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Grower summary

Headline

The formulated product Signum, (BASF, pyraclostrobin + boscalid) provides comparable protectant and substantially better curative activity than current standards and other fungicides that have been tested. The efficacy of this treatment appears to depend primarily on the active ingredient pyraclostrobin rather than boscalid. Tested under glasshouse efficacy experiments, and near-commercial conditions, no phytotoxic or deleterious effects on crop quality were found for this product, except for the deposit of visible residues on treated foliage.

Background and expected deliverables

This project aims to develop effective approaches to control chrysanthemum white rust (CWR), which recognize the importance of minimizing selection pressure for fungicide resistance and hence protects the armoury for fungicides available to growers.

The work is necessary because some UK populations of *Puccinia horiana*, the cause of CWR, may have evolved insensitivity to some important fungicide groups. Chrysanthemum growers are currently dependent on a limited range of fungicides to control this disease; so reduced efficacy of those products poses a serious threat to the profitability of the industry.
To address this threat, the project:

1. Determines the current extent of fungicide sensitivity in the UK white rust population - giving particular attention to measuring sensitivity to propiconazole and the extent of cross-resistance to other fungicides from the important triazole group;
2. Quantifies the efficacy of candidate fungicides, selected from across the mode of action groups, which have data packages on operator safety that permit, or could potentially permit, use on protected crops;
3. Develops clear treatment guidelines that enshrine best practice for product stewardship, so that an armoury of effective fungicides will be available to support the industry.

The expected deliverables from this work include:

- Quantification of fungicide efficacy against *P. horiana*
- Crop safety advice for key fungicides effective against the pathogen
- Best practice guidelines for sustainable CWR control programmes

Summary of the project and main conclusions

Fungicide efficacy

- Tests on isolates collected from white rust epidemics, which have proved difficult to control under commercial conditions, do not show evidence for insensitivity to the protectant fungicide azoxystrobin.
- The formulated product Signum, (BASF, pyraclostrobin + boscalid) provides comparable protectant and substantially better curative activity than current standards and other fungicides that have been tested.
- The efficacy of Signum is dependent upon pyraclostrobin. This fungicide is available as the commercial product Comet (BASF), but use is not currently on protected crops.
- Fungicide treatment is likely to be most effective when used in protectant situations.
- Curative activity is relatively short even for the best products.

Combating fungicide resistance

- Avoid using repeated applications of fungicides with the same FRAC (Fungicide Resistance Action Committee) code (see: www.fac.info/frac/index.htm), unless used in tank mixtures with products from a different group
- Use proprietary formulated mixtures, which are designed to avoid the build–up of resistance
- Use multi-site fungicides when appropriate
- Avoid repeated applications of very low doses
- Employ crop husbandry practices that reduce the build-up of inoculum
- Only apply fungicides when disease risk warrants treatment
- Use disease resistant or tolerant varieties where possible

Financial benefits

In the short-term

Improved control of chrysanthemum white rust. The recommendations by end of the project will allow growers to design fungicide schedules that are more dose efficient.

In the medium-term

An effective armoury of fungicide products to manage disease. Improved stewardship of important active ingredients will reduce selection pressure on the pathogen population, so that options for control are not eroded.

Action Points for Growers

- Growers should consider the mode of action groups of products included in their spray schedules. Consecutive and frequent applications of products from the same group increase the likelihood that the pathogen will develop fungicide insensitivity.

- Signum (BASF. pyraclostrobin + boscalid) provides improved curative activity compared with the current benchmark, propiconazole (e.g., c.p. Bumper). Glasshouse screening for fungicide activity at CSL and formal crop safety tests at Stockbridge Technology Centre have not found any detrimental effects to crop health from Signum.

Science Section

Introduction

Chrysanthemum white rust (CWR), caused by the obligate basidiomycete fungus *Puccinia horiana*, is a major disease of chrysanthemum (*Dendranthemum morifolium*) grown in all year round and natural season production systems. Disease pressure can be reduced by good crop hygiene and, under protection, by appropriate environmental management. However, fungicides remain one of the main pillars of control for most commercial crops, especially during periods when environmental conditions are favourable for disease development.

In 1999, UK growers began to report the occurrence of CWR that could not be controlled adequately using propiconazole (e.g. Tilt), which belongs to the DMI or azole/triazole group of fungicides. This chemical had provided effective protective and curative activity against the disease for at least 20 years. Tests of curative activity, against suspected fungicide insensitive strains, confirmed that disease control was not achieved with approved rates of propiconazole or myclobutanil e.g. Systhane, which also belongs to the DMI group. Further outbreaks of CWR that were not controlled by either propiconazole or azoxystrobin (e.g. Amistar from the QoI group) were reported in 2000. Tests at the Central Science Laboratory (CSL), (funded jointly by Defra and HDC) confirmed that these isolates of *P. horiana* were tolerant to up to five times the permitted concentration of both fungicides, whether applied in protective or curative situations (Cook, 2001). UK growers also use carboxamide fungicides within programmes targeted to control CWR. Insensitivity of *P. horiana* to this group has been reported on mainland Europe (Dirske et al., 1982; Grouet et al., 1981), indicating at least the potential for resistance to develop in the UK.

The limited range of fungicides currently approved for controlling CWR (Table 1) is causing considerable concern to growers. In addition, growers do not have access to any simple tools to screen *P. horiana* populations routinely for fungicide sensitivity. Since 2001, there has not been any systematic measurement of sensitivity within populations on commercial holdings.

Table 1. Fungicides approved in the UK for control of Chrysanthemum white rust

Active	Example Product	Crops		MOA[1]
		Protected	Outdoor	
Carbendazim	Bavistin	X	X	1
Iprodione	Rovral	X	X	2
Propiconazole	Bumper	X	X	3
Myclobutanil	Systhane	X	X	3
Prochloraz	Octave	X	X	3
Tebuconazole	Bezel	X	X	3
Oxycaboxin	Plantvax 75	X	X	7
Bupirimate	Nimrod	X	X	8
Azoxystrobin	Amistar	X		11
Kresoxim-methyl	Stroby	X	X	11
Tolclofos-methyl	Rizolex	X	X	14
Dinocap	Karathane Liquid	X	X	29
Fosetyl-aluminium	Cleancrop chicane	X	X	33
Cupric ammonium carbonate	Croptex Fungex	X	X	M1
Mancozeb	Karamate Dry	X	X	M3
Thiram	Thianosan	X	X	M3
Chlorothalonil	Bravo 500	X	X	M5
Dodine	Styllit		X	M7
Potassium hydrogen carbonate		X	X	M10
Carbendazim + prochloraz	Sportak Alpha	X		

[1]Mode of action. This defines how and where a fungicide works. Some fungicides, especially the older ones, affect many physiological processes within the target pathogen (multi-site). Others have very specific activity, perhaps affecting only one physiological process (site specific). Site-specific fungicides are generally at greatest risk of resistance development.

Materials and Methods

Isolate collection

Isolates collected in the mid-1990s that were designated insensitive to either, or both azoxystrobin and propiconazole are available as frozen specimens: but these cannot be revived for *in-planta* testing. Therefore, a priority for this work was to establish current baseline sensitivities for the UK populations. Ideally population isolates would be collected where fungicides have been used: either routinely, infrequently or never on the specified crop.

Isolates were sampled by selecting at least five stems showing fresh CWR symptoms from the same production area. The stems were cut into 20 cm sections containing the infected leaves and open flower heads were removed. These sections were wrapped together loosely in moist paper and placed in a plastic bag, which was inflated slightly and sealed. A full record was taken of the sample date, variety, and the fungicides treatments to the crop including their dates of application.

