PhD Summary Report No. 1

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Project No. RD-2001-2508

Effects of spring timings and rates of application of triazole fungicides on plant growth regulatory activity and control of light leaf spot (*Pyrenopeziza brassicae*) and phoma / canker (*Leptosphaeria maculans*) of oilseed rape (*Brassica napus*).

Anne Coules

Nottingham University, Sutton Bonington Campus

Loughborough EL12 5RD

Project aim:

To determine timings and rates of triazole fungicides to control oilseed rape diseases. Produce different canopy sizes and the responses to differing growth stages to five fungicide regimes. To establish the PGR activity of some fungicides determine the possible benefits on yield. Optimise application programmes to give maximum yield and disease control.

Use ELISA and GC-MS to measure fungicide residues on plants to help to determine length of time chemicals control disease.

Develop PCR as a molecular tool that can aid the identification of *P. brassicae and L. maculans*, before the occurrence of physical symptoms, and the possibility of using this in conjunction with forecasting systems

Key messages emerging from the project:

- Timing important for disease control.
- The PGR effect of tebuconazole important in the control of disease and the manipulation of the canopy.
- Improved yield and adequate disease protection can be achieved by using a spray programme that incorporated tebuconazole and flusilazole.

Summary of results from study:

Disease observations

Treatment 2 had the lowest percentage leaf area infected with Phoma in July 2003 and the highest with treatment 5. Treatments 2 and 4 had less than 2% infection.

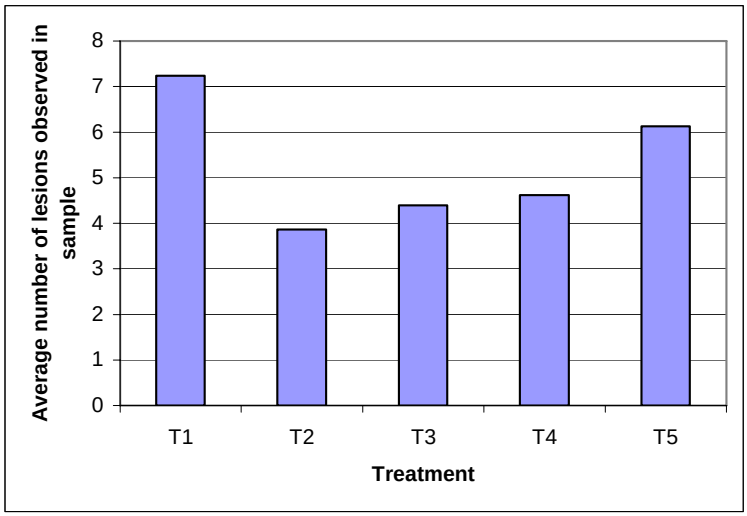


Figure 1: Number of lesions observed on stems in July 2003

There were more stem lesions observed on plants that had been sprayed with treatment 5 and those that were untreated in comparison to treatments 2, 3

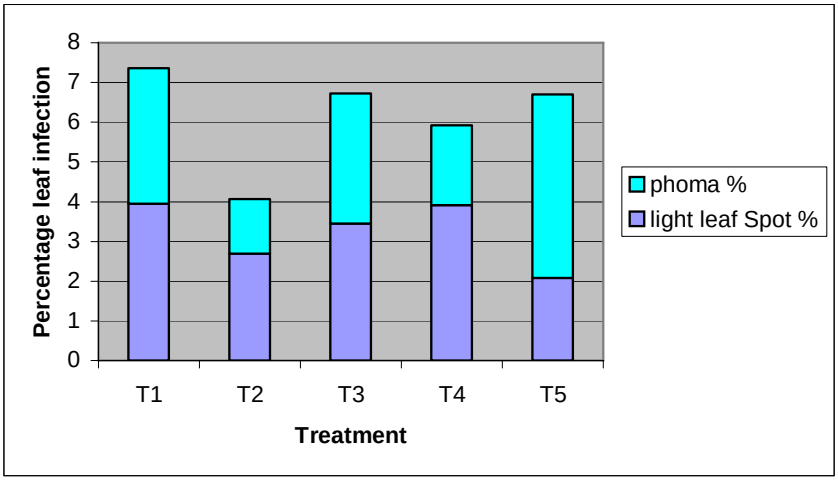


Figure 2: Contribution of each pathogen to total percentage leaf infection.

