PROJECT REPORT No. OS63

LIGHT LEAF SPOT (*PYRENOPEZIZA BRASSICAE*) IN OILSEED RAPE: EXTENT OF TRIAZOLE FUNGICIDE RESISTANCE IN SCOTLAND; FUNGICIDE STRATEGIES

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by

F J Burnett

Crop Science Department, SAC, West Mains Road, Edinburgh EH9 3JG

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1. ABSTRACT

In recent seasons the efficacy of triazole fungicides for light leaf spot control in oilseed rape has markedly declined in high disease pressure areas. The objective of this project was to determine the distribution of triazole resistance in light leaf spot (*Pyrenopeziza brassicae)* in Scotland and to establish how this problem could be managed in cost effective fungicide programmes. Isolates of *P. brassicae* were taken from commercial crops and trial sites throughout the main arable areas of Scotland and their sensitivity to Folicur (a.i. tebuconazole) and Punch C (a.i. flusilazole and carbenazim) determined. The sensitivity of isolates was very variable and some were up to forty times less sensitive than the most sensitive isolates. These less sensitive isolates were found in all the Scottish arable areas surveyed. There was no link between the sensitivity of isolates and the previous fungicide history of the crop from which they had been sampled.

The occurrence of less sensitive isolates within the *P. brassicae* population was critical in the efficacy of triazole fungicides in field trials. Tebuconazole residue analysis a month after autumn fungicide sprays were applied showed that the fungicide was detectable at more than background levels following reduced dose rate treatments. Even after a full dose rate treatment levels one month after treatment were low enough that less sensitive isolates would still have been able to develop. Disease control data from the trials showed that full and three quarter rate Folicur (tebuconazole) treatments tended to give significantly better control than half dose rate treatments. However there were crop safety issues involved in increasing the dose rate and a full dose rate caused significant losses in crop vigour that were reflected in yield. Punch C (flusilazole plus carbendazim) did not have the same crop safety issues at higher dose rates. Full rate treatment of Punch C, however, yielded less than a three quarter dose rate treatment, implying the three quarter rate was optimal. *P. brassicae* isolates assayed were generally more sensitive to Folicur than to Punch C but this was seldom translated into reduced efficacy compared to Folicur in the field trials. The addition of partner fungicides to the Folicur treatments with alternative modes of action such as Dithane, Thiovit or Bravo did not significantly improve disease control and consequently did not offer an effective anti-resistance strategy. Punch C may still offer an effective anti-resistance strategy in areas where sensitivity to MBC fungicides like carbendazim is still present. Triazole dose rates of below half as currently commonly used in commercial practice in Scotland will almost certainly not control light leaf spot effectively.

2. SUMMARY

In recent seasons the efficacy of triazole fungicides for light leaf spot control in oilseed rape has markedly declined in high disease pressure areas. The objective of this project was to determine the distribution of triazole resistance in the light leaf spot (*Pyrenopeziza brassicae)* population in Scotland and to establish how this problem could be managed in cost effective fungicide programmes.

The distribution of triazole resistance was determined by isolating the fungus from leaf samples taken from HGCA funded variety trials and from commercial crop. Field trials were established in the 2000/2001 and 2001/2002 seasons to determine the influence of dose rate on fungicide efficacy. Two trials were conducted in each season. Additional fungicides with alternative modes of action were evaluated to see if the efficacy of control from triazoles could be improved by their use in mixtures which would form an effective anti-resistance strategy. Fungicide residues were measured in field plants in the winter to determine the impact of fungicide dose on subsequent residues in new growth in the spring.

The results of the survey showed that the *P. brassicae* population was very variable in its sensitivity to the triazole fungicides Folicur (tebuconazole) and Punch C (flusilazole and carnendazim). The sensitivity of the isolates assayed in the course of this work ranged at least forty fold from an MIC (minimum inhibitory concentration to prevent fungal growth) value of 0.5 part per million (ppm) Folicur or Punch C to an MIC of 20 ppm. There was no link between area surveyed and the mean sensitivity of isolates assayed. Less sensitive isolates were isolated from commercial crops throughout Scotland and were as common in all the regions surveyed. There was no link determined between the recorded crop history and the sensitivity of the isolates – the variety sampled did not influence the sensitivity of the isolates and nor did previous fungicide history.

Comparison with historical data from DuPont (UK) Ltd shows that the proportion of isolates that will grow on a discriminatory dose of 10 ppm Punch C has increased since 1995, but this shift in sensitivity has not been large. This is in contrast to resistance to MBC fungicides like carbendazim where resistant isolates will continue to grow in concentrations in excess of 2000 ppm.

The range in sensitivity within the *P. brassicae* population was still large enough to cause problems in light leaf spot control in the field. Tebuconazole residue testing showed that a month after autumn applications to trials the half and three quarter dose rate tebuconazole treatments had levels of tebuconazole in the leaves that were no greater than the untreated control plots. Even in the full dose rate treatment sampled a month after spraying the tebuconazole levels were still low enough that the majority of the *P. brassica* isolates assayed would have been able to grow. This demonstrated that dose rate was very critical in the efficacy of control.

Similar results were found in the disease control seen in the field trials. Full and three quarter dose rate Folicur treatment tended to give significantly better control than half dose rate treatments. However there were crop safety issues involved in increasing the dose rate and a full dose rate caused significant losses in crop vigour that were reflected in yield. Given the current levels of reduced sensitivity to tebuconazole in the Scottish *P. brassicae* population, dose rates of below half dose rate Folicur will almost certainly be ineffective in controlling light leaf spot. A three quarter dose rate probably represents a compromise between this reduced disease efficacy and the crop damage seen at higher rates. There were no crop safety issues involved in increasing the dose rate of Punch C, although the full commercial dose rate yielded less than the three qaurter dose rate treatments, implying the three quartes rate treatment may be optimal. Although the *P. brassiace* population was generally less sensitive to Punch C than it was to Folicur the difference was seldom large enough to translate into a reduction in efficacy in the field. Only at one of the four trial sites which had a high proportion of tebuconazole sensitive isolates was Punch C significantly less effective in light leaf spot control when compared to Folicur. At the other three sites disease control from Punch C was comparable to the Folicur treatments and yield was equal or better.

There was no influence from dose rate on the sensitivity of isolates and isolates from reduced dose rate plots were no more or less sensitive than isolates from untreated or full commercial dose rate plots. The addition of partner fungicides with alternative modes of action such as Dithane, Thiovit or Bravo did not significantly improve disease control. As a consequence of this lack of efficacy they did not alter the sensitivity to triazoles of the *P. brassicae* isolates assayed from the trials and did not therefore represent an effective anti-resistance strategy. Sanction (straight flusilazole) was as effective as Punch C in controlling light leaf spot in trials and did not yield significantly more or less. This implies that the carbendazim component in Punch C was not contributing much to the disease control and yield benefits seen with this product. However the

straight carbendazim product, Bavistan, applied at a rate equivalent to that in full rate Punch C did show a small effect in reducing light leaf spot levels. This implies that it may still be effective against a small proportion of the light leaf spot population that remains sensitive to MBC fungicides like carbendazim and that Punch C may still represent an effective anti-resistance strategy where this is the case.

The lack of effective mixing partners demonstrated in this work and the widespread occurrence of MBC resistance makes the use of MBC as an alternative mode of action mixture strategy a questionable approach. It does however represent the only mixing partner with any efficacy at present and has to be given consideration because of this. Where there are still MBC sensitive isolates in the population it may still represent an effective anti-resistant strategy and may prolong the period of efficacy of the triazoles by avoiding an over reliance on the triazole component. Triazoles applied at dose rates of less than half the full commercial dose rate, as is common in commercial practice, will almost certainly not be effective at controlling light leaf spot in Scotland.

Technical detail

3. INTRODUCTION

3.1 Light Leaf Spot

Light leaf spot (*Pyrenopeziza brassicae*) is one of the most important diseases of winter oilseed rape (*Brassica napus* L. *oleifra* D.C.) in the UK. Oilseed rape is the main arable break crop in the United Kingdom with over 300 000 hectares grown in 2000, the season just prior to the start of this project. Over 30 000 ha of this were grown in Scotland (DEFRA, 2001, Scottish Executive 2001)

Each year approximately £9 million are spent on fungicides to control diseases (Fitt *et al*., 1997) but despite this, losses of as much as £40 million are attributed to light leaf spot (Gladders, 1998). Light leaf spot is the most important disease of oilseed rape crops in Scotland, being favoured by long, cool, wet winters. The disease first appears in the autumn, following infection from infected seed and stubble, and peaks in the early spring (Sutherland and Griffin-Walker, 1994). The symptoms are of pale green or bleached leaf blotches, often surrounded by a halo of white spore droplets. Stems may also develop lesions. Spores from the leaf spots are spread to other leaves, flower buds, pods and plants by rain splash.

3.2 Decision support systems

Fungicides for the control of light leaf spot do not always guarantee a yield response but omitting them in high pressure situations can result in large yield losses. Yield losses of up to 3 t/ha are possible as a result of infection with light leaf spot (Freer *et al*., 1998). Yield losses are due to decreased plant populations, decreased seed numbers per plant or decreased thousand seed weights (Doughty *et al.*, 1995). Losses are, however, very variable between crops and between seasons and recent work has focused on predicting the likely yield losses as a result of light leaf spot. This has been done in the hope of forecasting the likely cost benefits of applying fungicides to control the disease in order to optimise the use of fungicides for control of light leaf spot and avoid applications to crops that do not need them. The optimum timing for disease control is in the autumn (Sansford *et al*. 1996) but symptoms do not appear until later, usually between December and February (Sutherland *et al*., 1998). Su *et.al.* (1998a) found that the incidence of light leaf spot at growth stage 3,3 (flower buds visible) was closely related to final disease levels and yield loss. The severity of infection, however, differs between seasons, regions and individual crops (Fitt *et al*., 1997). This yield loss model has been incorporated into a forecasting model that also included, along with the disease incidence data, seasonal risk indexes based on

environmental data and crop risk indexes based on agronomic data (Fitt *et al.*, 1996.). The aim of these schemes has been to predict early in the season when sprays have to be applied and what the likely yield benefits of fungicide application are likely to be. Such risk predictions for oilseed rape required to be included into a scheme that managed all diseases and pests on winter oilseed rape (Fitt *et al*., 1997). This was subsequently developed as the PASSWORD project. The model developed by Fitt *et al*., in 1996 was validated by Sutherland *et al*. 1998. This showed that while the model worked well for predictions of high risk there was still a risk of infection in areas predicted to be at low risk where high levels of disease inoculum were present. The initial model by Fitt *et al*. (1996) suggested that early sowing and cultivar susceptibility increased the risk but that proximity to previous crop did not. The work by Sutherland *et al.* (1998) suggested that proximity to previous crop may be more important than previously thought. They concluded this from the occurrence of light leaf spot as a result of trash borne infection at otherwise low risk sites. This highlighted that growers should be advised to assess each individual crop even if a low risk has been predicted for their region.

Varietal resistance is also important in determining the likely response to fungicide sprays aimed at the control of light leaf spot. Yield losses of 3 t/ha were recorded for the susceptible variety Bristol in work by Freer *et al.* 1998. In contrast the resistant variety Rocket when untreated still out yielded a two spray fungicide treatment applied to Bristol.

Risk prediction at present is based on an assessment of seasonal risk to identify high risk seasons on the basis of rainfall and temperature, and on crop risk assessment based on sowing date, varietal resistance, previous cropping and regional disease incidence. In addition the presence of disease in the crop should be assessed every month from October to March so that the initial risk assessment can be modified and appropriate fungicide treatment applied (Fitt *et al*., (1997).

Based on the DEFRA crop survey for England and Wales, the forecast uses light leaf spot levels on pods at the end of the season to predict the percentage of crops with severe infections in the following spring. This in turn predicts the number of crops that would respond to fungicide treatments applied to control the disease in autumn. Levels of disease in the crop in spring can be used to predict the likely response to a spring fungicide treatment. The prediction scheme was extended into Scotland in 2000 (Sutherland *et al*., 2002). Initial results show that the scheme in its present form is likely to underestimate the risk of light leaf spot infection in Scotland and that it needs to be modified for Scottish conditions to allow for more accurate predications (Sutherland

et al., 2002). In general terms though crops in Scotland are far more likely to be sprayed routinely for light leaf spot and give far more consistent responses to fungicides controlling light leaf spot between season than is the case in England where crops are judged to be at much lower risk (Wale *et al*. 1990).

The light leaf spot forecast is available to growers via the Internet at http//www3.res.bbsrc.ac.uk/leafspot/forecast/. It allows growers to enter information on factors which influence severity such as region, variety and sowing date. It does not at present provide any information on product or dose rate selection or on what actions should be taken if weather and ground conditions prevent application at optimum times.

The development of the Pest and Disease Management System for Supporting Winter Oilseed Rape Decisions (PASSWORD) project aims to integrate as one Internet-based system the light leaf spot risk predication scheme described, with decision making information on other diseases such as phoma stem canker as well as integrating this with a key insect pest control decision support system (Sutherland *et al.,* 2002).

3.3 Chemical control

Growers in Scotland routinely apply fungicides to winter oilseed rape in the autumn and spring for control of light leaf spot and yield benefits of up to 3 t/ha are possible (Freer *et al*., 1998). SAC trials show that the most cost effective fungicide programmes for light leaf spot control consist of an autumn and spring application of a triazole fungicide such as tebuconazole (Folicur) or flusilazole + MBC (Punch C). In recent seasons, however, the efficacy of the triazole fungicides in areas of high light leaf spot pressure has declined (Figure 1). In 2000, there was a noticeable reduction in yield response compared to previous years. For example, Punch C at 0.4 l/ha, that had previously been a robust enough dose to control the disease, appeared to be less effective. There have also been widespread reports of disease control failure on farm. It is suspected that triazole resistance may be widespread throughout the UK and that it may be linked to the areas of most intense disease pressure and fungicide use such as Scotland. This is of great concern since growers rely wholly on triazoles for light leaf spot control.

The use of MBC fungicides for the control of light leaf spot was common in Scotland in the mid 1980s and early 1990s (Bowen *et al*., 1993). A study in 1987 (Ilott *et al*., 1997) demonstrated that

isolates were very sensitive to the MBC fungicide benomyl. All the field isolates surveyed at this time had MIC values (minimum inhibitory concentration of fungicide which will prevent growth) of less than 1ppm. By the early 1990s, however, disease control failures were common in high disease pressure areas like the north east of Scotland. This coincided with seasons of more intense disease pressure (Sutherland and Griffin-Walker, 1994). Sutherland and Griffin-Walker (1994) found that in contrast to the earlier work, 96% of field isolates assayed from the north east of Scotland grew on 1ppm carbendazim and 25% of these isolates could continue to grow on concentrations in excess of 2000ppm. They linked this with the failure of MBC fungicides to control light leaf spot in this area in 1993.