Isolates were collected from holdings where problems controlling disease have been reported (Table 2).

Table 2. Isolates collected for screening fungicide sensitivity and efficacy

Isolate	Source	Date	Cultivars	Comment
1	Hampshire, UK	June 2005	Euro	Discarded, because taken from same population isolate as 3 below
2	Canada	July 2005	Unknown	
3	Hampshire, UK	October 2005	Sheena Euro Fiji	Samples taken from severely infected glasshouse. Isolates from Sheena and Fiji discarded because believed to be same populations as obtained from Euro
4	Hampshire, UK	October 2005	Euro Universe Green Bird	Samples taken from moderate infections. Isolate from Green Bird discarded
5	Lincolnshire, UK	October 2005	Unknown	Grower reported difficulty controlling disease
6	West Sussex, UK	October 2005	Reagans	Initially, consistent problems transferring onto healthy plants, due to very little basidiospore release. Now bulked up and good levels of infection achievable because inoculum is no longer limiting.
7	Surrey, UK	August 2006	Mancetta-Jupiter	Samples taken from moderate infections

Maintenance of isolates

Isolates were bulked-up for maintenance and experiments using a method adapted from previous work. Infected leaf sections were suspended, pustule side downwards, above healthy receptor plants, (cv Sunny Margaret) sprayed with water and placed in a humidity chamber. The glasshouse was maintained at 18°C with natural light and, in-line with commercial practice, supplementary lighting to promote the growth of single stems. Blackout curtains were not available, so it was not possible to alter day length artificially. After 24 hours, inoculated plants were removed from the chamber, placed on the glasshouse bench and healthy uninoculated plants (sentinels) were placed amongst them to monitor potential cross contamination. Plants were watered from beneath as required.

Digital image measurement

A digital image process was developed in the open source statistical language R (www.r-project.org). This was used to provide objective measures of disease severity with explicit error bounds, i.e. an estimate of severity bounded by the absolute range for the maximum and minimum severity. The measurement and its error bounds are calculated using a defined convergence algorithm to score diseased areas, which avoids any subjective intervention by the observer (Plate A).

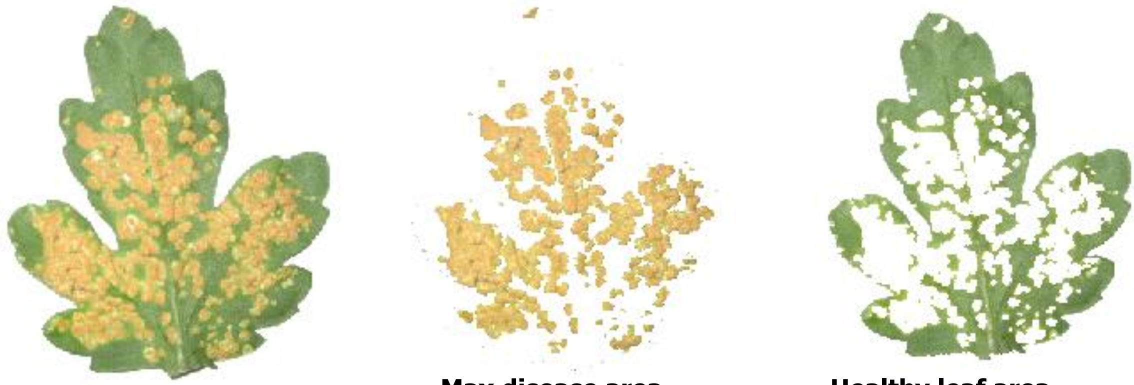

Plate 1. Photographs of leaves treated in experiments are decomposed to diseased and healthy areas and measured by images analysis

Quantitative sensitivity and efficacy testing

Fungicides were evaluated for efficacy against isolates by a two-stage process first by primary screening (2005-06), and then more detailed replicated experiments (2006-2008). Fungicides were tested to measure protectant and curative efficacy by adjusting the time of inoculation and spray application (Table 3).

Table 3. Treatment design to quantify fungicide efficacy

Treatment[1]	Notation	Control measured
Fungicide applied 10 days before inoculation	T-10	Protection
Fungicide applied 7 days before inoculation	T-7	Protection
Fungicide applied 4 days before inoculation	T-4	Protection
Fungicide applied 2 hours before inoculation	T0	Protection
Inoculation 4 days before fungicide applied	T+4	Curative
Inoculation 7 days before fungicide applied	T+7	Curative
Inoculation 10 days before fungicide applied	T+10	Curative

[1]Not all treatments were tested for every isolate x fungicide combination

Each treatment was applied to 3 plants (4 treatments x 3 reps plus 3 control plants =15). This was the maximum number of plants accommodated by a humidity box. The newest fully expanded leaf was tagged at the onset of treatment *i.e*., at inoculation for those testing curative efficacy, and at fungicide application for protectant treatments. Two to three weeks after inoculation (depending on disease development), the tagged leaf and the one immediately below it were cut from the stem, placed on dry absorbent paper and left to wilt for 2 hours. This reduced the amount of leaf curl, aiding the production of good quality photographs. Measurements of disease severity on the excised leaves were obtained using the digital image process described above. The leaves were photographed against a white square of known size contained within a black border. Standardization of the background size permits leaf and symptom areas to be measured on absolute (mm^2) or the usual disease severity (% leaf area) scales.

Curative (T-10, T-7 and T-4 ;Table 3) and protectant (T0, T+4 and T+7, T+10; Table 3) control was measured compared to infected plants that were untreated by fungicides:

$$Control(\%) = \frac{untreated - treated}{untreated} \times 100$$

Where $treated$ and $untreated$ are respectively, the disease severities with and without fungicides.

A total of 17 fungicides (Table 4) were tested to various levels of detail, dependant upon their efficacy and crop safety potential. Twenty-three replicated experiments have been completed using different white rust isolates (Table 5).

Table 4. Fungicides tested for efficacy

Product	Active ingredient	Mode of action [1]	Manufacturer
Amistar	azoxystrobin	11	Syngenta
Bravo 500	chlorothalonil	5	Syngenta
Bumper 250 EC	propiconazole	3	Makhteshim-Agan UK)
Citrox	natural biocide	N/A	Citrox
Comet	pyraclostrobin	11	BASF
Filan	boscalid	7	BASF
Opera	epoxiconazole/pyraclostrobin	3	BASF
Opus	epoxiconazole	3	BASF
Plover	difenoconazole	3	Syngenta
Proline	prothioconazole	3	Bayer
Rhino	flutolanil	7	Certis
Rocket	triflumizole	3	Certis
Shirlan	fluazinam	29	Syngenta
Signum	boscalid/pyraclostrobin	7	BASF
Systhane	myclobutanil	3	Landseer
Torch Extra	spiroxamine	5	Bayer
Twist	trifloxystrobin	11	Bayer