Green Area Index GAI - can indicate the amount of green area that is available to utilise the light. This information can also give an indication of plant / canopy size.

Specialist equipment is needed to measure GAI and can be time consuming, but can be an important tool for estimating whether or not the plant is large enough to be sprayed with a PGR fungicide.

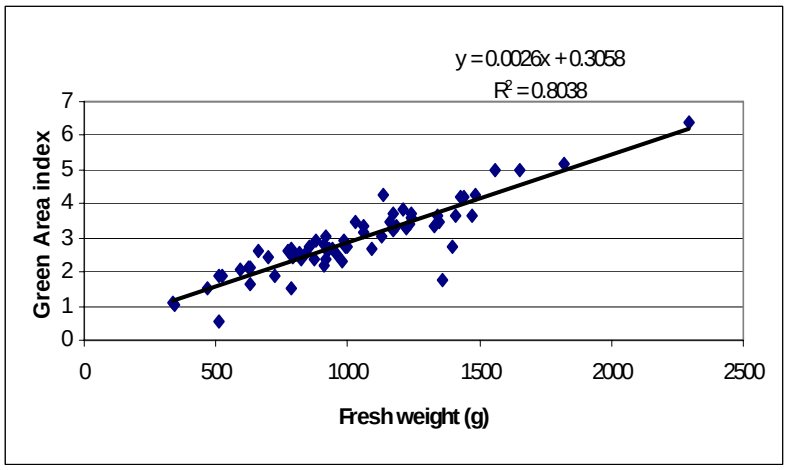


Figure 3 : Relationship between GAI and fresh weight

First year trials found a relationship between fresh weight and GAI, which gave an R^2 value of 0.8038. This would allow a grower to weigh a sample and without specialist equipment determine if the plant canopy was at the right stage to be sprayed with a fungicide containing a PGR or if the plant needed to be sprayed with a PGR.

Yield Responses

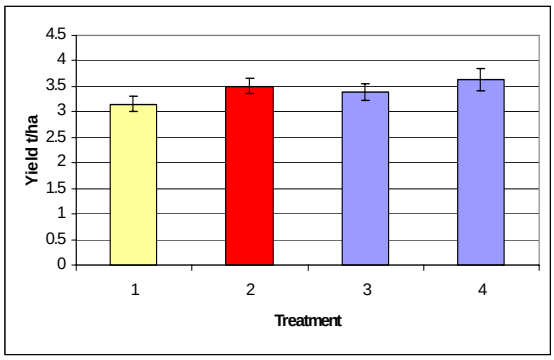


Figure 4 :Yield response to treatments -2002

Treatment	Stem Extension	Green bud
1	-	-
2	0.8 l/ha Punch C	-
3	1.0 l/ha Folicur	
4	0.8 l/ha Punch C	1.0l/ha Folicur

Table1: Summary of treatments and timings Year 1

2003:

Sutton Bonington. Treatment 5 had the highest yield in both varieties and the untreated plots yielded the least in both varieties.

The variety Recital had higher yields at each treatment during the trial at Sutton Bonington. Treatment 5 had the highest yield in both varieties and the untreated plots yielded the least in both varieties.

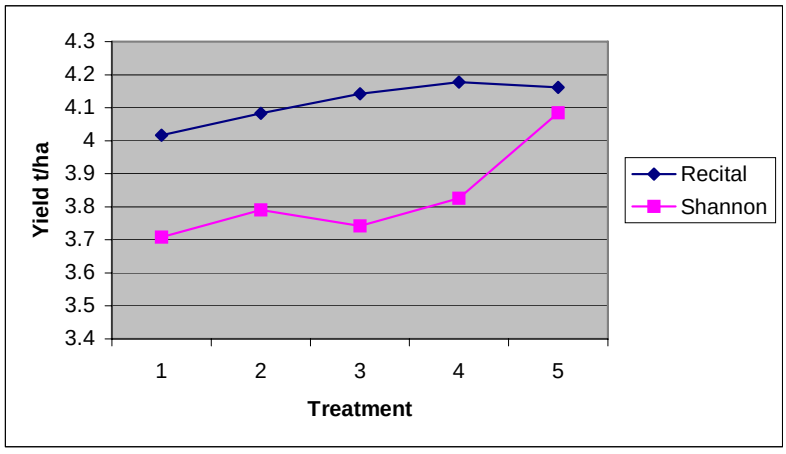


Figure 5. Yield responses to treatments and varietal variation 2003 (treatments see table 2).