MBC fungicides still appear to be effective against light leaf spot in England & Wales (Gladders, pers.comm.), so they may continue to play a part in mixtures to reduce any potential slide in the effectiveness of triazole fungicides in areas where MBC fungicides remain effective. However, Freer *et al*. (1998) found at a trial site in Suffolk that triazole fungicides were more effective than MBC fungicides. Straight MBC treatments did not reduce light leaf spot levels compared to the untreated control. No resistance testing was done as part of this study but the implication is that MBC resistance may have been present at the site.

In view of the widespread resistance to MBC fungicides, there was a need to revisit fungicides that in previous trials showed minimal effects e.g. sulphur and chlorothalonil. Such compounds may play a part in maintaining control with triazole fungicides if used in mixtures. Strobilurin fungicides that have transformed cereal fungicide programmes show little effect against light leaf spot control in winter oilseed rape (Simon Oxley, SAC trial report 1999), therefore current strobilurin fungicides are unlikely to gain approval for oilseed rape. Other oilseed rape fungicides, such as iprodione and vinclozolin, have limited effect on light leaf spot and as a result triazole fungicides will continue to be the main defence against light leaf spot in the near future.

Mixtures or alternations of fungicides with alternative modes of action in a spray programme are an important defense against the development of fungicide resistance (Jones, 1994). The use of such anti-resistance strategies are very important in prolonging the useful life of fungicides, even once resistance in the pathogen has started to develop. In an example borrowed from potato blight, the statutory requirement to use phenylamide fungicides in mixtures with fungicides with alternative modes of action meant that the phenylamides remained the mainstay of potato blight fungicide programmes despite resistance to phenylamides having been identified more than 20

years ago (Urech, 1994). Resistance to MBC fungcides in *P. brassicae* is already wide spread in high pressure areas and triazole fungicides are the only fungicide group with activity in these cases. As it is unlikely that any novel fungicides will be approved for use on oilseed rape in the near future, it is essential that the activity of triazoles is protected through sensible use strategies if yield losses from light leaf spot are to be minimised.

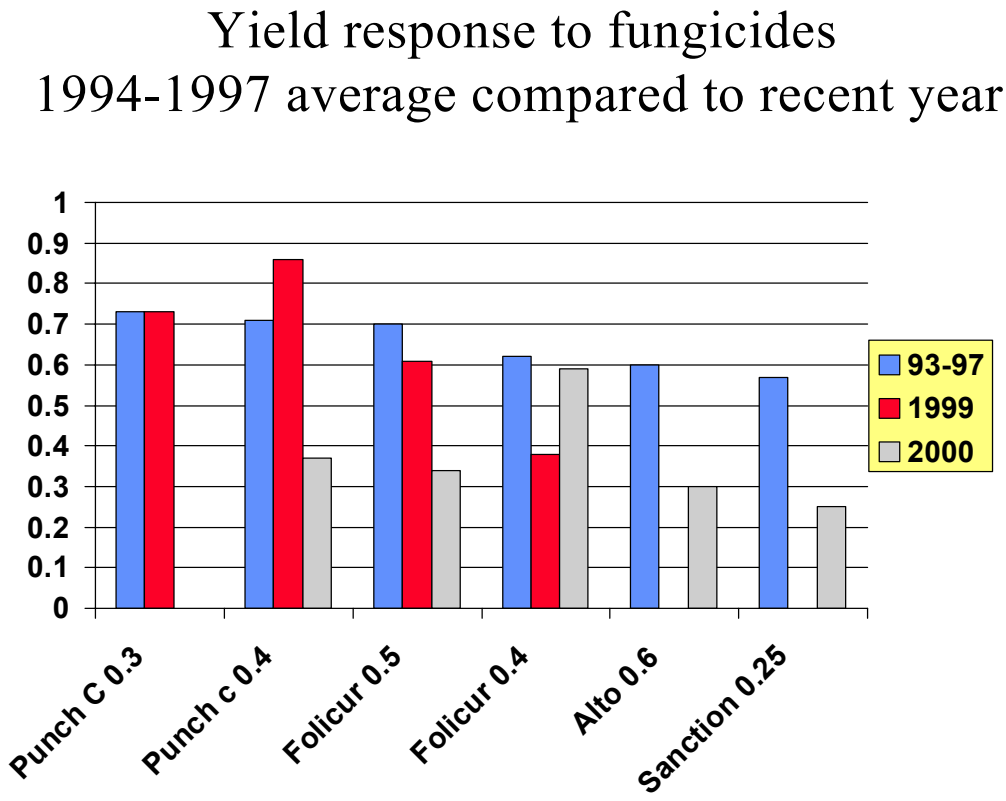

Figure 1. Average winter oilseed rape yields from SAC trials from 1999 and 2000 compared to 4 year average from 1993 - 1997

Manufacturers generally recommend a half dose or greater of triazole fungicide at any one spray timing, but with a fall in oilseed prices, there is great pressure on growers to reduce this dose further. Many are now applying one-third or one-quarter doses. An important question at the start of this project was whether triazole resistance was being encouraged by these low doses of

fungicide or whether poor disease control was as a result of reduced longevity of activity that is being construed as resistance.

The development of triazole resistance would have a direct effect on the levy payers, with less effective disease control and poor returns. Resistance will also have implications for other HGCA funded research such as variety testing and the PASSWORD project. The UK variety list is based on comparisons of varieties untreated and treated with triazole fungicides. If any of the variety test sites has a triazole resistant light leaf spot population then this could favour resistant but lower yielding varieties over susceptible but higher yielding varieties. It could also affect potential new variety screening.

3.4 Project aims

The aim of this project was to determine how triazole resistance in the *Pyrenopeziza brassicae* population is distributed in Scotland. Base line sensitivity data was supplied by DuPont, that would confirm if there has been a shift throughout Scotland or if this decline in triazole sensitivity affects only certain areas. It is critical that fungicide strategies are developed to minimise the risk of resistance and retain the efficacy of the triazole fungicides against light leaf spot.

In order to determine if dose rate was a factor in the reported problems of light leaf spot control, fungicide levels in leaves were tested. A recent HGCA project (Oxley, 1999) developed an accurate diagnostic to measure fungicide residues of tebuconazole in leaves. This test provides a valuable way to monitor the way different doses of tebuconazole are distributed in the crop over the winter and can indicate when residues are too low to provide further control in the spring. Testing also provides a useful method to directly compare effective fungicide doses in the laboratory with actual fungicide residues typically found in oilseed rape plants.

4. MATERIALS AND METHODS

The project experimental work was conducted over two years and there were two main sections to the work. The first involved a survey of winter oilseed rape crops around Scotland to determine the occurrence and distribution of resistance. The second investigated the influence of fungicide control strategies on the sensitivity of isolates through dedicated field trials. Leaf samples taken from these field trials were tested for tebuconazole residues to determine if dose rate applied was adequate for disease control purposes.

4.1 Light leaf spot survey

Samples of light leaf spot were collected from commercial farms throughout the main arable areas of Scotland. Samples of light leaf spot were also collected from leaves taken from the untreated plots in HGCA winter oilseed rape variety trials at sites located throughout Scotland. Additional isolates were collected across Scotland by sampling leaves from winter oilseed rape crops monitored for a SEERAD funded Crop Health Advisory Activity. Ten whole plants with roots or 50 infected leaves per crop were selected and sealed in plastic bags. Samples were taken by selecting leaves or plants with visible symptoms of light leaf spot. In the laboratory isolates were taken by selecting infected leaves and incubating these leaves in damp chambers at 18°C for 48 hours. After this time the white spore droplets were clearly visible under a dissection microscope. A sterile needle was used to pick spores from the leaf surface. Spores were plated onto either antibiotic amended Potato Dextrose Agar or onto antibiotic amended Malt Yeast Agar. In general the PDA had a higher recovery rate and was used exclusively in the second year of the project. Any plates showing fungal or bacterial contamination were discarded. The isolates recovered were kept at 18°C and were re-isolated onto fresh agar plates every four to six weeks as required until there was sufficient isolates to start fungicide resistance testing.

4.2 Fungicide Resistance testing

Plugs of the isolates to be tested were placed in the centre of agar amended plates and the sensitivity of the isolates determined as measured by the minimum inhibitory concentration needed to halt isolate growth.

Folicur (tebuconazole) was the test fungicide selected to determine triazole resistance. In the first year of the project the number of light leaf spot isolates that could be tested was limited by reductions in access to farms as a result of the foot and mouth epidemic. This meant that

resources were available to test isolates for sensitivity to Punch C (flusilazole + carbendazim), and this testing was continued in the second year of the project.

In year one the concentrations of Folicur and Punch C used in tests were, 0, 1,10, 100 and 1000 parts per million (ppm). In the second year, because of the range of sensitivities found in the first year, the concentrations were revised to 0.1, 0.5, 1, 5 and 10 ppm. In the second year any isolates that grew on 10 ppm were re tested on 20 ppm and 30 ppm amended agar. The concentrations used for Punch C were calculated for the flusilazole component.

Five replicate plates of each isolate were established at each concentration. The diameter of the colony was measured at the start of the test and again after 7, 14 and 28 days. The measurements after 28 days were then used to determine the minimum inhibitory concentration of the test fungicide.

4.3 Fungicide strategy field trials

Field trials were established in high risk areas in both years of the project to determine the efficacy of the fungicides and the effect of fungicide programmes on the sensitivity of the light leaf spot populations.

Isolates were taken from trials before in March or April, before spring applications of fungicide spray applications by sampling leaves as described in section 4.2 and their sensitivity to Folicur and Punch C tested, also as described in section 4.2. Included in the fungicide programmes was a study of the importance of dose rate to determine if low doses promote or reduce the likelihood of triazole resistance.

Four field trials were carried out in total, two in 2000/2001 and two in 2001/2002. The variety used was Synergy. There were four replicates of every treatment with the exception of Trial 2 where there were three replicates. Plot sizes ranged from 2 by 18 m to 2 by 20 m. The trial design was complete randomised blocks. Site details are shown in appendix C.

The treatment lists are shown in tables 1, 2, 3 and 4.

Table 1. Treatment list for Trial 1, Milrig site, West Lothian, 2000/2001

	Autumn treatment (T1) Dose rate as l/ha	Spring treatment (T2) Dose rate as l/ha
1	Nil	Folicur 0.5
2	Folicur 0.5	Folicur 0.5
3	Folicur 0.75	Folicur 0.5
4	Folicur 1.0	Folicur 0.5
5	Folicur 0.5 + Bravo 2.0	Folicur 0.5
6	Folicur 0.5 + Thiovit 10 kg	Folicur 0.5
7	Folicur 0.5 + Dithane 945 1.7 kg	Folicur 0.5
8	Bravo 2.0	Folicur 0.5
9	Thiovit 10 kg	Folicur 0.5
10	Dithane 945 1.7 kg	Folicur 0.5

Table 2. Treatment list for Trial 2, Blairnathort site, Kinross, 2000/2001

	Autumn treatment (T1) Dose rate as l/ha	Spring treatment (T2) Dose rate as l/ha
1	Nil	Nil
2	Punch C 0.4	Punch C 0.4
3	Folicur 0.5	Folicur 0.5
4	Folicur 1.0	Folicur 1.0
5	Foplicur 0.5 + Bravo 2.0	Foplicur 0.5 + Bravo 2.0
6	Folicur 0.5 + Thiovit 10 kg	Folicur 0.5 + Thiovit 10 kg
7	Folicur 0.5 + Dithane superflo 2.8 l/ha	Folicur 0.5 + Dithane superflo 2.8 l/ha
8	Bravo 2.0	Bravo 2.0
9	Thiovit 10 kg	Thiovit 10 kg
10	Dithane superflo 2.8 l/ha	Dithane superflo 2.8 l/ha

Table 3. Treatment list for Trial 3, Kames site, Borders, 2001/2002

	Autumn treatment (T1) Dose rate as l/ha	Spring treatment (T2) Dose rate as l/ha
1	Nil	Nil
2	Nil	Folicur 0.5
3	Folicur 0.5	Folicur 0.5
4	Folicur 0.75	Folicur 0.5
5	Folicur 1.0	Folicur 0.5
6	Punch C 0.4	Folicur 0.5
7	Punch C 0.6	Folicur 0.5
8	Punch C 0.8	Folicur 0.5
9	Bavisitin 0.2 l/ha	Folicur 0.5
10	Sanction 0.8	Folicur 0.5

Table 4. Treatment list for Trial 4, Blairnathort site, Kinross, 2001/2002

	Autumn treatment (T1) Dose rate as l/ha	Spring treatment (T2) Dose rate as l/ha
1	Nil	Nil
2	Nil	Folicur 0.5
3	Folicur 0.5	Folicur 0.5
4	Folicur 0.75	Folicur 0.5
5	Folicur 1.0	Folicur 0.5
6	Punch C 0.4	Folicur 0.5
7	Punch C 0.6	Folicur 0.5
8	Punch C 0.8	Folicur 0.5
9	Bavistin 0.2 l/ha	Folicur 0.5
10	Sanction 0.8	Folicur 0.5

Sampling and assessment details for trials:

Any crop tolerance effects such as vigour, scorch or differences in plant height were recorded at each site visit. Assessment for vigour was carried out by making a visual assessment on a whole

plot basis of the health , leaf colour, and biomass of plants. This was recorded on a scale of 1 – 9 with 9 representing high vigour. Scorch was also recorded on a whole plot basis on a 1 - 9 scale with 9 representing 100% scorch. The growth stage was also assessed (Sylvester-Bradley, 1985). Efficacy of fungicide was assessed by recording the mean % disease per plant and the % incidence of infected plants per plot.

Sampling for tebuconazole residues

Five plants per plot were sampled from trial 1 and 2 a month after autumn sprays were applied, and again at early stem extension in trial 1, prior to the second fungicide treatment. Trial 2 was over sprayed with fingicide before samples could be collected in the spring. Samples were stored at –20°C until they could be analysed using a competitive ELISA assay. The methodology used is detailed in Appendix A.