[1] See table 1 for further detail

Table 5. Fungicides tested against different white rust isolates

Run Number	Treatment	Isolate	Dose
1	Control		
1	Twist		2.0l/ha
1	Signum	3	1.0l/ha
1	Opera		1.5l/ha
1	Proline		0.8l/ha
2	Control		
2	Amistar		0.1l/ha
2	Bumper	3	0.4l/ha
2	Signum		1.0l/ha
2	Citrox		0.3l/ha
3	Control		
3	Bumper		0.4l/ha
3	Plover	3	0.3l/ha
3	Signum		1.0l/ha
3	Rocket		0.1l/ha
4	Control		
4	Signum		1.0l/ha
4	Filan	3	0.5kg/ha
4	Comet low rate		0.33l/ha
4	Comet high rate		1.25l/ha
5	Control		
5	Rhino		1.0l/ha
5	Torch Extra	3	0.9l/ha
5	Comet low rate		0.3l/ha
5	Comet very low rate		0.05l/ha
6	Control		
6	Comet high		1.0l/ha
6	Comet medium	3	0.5l/ha
6	Comet low rate		0.1l/ha
6	Comet very low rate		0.01l/ha
7	Control		
7	Amistar		0.1l/ha
7	Bumper	7	0.4l/ha
7	Signum		1.0l/ha
7	Comet low rate		0.33l/ha
8	Control		
8	Amistar		0.1l/ha
8	Bumper	5	0.4l/ha
8	Signum		1.0l/ha
8	Comet low rate		0.33l/ha
9	Control		
9	Amistar		0.1l/ha
9	Bumper	3	0.4l/ha
9	Signum		1.0l/ha
9	Comet low rate		0.33l/ha
10	Control		
10	Amistar		0.1l/ha
10	Bumper	6	0.4l/ha
10	Signum		1.0l/ha
10	Comet low rate		0.33l/ha

Runs 21 & 22 failed due to lack of disease on controls

Run Number	Treatment	Isolate	Dose
11	Control		
11	Amistar		0.1l/ha
11	Bumper	2	0.4l/ha
11	Signum		1.0l/ha
11	Comet low rate		0.33l/ha
12	Control		
12	Amistar		0.1l/ha
12	Bumper	4	0.4l/ha
12	Signum		1.0l/ha
12	Comet low rate		0.33l/ha
13	Control		
13	Shirlan		0.3l/ha
13	Systhane	3	0.225l/ha
13	Opus		1.0l/ha
13	Bravo 500		0.22l/ha
14	Control		
14	Proline 0.4		0.4l/ha
14	Proline 0.2	5	0.2l/ha
14	Proline 0.1		0.1l/ha
14	Proline 0.05		0.05l/ha
15	Control		
15	Comet high		1.0l/ha
15	Comet medium	5	0.5l/ha
15	Comet low rate		0.1l/ha
15	Comet very low rate		0.01l/ha
16	Control		
16	Comet high		1.0l/ha
16	Comet medium	6	0.5l/ha
16	Comet low rate		0.1l/ha
16	Comet very low rate		0.01l/ha
17	Control		
17	Comet high		1.0l/ha
17	Comet medium	7	0.5l/ha
17	Comet low rate		0.1l/ha
17	Comet very low rate		0.01l/ha
18	Control		
18	Comet high		1.0l/ha
18	Comet medium	2	0.5l/ha
18	Comet low rate		0.1l/ha
18	Comet very low rate		0.01l/ha
19	Control		
19	Comet high		1.0l/ha
19	Comet medium	4	0.5l/ha
19	Comet low rate		0.1l/ha
19	Comet very low rate		0.01l/ha
20	Control		
20	Amistar		0.1l/ha
20	Rhino	3	1.0l/ha
20	Torch 0.5		0.45l/ha
20	Torch 0.25		0.225l/ha
23	Control		
23	Signum		1.0kg/ha
23	Comet (6.7%)	3	0.33l/ha
23	Comet 1.0		1.0l/ha
23	Amistar		0.1l/ha

Crop Safety

Laboratory experiments

In addition to the direct measures of disease control made in the laboratory experiments designed to measure fungicide performance, observations of effects on plant health and appearance were also recorded.

Near commercial conditions

Four cultivars of chrysanthemum (cvs. Sunny Martin, Greenbird, Universe and Sunny Woodpecker), chosen for their white rust sensitivity and commercial popularity, were grown in a 150m^2 glass-house at Stockbridge Technology Centre, with one cultivar grown in each bay. The plants were propagated and grown in accordance with commercial practice. A total of 14 treatment regimes were tested for each cultivar. The treatments were applied at precise timings (Table 6) devised to provide a 'worse case scenario' for crop damage (*i.e.* stunting, scorching, twisting, reduced flowering, damaged flowers *etc)*, but based on standard commercial practice for fungicides.

Table 6. Application timings for crop safety tests

Application	Time
1	10-14 days post planting (long day period)
2	14 days after application 1 (start of short days)
3	Appearance of first colouring at bud development

Treatment rates and water volume

Each fungicide treatment was applied at the rates detailed in Table 7. In most cases, these were extrapolated from other protected crops, primarily lettuce and strawberry. Where this was impossible, estimated from commercial use in arable crops. The water rate varied at each spray timing. For example the water rate for the first application was 1000 l ha^{-1}, this increased to 1500 l ha^{-1} for the second application and to 2000 l ha^{-1} for the third application. However, the product concentration applied was kept constant, *i.e.* the product rate increased with the water rate. This ensured that all applications provided a 'worst case' scenario from a phytotoxicity perspective.

Table 7. Fungicide dose applied in experiments testing crop safety under near-commercial conditions

Treatment	Active Ingredient	Application rate	Rate taken from
1. Untreated	-	-	-
2. Commercial			Chrysanthemum
Programme:	azoxystrobin	1l/1000l water	
Amistar	azoxystrobin	1l/1000l water	
Amistar	propiconazole	0.4l/1000l water	
Bumper			
3. Signum 1N	boscalid + pyraclostrobin	1.5kg/ha	Protected Lettuce
4. Signum 2N	boscalid + pyraclostrobin	3.0kg/ha	Protected Lettuce
5. Filan 1N	boscalid	0.8kg/ha	Oilseed Rape*
6. Filan 2N	boscalid	1.6kg/ha	Oilseed Rape*
7. Comet 1N	pyraclostrobin	0.4l/ha	Spring Wheat*
8. Comet 2N	pyraclostrobin	0.8l/ha	Spring Wheat*
9. Rhino 1N	flutolanil	1.0l/1000l water	$
10. Rhino 2N	flutolanil	2.0l/1000l water	$
11. Torch extra 0.5N	spiroxamine	0.45l/ha	Spring Barley
12. Torch extra 1N	spiroxamine	0.9l/ha	Spring Barley
13. Nativo 1N	tebuconazole + trifloxystrobin	0.4kg/ha	Carrots
14. Nativo 2N	tebuconazole + trifloxystrobin	0.8kg/ha	Carrots

* actual rates adjusted to ensure equal rate of each active ingredient to that in Signum.

$ rate provided by Alan Horgan, Certis

1N = Normal rate, 2N = twice normal rate

Although primarily a crop safety trial, attempts to establish white rust were made to investigate the feasibility of establishing infection within the glasshouse for a future experiment scheduled later in the project. Infected plants obtained from CSL were placed in the guard rows and monitored for signs of infection spread.

All fungicide treatments were applied using an Oxford Precision sprayer with boom attachment with flat fan nozzles (BCPC code F110/1.2/3) operating at 2-bar pressure.

Crop health was inspected and recorded at 7-10 days after each application. Crop damage and plant height were measured in detail at the end of the experiment.

Fungicide Efficacy

Near commercial conditions

A chrysanthemum crop comprising the varieties Ruby Red Reagan (RRR) and Zembla White (ZW), selected because of their susceptibility to white rust, was grown in a 150m^2 glasshouse at STC during 2007. The plants were bought in as unrooted cutting material and propagated using a commercial protocol for 2 weeks prior to planting on the 15 Aug 2007. Post-planting the glasshouse temperature was maintained at 18°C with venting at 23°C (day and night). The glasshouse was lit for 6-8 hours in the middle of the night to provide 'long day' conditions for the 3-weeks post-planting to ensure stem elongation prior to flowering. The crop was irrigated using trickle tape. The two varieties were planted in alternate bays (see trial layout, Plate 2). Use of 2 varieties of chrysanthemum increased the likelihood of ensuring effective colonisation and establishment of white rust and to counter any potential strain variation in the isolate of white rust used.

