST) was used to trap ringspot ascosporic inoculum released from the developing ringspot lesions on the plants. The MTIST was operated at an air-flow rate of 57 litres min^{-1} (1.78 litres flow rate min-1 across each microstrip well) and with a sampling time period of three days. The microtitre strips (4x8 wells) of the MTIST spore trap were removed and replaced using fresh microtitre strips after each sampling period. The collected microtitre strips were stored prior to assay development at -20ºC.

Quantifying *M. brassicicola* ascospores in microtitre wells from CE cabinets

Exposed microtitre strips were blocked with 200µl of 1% Casein buffer (1% (w/v) casein PBS) and incubated at 37ºC for 45 min. Residual blocking buffer was removed and wells were washed four times for one min each with 200µl PBS, 0.05% Tween 20 and 0.1% Casein (PBSTw C). Wells 1-4 of each strip then received 100 µl of monoclonal Ab EMA 187 (raised at Warwick HRI to *M. brassicicola*), with the remaining wells of 5-8 each receiving 100µl of PBS. 0.05% Tween 20 and 0.1% Casein as a control. Following incubation in a Wellwarm shaker incubater (30ºC) for a period of 45 mins as above, wells were washed three times for one min each with 200µl PBSTincTw. A DAKO duet amplification system was used (DAKO Ltd, Angel Drive, Ely, Cambridge, UK; Cat no. K0492) to amplify the signal generated by bound tissue culture supernatant antibodies. Wells were washed as described above and 100µl of 3,3',5,5'- tetramethylbenzidene substrate (Sigma, Poole, Dorset, UK; Cat. No. T-3405 and P-4922) was then added to each well. The reaction was stopped by adding 25µl of a 20% 1M H_2S0_4 solution to each well. Absorbance at 450nm was determined with a Biohit BP800 ELISA plate reader (Alpha Laboratories, 40 Parham Drive, Eastleigh, Hampshire, UK).

Measurement of fungicide residues

Residue analysis was carried out with solvent extraction from sprayed leaf material. Samples were cleaned up by solid phase extraction and analysis was performed by hplc-uv methodology. Results were confirmed with lc-ms. Other samples were analysed by Scientific Analysis Laboratories where larger numbers of samples could be processed.

Glasshouse Experiments

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 or Cv Millenium. 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^{o}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^{o}C before inoculation on to the plant. The concentration of inoculum was adjusted to approximately 10 ^{4} zoosporangia ml^{-1} using a haemocytometer.

Plant inoculation

Plants were grown in FP9 pots and were inoculated as a single batch in each experiment. Plants were placed at 100% rh after inoculation at a temperature of approximately 12^{o}C in a misting tent for a 48 h period. After inoculation plants were removed from the misting tent and randomly assigned to spray treatments. At each treatment time after the 48 h inoculation period approximately six plants were removed and treated with fungicides. Unsprayed controls were used in each experiment.

Experimental Treatments used in glasshouse experiments

Sprays of Nativo, Amistar Top, Signum, and Folio Gold at recommended rates were applied as treatments for glasshouse experiments with white blister.

Disease assessment

The number of lesions on each leaf of each plant was recorded for each temperature at each assessment time. Disease assessments were taken at 0, 4, 7, 10, 14, and 21 days after sprays had been applied inoculation. At each assessment time new plants were assessed for disease and leaf area. Experiments were repeated on two separate occasions.

Field trials testing forecasts for white blister as criteria for spray applications

Trial Design of field experiment

The experiment comprised of twelve plots of Brussels sprouts (cv. Revenge) each 9m square grown at 50cm spacing. These were arranged as three replicate plots treatment^{-1} in a randomized block design with a 2m spacing between blocks. Untreated control plots were located within each replicate block however the within block interference between untreated and treated plots was reduced by adopting a semi-systematic trial design within within blocks (Figure 1). Seeds were sown in Hassy 308 trays (14 May 2008) and transplanted into the field during June 2008.

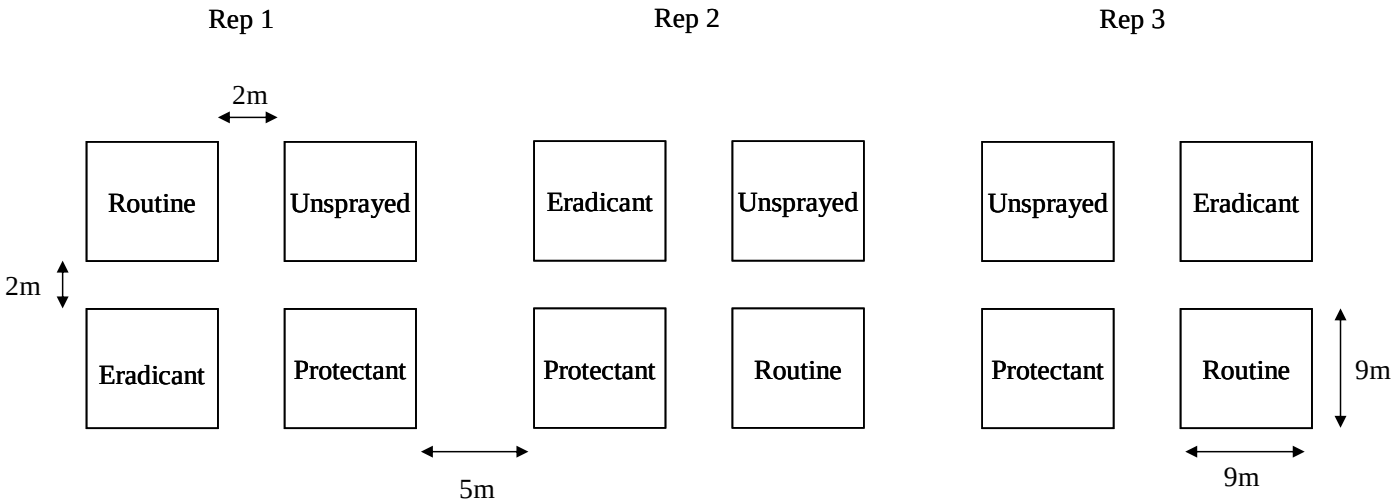

Figure 1: Trial design for the white blister Brussels sprouts experiment at Kirton 2008

Plot Inoculation of spray timing experiment

Plots were infected with *Albugo candida* by placing white blister infected seeding heads collected from an over wintered Brussels sprouts crop heavily infected with white blister which was planted during 2007 at Warwick HRI, Wellesbourne. Infected seeding heads were collected from Warwick HRI, Wellesbourne and taken to Warwick HRI Kirton on the 16 July 2008 and one infected seedling head was placed in the centre of each replicate field plot. Infected seeding heads were left in situ until the crop plants merged between rows at which point they were removed and discarded.

Spray Timing Treatments

Forecasts based on predictions of inoculum availability in the crop were used as the basis for

applying control sprays to the crop. The white blister Brassica$_{spot}$ forecasting system was used to predict the time taken for 5% or 50% of lesions in the crop to produce inoculum according to prevailing within crop weather conditions. These were particularly appropriate to test in relation to fungicide dosage. For example could a low dosage of fungicide be used if smaller amounts of inoculum (5%) had been predicted. Alternatively should higher dosages of fungicide be used if higher amounts of inoculum (50%) had been predicted.

The following treatments were used:

a) *Albugo* model Treatment (Time to 5% lesion appearance). Protectant spray
b) *Albugo* model Treatment (Time to 50% lesion appearance). Eradicant spray
c) Routine spray: Eradicant spray (Recommended dosage)
d) Unsprayed control

Experimental Treatments

The arrangement of treatments in the field is shown in Figure 1. The unsprayed plots remained unsprayed throughout the experiment. The routine treated plots were sprayed at 2-3 week intervals with Nativo. The Protectant treatment (Bravo) timings were designated according to the white blister spray timing model based on the time to 5% lesion appearance. The Eradicant treatment (Folio gold) timings were designated according to the white blister spray timing model based on the time to 50% lesion appearance.

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.

Tagging the assessed leaves

The layout of tagged plants and their location for each plot used for assessments is shown in Figure 2. A total of 8 plants were tagged for assessment in each plot. A total of six leaves selected at random were tagged on each plant. Three leaves were selected on the mid to lower section of the plant and three leaves were tagged on the upper part of each plant.

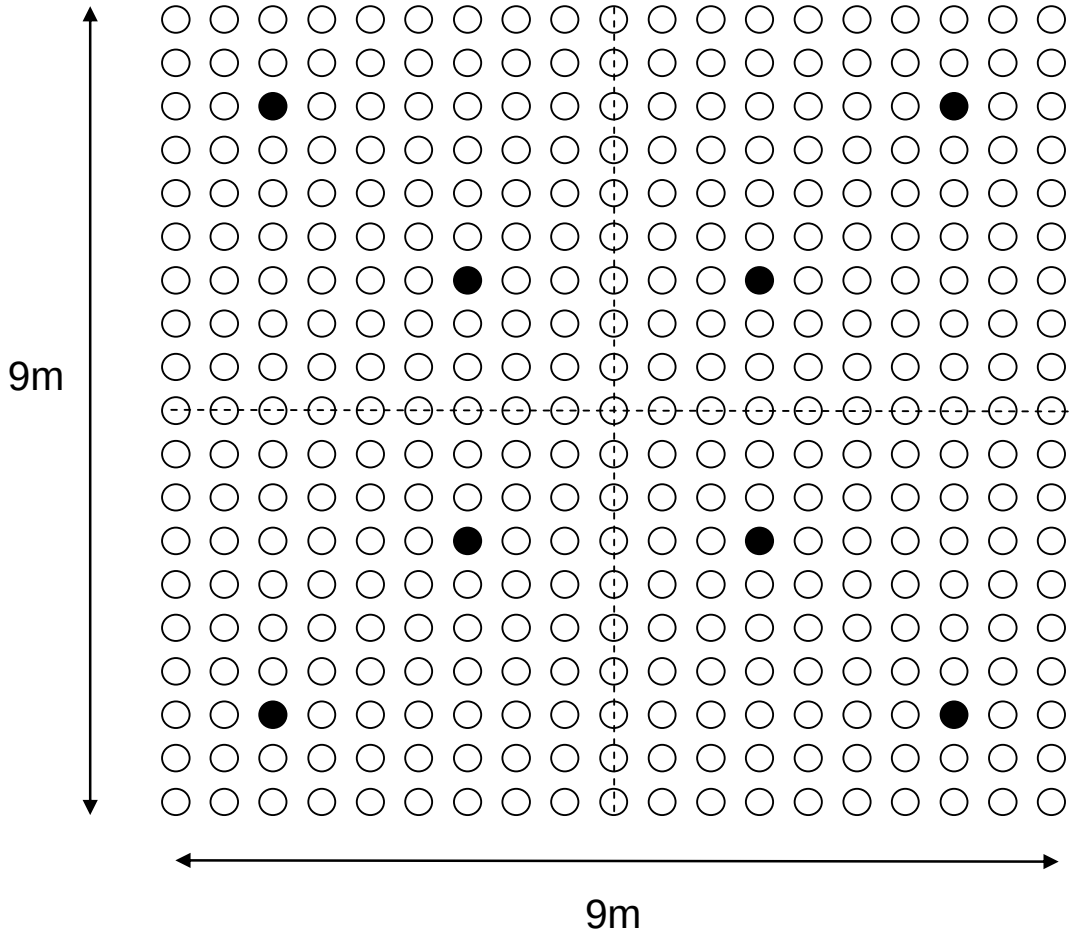

Figure 2: Layout of tagged plants in Brussels sprout plots

Assessment of white blister (*Albugo candida*) on Brussels sprout leaves

At each assessment the numbers of white blister lesions present on each tagged leaf were counted. Individual lesions where the pathogen had formed an individual blister were counted as one lesion. Often on more mature tissues these were formed in clumps. Leaves were assessed on a weekly basis on all plots. The results are expressed as the mean numbers of lesions present per tagged plant.

Assessment timings and intervals

At approximately 14 day intervals the tagged leaves were checked for white blister infection. Numbers of white blister lesions were counted on each leaf. Assessments began before the first sprays are applied for each treatment. Assessment timings on the unsprayed plots matched routinely treated plots. Assessments started first on routinely treated plots and unsprayed plots. Dropped leaves were not assessed but marked as missing.

Year One Results

Effect of post infection temperature and fungicide dosage on ringspot symptom appearance

Difenconazole (Plover) at recommended dosages (0.3 ml Litre water^{-1})

The effect of incubation temperature after infection on the efficacy of recommended dosage of difenconazole (plover) on ringspot infection at different incubation temperatures post inoculation is shown in Figure 3. The results show that at all incubation temperatures tested difenconazole at recommended dosages (0.3 litres) can be applied up to 5 days after infection and still give completed control of ringspot. However at application times over 5 day's incubation temperature greatly influenced the effectiveness of difenconazole in controlling ringspot. At a constant temperature of 5^{o}C difenconazole could be applied up to 14 days after inoculation and still reduce the occurrence of ringspot lesions. However at a constant incubation temperature of 15^{o}C sprays of difenconazole (at recommended dosages) became ineffective at between 5 and 8 days. At a constant incubation temperature of 10^{o}C sprays lost effectiveness when applied between 8 and 14 days after ringspot inoculation (Figure 3B). Results at 20^{o}C indicated that this incubation temperature was inhibitory for ringspot development after inoculation. At this constant temperature leaves infected with ringspot abcissed rapidly. Therefore ringspot development was lower as many leaves stopped growing and dropped off 8 days after inoculation. Application of difenconazole after 8 days at 20^{o}C produced lower numbers of ringspot lesions in comparison to untreated controls (Figure 3D). However the full interaction between application of difenconazole at recommended dosages and incubation at 20^{o}C post inoculation could not be ascertained within this experimental system.