The fungicides used in testing and in trials were as follows:-

Product name	Manufacturer	Active ingredient (a.i.)	Amount of a.i.	Full commercial dose rate	Formulation
Folicur	Bayer	tebuconazole	250 g/l	1.0 l/ha	Oil in water suspension (EW)
Bavistan FL	BASF	carbendazim	500g/l	2.0 l/ha	Suspension concentrate (SC)
Dithane 945	SumiAgro	mancozeb	80% w/w		Wettable powder (WP)
Sanction 25	DuPont	flusilazole	250 g/l	0.8 l/ha	Oil in water emulsion (EW)
Bravo 500	Syngenta	chlorothalonil	500 g/l		Suspension concentrate (SC)
Thiovit	Syngenta	sulphur	80% w/w		Water dispersible granules (WG)
Punch C	DuPont	carbendazim + flusilazole	125:250 g/l	0.8 l/ha	Suspension concentrate (SC)

The methods used in the course of this project and the data generated were covered by a quality assurance system operated by SAC Crop Science and Agronomy Departments which is both GLP and PSD (efficacy) compliant.

5. RESULTS

The minimum inhibitory concentrations (MIC) of Punch C and Folicur to prevent growth of the light leaf spot isolates that were collected in a survey of commercial crops and variety trials are shown in appendix B.

Tables 5, 6 and 7 shows the MIC values of Folicur and Punch C for the survey isolates according to their recorded field history.

Table 5. Influence of previous fungicide history on mean MIC value of survey isolates

Fungicide history	MIC Folicur ppm	MIC Punch C ppm
Untreated	8.28	23.3
Triazole treated	10.2	23.0
Triazole plus MBC treated	4.33	7.11
SED	9.073	15.96
P	Ns	Ns

Ns = not significant, $P>0.05$

Table 5 shows that there was no significant influence on the sensitivity of isolates when related to the previous fungicide history of the crop from which they were sampled. The most sensitive isolates were sampled from the crops that had been treated with a triazole and MBC mixture but this trend was not significant.

Table 6. Influence of variety of oilseed rape on mean MIC value of survey isolates

Variety sampled	MIC Folicur ppm	MIC Punch C ppm
Apex	0.50	0.50
Synergy	11.0	18.0
Bristol	10.0	10.0
Pronto	5.61	17.8
Lipton	6.06	8.19
Madrigal	9.00	27.0
Boston	10.0	100
Fortress	3.69	20.1
Herald	1.00	100
SED	8.501	16.74
P	Ns	Ns

The number of isolates collected for each variety was very variable (Appendix B). There was no significant influence of variety on the sensitivity of the isolates tested. It was observed that the least sensitive isolates were collected from one of the most popular varieties, Synergy but this trend was no statistically significant.

Table 7. Influence of location of oilseed rape on mean MIC value of survey isolates

Area sampled	MIC Folicur ppm	MIC Punch C ppm
Cambridgeshire	0.50	0.50
Kincardineshire	10.0	10.0
Aberdeenshire	5.31	5.38
Angus	5.12	23.0
East Lothian	17.6	27.1
West Lothian	5.40	28.1
Borders	5.31	22.3
Dumfriesshire	5.25	21.9
Ayrshire	4.13	11.2
Perthshire	8.56	37.9
Stirlingshire	5.50	10.2
Banff	30.1	8.75
Buchan	7.50	5.25
Fife	5.43	9.36
SED	8.542	16.22
P	Ns	Ns

There was no significant difference in the sensitivity of isolates as a result of the area from which they were sampled. Numbers sampled from each area were very variable (see Appendix B) and some areas are represented by only one or two isolates. The least sensitive isolates to Folicur were sampled from the north east of Scotland and the most sensitive isolate from Cambridgeshire.

There was no correlation between the sensitivity of isolates to Punch C and to Folicur in this survey (r value = 0.017,P= 0.873).

Tables 5, 6 and 7 show that the survey isolates tended to be more sensitive to Folicur than to Punch C.

2000 / 2001 FIELD TRIALS

Table 8. MIC values for isolates tested from Trial 1 Milrig, West Lothian site 2000/2001

Treat-ment code	Autumn treatment	MIC Folicur ppm	MIC Punch C ppm	Tebuconazole Residue levels 11 DEC 2000	Tebuconazole Residue levels at test 19 FEB 2001
1	Nil	10	10	0.24	0.15
2	Folicur 0.5	10	10	0.54	0.15
3	Folicur 0.75	10	55	1.40	0.10
4	Folicur 1.0	10	100	5.13	0.22
5	Folicur 0.5 + Bravo 2.0	100	10	0.36	0.09
6	Folicur 0.5 + Thiovit 10 kg	55	55	0.09	0.25
7	Folicur 0.5 + Dithane 945 1.7 kg	1	55	0.33	0.30
8	Bravo 2.0	10	10	0.41	0.26
9	Thiovit 10 kg	10	1	0.21	0.16
10	Dithane 945 1.7 kg	10	1	0.20	0.98
SED		29.47	24.45	1.662	0.469
P		Ns	Ns	<0.001	Ns

There were no significant differences in the sensitivity of isolates when related to the autumn fungicide treatment. There were significant differences in tebuconazole levels a fortnight after sprays were applied. There was significantly more tebuconazole in the full rate Folicur treatment (treatment 4) than in the other tebuconazole treatments applied at half or three quarter rate. These treatments did not have significantly more tebuconazole residue than any of the treatments that had not had Folicur applied. By mid February the tebuconazole levels had declined to background levels and there were no significant differences between treatments.

Table 9. MIC values for isolates tested from trial 2 Blairnathort, Kinross site 2000/2001

Treatment code	Autumn treatment	MIC Folicur *	Tebuconazole Residue levels 15 DEC 2000
1	Nil	1	0.44
2	Punch C 0.4	1	0.63
3	Folicur 0.5	1	0.83
4	Folicur 1.0	1	6.63
5	Foplicur 0.5 + Bravo 2.0	1	3.98
6	Folicur 0.5 + Thiovit 10 kg	-	0.21
7	Folicur 0.5 + Dithane superflo 2.8	-	0.12
8	Bravo 2.0	10	0.27
9	Thiovit 10 kg	1	0.42
10	Dithane superflo 2.8 l/ha	10	0.71
SED		-	2.005
P		-	0.074

- disease levels were so low in the trial that successful isolations of these treatments could not be made.

*as a result of low disease levels only one replicate per treatment was tested

The mean MIC of the isolates at this site was lower than at trial site 1 in this season. There were no significant differences in the sensitivity of isolates between treatments. The highest level of tebuconazole was detected in the full rate Folicur treatment (treatment 4) but differences in this trial were not significant. The trial was over sprayed in the spring before samples for a second tebuconazole test could be taken, as disease levels were too low.

Table 10. Disease and vigour assessment Trial 1 Milrig, West Lothian site 15 December 2000, GS 1,8

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Vigour 1-9 scale
1	Nil	0	0	5.6
2	Folicur 0.5 + Bravo 2.0	0	0	5.3
3	Folicur 0.75	0	0	5.3
4	Folicur 1.0	0	0	5.0
5	Folicur 0.5	0	0	6.0
6	Folicur 0.5 + Thiovit 10 kg	0	0	5.7
7	Folicur 0.5 + Dithane 945 1.7 kg	0	0	6.3
8	Bravo 2.0	0	0	6.0
9	Thiovit 10 kg	0	0	5.7
10	Dithane 945 1.7 kg	0	0	5.0
SED		0	0	0.39
P		Ns	Ns	Ns

There was no light leaf spot in the trial at this assessment timing. There were also no significant differences in vigour.

Table 11. Disease, scorch and vigour assessment Trial 1 Milrig, West Lothian site 15 February 2001, GS 1,8

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Scorch 1-9 scale	Vigour 1-9 scale
1	Nil	0.7	1.7	3.3	5.0
2	Folicur 0.5	0	0	2.7	6.3
3	Folicur 0.75	0	0	3.0	6.0
4	Folicur 1.0	0	0	3.0	6.3
5	Folicur 0.5 + Bravo 2.0	0	0	2.7	6.3
6	Folicur 0.5 + Thiovit 10 kg	0	0	3.0	6.3
7	Folicur 0.5 + Dithane 945 1.7 kg	0	0	3.0	6.3
8	Bravo 2.0	0	0	3.3	6.3
9	Thiovit 10 kg	0	0	3.3	6.3
10	Dithane 945 1.7 kg	0.7	1.7	3.7	6.0
SED		0.42	1.03	0.44	0.41
P		Ns	Ns	Ns	Ns

Light leaf spot was present at low levels in the untreated plots and in the straight Dithane treatment (treatment 10). There were no significant differences in scorch or vigour. The untreated control was the least vigorous but this trend was not significant.

Table 12 Disease, scorch and vigour assessment. Trial 1 Milrig, West Lothian site 26 March 2001, GS 2,4

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Scorch 1-9 scale	Vigour 1-9 scale
1	Nil	16.7	23.3	6.3	6.0
2	Folicur 0.5	7.7	35.0	6.7	5.0
3	Folicur 0.75	3.0	6.7	7.0	5.0
4	Folicur 1.0	3.0	6.7	6.7	5.0
5	Folicur 0.5 + Bravo 2.0	8.3	25.0	6.7	5.0
6	Folicur 0.5 + Thiovit 10 kg	3.0	6.7	6.7	5.3
7	Folicur 0.5 + Dithane 945 1.7 kg	6.0	8.3	6.7	5.0
8	Bravo 2.0	28.3	66.7	6.3	6.0
9	Thiovit 10 kg	15.0	36.7	6.6	5.7
10	Dithane 945 1.7 kg	18.3	43.3	6.3	5.7
SED		4.31	11.28	0.46	0.22
P		<0.001	<0.001	Ns	<0.001

There were significant differences in light leaf spot levels and in vigour at this assessment timing. Disease levels in the straight Thiovit and Dithane treatments were as in high as the untreated control. There was significantly more light leaf spot in the straight Bravo treatment. The lowest levels were recorded in the three quarter and full rate Folicur treatments and in the half rate Folicur plus Thiovit or Dithane treatments although the addition of Thiovit or Dithane did not significantly improve disease levels compared to the straight half rate Folicur treatment.

The vigour of plants was greatest in untreated control and was significantly reduced in all the Folicur treatments.

Table 13. Disease, scorch and vigour assessmentTrial 1 Milrig, West Lothian site 12 April 2001, GS 3,1

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Scorch 1-19 scale	Vigour 1-9 scale
1	Nil	20.0	93.3	5.0	7.0
2	Folicur 0.5	6.0	30.0	2.7	7.7
3	Folicur 0.75	4.3	13.3	2.7	7.0
4	Folicur 1.0	4.0	10.0	2.0	8.0
5	Folicur 0.5 + Bravo 2.0	7.7	26.7	2.3	7.0
6	Folicur 0.5 + Thiovit 10 kg	7.0	28.3	2.7	6.7
7	Folicur 0.5 + Dithane 945 1.7 kg	2.0	10.0	2.0	8.0
8	Bravo 2.0	21.7	80.0	6.0	5.7
9	Thiovit 10 kg	20.0	73.3	5.0	7.0
10	Dithane 945 1.7 kg	20.0	86.7	5.3	6.7
SED		3.09	10.26	0.79	0.70
P		<.001	<.001	<.001	0.08

There were significant differences in light leaf spot severity and in scorch at this assessment timing. The straight Bravo, Dithane and Thiovit treatments did not control light leaf spot compared to the untreated control. There was significantly less light leaf spot in the Folicur treatments compared to the untreated. The lowest levels of light leaf spot were recorded in the full rate Folicur treatment (treatment 4) and in the half dose rate Folicur treatment applied as a mixture with Dithane (treatment 7). The addition of Thiovit, Dithane did not significantly improve light leaf spot control compared to half dose rate Folicur and the addition of Bravo significantly reduced control.

The scorch score includes light leaf spot so the untreated control has high levels of scorch recorded. The levels of scorch recorded in the Folicur treatemnts 2 to 7 was significantly less.

Table 14. Disease and height assessment,Trial 1 Milrig, West Lothian site 27 April 2001, GS 3,3

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Height cm
1	Nil	11.7	90.0	81.7
2	Folicur 0.5	2.0	11.7	83.3
3	Folicur 0.75	8.0	60.0	95.0
4	Folicur 1.0	4.3	29.3	93.3
5	Folicur 0.5 + Bravo 2.0	5.0	35.0	98.3
6	Folicur 0.5 + Thiovit 10 kg	5.3	35.0	90.0
7	Folicur 0.5 + Dithane 945 1.7 kg	2.7	8.3	91.7
8	Bravo 2.0	12.7	60.0	90.0
9	Thiovit 10 kg	15.3	93.3	86.7
10	Dithane 945 1.7 kg	14.3	73.3	86.7
SED		2.931	21.35	6.681
P		<0.001	0.002	Ns

Disease levels were greatest in the untreated control and in the straight Thiovit, Bravo and Dithane treatments. The lowest level of disease was recorded in the half rate Folicur plus Dithane treatment (treatment 7) which was significantly better than the straight three quarter rate Folicur treatment (treatment 3), although the half rate Folicur treatment (treatment 2) had comparable levels of disease. Tebuconazole residues for these treatments (table 8) show more that residues were not significantly different between treatments 2 and 3. There were no significant differences in height although untreated plants were the shortest.

Table 15. Height (8 May 2001) and Yield (15 and 16 August 2001)Trial 1 Milrig, West Lothian site.

Treat-ment code	Autumn	Height 1-10 scale	T/ha 91% DM
1	Nil	5.3	3.90
2	Folicur 0.5	6.0	4.81
3	Folicur 0.75	7.3	4.86
4	Folicur 1.0	6.7	4.92
5	Folicur 0.5 + Bravo 2.0	7.0	4.84
6	Folicur 0.5 + Thiovit 10 kg	6.3	4.95
7	Folicur 0.5 + Dithane 945 1.7 kg	7.0	4.90
8	Bravo 2.0	6.3	4.40
9	Thiovit 10 kg	5.3	4.81
10	Dithane 945 1.7 kg	4.7	4.47
SED		0.871	0.218
P		Ns	<0.001

All the treatments had significantly boosted yield. All the Folicur treatment were significantly better than the straight Bravo or Dithane treatments. There was a larger yield benefit from straight Thiovit but this was not related to disease control and was probably a fertliser effect. There was not a significant yield improvement between half and full rate Folicur, or from the addition of Dithane, Thiovit or Bravo to half rate Folicur. There were no significant differences in height.