The three sowing dates showed different stages of development and height six months after sowing. The plant sizes had not caught up with the earlier planted plots. There was also differences in the heights of varieties, early sown Shannon was taller than Recital as was the late sown, However, the standard sowing date recital was taller than Shannon.

Treatment	Stem extension	Green Bud
1	-	-
2	0.4 l/ha Punch C	
3	0.4 l/ha Punch C	1.0l/ha Folicur
4	0.4 l/ha Punch C+ 1.0l/Folicur (tank mixed)	_
5	-	1.0l/ha Folicur

Table 2: Summary of sprays and timings 2003

Treatment three had the highest yield at York. Treatments 3, 4 and 5 were all significantly greater than treatment 2 and the untreated plots. Treatment 2 actually had the lowest yield of all.

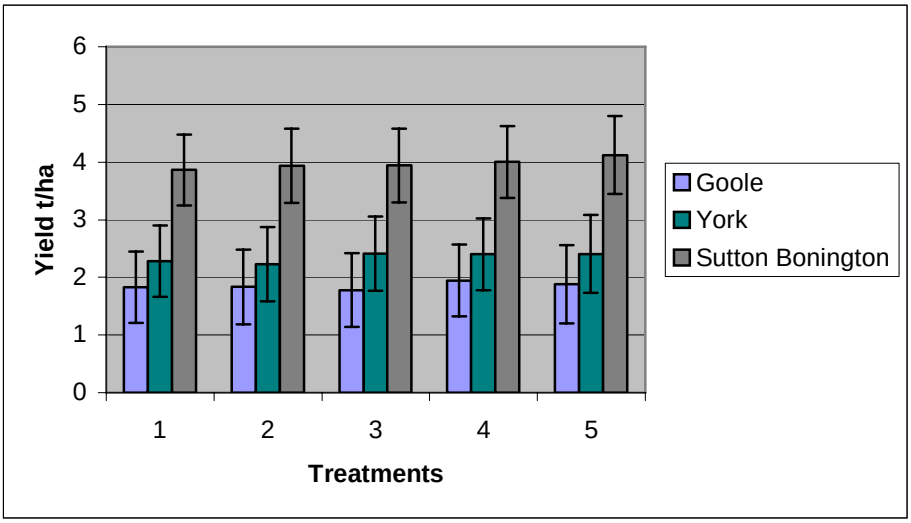


Figure 6: Differences in yield observed at three different sites in 2003

The yield values at Goole were much lower than those achieved in York. Treatment three had the lowest yield and treatment 4 had the highest yield.

Yields at Sutton Bonington were significantly higher than the two northern sites. Treatment 5 at Sutton Bonington had the highest yield of all treatments whilst treatment 3 at Goole was the lowest.

Logistical problems and weather had an effect on both the treatments applied and the subsequent growth analysis undertaken. During the autumn the weather was continually wet and or windy and the ground was too soft for machinery and other farm vehicles. The autumn spraying at all three sites was consequently delayed. The spring spraying at York and Goole was undertaken first and at the appropriate plant stages of stem extension and green bud. Again weather conditions and available resources hampered the correct spraying time for the site at Sutton Bonington. Many of the plots had started to flower, it was therefore decided to continue the sampling for growth analysis at York up to harvest. Pre harvest samples however, were still taken at Sutton Bonington and the analysis is currently on going.

Initial observations from second year trials

Sutton Bonington

Two varieties were grown at Sutton Bonington in the season 2002-3, there was a significant difference ($p<0.001$) in the yield responses of each variety. Recital is less susceptible to these two diseases and yields were higher.