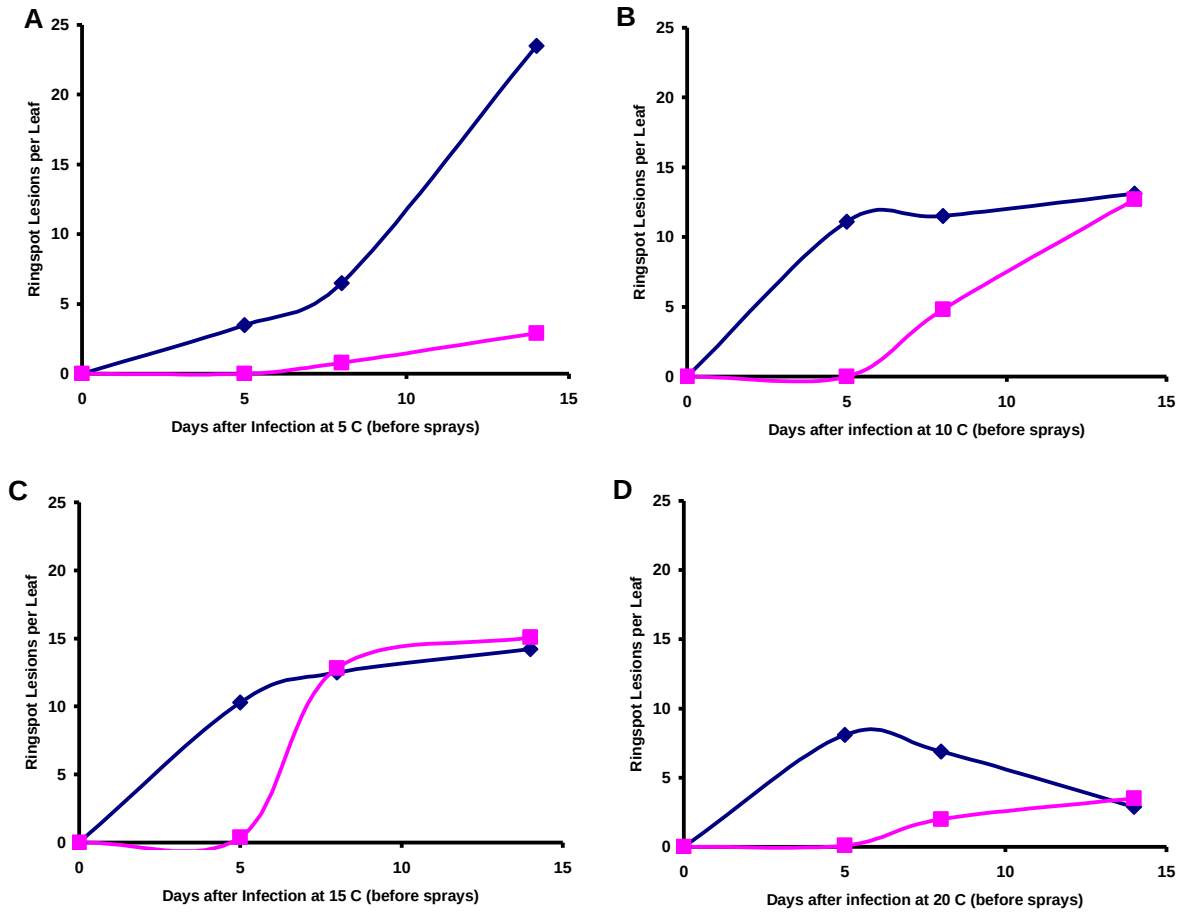

Figure 3: Ringspot infection on untreated plants (♦) and plants given sprays of Difenconazole (Plover) at 0.75 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

Difenconazole (Plover) at reduced dosages

The effect of incubation temperature after infection on the efficacy of reduced dosage of difenconazole (plover) on ringspot infection at different incubation temperatures post inoculation is shown in Figure 4 and 5. The results show that at all incubation temperatures tested difenconazole at reduced dosages (0.25 and 0.5 ml $litre^{-1}$) must be applied within 5 days after ringspot infection. Ringspot levels were low at all incubation temperatures if sprays were applied after 5 days post infection. At 5°C post infection there was very low levels of ringspot infection observed on plants treated with 0.25 and 0.5 ml $litre^{-1}$ difenconazole (Figure 3A and 4A). This suggests that mean temperatures could be used to determine effective control dosages of difenconazole. Dosage of Plover had negligible effect on disease levels at incubation temperatures of 10, 15 and 20°C when applied 8 days after inoculation (Figures 3

and 4). At 20°C incubation temperatures leave heavily infected with ringspot abcissed rapidly. regardless of treatment with reduced dosages of difenconazole. Many leaves stopped growing and dropped off 8 days after inoculation although this was less evident in treatments incubated at 20°C and sprayed with 0.5 ml litre^{-1} difenconazole (Figure 5D) .

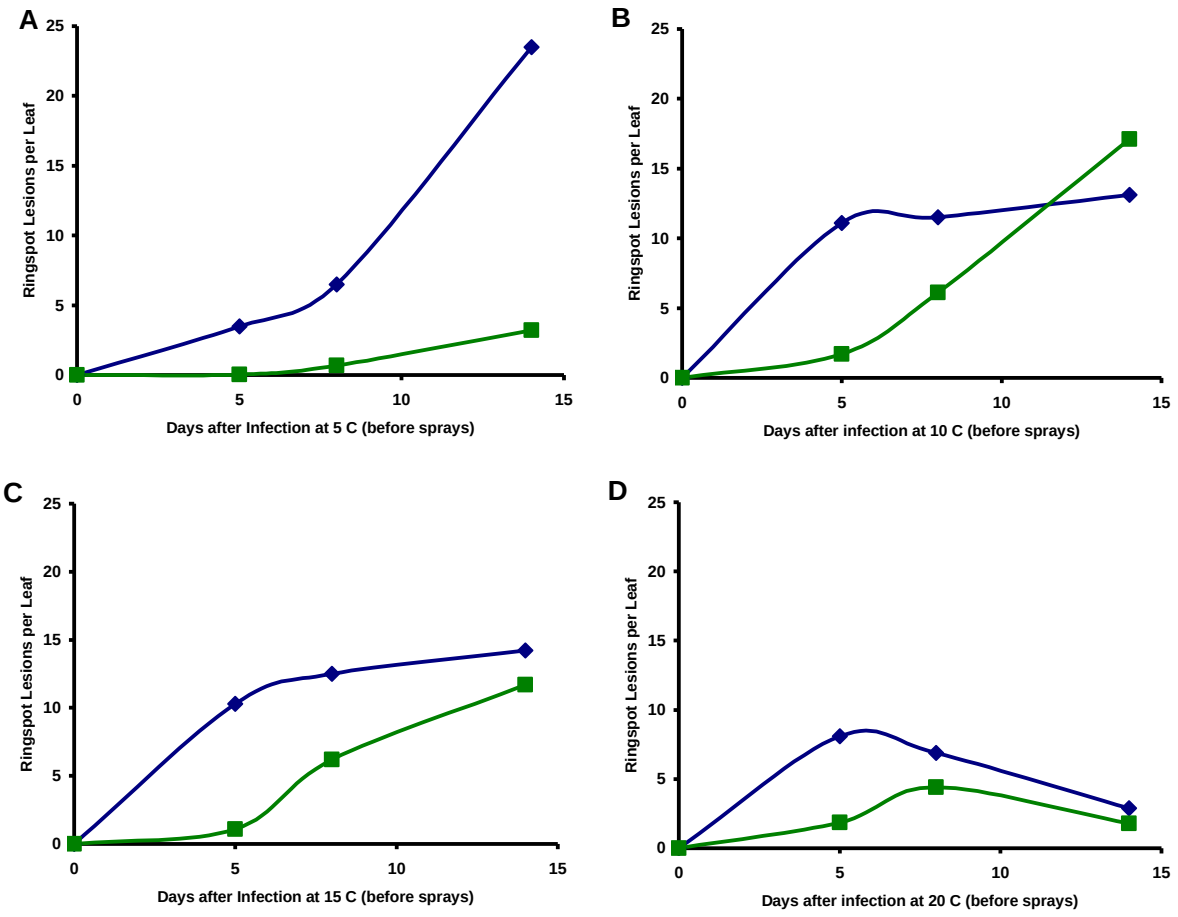

Figure 4: Ringspot infection on untreated plants (♦) and plants given sprays of Difenconazole (Plover) at 0.25 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

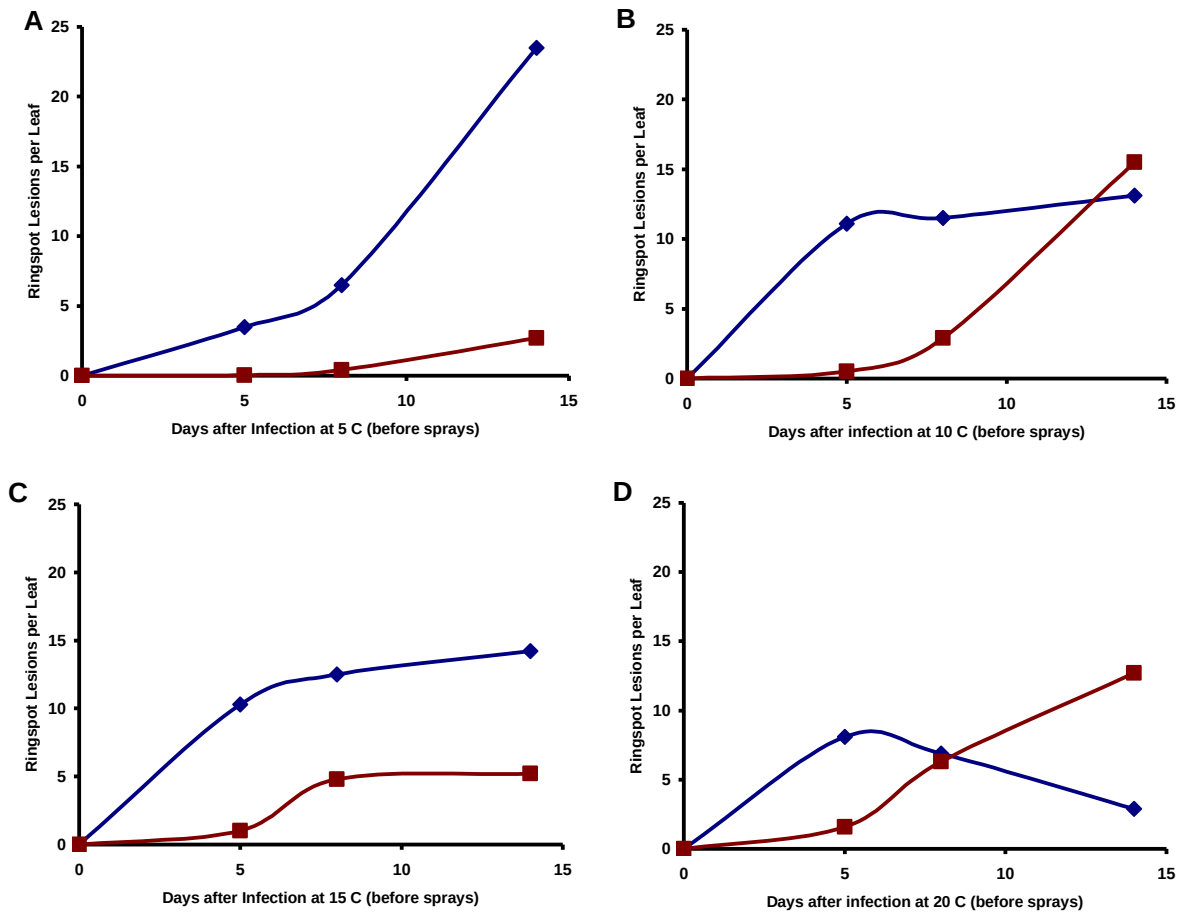

Figure 5: Ringspot infection on untreated plants (♦) and plants given sprays of Difenconazole (Plover) at 0.50 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

Chlorothalonil (Bravo) at recommended dosages (2.2 ml Litre water^{-1})

The effect of incubation temperature post-infection on the efficacy of recommended dosage of Chlorothalonil (Bravo) on ringspot infection is shown in Figure 6. The results show that at all incubation temperatures tested Chlorothalonil at recommended dosages did not control ringspot infection. At each incubation temperature, application of Bravo resulted in higher numbers of lesions present initially on plants. However numbers of ringspot lesions on unsprayed plants increased on inoculated plants incubated for over four days at each incubation temperature. There were lower numbers of ringspot lesions observed on sprayed and unsprayed treatments at 5°C and 20°C. In these experiments greater numbers of leaves dropped before assessments could be taken. This was observed at all temperatures especially on plants incubated at 15°C and 20°C. Inoculated plants treated with Bravo appeared to shed leaves slightly earlier than unsprayed plants (Figure 6B, C, D). At 10, 15, and 20°C some

symptoms of ringspot infection were already visible on plants and the high levels of ringspot infection resulted in abscission of the (small) leaves on young plants used in these experiments.

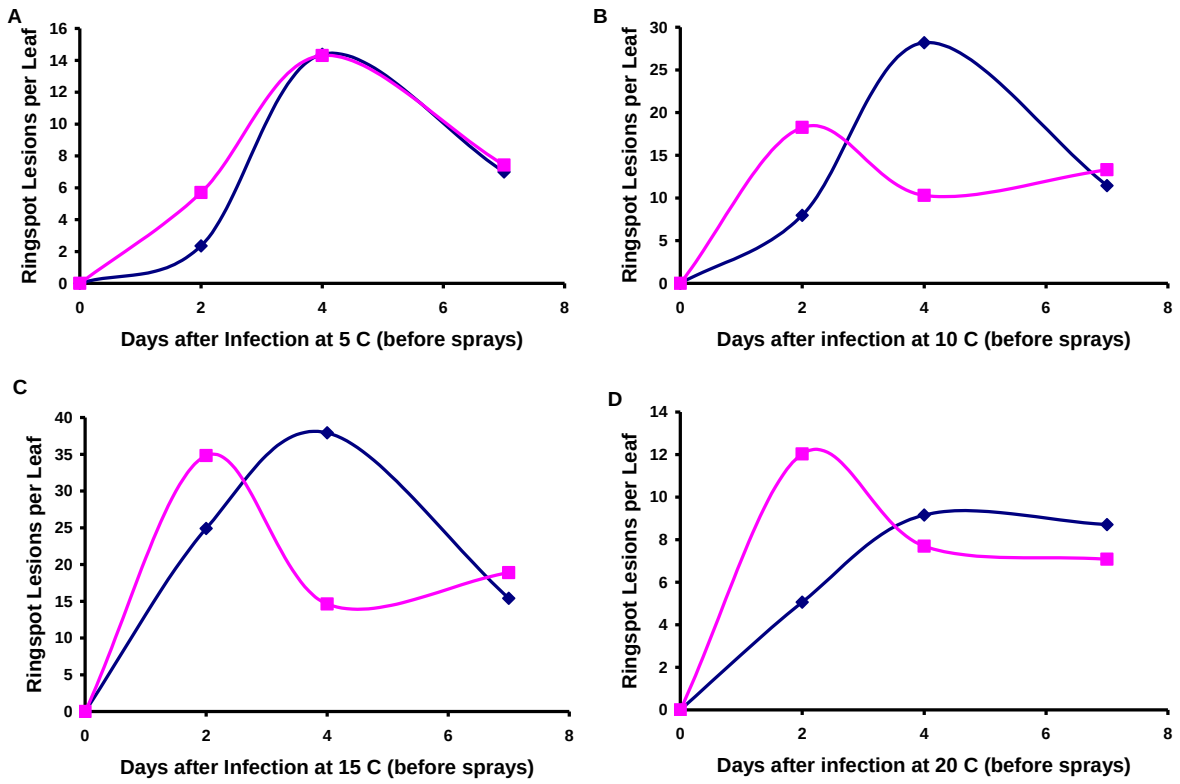

Figure 6: Ringspot infection on untreated plants (♦) and plants given sprays of Chlorothalonil (Bravo) at 2.2 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

Chlorothalonil (Bravo) at reduced dosages

The effect of incubation temperature post-infection on the efficacy of reduced dosage of difenconazole (plover) on ringspot infection is shown in Figure 7 and 8. The results show that at all incubation temperatures tested Bravo at reduced dosages (0.7 and 1.4 ml $Litre^{-1}$) did not control ringspot infection if applied 2 days after inoculation. At sub optimal temperatures application of Bravo appeared to stimulate the early appearance of ringspot lesions on the inoculated plants (Figure 8A, B, D). At 20°C incubation temperature leaves infected with ringspot abcissed rapidly regardless of treatment with reduced dosages of Bravo. As observed in other experiments leaves stopped growing and dropped off if heavily infected with ringspot. There was no effect of reduced dosages of Bravo on the severity of ringspot infection regardless of incubation temperature post-infection.