A range of 7 fungicides were used in the trial alongside an untreated control treatment. Three replicate plots of each treatment per variety were employed. Plot dimensions were 1m x 1.4m (144 plants/plot) with 2 guard rows of untreated plants between the plots.

Plate 2. General photograph of the trial layout during crop establishment

Inoculation

Potted plants of chrysanthemum with white rust symptoms, supplied by CSL, were placed into the centre of each plot 5 days post-planting. Overhead irrigation was used to provide optimum infection conditions by maintaining leaf wetness and high relative humidity. Clear polythene was used to cover the crop overnight for 1 week post-inoculation, again to increase relative humidity and aid the infection process.

Table 8: Fungicide dose applied in experiments testing fungicide efficacy under near-commercial conditions

Treatment	Active Ingredient	Application Rate	Rate sourced from
1. Untreated	-	-	-
2. Commercial Programme: Amistar Amistar Bumper	azoxystrobin (25% ai) azoxystrobin (25% ai) propiconazole (25% ai)	1l/1000l water 1l/1000l water 0.4l/1000l water	Chrysanthemum industry
3. Signum	boscalid (27% ai)+ pyraclostrobin (7% ai)	1.5kg/ha	Use on Prot Lettuce
4. Rhino	flutolanil (46% ai)	1.0l/1000l water	Certis (pers comm.)
5. Bumper	propiconazole (25% ai)	0.4l/1000l water	Chrysanthemum industry
6. Comet	pyraclostrobin (20% ai)	0.4l/ha	Use on Spring Wheat
7. Proline	prothioconazole (25% ai)	0.25l/ha	Use on S. Barley^
8. Opera	epoxiconazole (62% ai) & pyraclostrobin (8.5% ai)	1.5l/ha	Use on S. Barley

^ annual total permitted divided by 6

Sprays were applied using a battery operated knapsack sprayer at a constant 2 Bar pressure using F110-04 nozzles. The first application was made at the onset of white rust symptoms, with repeated applications at 10-14 day intervals, dependant on disease pressure. Fungicides were applied five times during the trial period.

Crop Diary

24.7.07	Unrooted cutting material received and potted up into 104 module trays and placed under milky polythene with mist irrigation throughout.
15.8.07	Plants well rooted, planted into glasshouse within wire-mesh supports.
20.8.07	Infector plants positioned in each plot.
12.9.07	First symptoms of CWR pustules observed on plants around infector plants,
13.9.07	Spray application 1
19.9.07	Disease assessment 1
27.9.07	Spray application 2
10.10.07	Spray application 3
16.10.07	Disease assessment 2
22.10.07	Spray application 4
30.10.07	Spray application 5
9.11.07	Final disease assessment – leaf samples collected for imaging technique.

The crop was monitored for pests and additional pathogens throughout. Insecticides to control caterpillars, aphids and thrips were applied as required during the trial period.

Disease Assessments

Disease was assessed three times during the trial period. At the first assessment, 19 September, the 10 lowest leaves of each sampled plant were examined and graded according to the EPPO grading scale (EPPO PP 1/173(2)) Table 9.

Table 9.

Grade of leaf	Number of pustules on the underside of the leaf
1	0
2	1
3	2-4
4	5-9
5	>10

During the second disease assessment, 16 October, a single leaf was sampled from approximately 1m above ground level, from each of 20 plants per plot. The number of pustules on each of these leaves was recorded.

The final disease assessment, 9 November, was a destructive harvest. Twenty plants per plot were cut at random from within a guard ‘picture frame’ area. Measurements of plant height, the number of flowers present, defects to flowers and the number of pustules on a single leaf 1m from the stem base, and on the 5 topmost leaves were recorded. Individual leaves from 1m and 1.2m from the stem base were removed for leaf area/disease imaging at CSL.

Results

Sensitivity and efficacy testing using replicated laboratory tests

A preliminary analysis to identify the most promising fungicides combined data from across a series of experimental runs. This provided an unbalanced design, with relatively low statistical power, but which was sufficient to distinguish differences in performance likely to be large enough to affect crop protection decisions. No difference was found between Amistar and Bumper under protectant situations. Moreover, under protectant situations, both Amistar and Bumper provided significantly worse disease control than most of the other fungicides tested (Tables 10 & 11). However, whilst providing greater control, Comet applied at the lowest dose tested (0.01 l ha^{-1}), was not significantly better than the benchmarks products. Similarly, compared to the two standards, Citrox, Filan, Plover and Rocket did not provide improved protectant efficacy (Tables 10 & 11). Under curative situations the efficacy of Amistar and Bumper were similar (Table 12 & 13).

For the more powerful balanced test of the most promising candidate fungicides Amistar and Bumper were shown to have relatively weak performance under both protectant and curative situations (Figures 1 & 2). Under protectant conditions, Bumper was the least effective product, significantly better control was achieved with Amistar and both Signum and Comet (0.33 l ha ⁻1) were found to be better than Amistar (Figure 1). Curative efficacy from Amistar and Bumper was negligible. However, curative performances of Signum and Comet were significantly better, with Comet providing significantly greater control than Signum. Apart from Comet, all the fungicides compared performed best as protectants. Efficacy of Comet did not differ significantly between protectant and curative conditions.

Table 10. Protectant efficacy of fungicides tested against Bumper (BASF; a. i. propiconazole). Differences are measured using an unbalanced ANOVA design.

Worse	Same[1]	Better
	(-) Citrox	Comet (all doses)
	(-) Plover	Filan
	(-) Rocket	Opera
		Proline
		Rhino
		Signum
		Torch

[1]symbols in parentheses indicates whether disease control was greater (+) or smaller (-) than achieved with Bumper, these differences are not significant at the level tested (p>0.067). The test level is adjusted according to the Bonferroni correction to allow multiple comparisons.

Table 11. Protectant efficacy of fungicides tested against Amistar (Syngenta; a. i. azoxystrobin).

Worse	Same	Better
Citrox	(-) Bumper	Comet (0.05 –1.25 l ha^{-1})
Plover	(+) Comet 0.01	Rhino
Rocket	(+) Filan	Torch
	(+) Opera	
	(+) Proline	
	(+) Twist	

[1]symbols in parentheses indicates whether disease control was greater (+) or smaller (-) than achieved with Bumper, these differences are not significant at the level tested (p>0.067). The test level is adjusted according to the Bonferroni correction to allow multiple comparisons.

Table 12. Curative efficacy of fungicides tested against Bumper (BASF; a. i. propiconazole.

Worse	Same	Better
	(+) Citrox	Comet (0.05 –1.25 l ha^{-1})
	(+) Comet (0.01)	Opera
	(+) Filan	Proline
	(+) Plover	Rhino
	(+) Rocket	Signum
		Torch
		Twist

[1]symbols in parentheses indicates whether disease control was greater (+) or smaller (-) than achieved with Bumper, these differences are not significant at the level tested (p>0.067). The test level is adjusted according to the Bonferroni correction to allow multiple comparisons.

Table 13. Curative efficacy of fungicides tested against Amistar (Syngenta; a. i. azoxystrobin).