The yield responses to the fungicide applications at Sutton Bonington were different in the second year. The tank mix of the two produced the highest yield and Folicur on its own also yielded well. The three sowing dates that were sown had produced plants and a canopy structure of differing sizes, but due to weather constraints the spraying regime could not be applied when intended and the effects on plants of different growth stages may

not be seen. Growth analysis however, is ongoing and differences between sowing dates will be examined.

York 2003 – Growth analysis

The heights observed showed that the Semi-dwarf hybrid HV-522 were noticeably shorter than the other two varieties as expected. Treatment 4 and 5 which both contained Folicur were the shortest, which demonstrated the PGR effect of the fungicide [Lunn *et al.,* 2002]. Light leaf spot incidence was high with treatment 4 but the best control was seen with folicur on its own. Punch C applied on its own gave the greatest disease control but higher yields were achieved with treatments containing Folicur. This would suggest that the benefits of a PGR treatment, whilst not increasing disease protection do contribute to increased yields. The hybrid variety had lower yields than had been anticipated this was mainly due to many of the plots experiencing losses over winter and spring from pigeon grazing. Other losses may have been expected from the adverse timing of PGR fungicides but in general it was treatment 2 that had the lowest yields in all varieties which could be due to the full dose being applied [Burnett, 2003].

Goole

The lowest yields of all three sites for 2003 were seen at Goole, this was unexpected as the growth within plots had looked uniform and there had been no significant problems with pigeon grazing that had been observed at York. The major causes for this were mainly due to weeds within the plots that were not adequately controlled. The main weed was Charnock which is similar in size to rapeseed quickly establishes itself and competes for space. The seed is also very similar in size and can present problems as it has very high glucosinolate composition.
Heights:

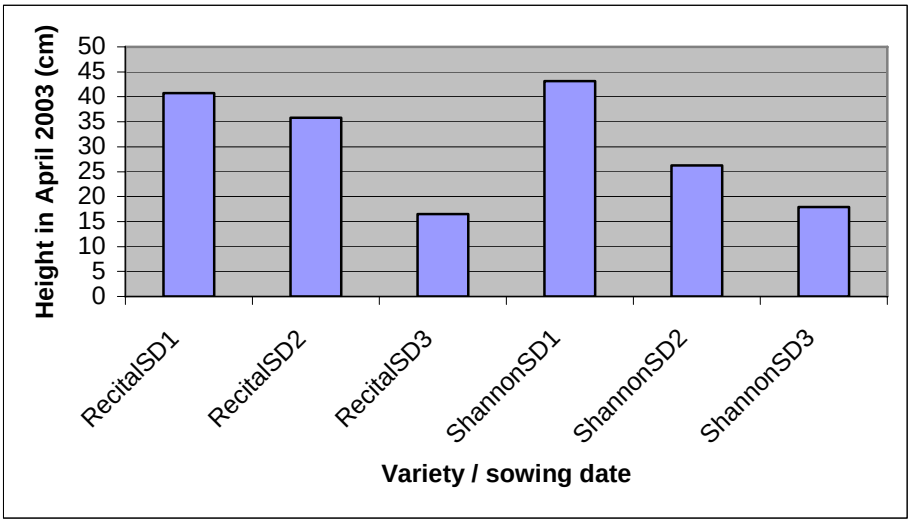


Figure 7 :Plant height differences observed between varieties and sowing date-2003.

The variety Shannon had taller plants than Recital at sowing dates SD1 and SD3. There was little difference observed between the heights of Recital plants at sowing date SD1 and SD2.

A field trail was conducted with the help of Pioneer in Yorkshire and conventional (Bristol and Lutin) and semi-dwarf hybrid varieties were used. The PGR effects of the fungicides were assed after spraying. After the initial spray applications there were some differences between varieties and the Semi-dwarf Hybrid HV522 was significantly shorter in height as was expected.

During the third year trial the PGR effects on height were observed and shown in Figure 6. Blue columns are treatments with no Folicur applied, Yellow are half rate application and red are full rate 1.0l/ha application.