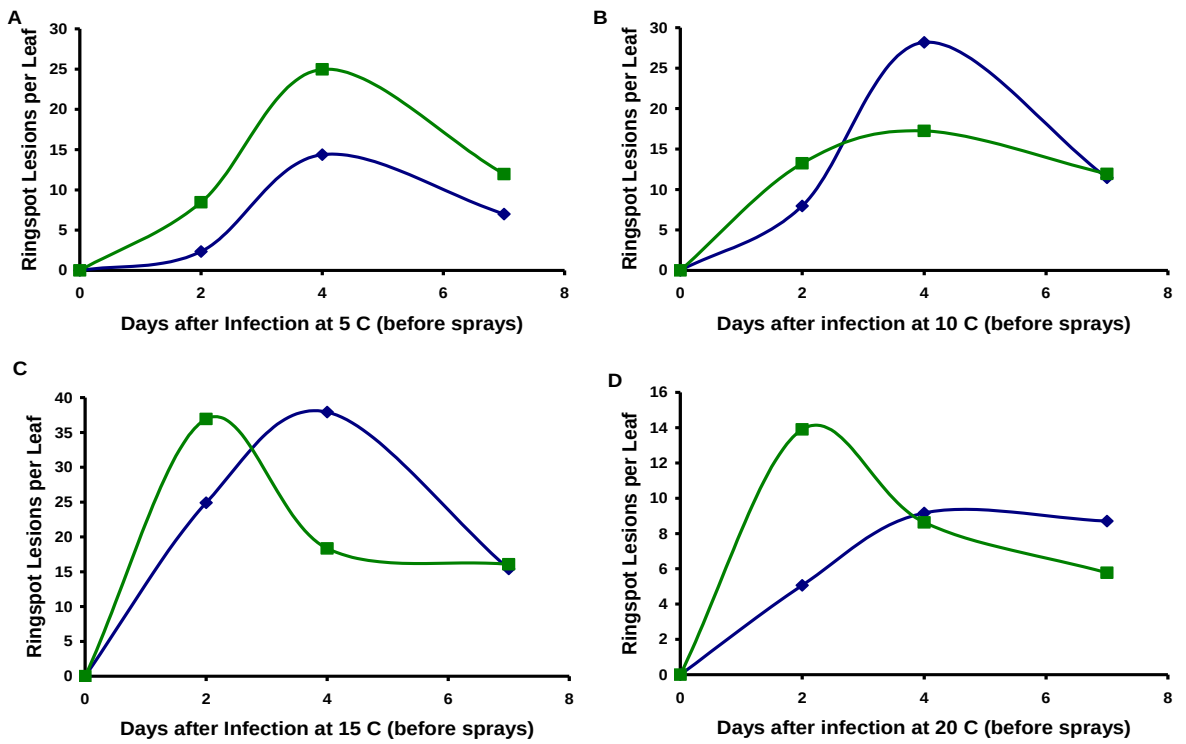

Figure 7: Ringspot infection on untreated plants (♦) and plants given sprays of Chlorothalonil (Bravo) at 0.7 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

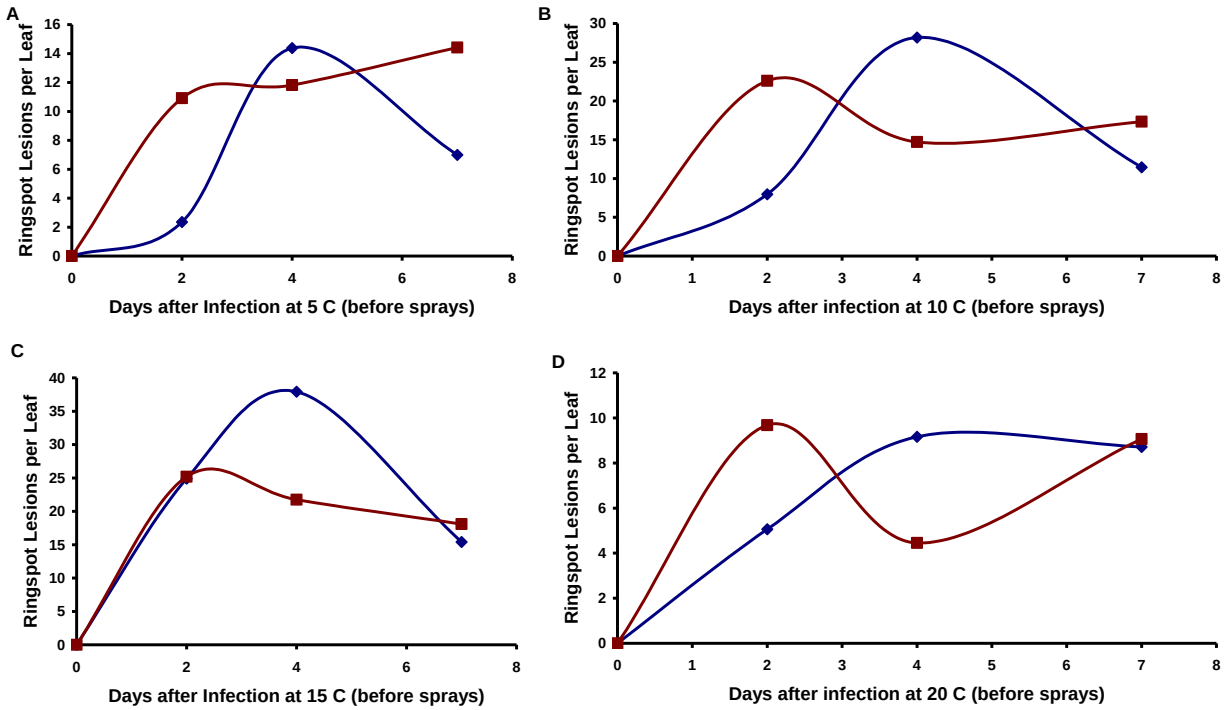

Figure 8: Ringspot infection on untreated plants (♦) and plants given sprays of Chlorothalonil (Bravo) at 1.4 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

Tebuconazole (Folicur) at recommended dosages (1.0 ml Litre water^{-1})

The effect of incubation temperature post infection on the efficacy of recommended dosage of Tebuconazole (Folicur) on ringspot infection is shown in Figure 9. Small amounts of ringspot infection were recorded at all incubation temperatures when sprays were applied 2 days post-infection. Application of Tebuconazole 7 days post-infection was still very effective at 5^{o}C incubation temperature and moderately effective at 10^{o}C. High levels of infection on untreated plants sprayed after 14 days post-infection at incubation temperatures of 5^{o}C resulted in leaf abscission before full leaf assessments could be taken (Figure 9 A). The result on this treatment should be higher as there were missing leaves. However at application times over 2 days at incubation temperatures of 15 and 20^{o}C were less effective (Figure 9 C, D). Tebuconazole was not very effective at controlling ringspot infection if applied at mean temperatures of 20^{o}C post-infection. Some control was observed at mean temperature of 15^{o}C post-infection. Results at 20^{o}C indicated that this incubation temperature was inhibitory for ringspot development after inoculation. At this constant temperature leaves infected with ringspot abcissed rapidly. Therefore ringspot development decreased at 14 days post-infection because disease was already visible and at high infection levels per leaf many leaves had stopped growing and dropped off.

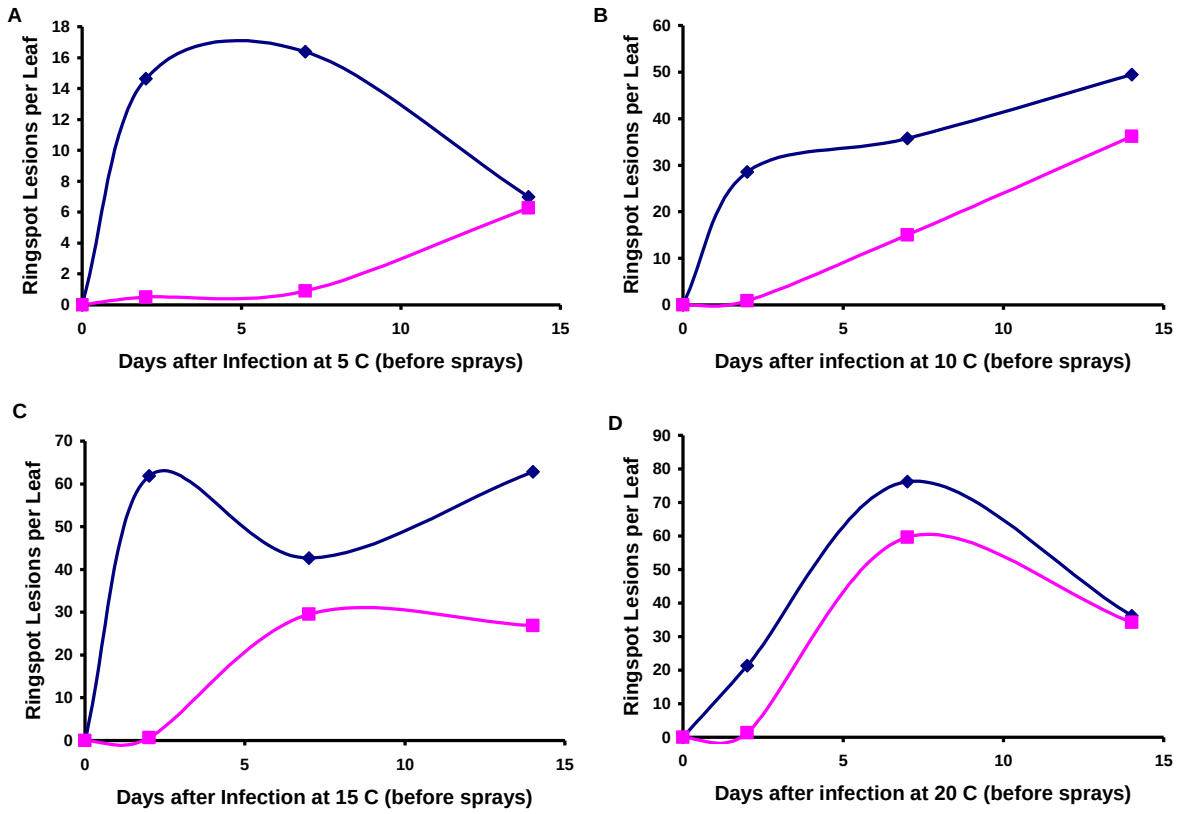

Figure 9: Ringspot infection on untreated plants (♦) and plants given sprays of Tebuconazole (Folicur) at 1 ml L^{-1} water (■) after incubation post-inoculation at 5^{o}C (A), 10^{o}C (B), 15^{o}C (C) and 20^{o}C (D)

Tebuconazole (Folicur) at reduced dosages[1]

The effect of incubation temperature post infection on the efficacy of reduced dosage of Tebuconazole (Folicur) on ringspot infection is shown in Figures 10 and 11. The results show that was little difference in the ringspot infection at all incubation temperatures tested using reduced dosages (0.3 and 0.6 ml Litre $^{-1}$) compared to the effect of using recommended dosages. Small amounts of infection were recorded on plants incubated at all temperatures for 2 days before spray applications. Ringspot lesion numbers were significantly lower at 5^{o}C post infection in comparison to other incubation temperatures used post-infection (Figure 10A and 11A). Lower numbers of ringspot lesions were observed on plants incubated for 7 days before applications of reduced dosages of Tebuconazole. Tebuconazole applications appeared more effective than difenconazole at lower incubation temperatures although the significance of results would need to be tested statistically.

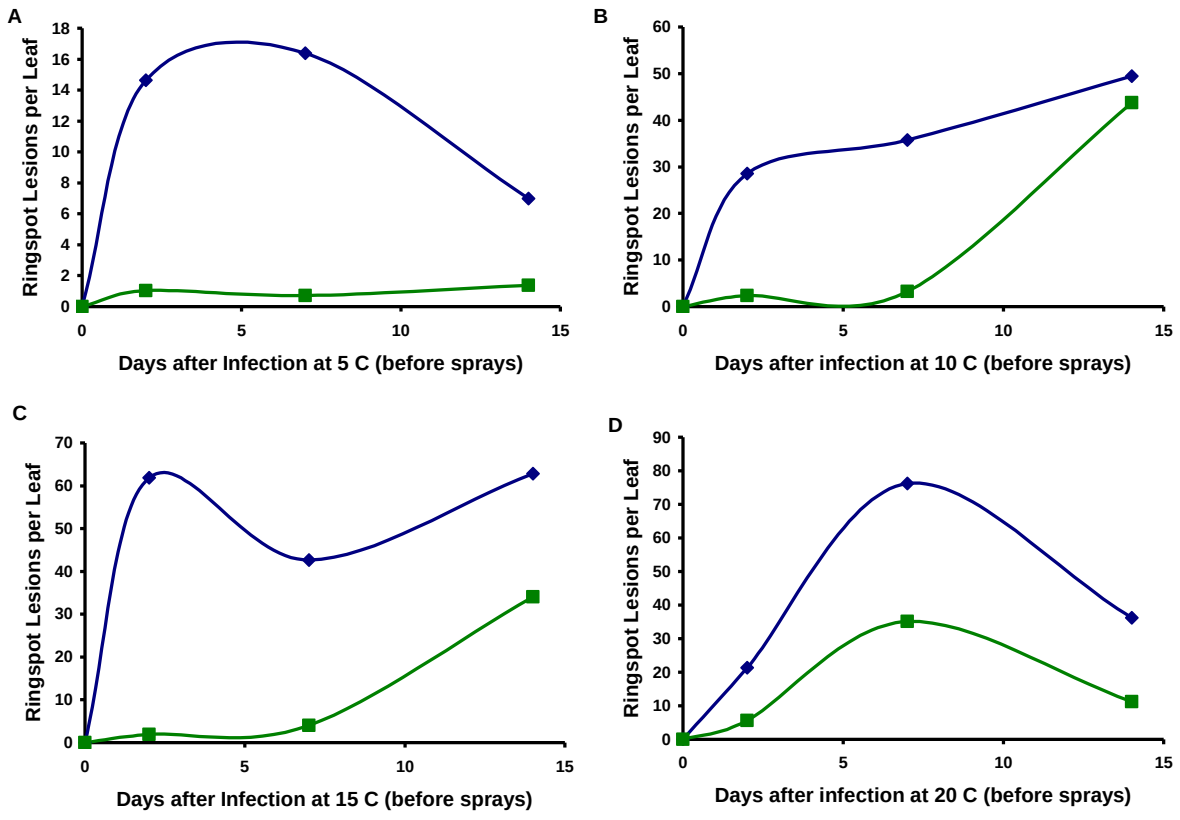

Figure 10: Ringspot infection on untreated plants (♦) and plants given sprays of Tebuconazole (Folicur) at 0.6 ml L^{-1} water (■) after incubation post-inoculation at 5^{o}C (A), 10^{o}C (B), 15^{o}C (C) and 20^{o}C (D)