Table 16. Disease, scorch and vigour assessment Trial 2 2000/ 2001 Blairnathort 3 April 2001 GS 3,1

	Autumn treatment	% Light leaf spot on plants	% light leaf spot in plots	Scorch 1-9 scale	Vigour 1-9 scale
1	Nil	13.3	85.0	8.3	4.5
2	Punch C 0.4	9.0	70.0	5.8	6.3
3	Folicur 0.5	4.3	17.5	3.8	7.8
4	Folicur 1.0	2.8	11.3	3.8	6.3
5	Folicur 0.5 + Bravo 2.0	4.3	21.3	4.5	7.5
6	Folicur 0.5 + Thiovit 10 kg	3.5	11.3	4.8	7.5
7	Folicur 0.5 + Dithane superflo 2.8 l/ha	10.3	28.7	3.8	7.5
8	Bravo 2.0	18.3	95.0	8.0	6.0
9	Thiovit 10 kg	13.5	82.5	8.0	4.5
10	Dithane superflo 2.8 l/ha	15.0	77.5	7.5	5.0
	SED	2.610	11.84	0.563	0.704
	P	<0.001	<0.001	<0.001	<0.001

Light leaf spot levels were highest in the straight Bravo treatments. Straight Thiovit and Dithane did not reduce light leaf spot levels compared to the untreated either. Folicur at a half rate plus Dithane was also not significantly better than the untreated control. The best control was seen in the full rate Folicur treatment. The addition of Bravo, Dithane or Thiovit to half rate Folicur did not significantly improve the control seen for half rate Folicur alone. Punch C at a half rate (treatment 2) was not as effective as a half rate Folicur (treatment 3).

Scorch levels were highest in the untreated and in the straight Dithane, Bravo or Thiovit treatments. There was more scorch in the half rate Punch C treatment than in the half rate Folicur treatment. The least scorch was seen in the full rate Folicur treatment, the half rate straight Folicur treatment and in the half rate Folicur plus Dithane treatment. The half rate Folicur treatemnt was however significantly more vigorous than the full rate Folicur treatment.

Table 17. Yield, Trial 2 Blairnathort 2001

	Autumn treatment	T/ha DM	91%
1	Nil	4.77	
2	Punch C 0.4	4.88	
3	Folicur 0.5	5.04	
4	Folicur 1.0	5.39	
5	Folicur 0.5 + Bravo 2.0	5.40	
6	Folicur 0.5 + Thiovit 10 kg	5.34	
7	Folicur 0.5 + Dithane superflo 2.8 l/ha	5.26	
8	Bravo 2.0	4.77	
9	Thiovit 10 kg	4.56	
10	Dithane superflo 2.8 l/ha	4.64	
SED		0.361	
P		<0.001	

The highest yielding treatment was treatment 5, half rate Folicur plus Bravo. However this was not significantly better than straight half rate Folicur. The only other treatment to yield significantly more than the untreated comtrol was treatment 4, the full rate Folicur treatment. There was no yield benefit from straight Bravo, Thiovit or Dithane at this site.

2001/2002 FIELD TRIALS

Table 18. MIC values for isolates tested from field trial 3 2002, Kames, Borders site

Treatment code	Autumn treatment	MIC Folicur ppm	MIC Punch C ppm
1	Nil	7.88	5.38
2	Nil	2.88	11.2
3	Folicur 0.5	5.50	4.12
4	Folicur 0.75	6.50	6.50
5	Folicur 1.0	4.12	7.62
6	Punch C 0.4	3.88	6.75
7	Punch C 0.6	9.00	11.2
8	Punch C 0.8	5.25	9.00
9	Bavisitin 0.2	2.00	8.75
10	Sanction 0.8	2.88	6.38
SED		3.661	4.889
P		Ns	Ns

There was no significant difference in the sensitivity of isolates when related to the Autumn fungicide treatment. Isolates were more sensitive to Folicur than they were to Punch C.

Table 19. MIC values for isolates tested in Trial 4 2001/2002 Blairnathort, Kinross site

Treatment code	Autumn treatment	MIC Folicur	MIC Punch C	% Disease control at sampling
1	Untreated	1.55	10.35	-

*Light leaf spot levels too low to assess sensitivity apart from in the untreated plots. Samples taken 15 May 2002

Isolates tended to be more sensitive to Folicur at this site compared to trial 3, the Kames, Borders site in this season. There was no light leaf spot in the autumn treated plots that could be sampled before the second sprays were applied.

Table 20. Disease and vigour assessment. Field trial 3 Kames, Borders site 11 January 2002 GS 1,6 – 1,8

Treat-ment code	Autumn treatment	% Light leaf spot per plant	Vigour 1-9 scale
1	Nil	0	7.8
2	Nil	0	8.3
3	Folicur 0.5	0	7.5
4	Folicur 0.75	0	6.8
5	Folicur 1.0	0	7.5
6	Punch C 0.4	0	7.8
7	Punch C 0.6	0	7.8
8	Punch C 0.8	0	8.0
9	Bavisitin 0.2	0	8.0
10	Sanction 0.8	0	8.3
SED		0	0.303
P		Ns	Ns

There was no light leaf spot at this timing and there were no significant differences in vigour.

Table 21. Disease, vigour and scorch assessment, Field trial 3 Kames, Borders site 27 March 2002 Gs3,1

Treat-ment code	Autumn treatment	% Light leaf spot plant	% Light leaf spot per plot	Vigour 1-9 scale	Scorch 1-9 scale
1	Nil	7.5	22.5	9.0	4.5
2	Nil	7.3	20.0	9.0	4.3
3	Folicur 0.5	0	0	5.8	2.0
4	Folicur 0.75	0	0	6.8	1.5
5	Folicur 1.0	0	0	4.8	0
6	Punch C 0.4	0	0	8.8	1.3
7	Punch C 0.6	6.3	1.3	8.8	2.0
8	Punch C 0.8	0.3	0.5	8.8	1.0
9	Bavisitin 0.2 l/ha	2.3	5.0	8.8	2.8
10	Sanction 0.8	0	0	9.0	1.5
SED		2.18	2.38	0.29	0.63
P		0.050	<0.001	<0.001	<0.001

There was no disease in straight Folicur treatments. Bavistan applied at 0.2 l/ha (the equivalent rate to that used in full rate Punch C) did reduce light leaf spot levels compared to the untreated control. There was no light leaf spot in the Sanction 0.8 l/ha treatment.

Vigour was significantly reduced in the Folicur treatments and the full rate was significantly worse than the three quarter rate Folicur treatment. There was significantly less scorch in all the treatments when compared to the untreated controls.

Table 22. Disease and vigour assessment, Field trial 3 Kames, Borders site 9 April 2002 Gs3,3

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Vigour 1-9 scale
1	Nil	12.5	80.0	9.0
2	Nil (spring Folicur 0.5)	4.5	28.8	9.0
3	Folicur 0.5	1.3	5.0	8.3
4	Folicur 0.75	0.0	0.0	6.8
5	Folicur 1.0	2.3	7.5	6.3
6	Punch C 0.4	0.5	2.5	8.5
7	Punch C 0.6	0.0	0.0	8.8
8	Punch C 0.8	0.3	1.3	8.8
9	Bavisitin 0.2	3.3	15.0	8.5
10	Sanction 0.8	0.5	1.3	9.0
SED		0.86	4.55	0.26
P		<0.001	<0.001	<0.001

There was no disease in the three quarter rate Folicur treatment and in the two third rate Punch C treatment. The Bavistan treatment was not significantly better than the untreated in autumn control (treatment 2) in terms of light leaf spot levels. Sanction gave equivalent control to full rate Punch C. The full rate Folicur treatment was significantly less vigorous that the half dose rate treatment. Increasing the dose of Punch C did not have this effect and vigour was comparable between all three treatments.

Table 23. Disease and vigour assessment, trial 3 Kames, Borders site 25 April 2002 Gs3,7 – 4,0

Treat-ment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Vigour 1-9 scale
1	Nil	9.8	70.0	8.3
2	Nil	6.0	60.0	8.8
3	Folicur 0.5	2.3	15.0	7.0
4	Folicur 0.75	0	0	7.3
5	Folicur 1.0	0	0	5.7
6	Punch C 0.4	1.3	6.5	8.0
7	Punch C 0.6	1.5	3.8	7.5
8	Punch C 0.8	0	0	8.5
9	Bavisitin 0.2 l/ha	1.0	5.0	7.5
10	Sanction 0.8	0.5	2.5	8.5
SED		0.84	5.18	0.42
P		<0.001	<0.001	<0.001

There was no disease in the three quarter rate and full rate Folicur treatments or in the full rate Punch C treatment. The other treatments were all significantly better than treatment 2 which had been untreated in the autumn. Vigour tended to be reduced in all the Folicur treatments and was significantly reduced in the full rate Folicur treatment.

Table 24. Crop Yields Field trial 3 Kames, Borders site.

	Autumn treatment	T/ha 91% DM
1	Nil	3.57
2	Nil	4.04
3	Folicur 0.5	4.12
4	Folicur 0.75	3.99
5	Folicur 1.0	3.85
6	Punch C 0.4	4.11
7	Punch C 0.6	4.12
8	Punch C 0.8	4.05
9	Bavisitin 0.2	3.80
10	Sanction 25 0.8	4.39
SED		0.206
P		0.043

None of the treatments gave a significant yield response compared to treatment 2 which was untreated in the Autumn. Yield was less in the full rate Folicur treatment compared to the other Folicur rates but this difference was not significant.

Table 25. Disease assessment, vigour and scorch, Trial 4, Blairnathort, Kinross site 15 March 2002 GS 1,12

Treatment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Vigour 1-9 scale	Scorch 1-9 scale
1	Untreated	0	0	7.0	8.0
2	Untreated	0	0	7.7	8.0
3	Folicur 0.5	0	0	8.0	7.7
4	Folicur 0.75	0	0	7.7	5.3
5	Folicur 1.0	0	0	8.3	6.7
6	Punch C 0.4	0	0	7.7	6.0
7	Punch C 0.6	0	0	8.0	4.3
8	Punch C 0.8	0	0	7.7	8.7
9	Bavistan 0.2	0	0	8.0	6.0
10	Sanction 25 0.8	0	0	7.7	6.0
SED		0	0	0.54	1.81
P		Ns	Ns	Ns	Ns

There was no disease at the site at this assessment timing and there were no differences in vigour or scorch levels.

Table 26. Disease and vigour assessment Trial 4, Blairnathort, Kinross site 9 May 2002 GS 4,1

Treatment code	Autumn treatment	% Light leaf spot per plant	% Light leaf spot per plot	Vigour 1-9 scale
1	Untreated	4.3	15.0	8.0
2	Untreated	1.7	16.7	7.7
3	Folicur 0.5	0.3	1.7	8.3
4	Folicur 0.75	0.1	0.0	7.7
5	Folicur 1.0	0.3	1.7	8.3
6	Punch C 0.4	0.0	0.0	8.0
7	Punch C 0.6	0.0	0.0	8.3
8	Punch C 0.8	0.0	0.0	8.0
9	Bavistan 0.2	0.7	1.7	8.0
10	Sanction 25 0.8	1.7	1.7	8.7
SED		1.15	6.42	0.35
P		Ns	Ns	Ns

There were very only low levels of disease in the trial apart from in the untreated controls. Differences in disease levels between fungicide treatments were not significant. There were no significant differences in vigour.

Table 27. Crop yield, Trial 4, Blairnathort, Kinross site

Treatment code	Autumn treatment	T/ha DM	91%
1	Untreated	4.39	
2	Untreated	4.05	
3	Folicur 0.5	4.64	
4	Folicur 0.75	4.44	
5	Folicur 1.0	4.53	
6	Punch C 0.4	4.74	
7	Punch C 0.6	4.72	
8	Punch C 0.8	4.49	
9	Bavistan 0.2	4.34	
10	Sanction 25 0.8	4.5	
SED		0.147	
P		0.035	

There was a significant yield boost from the spring fungicide application (treatment 2) when compared to the untreated control (treatment 1). All the fungicide treatments boosted yield when compared to the untreated in autumn control. There was no rate response however to increasing the dose of Folicur or Punch C. The use of Bavistan at a comparable rate to full rate Punch C did significantly increase yield and but Sanction at 0.8 l/ha yielded the same as Punch C at 0.8 l/ha.

6. DISCUSSION

There were two main areas of testing in this project. The first was a survey of commercial crops to determine the incidence and spread of triazole resistance to fungicides. The second area was the use of field trials to generate fungicide efficacy data and to determine if disease control was related to the sensitivity of the light leaf spot to the triazole fungicides and to determine if non triazole mixing partners could improve control.

6.1 Survey of crops to test for triazole sensitivity in light leaf spot

The survey covered selected commercial crops throughout the main arable areas of Scotland. A limited number of samples were also collected from variety trials as part of this survey to increase the number of varieties sampled within the survey. Isolates were tested for sensitivity to both Folicur (tebuconazole) and Punch C (flusilazole plus MBC). Fewer isolates were collected in the 2000/2001 season as the outbreak of Foot and Mouth Disease restricted access to farms.

The range of sensitivities measured was large and ranged from 0.5 to 20 ppm. In the first year of testing the highest concentrations used for testing were 10 and 100 ppm so that isolates that grew on 10 ppm were classed as having a MIC value of 100. In the second year of testing the highest concentrations were revised as isolates were generally more sensitive than expected. No isolates in 2002 grew on fungicide concentrations of greater than 20 ppm and it is likely that the 2001 isolates which grew on 10 ppm would have shown no growth on 20 ppm. It would therefore be misleading to imply that sensitivity ranged 200 fold. A 40 fold range in sensitivity from 0.5 ppm to 20 ppm is more accurate.

Isolates with very low MIC values would be very sensitive to the fungicides and isolates with MIC values of 20 ppm would much less sensitive and therefore harder to control in practice. The survey showed that there was no significant link between the sensitivity of isolates and the location from which they were sampled. There was an interesting trend in that isolates from the north east where light leaf spot pressure is high were amongst the least sensitive, whilst the isolate collected from Cambridge was the most sensitive with an MIC value of 0.5.

There was also no link between the sensitivity of isolates and the variety from which they had been sampled which suggests there was no selection for different resistance genotypes with

different varieties. Previous fungicide history on the crop from which light leaf spot samples were taken did not influence fungicide sensitivity and there was no evidence that samples from untreated crops were more or less sensitive than those from treated crops. Similarly there was no evidence that isolates taken from MBC plus triazole treated crops were any more or less sensitive than isolates sampled from straight triazole treated crops. There was also no correlation between area sampled and the sensitivity of isolates. Isolates with reduced sensitivities were noted in all the Scottish geographic regions sampled.