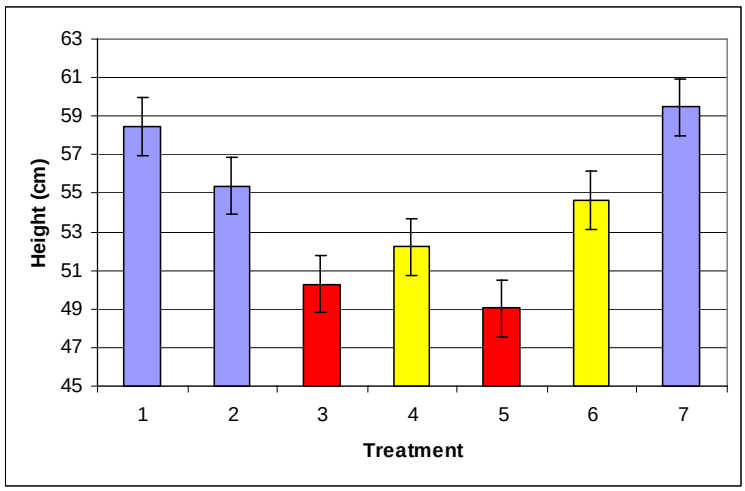


Figure 8: Height observations April 2004

Treatment	Autumn spray	Stem extension	Gren bud
1	-	-	-
2	0.4 l/ha Punch C	0.4 l/ha Punch C	
3	0.4 l/ha Punch C	1.0l/ha Folicur	
4	0.4 l/ha Punch C	.05/ha Folicur	.05/ha Folicur
5	0.4 l/ha Punch C	0.4 l/ha Punch C+ 1.0l/Folicur (tank mixed)	
6	0.4 l/ha Punch C	.05/ha Folicur	
7	0.4 l/ha Punch C		

Table 2: Summary of Rates and timings - 2004

Seed oil quantity and the use of Near infra-red spectroscopy (NIR) for determination of percentage oil in seed

The extraction and measurement of oil percentage in oilseeds can be both timely and expensive, involving many chemicals and the risk of losses of oil if done on a small scale in the laboratory. A method was devised to utilise NIR to measure the percentage of oil in the samples from field trials. Laboratory extraction was first performed on samples and these were used to calibrate the NIR machine for future use, a correlation of 0.7214 was found between the actual and predicted amount of oil in the sample.