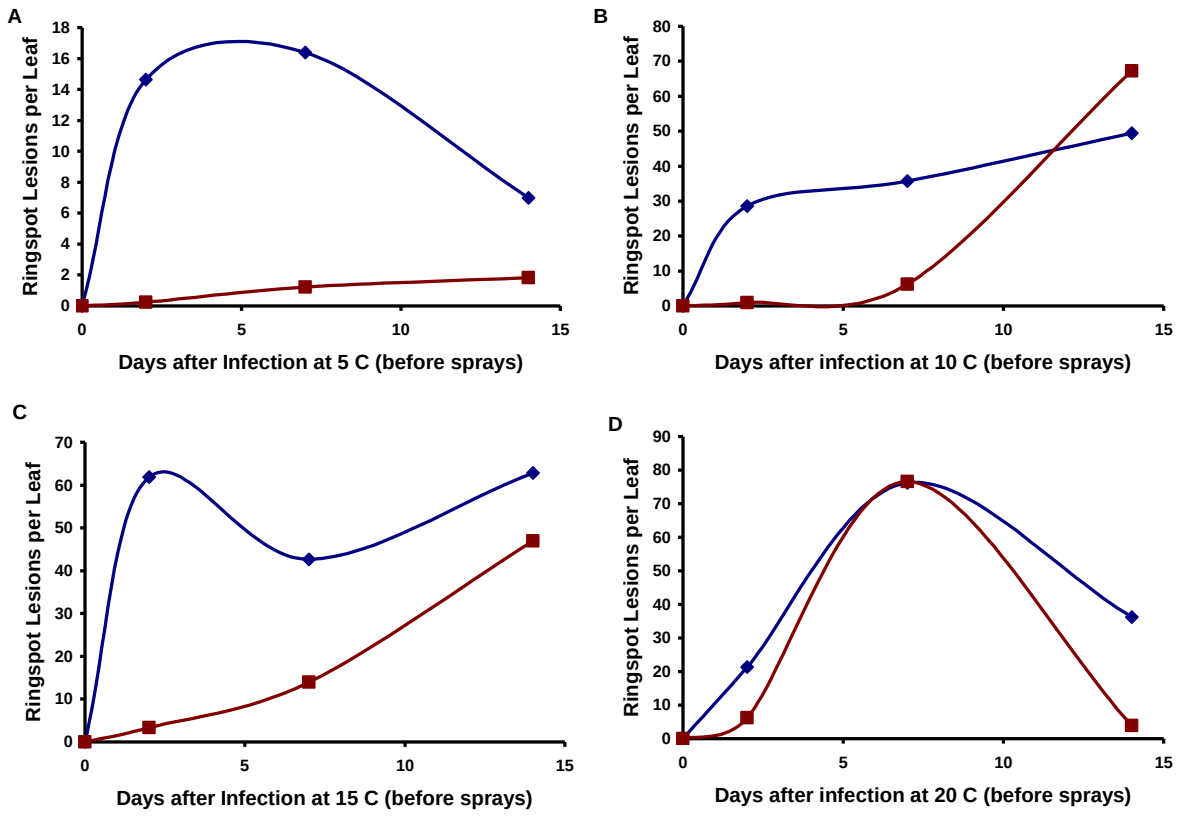

Figure 11: Ringspot infection on untreated plants (♦) and plants given sprays of Tebuconazole (Folicur) at 0.3 ml L^{-1} water (■) after incubation post-inoculation at 5°C (A), 10°C (B), 15°C (C) and 20°C (D)

Ringspot infection at incubation temperatures of 20°C were similar on unsprayed and sprayed plants regardless of Tebuconazole dosage used. At 20°C some ringspot infection was visible before sprays were applied. Applications of reduced dosages of Tebuconazole at increasing time after inoculation had little effect on the number of new ringspot lesions appearing (Figure 10D and 11D).

Testing forecasts for white blister as criteria for spray applications

Prediction of white blister development in the field at Warwick HRI Kirton

The results of using the Brassica$_{spot}$ disease forecasting system for white blister are shown in Figure 12. The bar chart shows the prediction of white blister infection conditions. The line graph shows the time to symptom appearance from particular periods of high risk infection conditions.

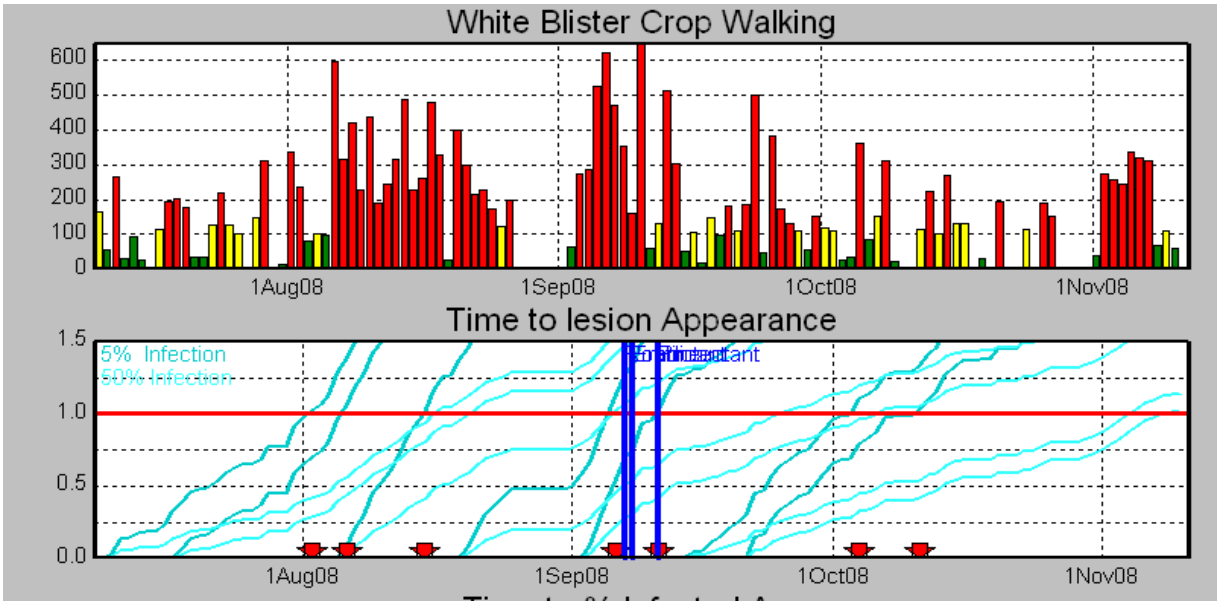

Figure 12: Prediction of dark leaf spot and ringspot infection in the field at Warwick HRI 2006 red coloured bar (high risk) yellow bar (moderate infection risk) green bar (no risk). Time to 5 % (■) and 50 % (■) lesion appearance

The results show that during 2008 white blister infection conditions were not limiting at WHRI Kirton. There were many periods when white blister infection was predicted in the field using environmental data from the data logger in conjunction with the white blister Brassica$_{spot}$ prediction model. Infection conditions for white blister were fulfilled when an infection score of greater than 150 was recorded as designated with a red coloured bar (high risk) on the day where infection was predicted as having occurred. Days represented by a yellow bar (moderate infection risk) also occurred. High risk period occurred on the 12, 18, 19, 20, 24 and 29 July 2008, and the 1, 2, 6 – 19, 21-26 and the 28 August 2008. High risk infection periods also occurred on the 3 – 10, 13, 14, 20, 22, 23, 25, 26, 27, and 30 September 2008 and on the 5, 8, 13, 15, 21, 27 and 28 October and the 2- 7 November 2008. Other moderate risk periods occurred on the 10, 17, 23, 25, 26 and 28 July 2008 and the 4, and 25 August 2008. The 12, 16, 18, 21, and 28 September with the 5, 8, 13, 15, 21, 27, and 28 October 2008 were also moderate risk periods for white blister infection.

White blister lesions counts from leaves in a Brussels sprout crop at Warwick HRI Kirton sprayed according to the Brassica$_{spot}$ white blister model

There were low numbers of white blister lesions observed on crop plants during the trial. Older leaves showed higher levels of infection (Figure 13) in comparison to the younger leaves (Figure 14) in the crop. White blister did not increase on lower unsprayed leaves during the

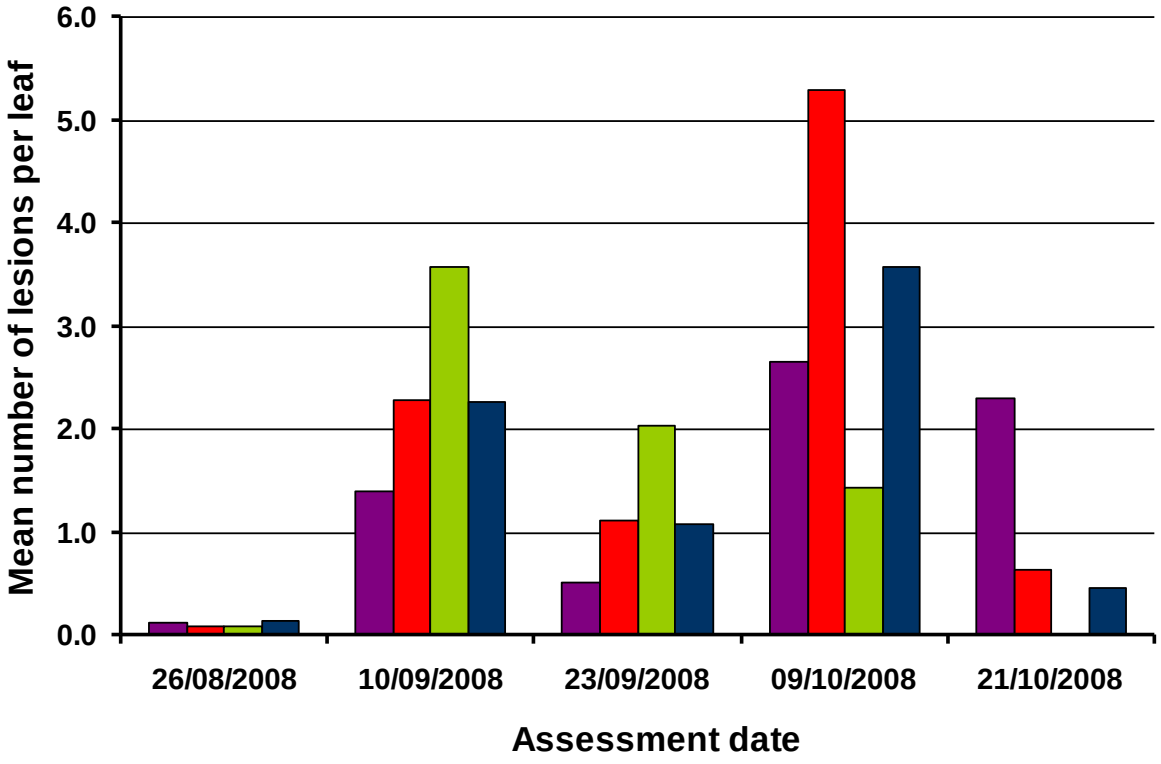

Figure 13: Mean total number of white blister lesions per leaf on lower plant leaves sprayed with a routine spray of Nativo (■), Folio Gold applied according to the output from the white blister model (■), Bravo applied according to the output from the white blister model (■) and unsprayed (■) in replicated plots at Warwick HRI Kirton

Trial period. Approximately 2–3 white blister lesions were observed on older leaves from crop plants. White blister lesion number increased on upper crop plant leaves reaching approximately 5 lesions per leaf on the 21 October 2008. Application of Folio Gold (in conjunction with the white blister model) controlled white blister infection on both lower and upper leaves (Figure 13 & 14). The 6 August 2008 was used as the start date for computing the time to 5% and 50% lesion development. The 20 August 2008 was used as an additional date for computing the time to 5% and 50% lesion development. Sprays of Bravo were applied at the estimated time to 5% lesion development with Folio Gold applied at the estimated time to 50% lesion development. There was little effect on white blister lesion numbers of applying Nativo as a routine spray to lower and upper leaves (Figure 13 & 14). Application of Bravo (in conjunction with the white blister model) was not effective in controlling white blister on lower leaves (Figure 13) but was more effective in controlling white blister lesion number on upper leaves (Figure 14).

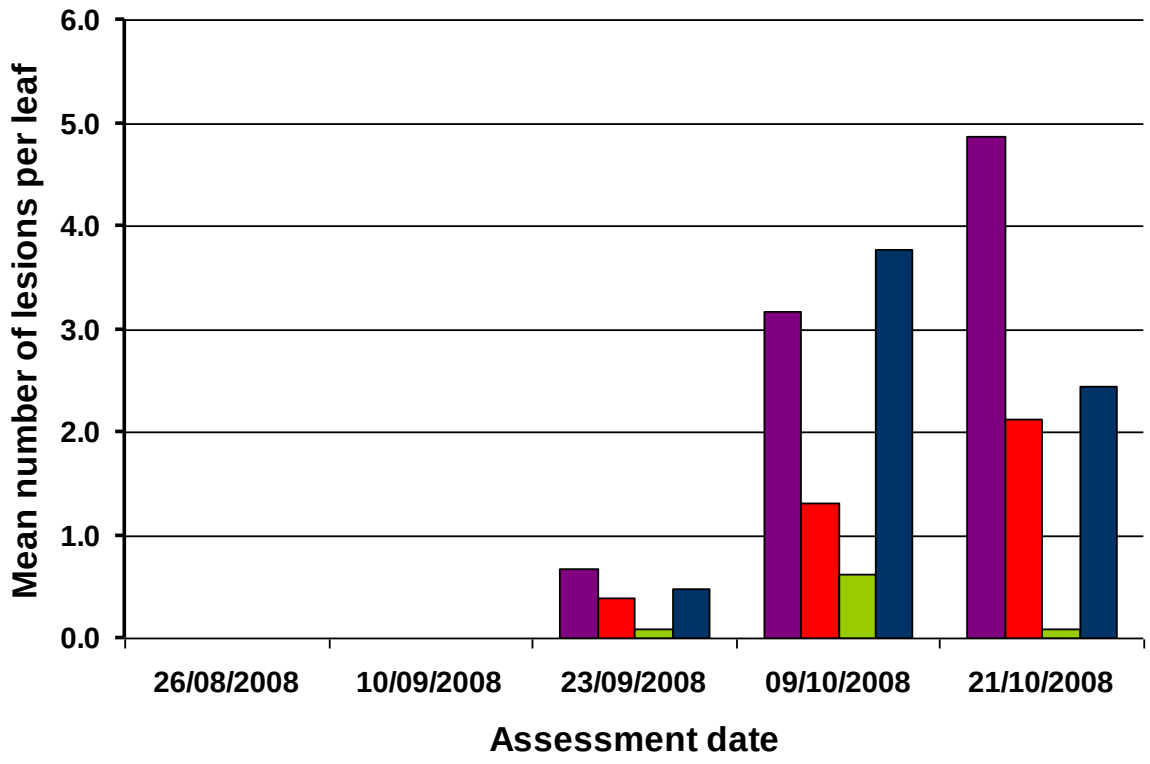

Figure 14. Mean total number of white blister lesions per leaf on younger leaves sprayed with a routine spray of Nativo (■), Folio Gold applied according to the output from the white blister model (■), Bravo applied according to the output from the white blister model (■) and unsprayed (■) in replicated plots at Warwick HRI Kirton

Spray dates used in white blister field trial

White blister development at Warwick HRI Kirton on crop plants was relatively low. Secondary spread of white blister within the crop was not observed within the trial until August/September 2008. Additionally application of sprays within the crop was delayed as a result of the very wet conditions which occurred during August 2008. As a result of these factors only two applications of Nativo were possible in the routinely sprayed plots on the 8 September 2008 and the 6 October 2008. A 3 week spray application interval was used in this treatment. Conditions became unfavourable for white blister after October 2008. Folio Gold was applied on the 12 September 2008 in response to the model output. The time to 50% symptom appearance from infection conditions observed on the 6 August 2008 (Figure 12). This model condition was fulfilled on approximately the 12 September 2008 when spray application conditions permitted the spray to be applied. A spray of Bravo at recommend dosage was applied to the protectant treatments on the 12 September 2008 in response to model output. The time to 5% symptom appearance from infection conditions occurring on

the 3 September 2008 (Figure 12). A further spray of Bravo was applied to the protectant treatment on the 6 October in response to the time to 5% symptom appearance from infection conditions occurring on the 14 September 2008.