Isolates were tested for sensitivity to Folicur and to Punch C. Isolates tended to be more sensitive to Folicur (mean MIC 8.22 ppm) than to Punch C (mean MIC 19.2ppm). There has been very little base line testing of triazole fungicides carried out for light leaf spot and it is therefore hard to determine if the sensitivity to triazoles have declined markedly since their introduction for light leaf spot control. Data supplied by DuPont (UK) Ltd shows that for a limited number of isolates tested in 1995 from England, none grew on 10 ppm Punch C amended agar. For Scottish isolates tested at the same time, 40% grew on 10 ppm amended agar. None of the isolates tested in the 1995 survey had an MIC value of greater than 25 ppm. For the current project, in the survey completed in 2001 and 2002, 68% of isolates showed some growth on 10 ppm Punch C, but in 2002 none of the isolates showed any growth on 20 ppm Punch C amended agar. There is therefore an indication from past data that more isolates will now grow on a 10 ppm discriminatory dose now compared to 1995 but this shift has not been dramatic as isolate growth was still halted at 20 ppm.

The results of the survey show that there was a wide distribution in the minimum inhibitory concentration (MIC) values for Folicur and Punch C (Figure 2). A range of sensitivities is characteristically seen for fungicides to which resistance in the pathogen is controlled by multiple genes. It is typical for the triazole fungicides with other pathogens such as Rhynchosporium in barley (HGCA Project 2322, unpublished). Fungicides to which pathogens have a single gene conveying resistance would tend to be distributed in two separate groups – with a clear distinction between sensitive and resistant isolates. This is characterised by MBC resistance where isolates of *P. brassicae* are either very sensitive with MIC values of 1 ppm or less or very resistant with MIC values of greater than 2000ppm (Sutherland and Griffin Walker, 1994). As has been seen with other pathogens like mildew and Rhynchosporium in cereals the move towards resistance to triazoles tends to be a slow decline in efficacy rather than an all or nothing effect such as has been seen in cereals to the strobilurin group of fungicides.

The results in Figure 2 do not show a normal distribution. There are more isolates grouped at the sensitive end of the scale than would be expected if a normal distribution curve was fitted. This is also typical of the triazole group of fungicides and has been seen for other pathogens. For example in HGCA project 2322 (unpublished) on Rhynchosporium there was a similar pattern in the sensitivity data generated by Queens University, Belfast. In the Rhynchosporium work some isolates of the pathogen population remained sensitive, while a second, overlapping group within the population were less sensitive and formed a second distribution curve. In the light leaf spot survey this pattern is seen in the incidence of isolates recorded as highly sensitive in the survey which are grouped at the left hand side of the axis and in an overlapping group which are less sensitive and form a second distribution curve further along the axis. Very few isolates had MIC values above 10 ppm which shows that the population is still generally sensitive to triazoles but the variation is such that differences in the efficacy of triazole fungicides will be apparent between sites in Scotland.

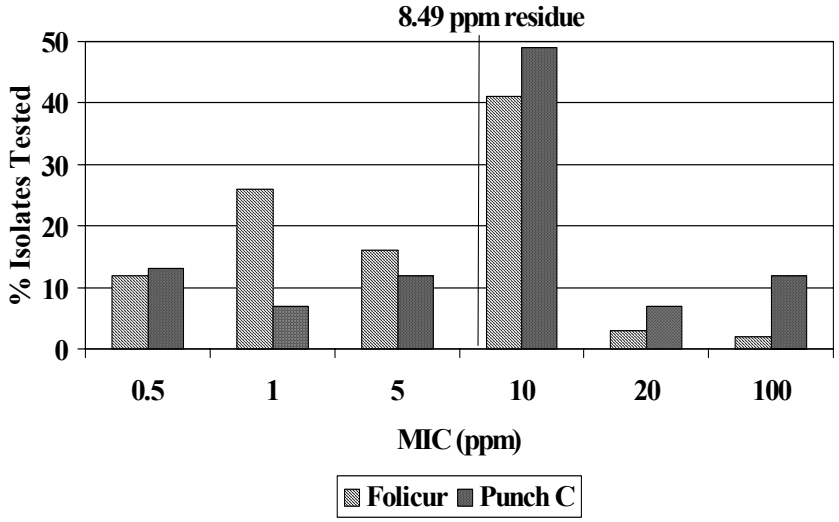

Figure 2: Distribution of sensitivity for Folicur and Punch C by MIC value.

The issue of variable sensitivity leading to variable control with fungicides is very critical when the data from the tebuconazole residue testing is added to the data in Figure 2. The highest residue recorded in the trials a month after a spray with a full dose rate of Folicur (a.i. tebuconazole) was 8.49 ppm.

The growth habit of the light leaf spot pathogen is different *in planta* than it is when isolated onto agar. On amended agar *P. brassicae* is more directly exposed to the fungicide than would be the case *in planta* , and there will be differences in the breakdown and metabolism of fungicides between the two scenarios. It is not, therefore, possible to compare directly between fungicide efficacy in the plant and fungicide efficacy in the amended agar used to determine the isolate sensitivity. Making the assumption that they are related the survey data shows that two weeks after spray application, potentially 68% of the light leaf spot population in Scotland would not be controlled by this level of tebuconazole. This raises very important questions about the dose rates used in light leaf spot control programmes in terms of the persistence of the fungicide sprays applied and the protectant effect that is required.

A full dose rate of Folicur in 200 litres of water equates to 1250 ppm. One half of the full dose rate equates to 625 ppm as shown in Figure 3. The residue levels detected in the trial sites therefore represent a small fraction of the dose rate applied to the treatment. Punch C in 200 litres of water gives a concentration of 1000 ppm flusilazole. The line at the bottom of the graph in Figure 3 represents the highest residue level of 8.49 ppm tebuconazole that was detected in the trials. The wide range in sensitivity to Folicur and to Punch C that has been quantified in this project, coupled with the residue data showing tebuconazole residues in plants following treatment implies that there will be variable efficacy in triazole fungicides across Scotland. Dose rate maybe very critical in determining the degree of control seen on many farms.

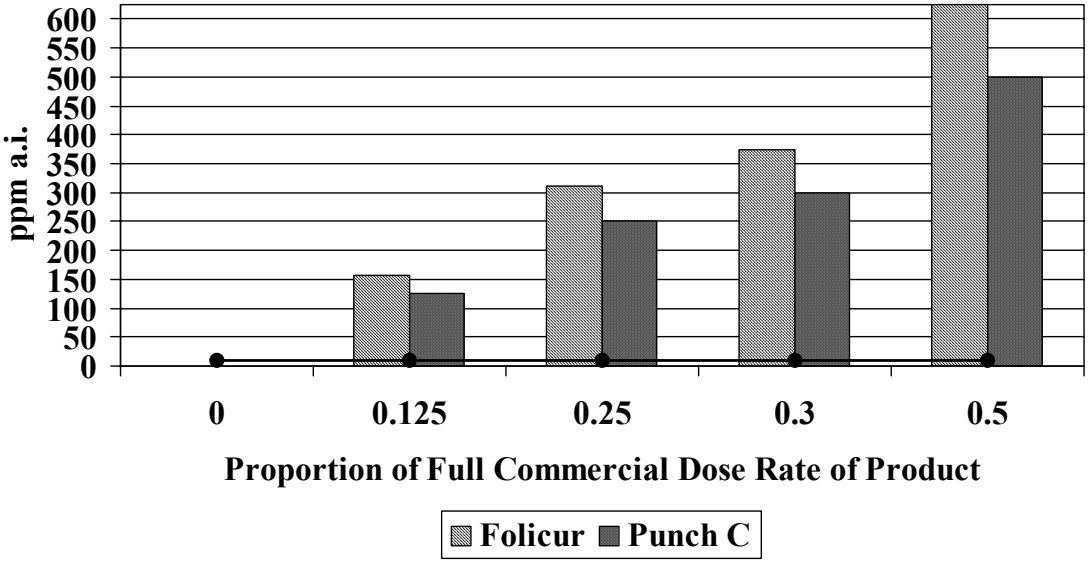

Figure 3. The relationship between full commercial dose rate and ppm of active ingredient with a line to indicate the maximum residue level detected in leaf tissue following full dose rate treatment.

6.2 Fungicide efficacy in field trials

The issue of dose rate was one of the factors investigated in the series of four trials that were carried out as part of this project. One aim was to investigate if effective control of light leaf spot was being compromised by the reduced dose rates that are common in commercial practice and to determine if increasing the triazole dose rate would improve disease control and result in yield increases. The trials also aimed to determine if increasing the dose rate would influence the sensitivity of light leaf spot isolates collected from the trials and to see if mixing fungicides with alternative modes of action would increase the efficacy of the triazole partner and manage any shifts in the sensitivity of light leaf spot.

There was no evidence of any shifts in sensitivity in the trials related to the dose rate of triazole used. Isolates collected from high dose rate treatments were not significantly more or less sensitive than isolates sampled from low dose rate treatments. The addition of mixing partners with alternative modes of action like Thiovit, Bravo and Dithane did not influence the sensitivity of the light leaf spot in this study, and none of the fungicide treatments altered the mean sensitivity of the isolates when compared to the untreated control plots.

There were, however, differences in the sensitivity of the isolates between sites (Figure 4). In the first year of the trials (2000 / 2001) the isolates sampled from trial site 1, Milrig in West Lothian

were much less sensitive to both Punch C and Folicur than the isolates sampled from trial site 2, Blairnathort, by Kinross. In the second year of the study, 2001/2002, trial site 3, Kames in the Borders region was less sensitive than the Blairnathort site (trial site 4). Sensitivity to Punch C was not tested for the Blairnathort site in 2000/2001.

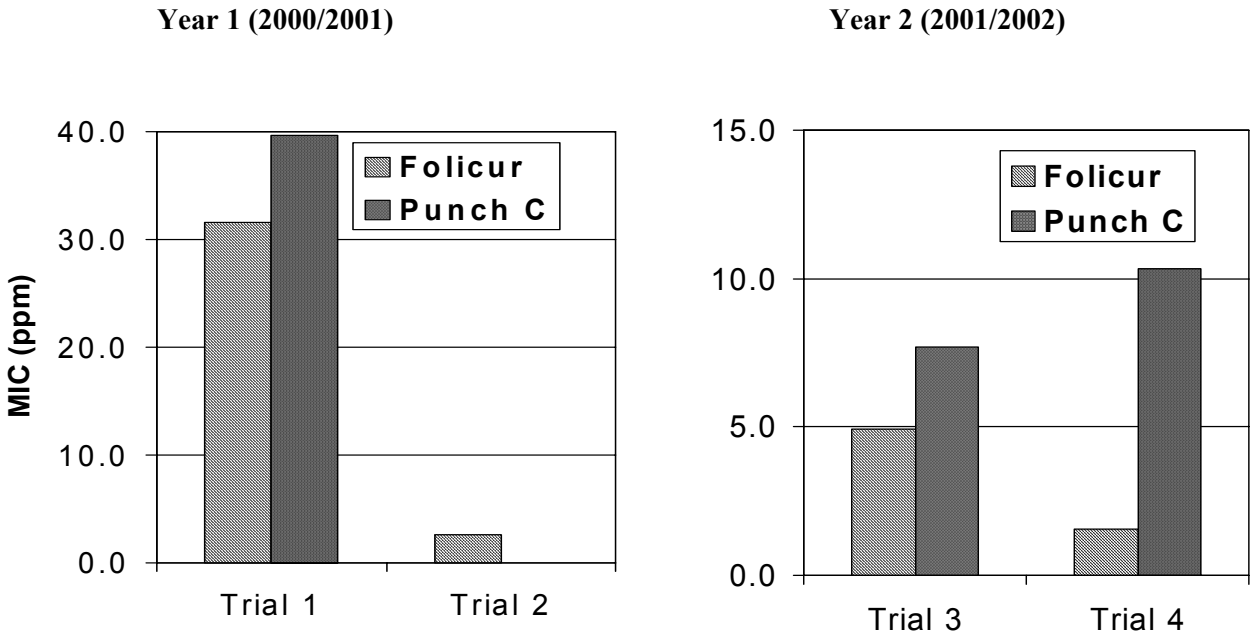

Figure 4. The mean sensitivity (MIC) of light leaf spot from the four trial sites.

The levels of disease also varied between sites and did not appear at any more than trace levels in all the trial sites until spring. Figure 5 shows the levels of disease in the untreated controls throughout the season. Trials were treated in the autumn and again in the spring. Isolates of light leaf spot tested for sensitivity were collected from the trials just before the second spray was applied, which was in March or April. This was generally when light leaf spot was just starting to become visible in the trials. In trial 2 it was not possible to isolate enough light leaf spot to test more than one replicate of each treatment and in trial 4 there was only enough disease in the untreated controls to allow testing before the spring spray was applied.

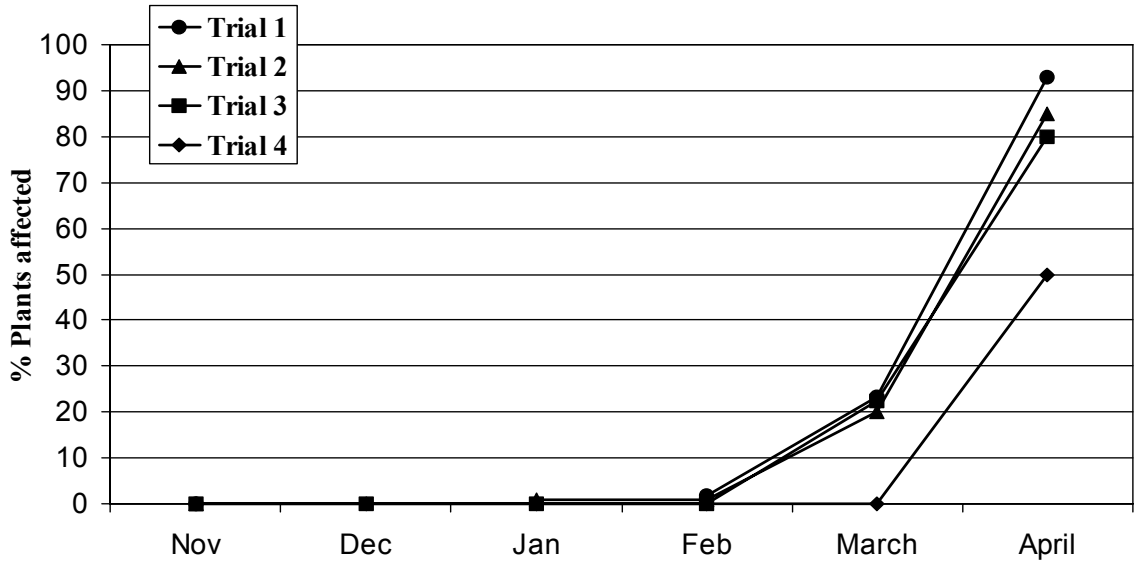

Figure 5. Light leaf spot levels in the untreated controls at the field trial sites

In the first season of the trials in 2000/ 2001, a range of triazole dose rates were evaluated as well as a range of mixing partners with alternative modes of action.