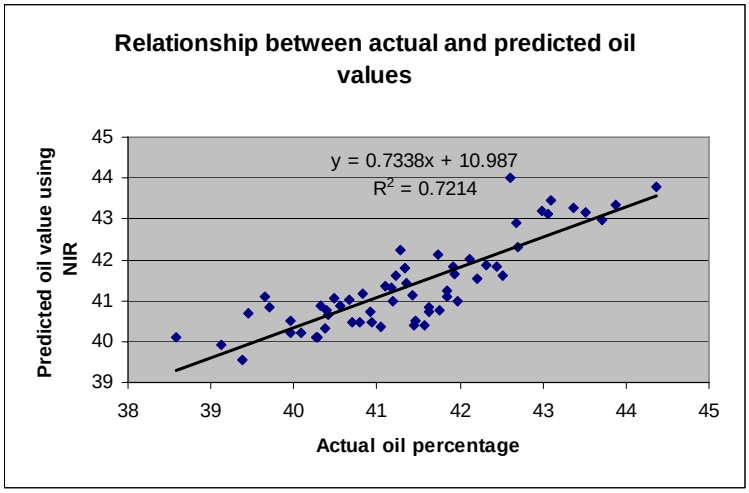


Figure 9 : Relationship between actual and predicted oil values

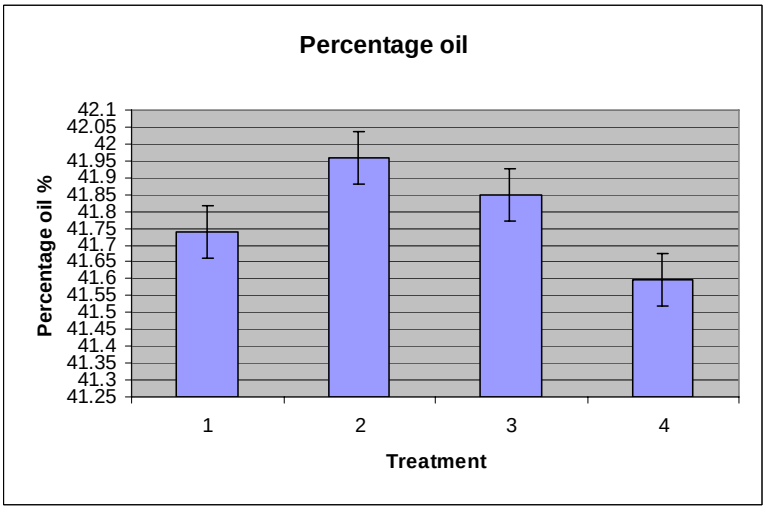


Figure 10: Percentage oil measured in seed after four spring treatments 2002

The first year filed trials showed that there was no significant difference between the oil quantity in those samples that were untreated compared with those that had been treated. However, treatment four had significantly less oil than treatments two and three.

Molecular approach – PCR identification of spore trap samples

This investigation took place to examine methods for forecasting disease before disease symptoms are visibly identifiable. It is also important when looking at the control of plant pathogens that they are accurately identified, especially when disease symptoms are difficult to recognise or similar to other symptoms. This is particularly true with light leaf spot as it is difficult to identify and can be confused with fertilizer scorch. One possibility examined was the use of PCR as a molecular tool in the identification of disease on spore trap samples collected in the field. It was important to establish if the disease could be accurately identified against a background of many other spores and debris collected. The level of sensitivity was also investigated as levels of inoculum in the field that would be adhered to the spore trap tape could be very low. Levels of sensitivity accurately identified were 20 spores in 20μl

Root responses

Investigations were undertaken to determine the effect of different fungicides on the roots of oilseed rape seedlings. After initial attempts at growing large plants in soil medium, it was found difficult to isolate the roots and clean up sufficiently for scanning the roots. A hydroponic system was set up so the roots could be easily measured.

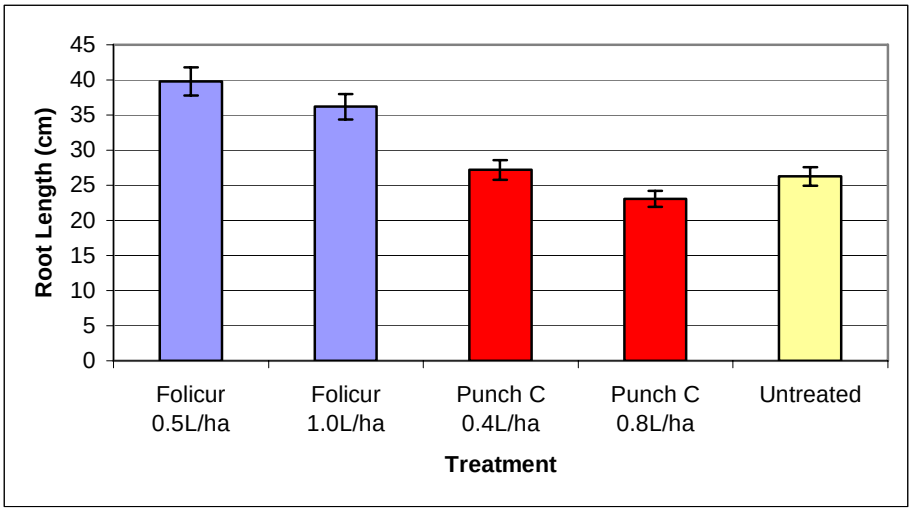


Figure 11 : Root lengths observed on seedlings with two true leaves

Roots on oilseed seedling plants with five true leaves were also examined and the results seen in the figure below.