Worse	Same	Better
	(+) Bumper	Comet (0.05 –1.25 l ha^{-1})
	(+) Citrox	Opera
	(+) Comet 0.01	Proline
	(+) Filan	Rhino
	(+) Plover	Signum
	(+) Rocket	Torch
		Twist

[1]symbols in parentheses indicates whether disease control was greater (+) or smaller (-) than achieved with Bumper, these differences are not significant at the level tested (p>0.067). The test level is adjusted according to the Bonferroni correction to allow multiple comparisons.

Figure 1. Protectant performance of fungicides against white rust. Differences are measured using a balanced ANOVA design and are significant for position along the arrow (P<0.05)

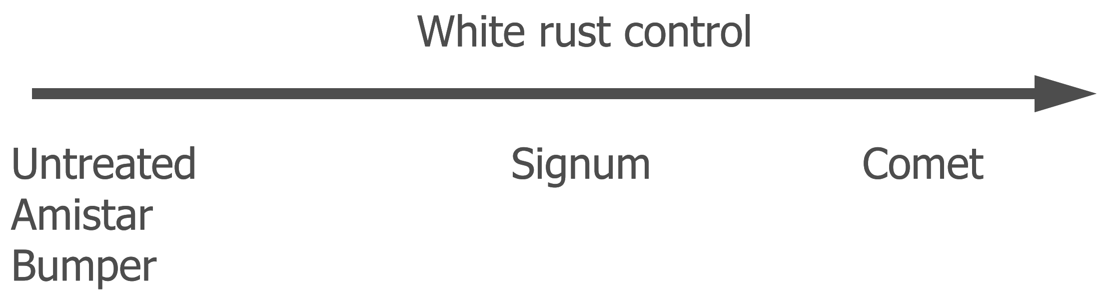

Figure 2. Curative performance of fungicides against white rust. Differences are measured using a balanced ANOVA design and are significant for position along the arrow (P<0.05)

Amistar, Bumper, Signum and Comet were tested against isolates listed in Table 2. Both Amistar (azoxystrobin) and Bumper (propiconazole) provided a degree of protectant activity against all isolates but eradicant activity was poor. Both fungicides were effective when applied up to seven days before inoculation. However, very little curative activity was provided by applications of either fungicide when applied 4 and 7 days after inoculation. Although Amistar did not give complete protectant control with all the isolates, it gave 100% control when used against isolates 2, 3,4 and 6 (Figures 3-6).

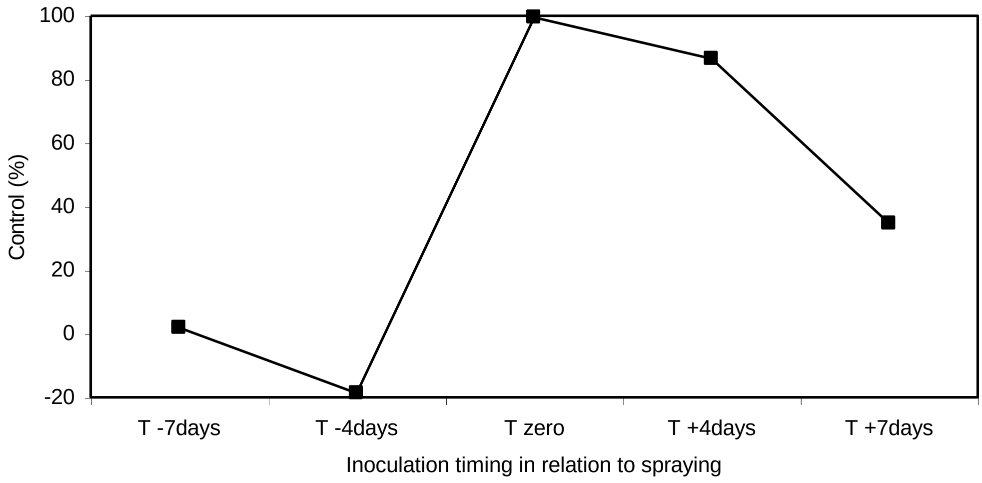

Figure 3. Control compared to Untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 3 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

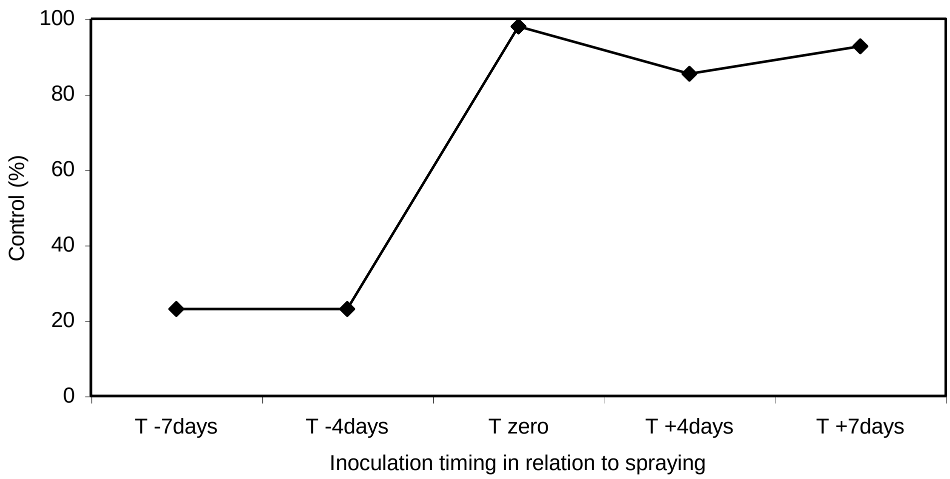

Figure 4. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 6 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

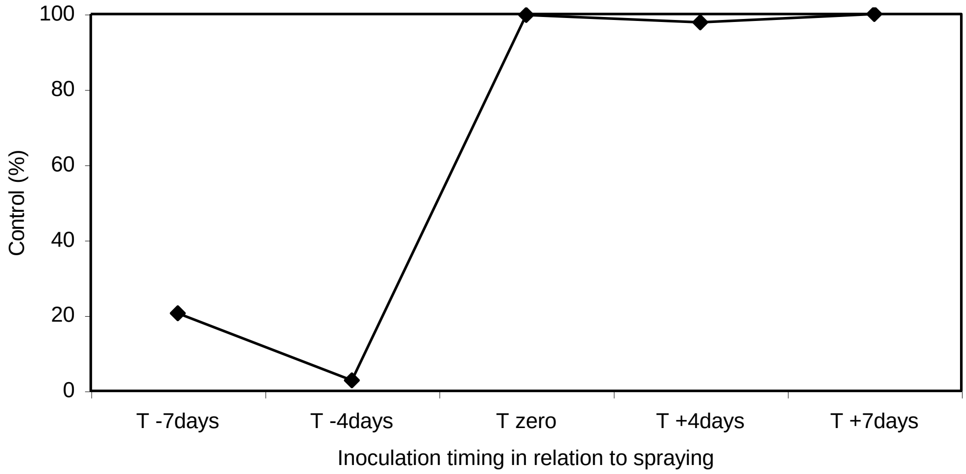

Figure 5. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 2 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