There was no significant yield improvement at either site when a half dose rate of Folicur was compared to a full dose rate in 2000/2001. At trial site 1, Milrig, disease control was significantly better in March (Figure 6 and Table 12) following treatment with three quarter or full rate Folicur when compared to the half dose rate treatment. This difference was not apparent at trial site 2, (Blairnathort) where Folicur at 0.5 l/ha was as effective as a full rate treatment of 1.0 l/ha. It is possible that this difference in efficacy is related to the sensitivity of the light leaf spot at the trial sites. The measured MIC at Blairnathort (Site 2) was much lower than at Milrig (Site 1). This difference in efficacy of the half dose rate Folicur between the two sites further underlines the implications drawn from the tebuconazole testing work which show that many isolates within the Scottish light leaf spot population will not be controlled by reduced rate tebuconazole treatment.

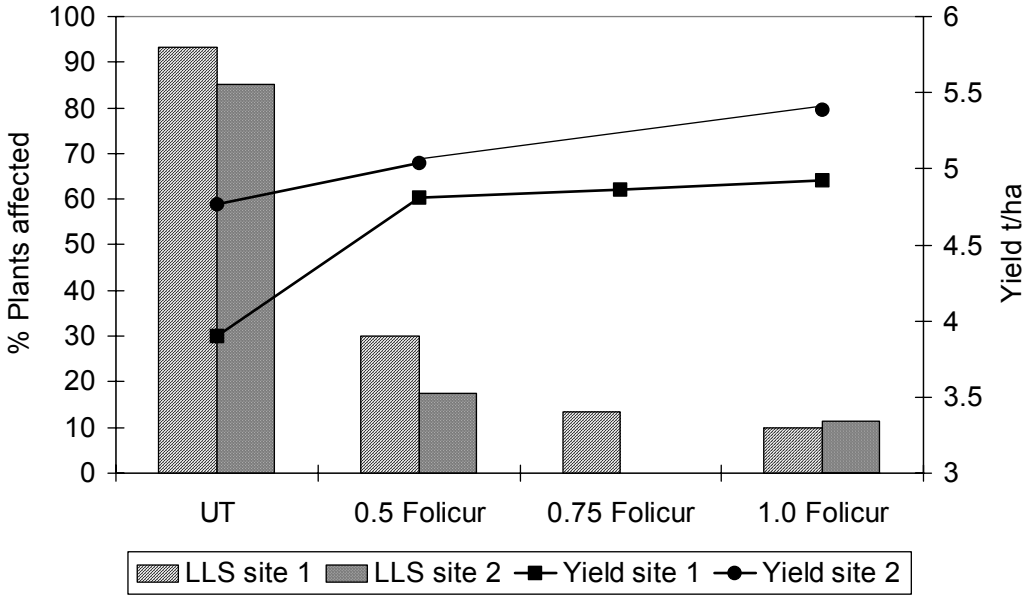

Figure 6. The influence of dose rate of Folicur on light leaf spot control at Gs 3,1 and yield, sites 1 and two.

In both trials carried out in 2000/2001 (Trial sites 1 and 2) there was no significant improvement in disease control with the addition of either Thiovit, Bravo or Dithane to half dose rate Folicur when compared to a straight half dose rate Folicur treatment. Disease control from straight Thiovit, Bravo or Dithane was not significantly better than the untreated controls and one assessment timings Bravo was significantly worse. There was a small but just significant (P = 0.05) yield benefit, however, at trial site 1 at Milrig, West Lothian from straight Bravo and Dithane when compared to the untreated control (Figure 7). There was a larger yield benefit to straight Thiovit at this site but the site was of a lower sulphur status and the yield response was probably a fertiliser response and was not noted at the other site.

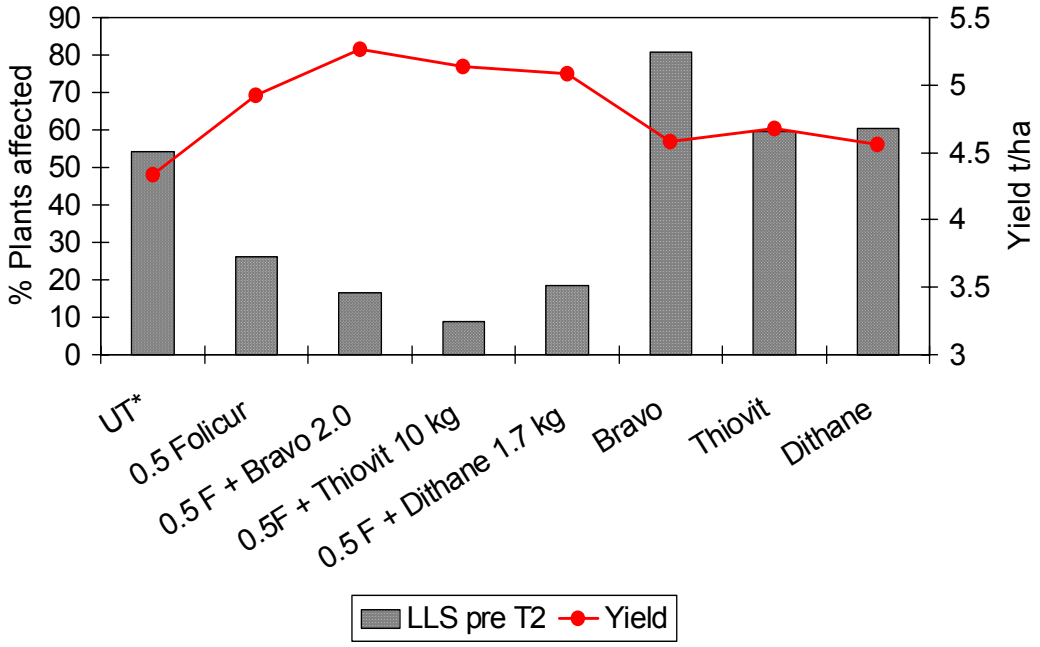

Figure 7. The mean effect of partner fungicides to Folicur on yield and light leaf spot control before second sprays were applied (T2), Sites 1 and 2.

In the second season in which trials were conducted (2001 / 2002) the additional mixing partners used in the first season were not re-evaluated because of the poor disease control demonstrated and their lack of influence on the sensitivity of the light leaf spot to triazole fungicide. There was no evidence that they increased the sensitivity of light leaf spot in these treatments.

The focus of the trials in 2001/ 2002 was on the influence of dose rate. Folicur (tebuconazole) and Punch C (flusilazole and carbendazim (MBC)) were evaluated at two trial sites, site 3 at Kames in the Borders region of Scotland and site 4 at Blairnathort, Kinross. Sanction (flusilazole) and Bavistan (MBC) were also evaluated at dose rates equivalent to those present in full dose rate Punch C.

Full rate tebuconazole (as Folicur) significantly ($P = 0.001$) reduced the crop vigour (table 21 and 23) compared to the half or three quarter dose rate treatments. Three quarter and full dose rate Folicur significantly ($P = 0.001$) reduced the incidence of light leaf spot at an assessment at GS 3,7 – 4,0 (table 23) compared to the half dose rate treatment.. Three quarter dose rate Folicur did cause scorch at GS 3,3, however (table 22). Although there were significant differences in disease

levels between crops there was no significant difference in yield. The lowest dose rate of Folicur yielded best but was not statistically better than the higher dose rates. The full dose rate Folicur was the lowest yielding of the Folicur treatments which may reflect the reduction in vigour noted from this treatments at this site.

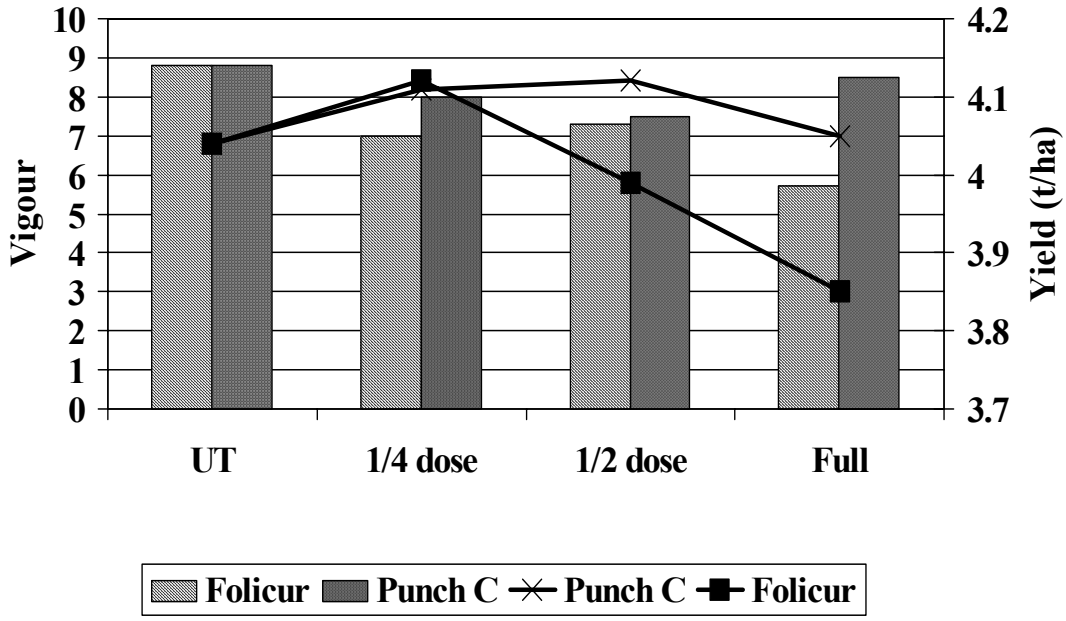

Figure 8. The influence of crop vigour on yield at Site 3, 2002.

Full dose rate (0.8 l/ha) Punch C was no more effective in controlling light leaf spot at this site compared to a half dose rate, apart from at GS 3,7 – 4 (table 23), but this increase in control was not significant. There was no corresponding increase in yield from increased dose rate of Punch C but in contrast to the Folicur treatments there were no reductions in vigour from the higher dose rate treatments of Punch C. Bavistan applied at a dose rate equivalent to the dose of carbenazim present in Punch C did reduce the levels of light leaf spot at most assessment timings however Sanction at a dose rate equivalent to the flusilazole rate in Punch C was never significantly different in terms of disease control from the full dose rate treatment of Punch C (Figure 9). This implies that the MBC or carbenazim component in the Punch C is adding very little in terms of light leaf spot control and yield. However, carbendazim at a rate equivalent to that in Punch C, applied as the straight product Bavistan, did give a small reduction in light leaf spot suggesting that a small proportion of the *P. brassiace* population remains sensitive. The results would suggest that the *P. brassicae* population in Scotland is still largely resistant to MBCs and that

MBC fungicides will only control a small percentage of the population. This is similar to the findings of Sutherland and Griffin-Walker (1994) who found that there were high levels of MBC resistance present in the light leaf spot population.

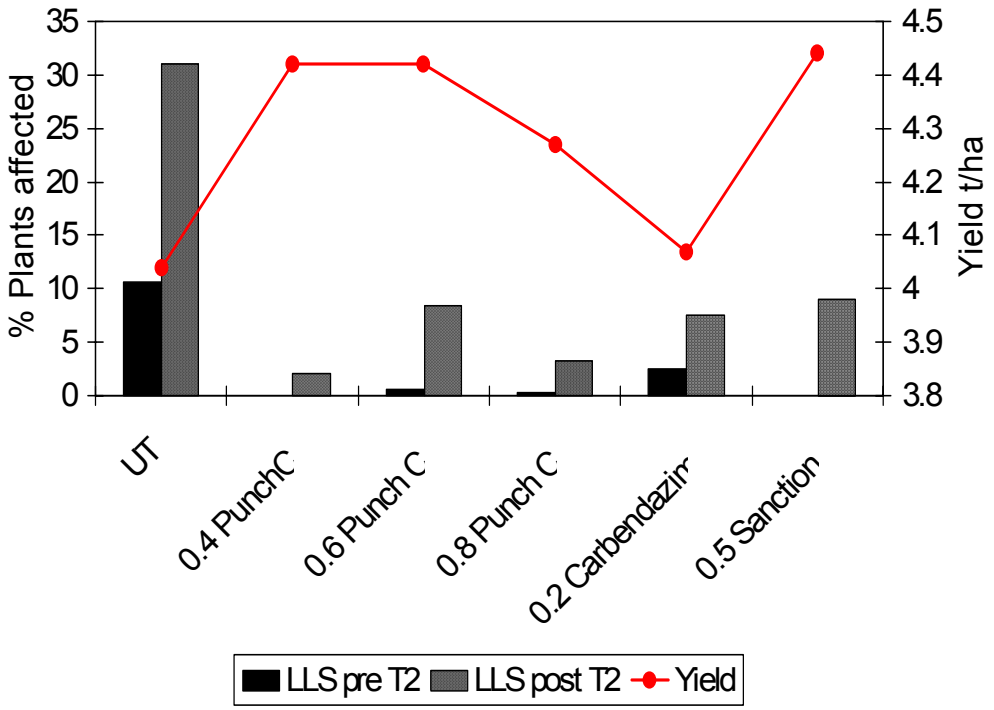

Figure 9. Light leaf spot control and yield as a result of Sanction or Bavistan treatment in comparison to Punch C

Disease levels were very low at the other site used in 2001/ 2002 (site 4 Blairnathort). There were no differences in light leaf spot control at either assessment timing. All the Punch C treatments were disease free throughout the season. There were traces of disease at the end of the season in the Folicur treatments but this was not significantly different from the Punch C treatments. There were no differences in vigour from any of the treatments. Despite the low disease levels present at this site there were significant ($P = 0.035$) yield responses from the autumn fungicide treatments. Full rate Folicur yielded significantly less than the three quarter or half rate treatments. The half dose rate of Punch C was the highest yielding of the Punch C treatments but this yield increase was not significantly better than the three quarter or full dose rate treatments.

These yield responses (shown in table 24) from fungicide treatments were significantly ($P = 0.035$) better than the control treatment that was untreated in the autumn and over sprayed in the

spring with the treatment plots (treatment 2). The totally untreated control (treatment 1) that was not sprayed in either the autumn or the spring however yielded significantly better than the control that was over sprayed in the spring implying that the Folicur over sprayed at half dose rate may have had a yield reducing effect. It is interesting that the half and three quarter dose rate Punch C treatments yielded better than the untreated control plots as well as the untreated in autumn only control. This further demonstrates that dose rates of triazoles need to be carefully selected and that despite the variable disease control demonstrated at reduced dose rates, increasing the dose rate is not necessarily the answer to this reduced efficacy. There were significant negative impacts on the vigour of the crop as a result of high rate Folicur treatments.