Table 1 Fungicide spray dates in the white blister field trial at WHRI Kirton

Spraying date (Actual)	Scheduled	Product	Plots
8/9/08		Nativo	Routine
12/9/08		Folio Gold	Eradicant
		Bravo	Protectant
6/10/08	29/9/08	Nativo	Routine
	6/10/08	Bravo	Protectant

Year Two Results

Effect of post infection temperature and fungicide dosage on ringspot symptom appearance

Effect of Boscalid and Pyraclostrobin *(Signum)* application at recommended dosages after ringspot infection

The effect of incubation temperature post infection on the efficacy of recommended dosage of Boscalid and Pyraclostrobin (Signum) on ringspot infection is shown in Figure 5. The results show that 5, 10 and 15^{o}C reduced the development of ringspot lesions when applied after infection in comparison to 20^{o}C. Temperature of 5^{o}C post inoculation improved the efficiency of Signum in comparison to other incubation temperatures. Signum applied up to 3 days post inoculation at 5^{o}C reduced ringspot infection significantly. The results show that Boscalid and Pyraclostrobin applied as Signum was effective in reducing new ringspot lesion development up to 7 days after infection at 5, 10 and 15^{o}C in comparison to 20^{o}C. However, at application times over 7 days were less effective but still controlled ringspot lesion number in comparison to plants incubated at 20^{o}C . Results show the cumulative development of ringspot lesions at different incubation temperature so care must be taken in the interpretation of the results (Figure 15). However the full interaction between application of Signum at recommended dosages and incubation temperatures of at 5, 10, 15 , and 20^{o}C post inoculation could not be

ascertained within this experiment because the control plants used lost their leaves and therefore did not express any ringspot symptoms which could be counted. The numbers of ringspot lesions per plant were low at approximately 3 by the end of the experiment. It is possible that higher amounts of ringspot on the control plants caused leaf abscission. If this was correct the results may indicate that Signum reduces ringspot infection on plants when applied up to 21 days post inoculation.

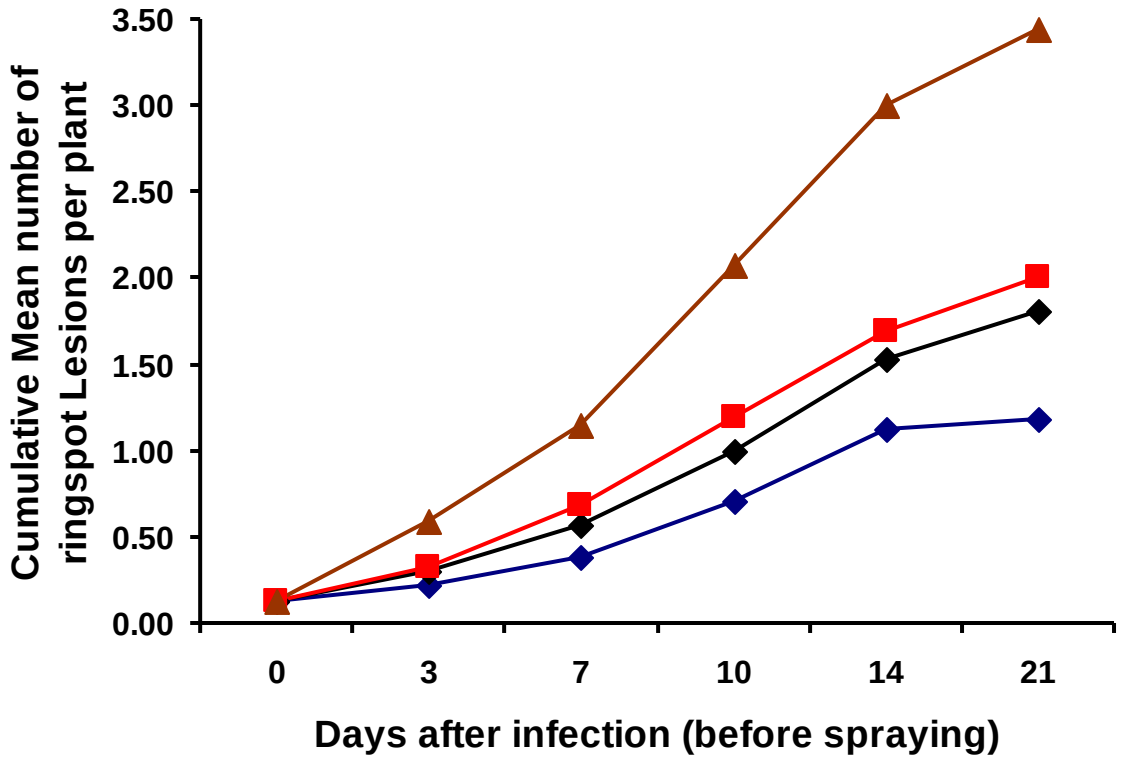

Figure 15 Cumulative ringspot lesions on plants given sprays of Signum (at recommended dosage) at increasing days post infection at 5°C (♦), 10°C (♦),15°C (■), and 20°C (▲)

Effect of temperature on fungicide content in leaves (First Replicate Experiment)

Experiments investigating the effect of temperature on fungicide content in leaves sprayed with of Tebuconazole (Folicur), difenconazole (Plover), Boscalid and Pyraclostrobin (Signum) were carried out under controlled temperatures conditions. The sprayed leaf material was analysed for total content in 30 g of leaf material harvested. The experiments were repeated on three occasions. The actual residue data for one replicate experiment over the 5 – 20°C temperature range is shown in Figure 16, 17, 18, and 19. There was little difference in the Tebuconazole content.

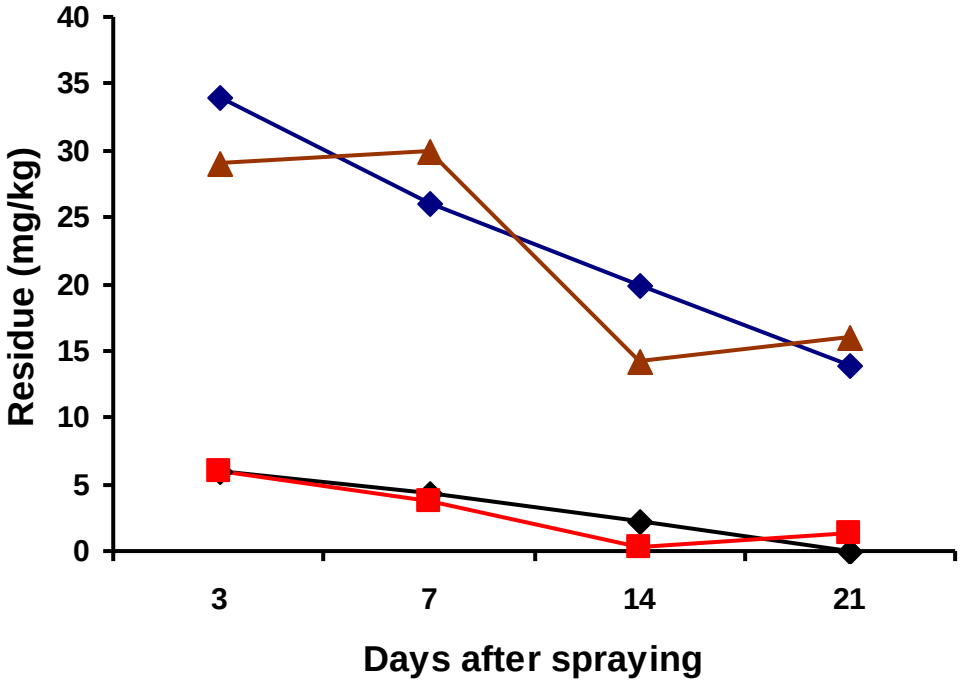

Figure 16 Fungicide residue on seedlings at 5°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

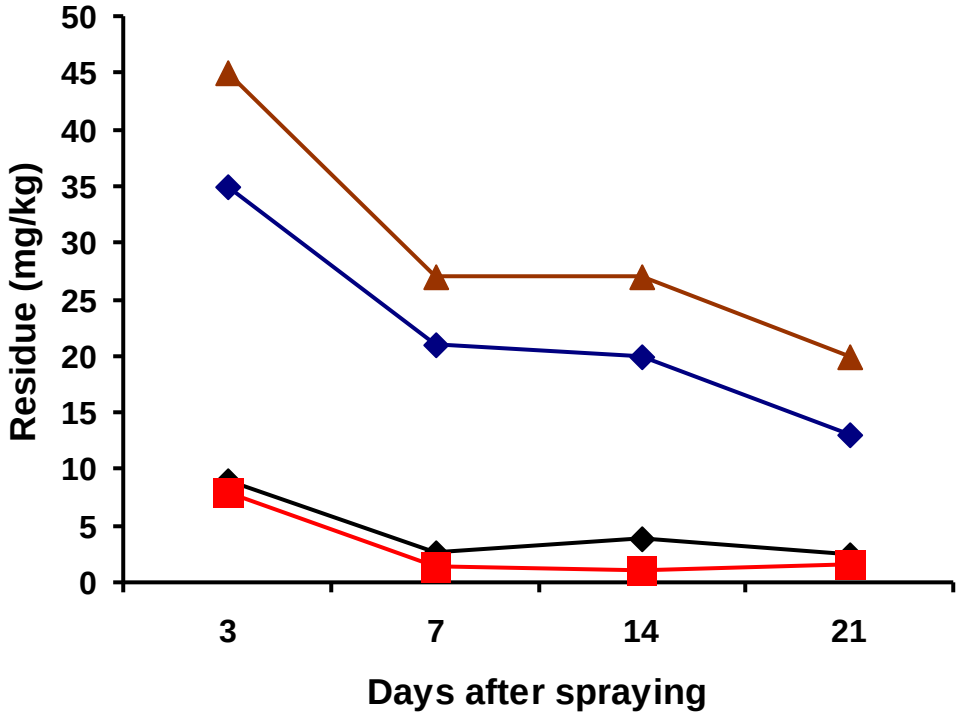

Figure 17 Fungicide residue on seedlings at 10°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

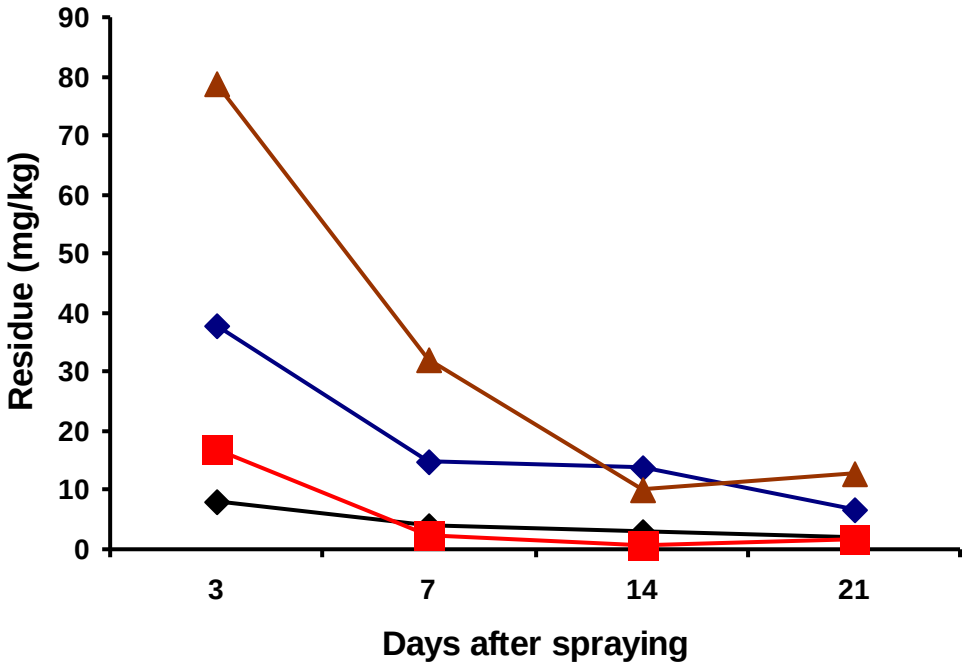

Figure 18 Fungicide residue on seedlings at 15°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

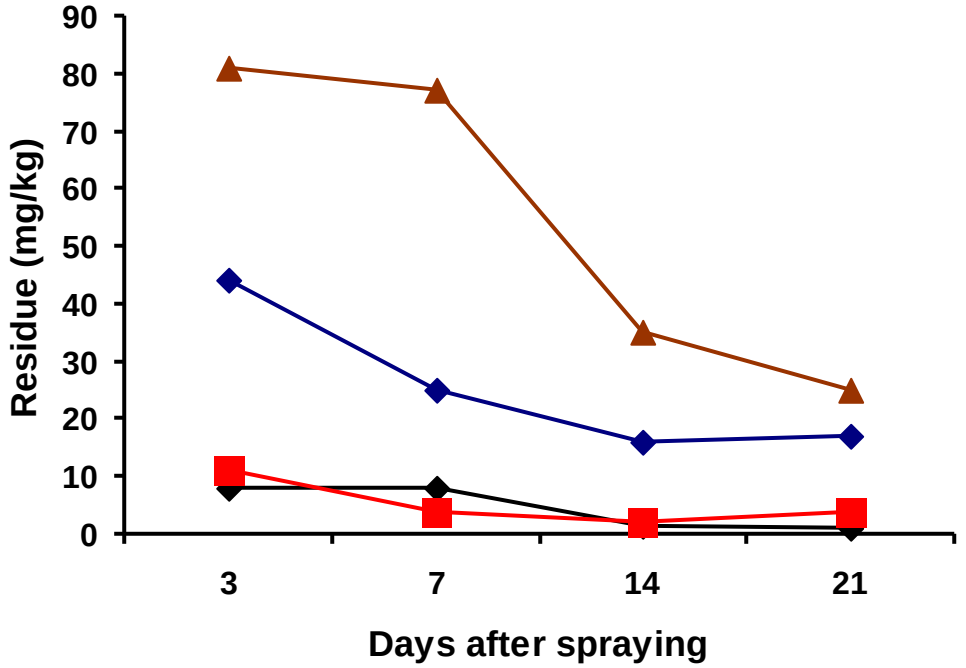

Figure 19 Fungicide residue on seedlings at 15°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

Between temperatures over the 21 day assessment period. Boscalid in leaves sprayed with recommended dosages remained high regardless of incubation temperature (Figures 16 – 19). However difenconazole and Pyraclostrobin declined rapidly after application and by had dropped significantly 7 days post application. The rate of decline of these chemicals in leaf samples was more rapid at temperatures of 15 and 20^{o}C (Figures 18 and 19). This was observed for Pyraclostrobin in particular. Levels of Tebuconazole in leaf samples were higher over the 21 day experimental period declined at the same approximate rate regardless of temperature. Low levels of difenconazole were present in leaf samples regardless of incubation temperature or days post application. The initial uptake of Boscalid by plants was increased at temperatures of 15 and 20^{o}C. On the basis of previous results, the content of difenconazole, should be above 5 ng/ml, for effective control of ringspot to be maintained. However it is unclear if the enhanced response of difenconazole results from the direct effect of the temperature on the chemical content of the leaf or whether it is an interaction between chemical content and effect of temperature on the rate of infection by the ringspot pathogen. The results plotted as a percentage of the initial chemical residue found on control plants are shown in Figures 20 – 23.