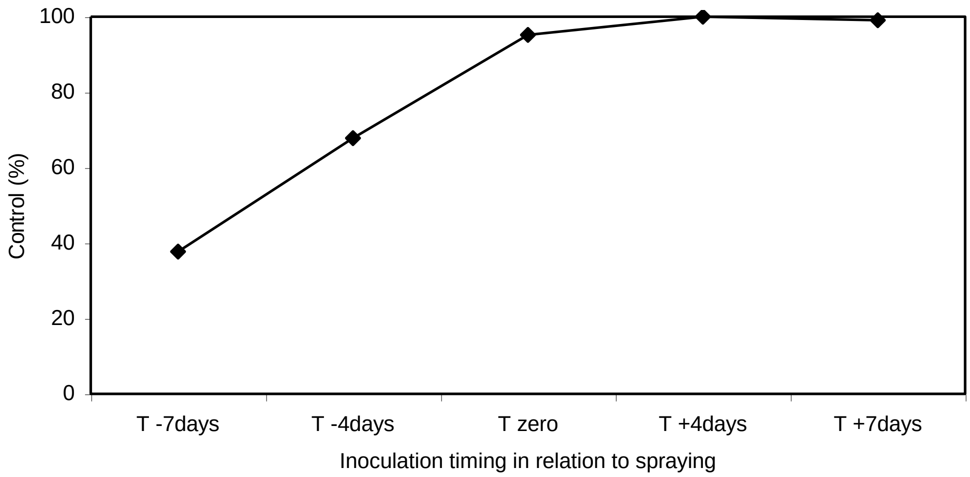

Figure 6. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 4 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

The proprietary formulation Signum (pyraclostrobin + boscalid) offered slightly greater protectant control across isolates than azoxystrobin. It was also substantially more effective under curative situations. The fungicide with the single active ingredient, Comet (pyraclostrobin) proved to be the most effective in both its protectant and eradicant properties: many of the plants assessed had very little or no disease symptoms when a dose of 0.33l ha^{-1} was used.

Comet (pyraclostrobin) was tested at 4 rates (1.0, 0.5, 0.1, 0.01l ha^{-1}). The high and medium rates produced consistent results across the isolate range with no disease recorded on plants in either protectant or eradicant situations. When used at 0.1l ha^{-1} some disease was recorded, mainly when the spray timing was +/- 7days. This trend was repeated when using the very low rate of 0.01l ha^{-1} with disease also occurring on the +/-4 day plants, highlighting a progressively narrower spray interval with a reduction in dose (Figures 7 & 8).

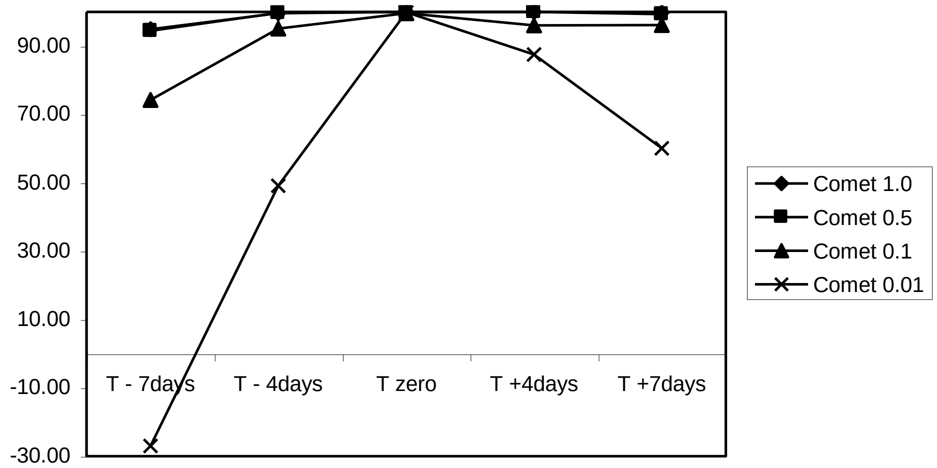

Figure 7. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 3 treated with Comet. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

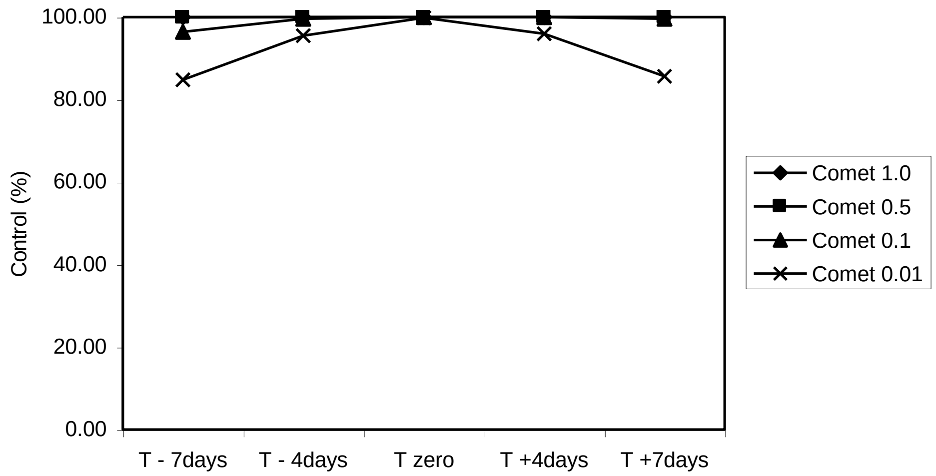

Figure 8. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 4 treated with Comet. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

Opus (epoxiconazole) and Bravo (chlorothalonil) provided good protectant control with Shirlan (fluazinam) somewhat less effective. Even under protective situations Systhane (myclobutanil) showed little difference in disease severity from the untreated control.

Rhino (flutolanil) and Torch (spiroxamine) were tested in comparison against Amistar. Rhino had less curative efficacy than Amistar, but performed well as a protectant (Figure 9). Torch was tested at two relatively small application rates (Run 20, Table 5), which provided both good protectant and erradicant control.

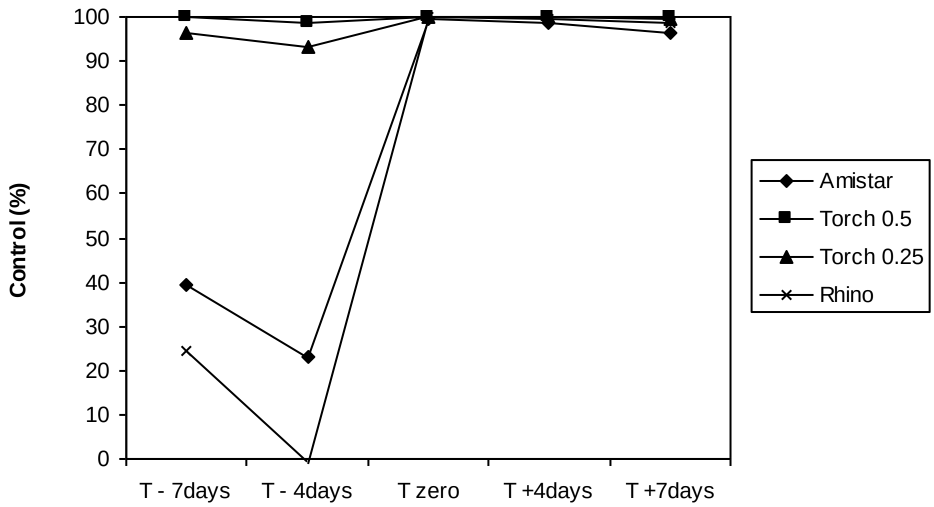

Figure 9. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 2 with 4 treatments. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

The duration of protectant and curative performance was tested for the products that were most effective in previous experiments, specifically, Comet (pyraclostrobin), Signum (pyraclostrobin + boscalid) and Amistar (azoxystrobin). All provided full protectant control for 10 days (Figure 10). However, curative efficacy from Signum and Amistar declined significantly when tested beyond seven days (Figure 10). For Comet, at both the rates tested, curative efficacy was also reduced when extended beyond seven days, but to a much lesser extent than for Signum and Amistar (Figure 10). For the durations of protectant and curative control tested (both up to 10 days), no significant difference was found between Comet applied at a rate equivalent 1 l ha $^{-1}$ and 0.33 l ha $^{-}$1 (Figure 10).