Reviewing the efficacy, crop safety and yield data generated in the course of this project it is clear that because of the range of sensitivities in the *P. brassicae* population to triazole fungicides, the efficacy of these fungicide treatments will be variable within and between sites. It was apparent from the tebuconazole residue testing that reduced dose rate treatments will result in levels of fungicide in the leaves that will not be effective at controlling the majority of *P. brassicae* isolates that were assayed for sensitivity in the course of this work. Unfortunately increasing the dose rate is not as straight forward a solution to this as might be thought. High dose rates of Folicur had significant impacts on the vigour of the crop and caused visible stunting of plants at the Kames site. This reduction in vigour was reflected in reduced yields from the full does rate treatments and there was also evidence that even lower doses can cause yield reductions where the benefits in disease control are not sufficient to mitigate the vigour effect (Site 4). In contrast, although the *P. brassicae* isolates assayed in this work were generally less sensitive to Punch C than they were to Folicur there were no crop safety issues with this product at the full dose rate treatments evaluated. Disease control in the field trials was generally comparable to that seen with Folicur which implies that the difference in sensitivity is not often large enough to be noted in a reduction in efficacy. Folicur did give significantly better control than Punch C at the Blairnathort site in 2001 (Site 2). Isolates from this site were amongst the most sensitive to tebuconazole of the isolates tested in the project and this may explain the increased efficacy of Folicur at this site. Punch C out yielded Folicur at both sites in 2002, although not significantly.

The addition of additional partner fungicides with alternative modes of action such as Dithane, Thiovit or Bravo did not significantly improve disease control or alter the sensitivity of the *P. brassicae* isolates assayed from the trials. This is not to imply that a mixture stategy of alternative mode of action fungicides is not an effective way to manage fungicide resistance. Rather it

probably reflects the lack of efficacy of the partner fungicides evaluated. Thiovit, Dithane and Bravo showed no significant disease control and therefore as a result add nothing to the light leaf spot control shown from triazole fungicides and cannot therefore be expected to be an effective anti-resistance strategy.

MBC as a mixing partner was slightly different. As straight Bavistan very little light leaf spot control was demonstrated in the trials and straight Sanction was not significantly better or worse than the formulated mixure product Punch C. In contrast to Thiovit, Bravo and Dithane, however, there may be isolates of *P. brassicae* that are sensitive to the MBC component in Punch C and Bavistan, particularly in lower disease pressure areas of the UK. The low levels of disease control seen in these trials from the MBC component imply that a significant proportion or possibly the majority of the *P.brassicae* population was resistant to MBC fungicides at the sites utilised, but it may still represent a potential mixing partner fungicide strategy for managing resistance in areas where efficacy is retained. Previous work (Sutherland and Griffin-Walker, 1994) has shown how widespread resistance to MBC fungicides is in the *P. brassicae* poulation, which is unfortunate as there are no other effective potential mixing partners to form alternative anti-resistance strategies with. The strobilurin fungicides do not generally show much efficacy against light leaf spot and are unlikely to gain Approval for use of the crop.

The survey of light leaf spot, sampled from winter oilseed rape across Scotland and tested for sesnitivity, showed that isolates with reduced sensitivity (higher MIC values) were as likely to be found in all the main arable regions of Scotland and were not restricted to high pressure areas such as the north east. The recommendations drawn from this project are therefore applicable to all regions in Scotland. Dose rates of half the full commercial dose rate or less are likely to show very variable degrees of efficacy and will show poor disease control in many cases. This is because a significant proportion of the *P. brassiace* population in Scotland will not be controlled by the levels of fungicide residues that such reduced dose rate treatments will deposit in the crop. Increasing the dose rate of Folicur too far caused crop damage. Three quarter does rate sprays were generally significantly more effective than the half dose rate sprays and did not cause such significant crop damage. Punch C did not have the same crop safety issues at higher dose rates, although a full dose rate yielded less than the three quarter dose rate, suggesting that the three quarter rate is optimal. There were no effective mixing partners demonstrated in this work and the widespread occurrence of MBC resistance makes the use of MBC in mixtures with triazoles as an alternative mode of action anti-resistance strategy a questionable approach. It does, however,

represent the only mixing partner with any efficacy at present and has to be given consideration because of this.

The development of triazole resistance is having a direct effect on the levy payers, with less effective disease control and poor returns. Resistance will also have implications for other HGCA funded research such as variety testing and the PASSWORD project. The UK variety list is based on comparisons of varieties untreated and treated with triazole fungicides. If any of the variety test sites has a triazole resistant light leaf spot population then this could favour resistant but lower yielding varieties over susceptible but higher yielding varieties. It could also affect potential new variety screening. The PASSWORD project aims to develop a pest and disease management system for supporting winter oilseed rape decisions. If triazole resistance is recognised on a farm this will affect the management decisions taken. The extent of triazole resistance needs to be established so that this can be built into the PASSWORD project.

A future area of study, leading from this project, has to be a determination of the distribution of triazole and MBC resistance throughout the whole of the UK so that the efficacy of the very limited range of products which control light leaf spot can be protected. Limited evidence shows that *P. brassicae* may still be more sensitive to triazole fungicides in England. An isolate from Cambridgeshire tested in this project was amongst the most sensitive assayed. Similarly more isolates from England may remain sensitive to MBC fungicides. If this is the case then the use of triazole plus MBC mixtures may represent an effective anti-resistance strategy. In the past growers have placed very little value on the selection of fungicides for anti-resistant reasons. Yield and disease control properties are far more important to individual growers than any longer term protection strategies designed to protect the fungicides they rely on. It is critical that anti-resistance strategies are promoted by consultants and advisors, particularly in cases like this where the cost and efficacy penalties in the short term of using such a strategy compared to a straight triazole programme are minimal, if present at all.

7. CONCLUSIONS

The *P. brassicae* population is very variable in its sensitivity to triazole fungicides. The sensitivity of the isolate assayed in the course of this work ranged at least forty fold from an MIC values of 0.5 ppm to 20 ppm tebuconazole. There was no link between area surveyed and the mean sensitivity of isolates assayed. Less sensitive isolates were isolated from commercial crops throughout Scotland and were as common in all the regions surveyed. There was no link determined between the recorded crop history and the sensitivity of the isolates – the variety sampled did not influence the sensitivity of the isolates and nor did previous fungicide history. Previous treatment with a triazole fungicide or a triazole plus fungicide mixture did not significantly alter the sensitivity of isolates compared to isolates assayed from untreated crops. This may reflect the almost ubiquitous use of triazoles or triazole plus MBC mixtures to control light leaf spot in oilseed rape and the consequent high levels of expose of the whole population to these fungicide groups.

Isolates were generally slightly less sensitive to Punch C than they were to Folicur. This would tend to demonstrate that the MBC component of the mixture is largely ineffective as a result of *P. brassiace* resistance to the MBC group of fungicides. The field trials showed however that the difference in sensitivity between Punch C and Folicur seen in the assays for sensitivity was seldom translated into a significant difference in disease control in the field. Only at a site with a high proportion of tebuconazle sensitive isolates was Punch C significantly less effective in light leaf spot control.

Comparison with historical data from DuPont (UK) Ltd shows that the proportion of isolates that will grow on a discriminatory dose of 10 ppm flusilazole in 1995 has increased in 2000 to 2002, but that the shift in sensitivity has not been large. No current isolates in this project grew on a 20ppm discriminatory dose. This is in contrast to MBC resistance where resistant isolates will continue to grow in concentrations in excess of 2000 ppm.

The range in sensitivity within the *P. brassiacae* population was still large enough to cause problems in light leaf spot control in the field and the reason for this was clearly demonstrated with the tebuconazole residue testing that was done. A month after treatment with reduced dose rate Folicur levels of tebuconazole in the leaves were no greater than in untreated control plots. Even in the full dose rate treatment sampled a month after spraying the tebuconazole levels were

still low enough that the majority of the *P. brassicae* isolates assayed would have been able to grow. This demonstrated that dose rate is likely to be very critical in the efficacy of fungicides in the control of light leaf spot.

Similar results were found in the field trials. Full and three quarter rate Folicur treatment tended to give significantly better control than half dose rate treatments. However, there were crop safety issues involved in increasing the dose rate and a full dose rate treatment caused significant losses in crop vigour that were reflected in yield. Given the current levels of reduced sensitivity to tebuconazole in the Scottish *P. brassicae* population dose rates of below half dose rate Folicur will almost certainly be ineffective in controling light leaf spot. A three quarter dose rate probably represents a compromise between this reduced disease efficacy and the crop damage seen at higher rates. There were no crop safety issue involved in increasing the dose rate of Punch C and although the *P. brassiace* population is generally less sensitive to this product than it is to Folicur the difference is seldom large enough to translate into a reduction in efficacy in the field. Three quarter dose rate of Punch C was optimal for yield in the trials.

It was not possible to recommend a successful anti resistance strategy through the use of partner fungicides with alternative modes of action in the course of this work. This was because of the lack of any alternative fungicides with any efficacy against light leaf spot. Thiovit, Barvo and Dithane were evaluated but did not show any significant efficacy against *P. brassicae*. MBC fungicides are similarly weak as an anti-resistant strategy because of their widespread lack of efficacy where resistant strains of *P. brassiacae* are present. Where there are still resistant isolates in the population they may still represent an effective anti-resistant strategy and may prolong the period of efficacy of the triazoles by avoiding an over reliance on the triazole component which will select for less sensitive members of the population which is probably what has happened in the Scottish situation where triazole fungicides have been used almost exclusively for a number of years.

Limited evidence shows that *P. brassicae* may still be more sensitive to triazole fungicides in England. An isolate from Cambridgeshire tested in this project was amongst the most sensitive assayed. Similarly more isolates from England may remain sensitive to MBC fungicides. If this is the case then the use of triazole plus MBC mixtures may represent an effective anti-resistance strategy. In the past growers have placed very little value on the selection of fungicides for anti-resistant reasons. Yield and disease control properties are far more important to individual

growers than any longer term protection strategies designed to protect the fungicides they rely on. It is critical that anti-resistance strategies are promoted by consultants and advisors, particularly in cases like this where the cost and efficacy penalties in the short term of using such a strategy compared to a straight triazole programme are minimal, if present at all.

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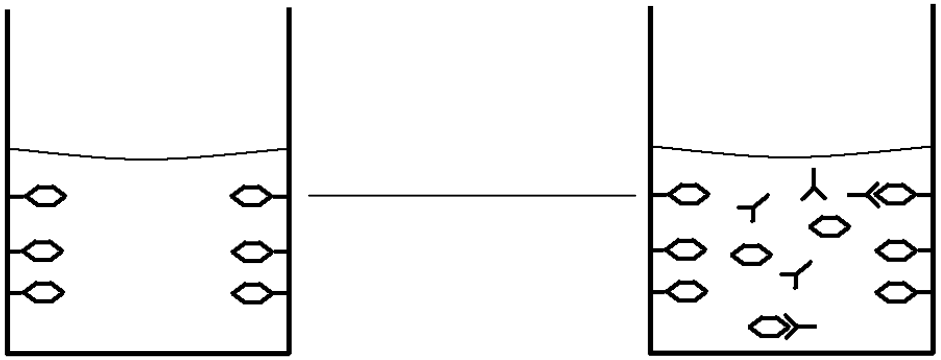
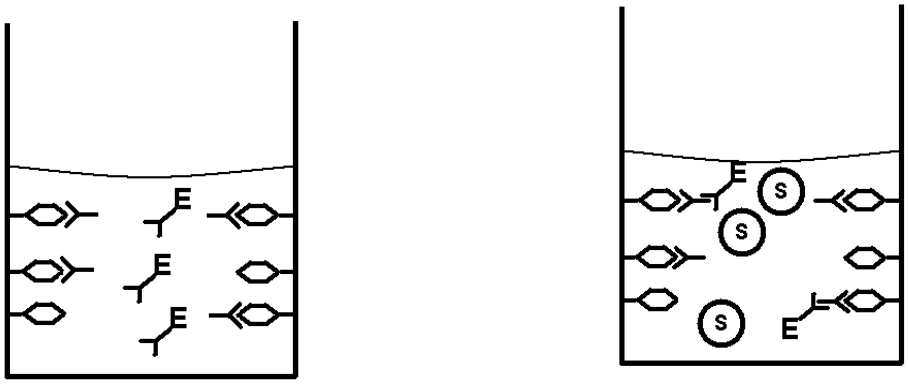
9. APPENDICES

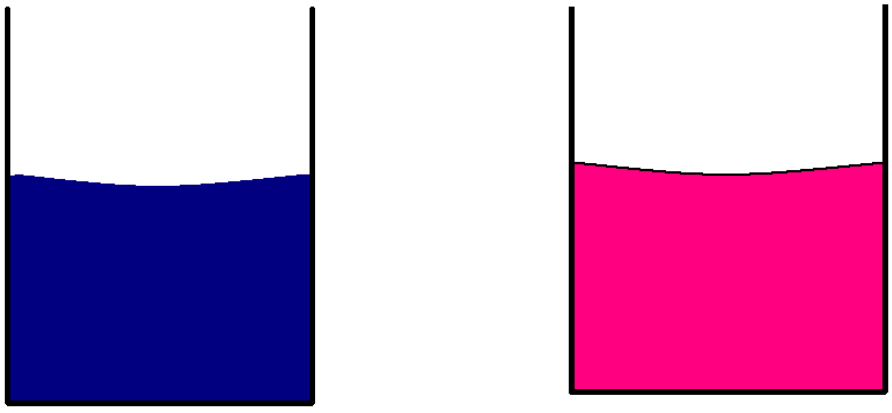
Appendix A. Tebuconazole competative assay



1. **2.**



3. **4.**



LIGHTER COLOUR = MORE TEBUCONAZOLE IN THE SAMPLE

Appendix A. Summary of competitive ELISA

1. 1.Coat wells with a known concentration of tebuconazole. In the kit the plate is supplied pre-coated.

2. Add sample and specific antibody. If the sample contains tebuconazole it will compete with the tebuconazole coated onto the wells to bind the antibody. Some antibody molecules will bind to the tebuconazole on the well and some to the tebuconazole in the sample. Antibody bound to tebuconazole in the sample is removed by washing.