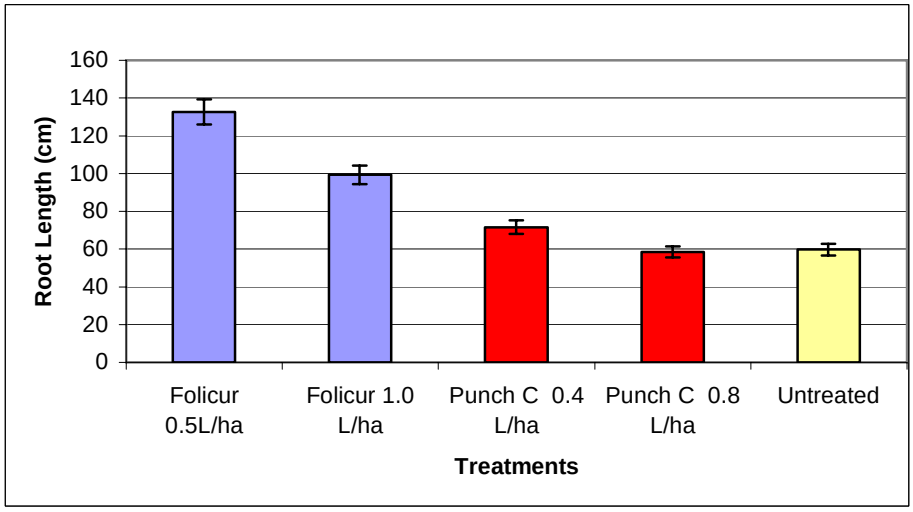
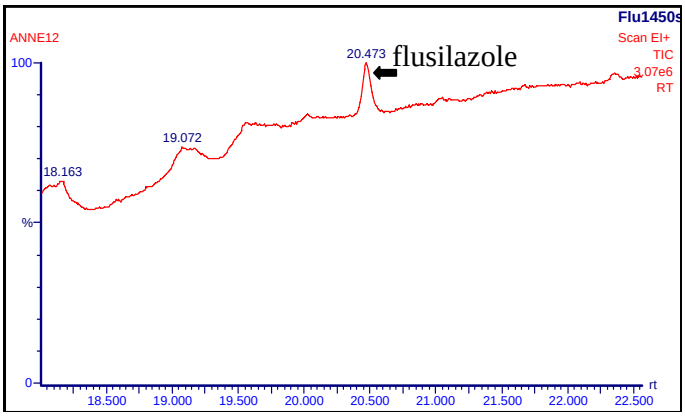


Figure 12: Root lengths of five true leaf seedlings sprayed with fungicides

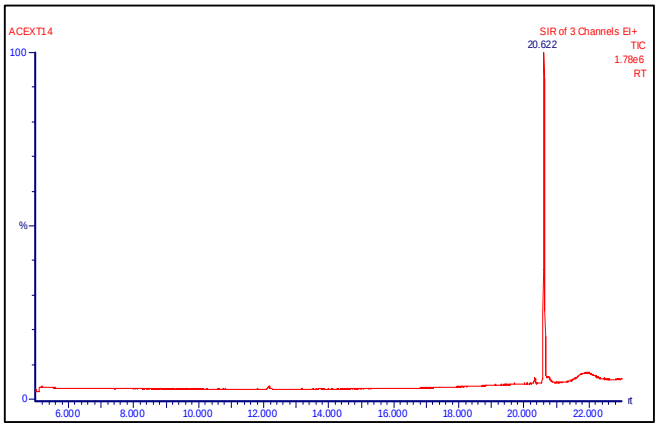
Systemic and longevity investigations of flusilazole and tebuconazole

To fully understand and maximise the control of plant pathogens it is important to not only understand the pathogen but also the activity of the

control agent. Therefore, fungicide persistence and systemic activity were also investigated during the third year. Leaves were sprayed with each fungicide used in the field trials and then at various time periods after spraying gs were observed.



Sample washings of flusilazole
14 days after spraying



Sample extraction after washings

Sample 14 days after inoculation extracted from point of inoculation

Summary

Tebuconazole has a PGR effect on plant height and care needs to be taken when applying to ensure that the canopy is not too small. There also appears to be an effect on roots of seedlings.

The most ideal spraying regime used a combination of Punch C and Folicur with autumn and spring applications to control the two major oilseed rape diseases.

Identification of pathogen in field before the occurrence of visible symptoms- PCR was used to successfully identify light leaf spot and phoma spores amidst other spores adhered to spore trap tape from samples collected in the field.

Identification of systemic activity of fungicides and duration of chemical activity-

GC-MS has now been successfully used to detect tebuconazole and flusilazole both on the leaf surface and *in planta*.