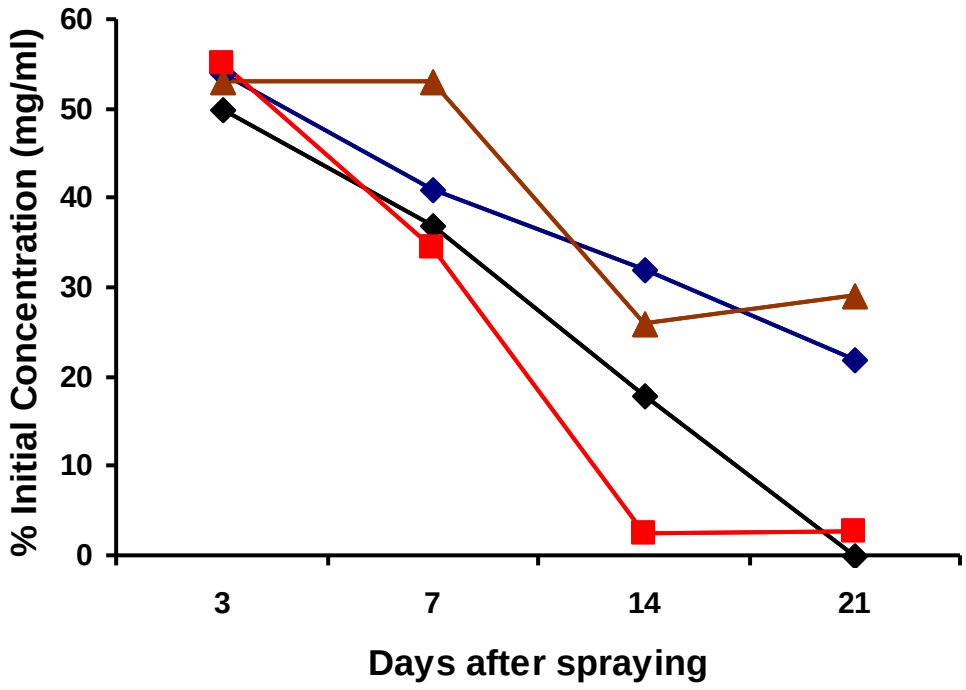

Figure 20 Fungicide residue on seedlings as a percentage of that occurring on control plants at 5^{o}C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

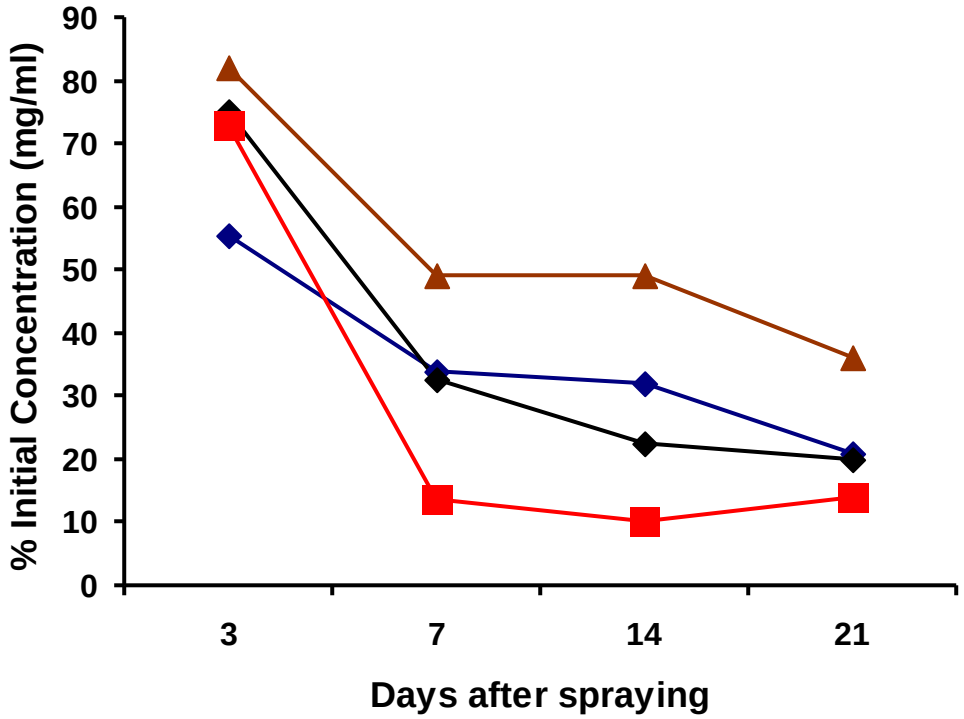

Figure 21 Fungicide residue on seedlings as a percentage of that occurring on control at 10°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

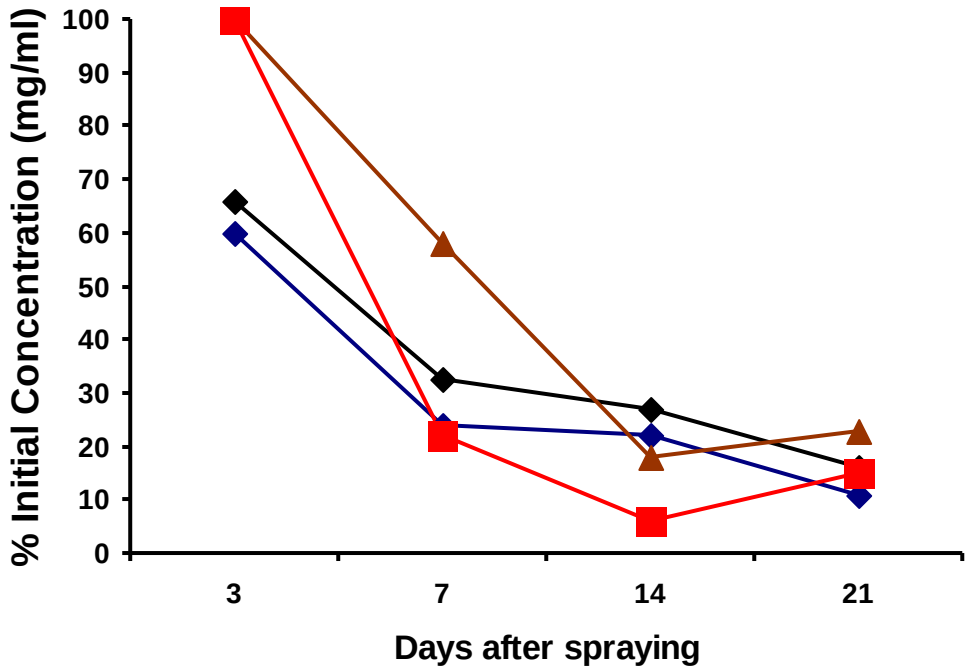

Figure 22 Fungicide residue on seedlings as a percentage of that occurring on control at 15°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

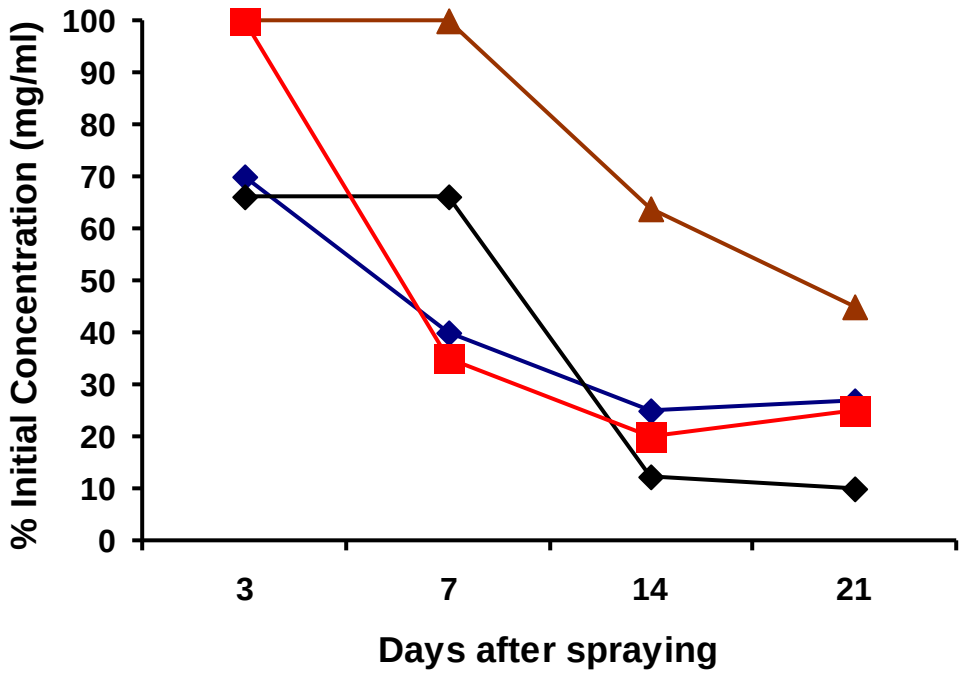

Figure 23 Fungicide residue on seedlings as a percentage of that occurring on control at 20°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

Results show that difenconazole content in leaves declined more rapidly in comparison to Tebuconazole at temperatures of 5°C (Figure 20). After approximately 21 days Difenconazole levels in the sprayed plants had declined to 0% of that found in the control plants after chemical application. However 30% of the initial level of Tebuconazole was present after 21 days at 5°C (Figure 20). At higher temperatures (10, 15, and 20°C) the levels of difenconazole were much higher as a percentage of the initial concentration (Figures 21, 22 and 23). Levels of difenconazole were similar to those of tebuconazole found at each of the temperatures tested. The levels of the chemical boscalid, in sprayed leaf samples, dropped by 50%, regardless of incubation temperature over the experimental period. However pyraclostrobin in leaf samples were reduced to approximately 30 – 40 % of that found in controls after seven days at 10, 15 and 20°C. At 5°C Pyraclostrobin was detected in higher amounts 7 days after spraying but dropped to very low levels 14 days after sprays were applied.

Effect of temperature on fungicide content in leaves (Third Replicate Experiment)

The results of the third controlled environment experiment plotted as a percentage of the initial chemical levels found on control plants are shown in Figures 24 – 27. The results show similar

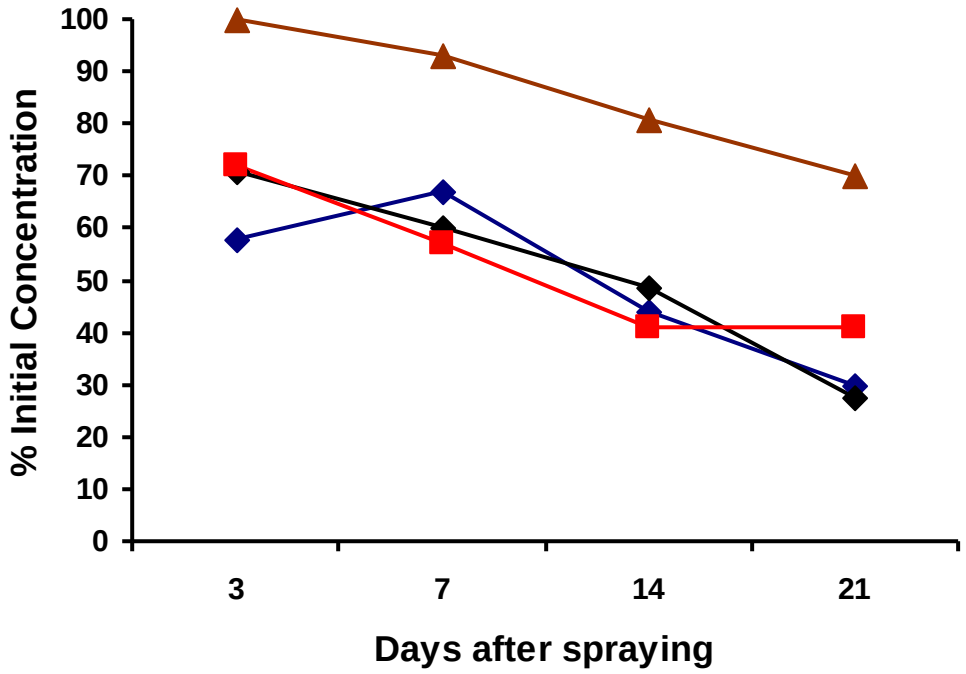

Figure 24 Fungicide residue on seedlings as a percentage of that occurring on control plants at 5°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

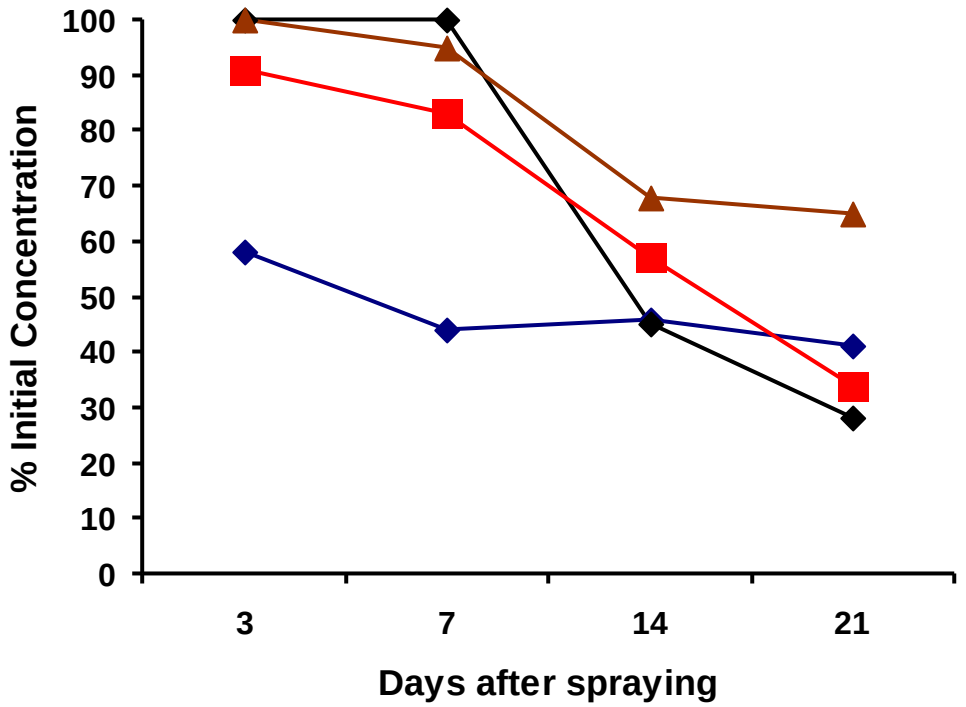

Figure 25 Fungicide residue on seedlings as a percentage of that occurring on control at 10°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