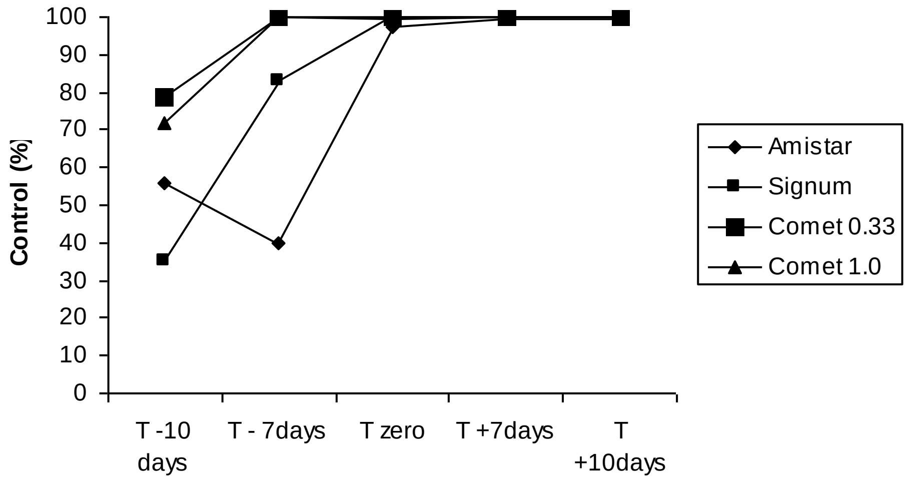

Figure 10. Control compared to untreated (%) measured from the mean of the first 3 leaves below the tag for isolate 3 with 4 treatments. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

Crop safety - glasshouse tests

Proline (prothioconazole; Run 12) was tested at 4 rates (0.4, 0.2, 0.1, 0.05l/ha) and produced some control at the two greatest 2 rates, although significant stunting in plant growth resulted (Plate 3). This growth regulatory effect was less marked in the smallest application rates, but disease severity was controlled less effectively.

Plate 3. Dose of Proline increases in direction of arrow (increment 0.05, 0.1, 0.2, 0.4 l ha^{-1}). Plants on the left side of the tray are untreated

Despite good levels of control Opus had a similar stunting effect to Proline. Shirlan and Bravo did not produce any stunting, but residues were observable on the leaves after sprays were applied. Residues were also evident for Signum, but not for Comet (Plate 4).
Whilst Torch (spiroxamine) provided good curative and protectant activity (Figure 9), some phytotoxic effects were observed, even at the smallest rate (see plates 5 & 6).

Plate 4. Deposits From use of Signum (left) compared with from Comet (right)

Plate 5

Plate 6

Plate 5 & 6. Scorch symptoms caused by Torch Extra.

Table 13 summarises plant damage observed over the series of efficacy experiments at CSL.

Table 13. Damage to plants from tested chemicals.

Product	Fungicide activity tested		
	Protectant	Curative	Damage
Proline	✔	✔	Slight stunting
Corbel	✔	✔	Stunting
Fandango	✔	✔	Stunting
Prosaro	✔	✔	Stunting
Sonata	✔	✔	Residues
Elvaron Multi	✔	✕	Residues
Folicur	✔	✕	Stunting
Torch	✔	✔	Pytotoxic effects
Shirlan	✔	✔	Residues
Bravo	✔	✔	Residues
Signum	✔	✔	Residues

Crop safety in a semi-commercial trial (STC)

At flowering all the plant heights were measured. Differences in height across the fungicide programmes were small, and unlikely to cause significant problems for production (Table 14 and Figures 11 - 14)

Table 14. Average plant height (m)

Treatment	Greenbird	Sunny Martin	Sunny Woodpecker	Universe
1.Untreated control	1.44	1.34	1.40	1.15
2.Comm programme	1.40	1.28	1.36	1.08
3.Signum 1N	1.40	1.26	1.30	1.13
4.Signum 2N	1.49	*	1.35	1.12
5.Filan 1N	1.46	1.32	1.31	1.17
6.Filan 2N	1.40	1.24	1.34	1.12
7.Comet 1N	1.42	1.17	1.34	1.14
8.Comet 2N	1.41	1.33	1.27	1.13
9.Rhino 1N	1.44	*	1.39	1.15
10.Rhino 2N	1.41	*	1.33	1.16
11.Torch Extra ½ N	1.39	1.16	1.29	1.11
12.Torch Extra 1N	1.46	*	1.24	1.16
13.Nativo 1N	1.43	*	1.26	1.19
14.Nativo 2N	1.45	1.16	1.29	1.19

*Treatments unrepresentative due to caterpillar damage

The occurrence of phytotoxic effects, expressed as leaf scorch, flower damage, twisting, stunting etc, were recorded as presence/absence (Table 15). Damage to foliage ('scorching') was caused by Torch Extra (Plates 5 & 6).

Table 15. Occurrence of phytotoxic effects in varieties treated by fungicides.

Treatment	Cultivar			
	Greenbird	Sunny Martin	Sunny Woodpecker	Universe
1.Untreated control	-	-	-	-
2.Comm programme	-	-	-	-
3.Signum 1N	-	-	-	-
4.Signum 2N	-	-	-	-
5.Filan 1N	-	-	-	-
6.Filan 2N	+	-	-	-
7.Comet 1N	-	-	-	-
8.Comet 2N	-	-	-	-
9.Rhino 1N	-	-	-	-
10.Rhino 2N	-	-	-	+
11.Torch Extra ½ N	+		+	+
12.Torch Extra 1N	+	+	+	+
13.Nativo 1N	-	+	-	-
14.Nativo 2N	-	-	-	-

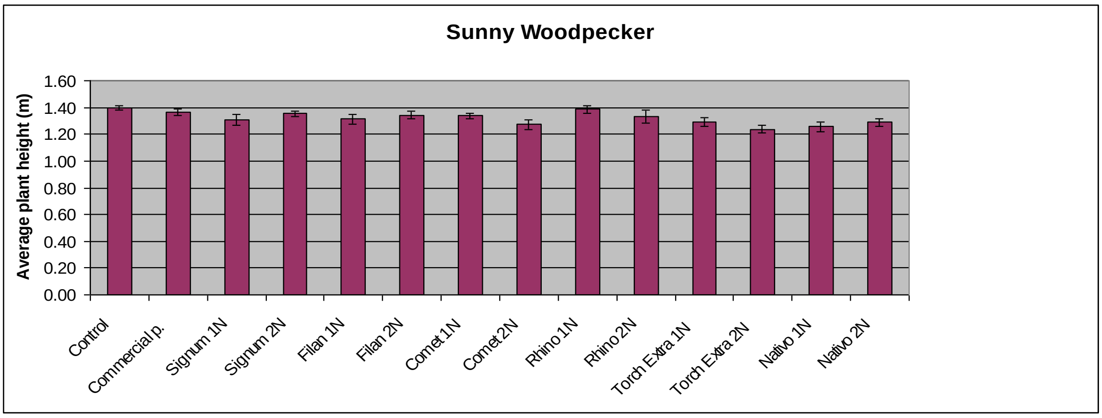

Figure 11. Effect of treatment on plant height for CV Sunny Woodpecker. Error bars show SE around the mean.