3. A conjugate, which in this case is an antibody labelled with an enzyme, is added to the wells. The conjugate is able to recognise and bind to the specific antibody that is bound to the tebuconazole in the well.

4. Unbound conjugate is removed by washing. A substrate for the enzyme is added. The product of the enzyme-substrate reaction is coloured and can be visualised or the absorbance measured with a spectrophotometer.

The 'free' tebuconazole and the bound tebuconazole compete for the antibody. If there is a high level of tebuconazole in the sample most of the antibody will bind to the 'free' tebuconazole and be removed by washing. Thus, when the conjugate is added there is no, or very little, antibody bound to the tebuconazole in the wells. Therefore there is nothing for the conjugate to bind to and the colour production is very low.

High level of tebuconazole in the sample = low colour.

Low level of tebuconazole in the sample = high colour

Sample preparation and competition ELISA protocol

A. Preparation of extract

1. Place 1g of leaf material in a heavy duty polythene bag. Add 4ml of 80% methanol and homogenise using a hand held homogeniser (Bioreba) for 3 - 4 minutes.
2. Heat seal the bag and incubate at 4^0C overnight (for at least 16 hours).
3. Add 60ml distilled H_2O to the extract in the bag and shake gently to mix.
4. Filter the sample through a double layer of muslin.
5. The sample(s) is/are then ready for use in the tebuconazole assay.

B. Competition ELISA

1. Coat plates with Tebuconazole-NH-BSA conjugate 1µg/ml in coating buffer (9µl of stock in 100mls). Add 100µl per well. Incubate overnight at 4^0C.

2. Wash 4 times with PBST and pat dry on paper towels.

3. Block with PBSTM (5% non-fat milk powder) at 200µl/well. Incubate at 37^0C for 2 hours.

4. Meanwhile cross-adsorb the sera in PBST-4%BSA for 30 - 60 minutes at 37^0C.

5. Wash plate 4 times with PBST and pat dry on paper towels.

6. Add to plate (i) 50µl of cross-adsorbed sera diluted at 1/5000
   (ii) 50µl of standard/samples in solvent.
   Cover plate with plastic film and place on shaker at room temperature for 2 hours.

7. Wash 4 times with PBST and pat dry on paper towels.

8. Add 100µl/well of goat anti-Rabbit IgG-HRP conjugate in PBST and incubate at 37^0C for 1 hour.

9. Wash plate 4 times with PBST and pat dry on paper towels.

10. Add 100µl/well of K-blue substrate (or alternative TMB substrate) to the wells. Incubate in the dark at room temperature for 15 minutes.

11. Stop the reaction by adding 50µl/well H_2SO_4 (10%). Gently shake the plate to mix the substrate and stop solution and read at 450nm.Appedices

Appendix B. Fungicide sensitivity of isolates tested in survey

Isolate code	Fungicide history 0, 1 or 2 sprays MBC or T	Location	Variety	Year sampled and tested	MIC value Folicur	MIC value Punch C
ED01	0	Angus	Pronto	2001	1	100
ED02	0	Angus	Synergy	2001	10	10
ED04	1T	Angus	Synergy	2001	10	100
ED10	0	East Lothian	Synergy	2001	10	10
Ed11	IT	East Lothian	Synergy	2001	10	100
Ed12	IT	East Lothian	Pronto	2001	10	100
ED14	IT	Borders	Madrigal	2001	10	100
Ed15	IT+MBC	Borders	Pronto	2001	1	10
Ed16	IT	Borders	Pronto	2001	10	10
Ed17	0	Borders	Synergy	2001	10	10
Ed18	0	Borders	Synergy	2001	1	10
Ed19	0	Borders	Boston	2001	10	100
ED24	IT	Dumfriesshire	Fortress	2001	10	100
ED25	IT+MBC	Dumfrieshire	Synergy	2001	10	10
ED27	0	Ayrshire	Synergy	2001	1	10
ED28	0	Perthshire	Herald	2001	1	100
ED30	IT	Perthshire	Synergy	2001	10	10
ED31	IT	Perthshire	Synergy	2001	10	100
ED32	0	Perthshire	Synergy	2001	10	100
ED33	0	Perthshire	Pronto	2001	10	10
ED35	0	Perthshire	Pronto	2001	10	10
ED37	IT	Stirlingshire	Synergy	2001	10	10
ED38	IT	Banff	Synergy	2001	100	10
ED39	IT+MBC	Banff	Lipton	2001	10	10
ED40	IT+MBC	Buchan	Lipton	2001	10	10
ED47	IT	Stirlingshire	Fortress	2001	1	10

ED48	0	East Lothian	Synergy	2001	100	10
ED49	IT	East Lothian	Synergy	2001	10	10
ED50	0	West Lothian	Madrigal	2001	10	100
ED53	0	West Lothian	Madrigal	2001	10	10
ED56	IT	Borders	Madrigal	2001	10	10
ED57	IT	Borders	Pronto	2001	1	10
B13/02	0	Cambridge	Apex	2002	0.5	0.5
Laur/02/1	0	Kincardinshire	Synergy	2002	10	10
TC/02/9	0	Aberdeenshire	Synergy	2002	5	10
AB/B/S1N1/ Tr	1T	Aberdeenshire	Bristol	2002	10	10
02/37	1T +MBC	Dundee	Lipton	2002	1	10
02/38	1 T+MBC	Dundee	Lipton	2002	0.5	10
02/41	1T	West Lothian	Pronto	2002	5	20
02/31	0	Errol	Fortress	2002	1	20
02/32	0	Errol	Fortress	2002	5	5
02/30	0	Fife	Synergy	2002	10	20
02/29	1T	Fife	Pronto	2002	1	5
ED60	0	Borders	Synergy	2002	5	0.5
ED61	0	Borders	Synergy	2002	0.5	10
ED63	1I	Borders	Madrigal	2002	5	10
ED64	IT+MBC	Aberdeenshire	Pronto	2002	10	0.5
ED65	IT	Aberdeenshire	Synergy	2002	0.5	0.5
ED70	IT	Aberdeenshire	Fortress	2002	10	1
ED71	IT+MBC	Borders	Pronto	2002	5	5
ED72	IT+MBC	Fife	Synergy	2002	1	0.5
ED73	0	Fife	Synergy	2002	10	10
ED74	IT	Fife	Pronto	2002	10	5
ED75	IT+MBC	Borders	Synergy	2002	0.5	5
ED76	IT	Aberdeenshire	Madrigal	2002	5	1
ED78	0	Aberdeenshire	Synergy	2002	1	10
ED79	IT+MBC	Ayrshire	Pronto	2002	10	20
ED81	IT+MBC	Ayrshire	Synergy	2002	5	5

ED82	IT+MBC	Dunfriesshire	Pronto	2002	0.5	0.5
ED83	IT	East Lothian	Synergy	2002	20	5
ED84	IT	East Lothian	Pronto	2002	1	1
ED87	IT	East Lothian	Synergy	2002	5	5
ED88	0	Banff	Fortress	2002	0.5	5
ED91	IT+MBC	Banff	Synergy	2002	10	10
ED92	0	Angus	Fortress	2002	1	10
ED93	IT	Angus	Synergy	2002	1	5
ED94	0	Angus	Pronto	2002	10	10
ED95	IT+MBC	Angus	Madrigal	2002	1	1
ED97	0	Buchan	Pronto	2002	5	0.5
ED98	IT	Dumfriesshire	Synergy	2002	0.5	10
ED99	0	Dumfriesshire	Synergy	2002	10	10
ED100	0	Aberdeenshire	Fortress	2002	1	10
ED102	IT+MBC	Angus	Pronto	2002	1	10
ED103	IT	Angus	Madrigal	2002	20	10
ED104	IT	Angus	Synergy	2002	5	0.5
ED105	IT+MBC	Perthshire	Lipton	2002	1	0.5
ED107	IT	Dumfriesshire	Synergy	2002	0.5	1
ED108	IT+MBC	Ayrshire	Synergy	2002	0.5	10
ED109	0	West Lothian	Lipton	2002	1	10
ED110	0	West Lothian	Synergy	2002	5	20
Ed114	IT	East Lothian	Lipton	2002	5	10
ED115	IT	West Lothian	Synergy	2002	1	0.5
ED116	0	Stirlingshire	Synergy	2002	1	20
ED118	IT	Stirlingshire	Madrigal	2002	10	1
ED119	0	Perthshire	Pronto	2002	5	10
ED121	IT	Perthshire	Synergy	2002	20	0.5

Appendix C. Field trials site details

Field trial site 1 details

FARM		Milrig						
ADDRESS		Kirkliston, West Lothian						
GRID REF:-		NT 103 731		ELEVATION		50m		
SOIL SERIES:-		Woodhead		SOIL TEXTURE:-		Sandy Clay Loam		
pH:-	6.3	AVAILABLE (mg/l)	P	Mod	K	Mod	Mg	High
			S	Low	Mn	Mod		
PREVIOUS CROPPING		2000 S Barley 1998 W Wheat			1999 S Barley 1997 S Barley			
DESIGN	Randomised Block		NUMBER OF REPLICATES		3			
VARIETY	Synergy		DATE SOWN		25 Aug 00			
			DATE HARVESTED		15-16 Aug 01			
SEED RATE	70 seeds/m^2		PLOT SIZE		22m x 2m			

FERTILISER APPLIED (Kg/ha)	N	P_2O_5	K_2O	S	DATE	GS
	50	60	60		19 Sep 00	1.2
	80	0	0	21	22 Feb 01	1.8
	80	0	0		21 Mar 01	3.0

HERBICIDE APPLIED	RATE	PRODUCT	DATE	GS
	2l/ha	Katamaran	15 Sep 00	1.2
	1.1kg/ha	Barclay Piza 500	16 Oct 00	1.4

FUNGICIDES APPLIED	RATE	PRODUCT	DATE	GS
As per protocol			10 Nov 00	1.6
			30 Mar 02	3.3

OTHER TREATMENTS	RATE	PRODUCT	DATE	GS
	5.5kg/ha	Draza	07 Sep 00	1.0
	5.5kg/ha	Draza	18 Sep 00	1.2

Field trial site 2 details

FARM	Blairnathort
ADDRESS	Kinross, Tayside

GRID REF:-	NT 138 066

SOIL TEXTURE:-	Sandy Loam

PREVIOUS CROPPING	2000 Set-A-Side

DESIGN	Randomised Plots	NUMBER OF REPLICATES	3

VARIETY	Synergy	DATE SOWN	29 Aug 00
		DATE HARVESTED	22 Aug 01

SEED RATE	70 seeds/m^2	PLOT SIZE	2 x 18 metres

FERTILISER APPLIED (Kg/ha)	N	P_2O_5	S	K_2O	Date	GS
10	32	96		96		
Top Dressing	30		7.5			
Top Dressing	80		20			
Top Dressing	120		29			
HERBICIDE APPLIED	RATE	PRODUCT			Date	GS
		Butisan S			01 Sep 00	
FUNGICIDES APPLIED	RATE	PRODUCT			Date	GS
As per protocol					15 Nov 00	1,6 – 1,8
					16 Apr 01	2,1 – 2,3

GROWTH EGULATORS APPLIED	RATE	PRODUCT	Date	GS

OTHER TREATMENTS	RATE	PRODUCT	DATE	GS
		Hallmark	01 Sep 00	
		Ronilan/Zeon Hallmark	21 May 01	
	12.5kg/ha	Metaldehyde	Pre drilling	

Files trial site 3 details

FARM	Kames East Mains
ADDRESS	Leitholm, Berwickshire

GRID REF:-		NT 785 445		ELEVATION		75 metres		
SOIL SERIES:-		Whitsome Complex		SOIL TEXTURE:-		Clay Loam		
pH:-	7.0	AVAILABLE (mg/l)	P	8.2	K	267	Mg	210
			S	77	Mn			

PREVIOUS CROPPING	2001 S Barley	2000 W Wheat
	1999 W Wheat	1998 Grain Peas

DESIGN	Randomised Block	NUMBER OF REPLICATES	4

VARIETY	Synergy	DATE SOWN	01 Sep 01
		DATE HARVESTED	13 Aug 02

SEED RATE	kg/ha100 seeds /m^2	PLOT SIZE	20 metres x 2 metres

FERTILISER APPLIED (Kg/ha)	N	P_2O_5	K_2O	DATE	GS
	50	60	60	05 Oct 01	1,3
	90	0	0	04 Mar 02	1,6
	80	0	0	25 Mar 02	3,1
HERBICIDE APPLIED	RATE	PRODUCT		DATE	GS
	2.0 l/ha	Katamaran		19 Sep 02	1,2
	1.0 kg/ha	Benazalox		01 Nov 02	1,6
FUNGICIDES APPLIED	RATE	PRODUCT		DATE	GS
As per protocol				01 Nov 01	1,6
				25 Mar 02	3,1
GROWTH REGULATORS APPLIED	RATE	PRODUCT		DATE	GS
OTHER TREATMENTS	RATE	PRODUCT		DATE	GS
	5.5 kg	Draza		01 Sep 01	0,0
	23kg	Sulphur		04 Mar 02	1,6
	20 kg	Sulphur		25 Mar 02	3,1

Field trial site 4 details

FARM	Blairnathort
ADDRESS	Kinross, Tayside

GRID REF:-	NT 136 062

SOIL TEXTURE:-	Sandy Loam

PREVIOUS CROPPING	2001 Set-a-Side

DESIGN	Randomised plots	NUMBER OF REPLICATES	4
VARIETY	Synergy	DATE SOWN	25 Aug 01
		DATE HARVESTED	13 Aug 02

SEED RATE	70 seeds/m^2	PLOT SIZE	2 x 18 metres

FERTILISER APPLIED (Kg/ha)	N	P_2O_5	K_2O	S	DATE	GS
10	32	96	96		30 Aug 01	
Top Dressing	80			20	14 Feb 02	
Top Dressing	120			30	26 Mar 02	

HERBICIDE APPLIED	RATE	PRODUCT	DATE	GS
		Butisan S	30 Aug 01	
		Butisan S	17 Sep 01	

FUNGICIDES APPLIED	RATE	PRODUCT	DATE	GS
As per protocol			07 Nov 01	1,4 – 1.7
			14 Mar 02	2,0 – 2,5

GROWTH REGULATORS APPLIED	RATE	PRODUCT	DATE	GS

OTHER TREATMENTS	RATE	PRODUCT	DATE	GS
		Cypermethrin	30 Aug 01	
		Cypermethrin	17 Sep 02	
	12.5kg/ha	Metaldehyde	Pre drilling	
	10kg/ha	Metaldehyde	21 Sep 02	