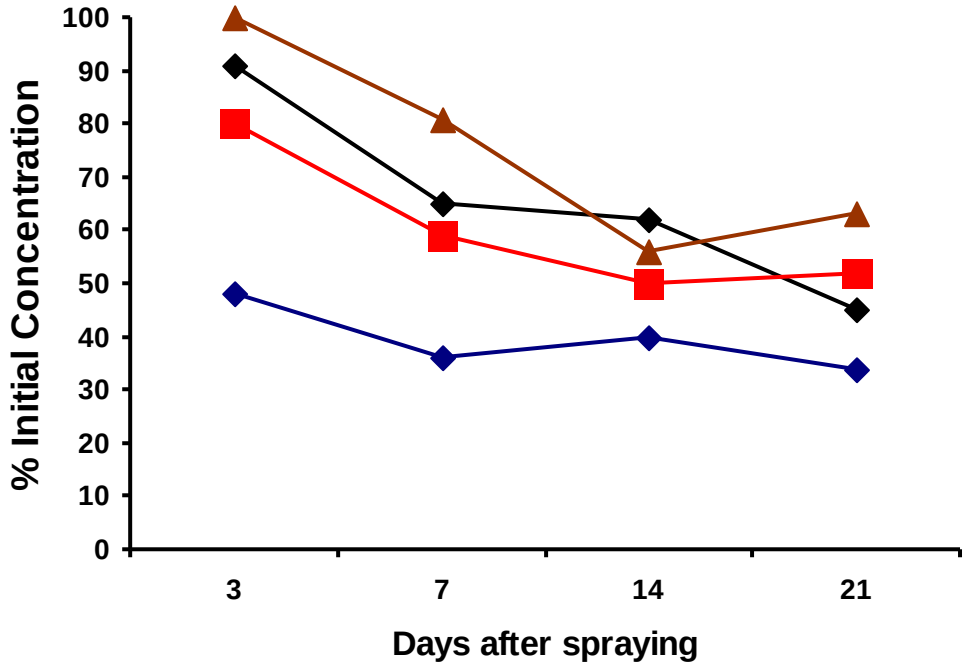

Figure 26 Fungicide residue on seedlings as a percentage of that occurring on control at 15°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

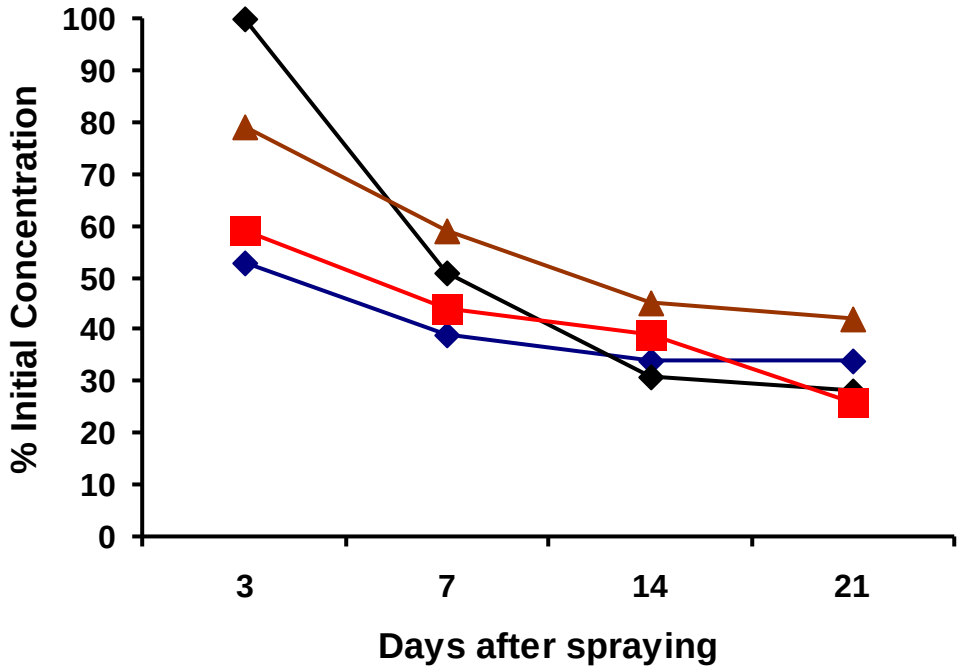

Figure 27 Fungicide residue on seedlings as a percentage of that occurring on control at 20°C sprayed with Tebuconazole (♦), Difenconazole (♦), Pyraclostrobin (■), and Boscalid (▲) at recommended dosage

levels of decline in chemical content in leaves sprayed with Boscalid and Tebuconazole as that observed in the second replicate experiment. However the levels of difenconazole and Pyraclostrobin were not reduced to the same levels observed in the second replicate experiment at 10 and 15°C incubation temperature. There were approximately 30% of the levels of difenconazole found in control plants regardless of temperature after 21 days. There were similar levels of Tebuconazole present in the leaves after 21 days incubation at all incubation temperatures between replicate experiments. Higher levels of Boscalid were observed after 21 days incubation at 5 and 10°C in the third replicate experiment in comparison to the second replicate experiment.

Measuring the effect of fungicides on inoculum production by the ringspot pathogen

The effect of spraying recommended dosages of difenconazole (Plover) on inoculum production by established lesions of the ringspot pathogen is shown in Figure 28. The experiment is being repeated with sprays of Plover and other fungicides. The results of the initial experiment show that sprays of difenconazole initially depressed the level of inoculum production. However 7 days after fungicide application inoculum production by lesions began to rise to the levels observed on control plants (Figure 28).

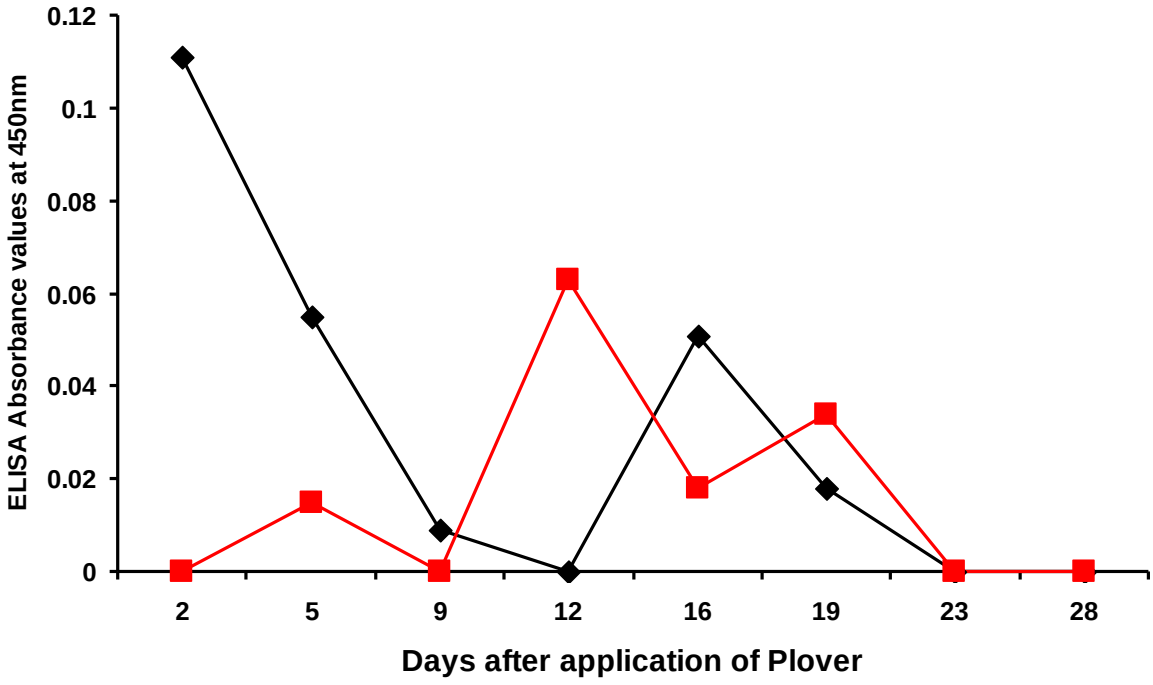

Figure 28 Inoculum production from ringspot lesions on Brussels sprouts seedlings at 20°C unsprayed (♦), and sprayed with difenconazole (■) at recommended dosage

The pattern of inoculum production thereafter followed that observed from unsprayed control lesions. This initial experiment suggests that applications of difenconazole on to established lesions reduced inoculum production from those lesions up to 9 days after application. The experiments with difenconazole are currently being replicated. The application of tebuconazole (as Folicur) and boscalid and pyraclostrobin as Signum on inoculum production from ringspot lesions will also be investigated.

Year Three Results

Effect of field exposure to fungicide content of sprayed leaves

Brussels sprouts cv. Millennium were raised under glasshouse conditions and transplanted into large pots of 20 – 30 cm diameter. Plants were grown to the fifteen to twenty leaf stage in under 12 – 14 ^{o}C day temperatures. Plants were divided into four equal groups and either left unsprayed or sprayed with either difenconazole (as Plover), or tebuconazole (as Makhteshim AGAN tebuconazole generic product) or Boscalid/ Pyraclostrobin (as Signum). All plants were placed under field conditions and watered daily. Existing leaves at the time of spraying were marked with marker pen. Leaves were harvested from plants in each treatment after spray application and subsequently at 3, 7, 14, 21 and 42 days post spray application. The leaves were frozen at – 20 ^{o}C after harvest and residue analysis was carried out with solvent extraction from sampled leaf material. Samples were cleaned up by solid phase extraction and analysis was performed by hplc-uv methodology. Results were confirmed with lc-ms by Scientific Analysis Laboratories where larger numbers of samples could be processed. Each set of harvested leaves from each plant were separated and analysed separately in each experiment. There were ten replicated sprayed plants per fungicide treatment and ten unsprayed control plants. Plants were sprayed on the 21 August 2009 and leaves harvested during September and October 2009 for analysis.

Fungicide levels in sprayed plants in the field

The results of the first replicated residue field experiment are shown in Figure 29. The results show a rapid decline in residue levels during the first 7 days post application of Difenconazole, Tebuconazole and Boscalid. There was some reduction in the amount of Pyraclostrobin in leaf samples 7 days post application however levels were only marginally lower. There was a further reduction in levels of all fungicides in leaf samples taken between day 7 and day 42

post application. After 42 days post fungicide application levels of Difenconazole, Boscalid. and Pyraclostrobin were reduced to virtually zero. Tebuconazole however remained relatively high in sprayed leaf samples after 42 days (Figure 29).

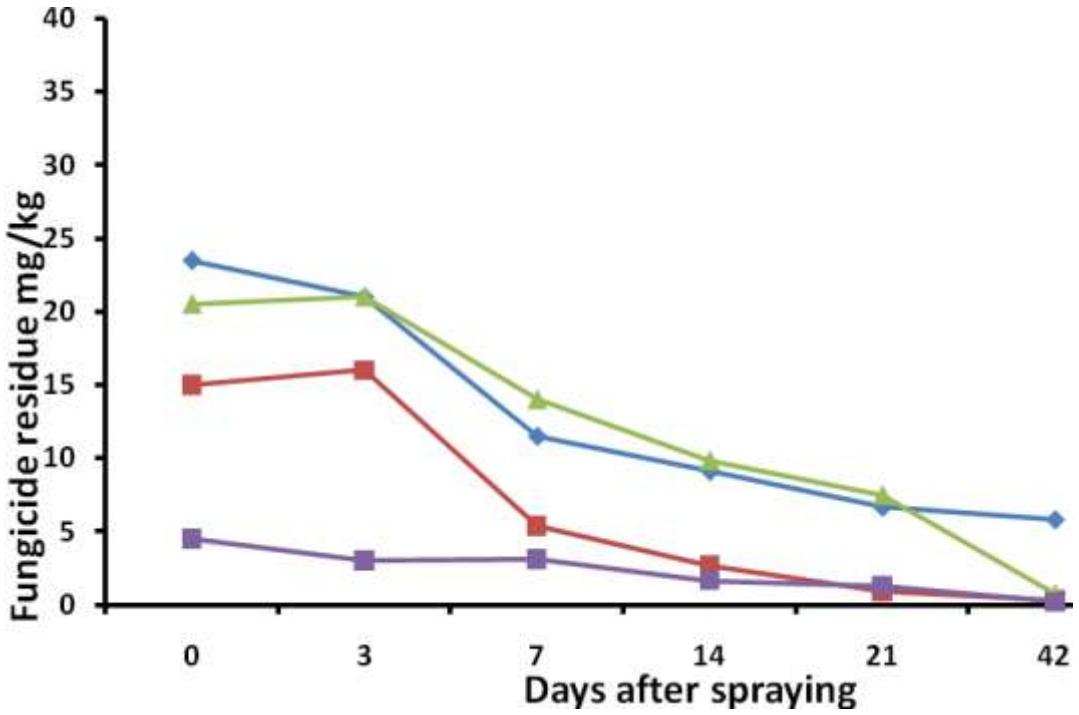

Figure 29 Fungicide residue on leaves of field exposed Brussels sprout plants sprayed with Tebuconazole (Generic Folicur) (♦), Difenconazole (Plover) (■), Pyraclostrobin (Signum) (■), and Boscalid (Signum) (▲) at recommended dosage

Similar results were obtained in a repeat experiment (Figure 30). Similar uptake of fungicide sprays was observed as in the first experiment. After 42 days post fungicide application levels of Difenconazole, Boscalid. and Pyraclostrobin were reduced to virtually zero. Tebuconazole levels remained relatively high in sprayed leaf samples after 42 days (Figure 30). Difenconazole levels in sprayed leaves were reduced significantly 7 days post spray application. The results appeared to confirm results observed using ringspot inoculation after spray application with Difenconazole applied as Plover.

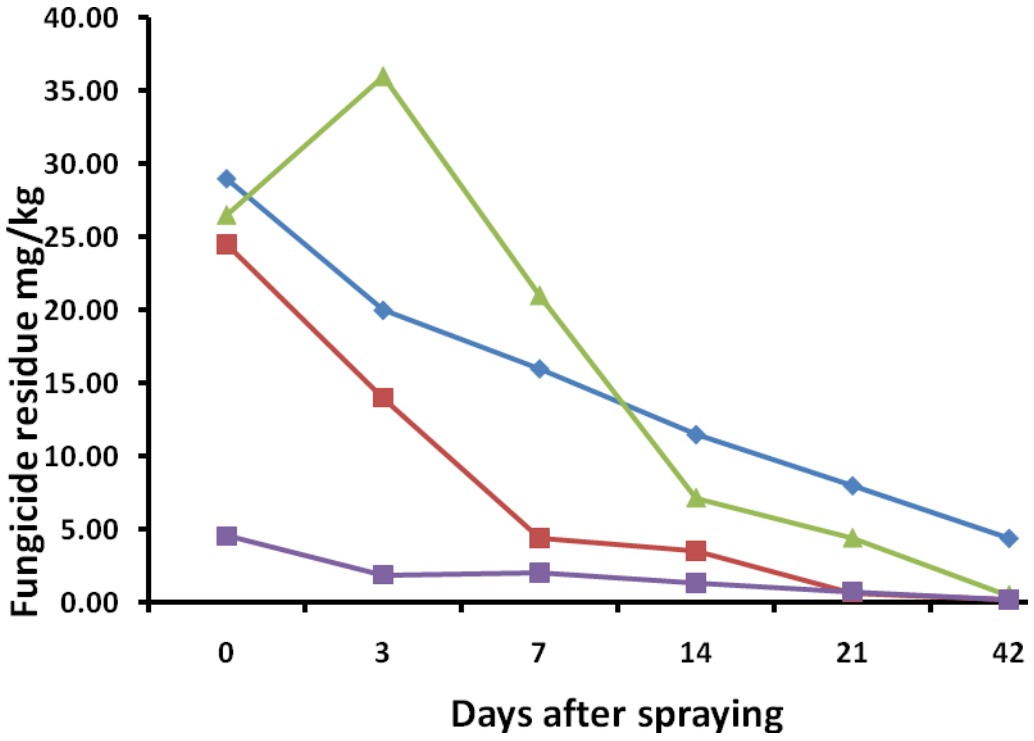

Figure 30 Fungicide residue on leaves of field exposed Brussels sprout plants sprayed with Tebuconazole (Generic Folicur) (♦), Difenconazole (Plover) (■), Pyraclostrobin (Signum) (■), and Boscalid (Signum) (▲) at recommended dosage

Effect of fungicides on ringspot inoculum production

The effect of fungicides on ringspot inoculum production is shown in Figure 31. The effect of application of Difenconazole (Plover), Tebuconazole (Nativo) , Boscalid. and Pyraclostrobin (Signum) could not be measured as few ascospores were produced in the unsprayed control treatment. Despite the appearance of ringspot lesions on the plants used in this experiment unsprayed control plants failed to produce inoculum within the cabinets where ascospore trapping was used to quantify inoculum production. Variation in experimental conditions between cabinets used in the experiment can also be ruled out in explaining the results. The absorbance values in all cabinets were very low to negligible indicating zero or very low levels of ringspot ascospores present regardless of fungicide treatment (Figure 31).

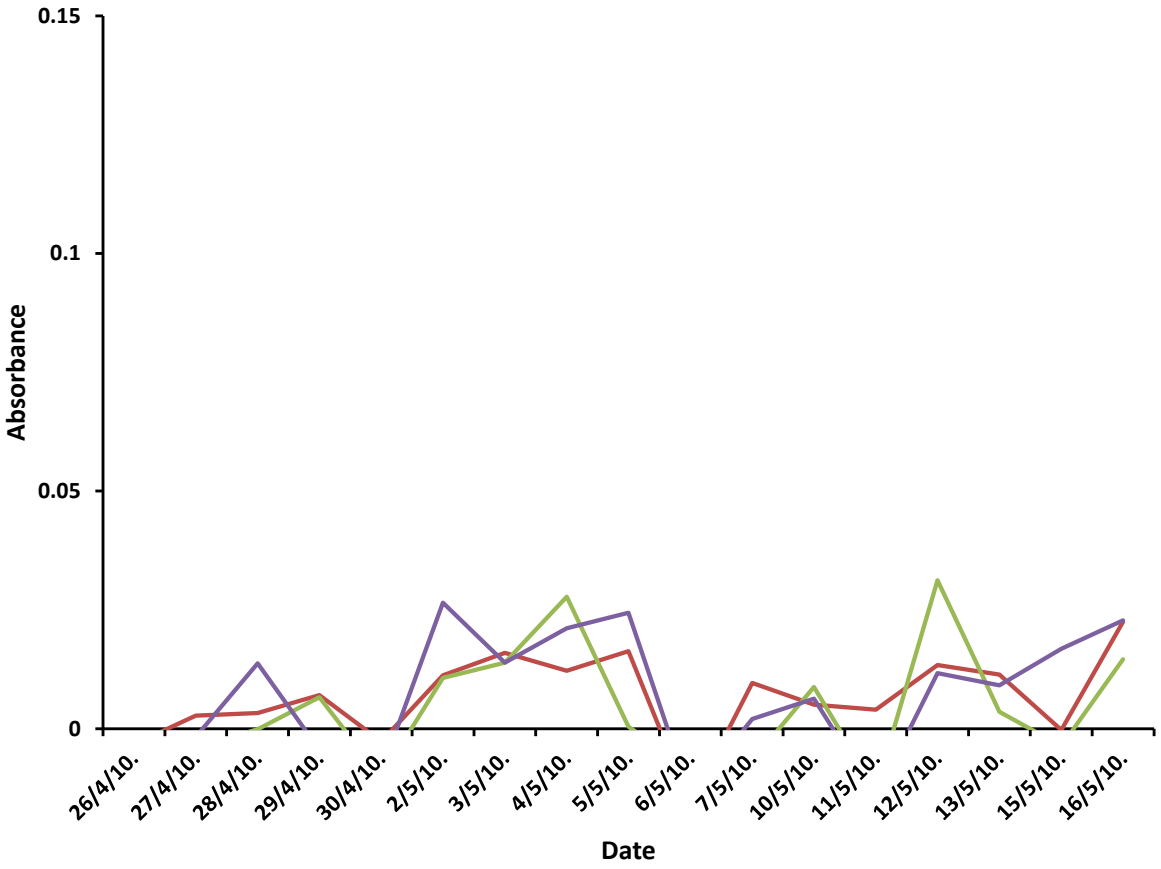

Figure 31 Fungicide residue on leaves of field exposed Brussels sprout plants sprayed with Tebuconazole (Generic Folicur) (■) Difenconazole (Plover) (■), Pyraclostrobin and Boscalid (Signum) (■), at recommended dosage.

Discussion

Fungicidal control of foliar diseases of vegetable Brassica crops

There are many fungicides which hold approval for control of leaf spots of vegetable Brassicas. However many of these compounds originate from the same chemical family and hence have the same activity against important leaf spots such as ringspot. However there is little information on how these chemicals behave in the environment. Many vegetable Brassica crops are grown under a variety of field conditions. Some crops are predominantly grown under summer conditions however many long season crops are planted in the summer and harvested in the winter. Over wintered Brassica crops have been a feature of Brassica production in the UK for many years.

Fungicide application is the only option for controlling diseases in vegetable Brassica crops at present. With information now available on the variation in activity of fungicides in relation to post infection temperature more appropriate control options for leaf spots in Brassicas can be identified. Optimal timing of white blister sprays is now available with a disease forecasting system which covers all the major vegetable Brassica diseases which occur in the U.K. 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. The year one results of this project can be integrated more fully with the forecasts. Plover can be applied effectively up to 8 days after infection has occurred especially at mean temperatures post infection of up to 10^{o}C. Folicur is also equally effective at these lower temperatures, which indicates that more reliance might be placed on Plover early in the season and if necessary Folicur could be used later in the season. The addition of Chlorothalonil to these other chemicals is ineffective in reducing ringspot after infection has occurred. During the work on this project Chlorothalinil has lost its approval for control of leaf spot diseases of vegetable Brassicas. It is unlikely that the other fungicides used in this study will lose their approval. The azole type fungicides (Difenconazole and Tebuconazole) form the backbone of fungicidal control of vegetable pathoges.

Problems and practical usage of forecasts in cropping system

Disease forecasting systems can be divided into those systems which forecast the need for future fungicide applications and those which predict fungicide application based on weather

conditions which have already occurred in the crop. Systems which predict the need for future applications of fungicide are rarely used in practice for designating the need for fungicide usage. This is because they can be extremely inaccurate and are only as accurate as weather forecasts they are based on. Many leaf spots depend on periods of leaf wetness and its interaction of temperature. Many future forecasts cannot accurately predict temperature and certainly cannot predict how long wetness durations will occur often due to local conditions. The usage of weather forecasts to time fungicide applications has often lead to the massive over usage of fungicides and resulted in phytotoxic damage to crops for no disease control advantage. Additionally costs involved in high fungicide usage make these practices uneconomic.

Forecasts based on past weather collected in the crop are more accurate and provide local forecasts of conditions which can lead to disease outbreaks. One common problem in using past weather within disease forecasts has been the lag time between determining risk and applying fungicide applications in response to it. This means that information on the effect of delaying fungicide sprays after infection has occurred would be of practical value in making appropriate choices of approved products for control of leaf spots on vegetable Brassicas. Many systemic fungicides exist which can be effective in controlling disease when applied post infection. However in the field temperatures are not constant and there is little information on the effect of temperature on determining the length of the period after infection during which fungicide application still might be effective.

Timing fungicide applications is of great importance in determining successful control of fungal pathogens. Systems exist whereby mathematical modelling can be used to determine risk of fungal infection. These approaches have now been adopted within crop disease management systems. Use of mathematical models within disease forecasts enable predictions to be made about the occurrence of plant diseases at economically important levels. Within vegetable production systems economically important levels of disease are usually low as many diseases cause cosmetic damage to produce resulting in a down grading in quality. The presence of only one or two lesions, on many vegetable Brassicas is enough to result in a down grading in quality. For this reason disease predictions in vegetable crops must be of a higher accuracy in comparison to other disease forecasting systems. As infection risk can be determined using models the weather conditions post infection could be used to determine the effectiveness of fungicides applied at increasing time after infection in the field. This information will also overcome problems in scheduling fungicide applications when field conditions make spray application difficult.

Effect of incubation temperature on fungicide control with increasing application time after infection by the ringspot pathogen

The results of experiments conducted during year one of this work has shown that there are differences in the effectiveness of fungicides when applied at increasing time periods after infection. The results also indicate that this varies according to the mean temperatures after infection has occurred. The results show that at recommended dosages difenconazole could be applied up to 5 days post infection and still result in complete control of ringspot. However if reduced dosages of difenconazole were used complete control of ringspot was not observed in treatments where difenconazole was applied 5 days after infection had occurred. If recommended dosages of difenconazole were applied up to 8 days after infection, ringspot control was observed only where the mean temperature had been 5 and 10^{o}C constantly after infection. The effect was not observed at mean temperatures above 15^{o}C. Temperatures of 20^{o}C were inhibitory to ringspot post-infection due largely to the degree of leaf fall before symptoms appeared. There appeared to be differences between difenconazole (Plover) and tebuconazole (Folicur) in their effectiveness when applied at increasing time after infection. Small amounts of disease were recorded if tebuconazole was applied only 2 days after infection regardless of incubation temperatures. Although 5 days was not used as an application timing in these trials the results indicate a reduced response between tebuconazole in comparison to difenconazole if applied at 5 days after infection by the ringspot pathogen. Conversely tebuconazole treatments in which constant incubation temperatures of 5 and 10^{o}C were used post application appeared more effective in controlling ringspot in comparison to difenconazole. One potential explanation for this observation maybe differences in the growth stimulatory effects of difenconazole and tebuconazole on Brassica leaves. Additionally care must be taken in the interpretation of the results as the experiments were carried out using young seedlings which may react in different ways to ringspot infection in comparison to larger crop plants.

Experiments with chlorothalonil as Bravo applied after infection had occurred had little or no effect on the control of ringspot regardless of temperature after infection. This was not surprising as chlorothalonil is a protectant with no systemic action. Chlorothalonil could not be used after infection to prevent fungal infection. The effect of applying boscalid and pyraclostrobin as Signum has also been investigated (at recommended dosages only). The results indicated the effectiveness of Signum as a post infection control treatment. Signum applied 3 days after infection did not prevent a small amount of ringspot developing on Brussels sprouts seedlings. However the control was much greater at constant temperatures

of 5, 10, and 15^{o}C in comparison to 20^{o}C. This indicates that Signum, could be applied at up to 7 days post infection, and still achieve good control during times of the year when lower temperatures occurred.

Effect of incubation temperature on fungicide content in leaves

During year two the project the effect of temperature on spray content in leaves after spray application was investigated. The results showed differences between chemicals on the effect of temperature on the decline of chemical levels in plants. Difenconazole levels in leaves decline more rapidly in comparison to tebuconazole at temperatures of 5^{o}C. After approximately 21 days difenconazole levels in the sprayed plants had declined to 0 % of that found in the control plants after chemical application. However 30% of the initial level of tebuconazole was present after 21 days at 5^{o}C. This may explain some of the differences between the two fungicides in their activity post infection. Difenconazole levels declined rapidly indicating that after 5 – 7 days there was not enough chemical present in the leaves to stop all infection. This suggests that higher rates of chemical might be required to control infection at increasing time post inoculation. This effect could change depending on the temperature after infection. Levels of tebuconazole appeared to remain relatively high over longer periods post application. Based on these figures the application interval for tebuconazole would appear to be longer than difenconazole and is not affected by temperatures after spray application in the same way that difenconazole is. The levels of boscalid, in sprayed leaf samples, dropped by 50% regardless of incubation temperature over the experimental period. However pyraclostrobin in leaf samples were reduced to approximately 30 – 40% of that found in controls after seven days at 10, 15 and 20^{o}C. At 5^{o}C pyraclostrobin was detected in higher amounts 7 days after spraying but dropped to very low levels 14 days after application. Signum would therefore appear to have a dual activity mirroring the short term like effect of difenconazole (as Pyraclostrobin) combined with the longer term like effect of tebuconazole (as Boscalid). Experiments conducted during year three of the projects showed how fungicide content of leaves changes under changing temperatures. Sigmum and Plover levels in leaves declined to zero 42 days after application however generic Folicur did not. This might explain some of the problems associated with application of this chemical. With repeated applications on the same leaf it is possible that levels of Tebuconazole (as generic Folicur) could build up during the season. This may lead to phytotoxic reactions on leaves after a certain number of spray applications. Phytotoxic reactions have in the past been associated with this chemical. It's possible that formulations which promote rapid uptake may lead to these phytotoxic reactions if application intervals are short.

Effect of recommended dosages of fungicides on inoculum production by the ringspot pathogen

Previous studies showed that inoculum release occurs in the presence of light and wetness (Cullington, 1995). The results of preliminary trials showed that sprays of Plover applied directly to established lesions of ringspot were effective in reducing inoculum production. It is possible that this is directly related to the chemical content of Plover in the leaves as inoculum production increased to that observed in the control after 7 – 9 days. The content of difenconazole in the leaves would have dropped too much lower levels at this time (regardless of incubation temperature) after application. The dosages applied were those recommended on the label indicating that this chemical at the recommended doasage has anti sporulant activity for ringspot. Lesions tended to stop sporulating after 28 days indicating that the ringspot fruiting bodies were declining after this time. However follow up experiments conducted in year three of the project were unsuccessful in comparing potential antisporulant activity of Plover, Generic Folicur and Signum. This was largely due to the low levels of disease on plants used in these experiments. I could not be conclusively proved that Signum and generic Folicur had the same level of anti sporulant activity in comparison to Plover. However information on antisporulant activity could prove useful in crop protection strategies for controlling ringspot.

Spray timing studies with white blister using new disease forecasting models in vegetable Brassica crops in the field

The white blister infection and white blister disease development models have been programmed within the Brassica$_{spot}$ (MORPH based) system and used within year one field trials to time fungicide sprays for controlling this pathogen. The results of trials showed that the white blister model can be used effectively to reduce white blister infection in crops of Brussels sprouts. White blister disease development was lower than expected during 2008. Lower temperatures and unusual crop growth gave only low levels of white blister within inoculated Brussels sprouts crops at Warwick HRI Kirton. Despite this white blister did develop in the crop. Two routine sprays with Nativo at recommended dosages did not appear to control white blister in comparison to one spray of the industry standard Folio Gold applied according to the white blister disease development model. Additionally applying Chlorothalonil at the recommended dosage according to the white blister disease development model also reduced the level of white blister in the crop. White blister was assessed only on the leaves of the plants and no infection was observed on harvested buttons. The results show the fungicidal activity of both Metalaxyl and Chlorothalonil (as Folio Gold) when applied at optimal timings for control of white blister.

Further work on integrating fungicide information within disease forecasting systems

The information presented in this report could be fully integrated into the Brassica$_{spot}$ disease forecasting system. By fitting mathematical equations to the data a component of the infection model could be created which would use weather data to predict when further applications of chemical would be necessary to crops. Such a system could also be used to manage potential residue issues more effectively. The “in field” weather data collected for the assessment of infection using the pathogen models could be used to compute the fungicide level in the crop and determine if this was still active. The temperature data could also be used to determine the spray window post infection. In this way the efficiency of fungicides could be further improved. As crop move to lower input regimes this added benefit could prove valuable for growers to further optimize their growing systems.

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Kennedy, R., Wakeham, A.J., Byrne, K.G., Meyer, U.M. and Dewey, F.M. (2000). A new method to monitor airborne inoculum of the fungal plant pathogens *Mycosphaerella brassicicola* and *Botrytis cinerea*. *Applied and Environmental Microbiology*, **66**: 297-307.