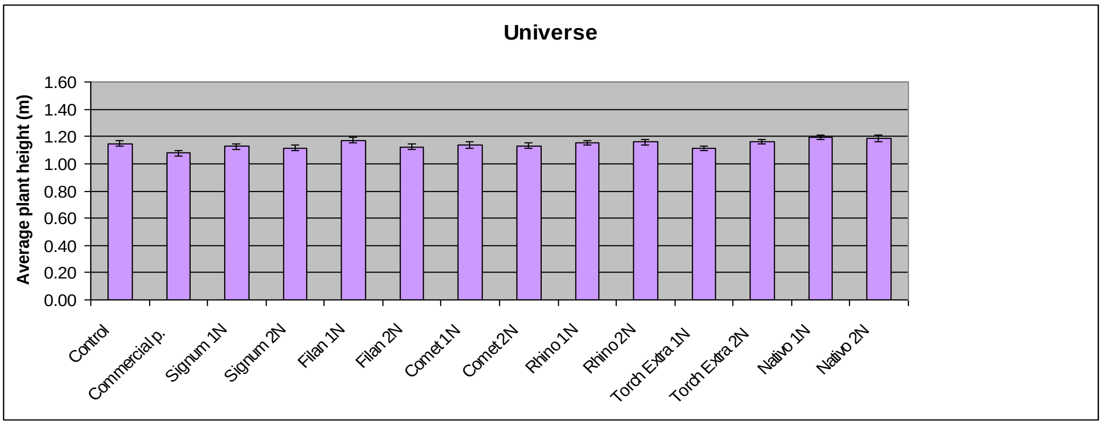

Figure 12. Effect of treatment on plant height for CV Universe. Error bars show SE around the mean.

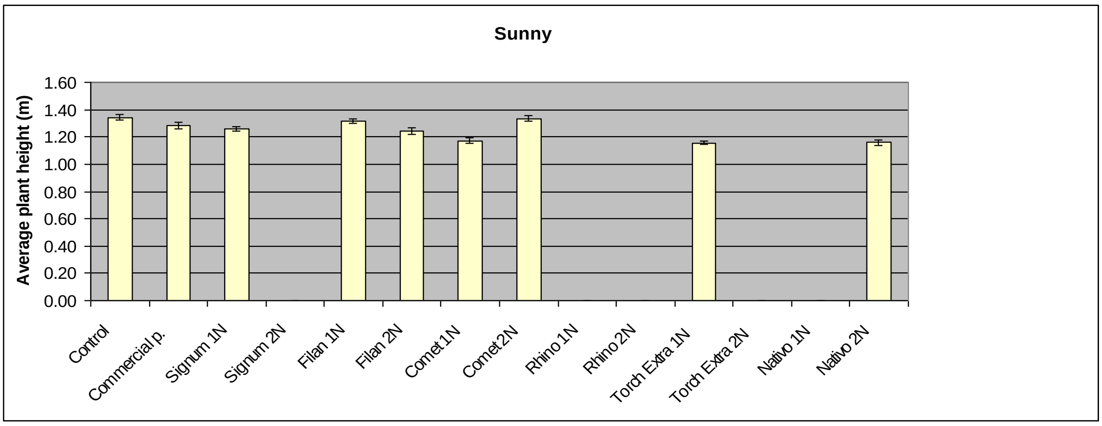

Figure 13. Effect of treatment on plant height for CV Sunny. Error bars show SE around the mean.

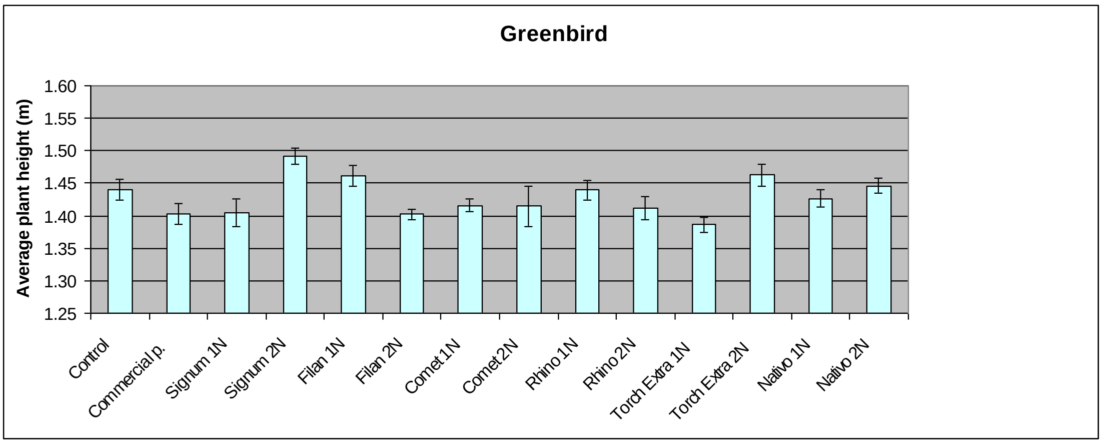

Figure 14. Effect of treatment on plant height for CV Greenbird. Error bars show SE around the mean.

Crop efficacy in a semi-commercial trial (STC)

Both varieties established and grew well. Early signs of CWR pustules were observed on the plants closest to the infector plants approximately 3 weeks following their introduction (Plate 7)

Plate 7. Early development of white rust symptoms on cv Ruby Red Reagan

Significant amounts disease were observed initially in the untreated plots of both varieties, indicating that the use of inoculator plants had been successful in establishing crop infection. Ruby Red Reagan (RRR) proved considerably more susceptible to the pathogen isolate used than Zembla White (ZW). Severe symptoms of white rust developed during the period of the trial, particularly on RRR.

The first quantitative assessment of disease was completed on 19 September (Table 16).

Table 16. Incidence of CWR on 19th September 2007

Treatment	**Ruby Red Reagan Mean Disease Score[a] (1-5)**	**Zembla White Mean Disease Score[a] (1-5)**
1. Untreated	1.49[a]	1.68[a]
2. Commercial Programme	1.38[a]	1.46[a]
3. Signum	1.26[a]	1.44[a]
4. Rhino	1.68[a]	1.59[a]
5. Bumper	1.40[a]	2.21[a]
6. Comet	1.39[a]	1.53[a]
7. Proline	1.23[a]	1.75[a]
8. Opera	1.25[a]	2.09[a]
LSD (P=0.05)	0.52	0.82
Standard Deviation	0.30	0.47
Coefficient of Variance	21.5	27.11

[a] Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

For this first assessment, no significant differences were found in amounts of disease between the treatments.

A second assessment of disease was completed on the 16 October 2007, by which time three sprays had been applied. One leaf per plant was selected at approximately 1m high and the number of CWR pustules was measured (Table 17).

Table 17. Incidence of CWR on 16th October 2007

Treatment	Ruby Red Reagan		Zembla White	
	Mean no. pustules/leaf	Percentage difference from untreated control	Mean no. pustules/leaf	Percentage difference from untreated control
1. Untreated	4.5[a]	0.00[f]	0.7[a]	0.0[e]
2. Commercial Programme	3.7[a]	17.8[d]	1.1[a]	-61.9[g]
3. Signum	3.4[a]	24.9[b]	0.4[a]	50.0[c]
4. Rhino	4.7[a]	-5.6[g]	0.4[a]	40.5[d]
5. Bumper	3.9[a]	11.9[e]	1.2[a]	-64.3[h]
6. Comet	4.5[a]	0.0[f]	0.2[a]	65.8[a]
7. Proline	3.4[a]	24.2[c]	0.7[a]	-2.4[f]
8. Opera	1.7[a]	61.3[a]	0.3[a]	57.1[b]
LSD (P=0.05)	3.1	-	0.7	-
Standard Deviation	1.8	-	0.4	-
Coefficient of Variance	47.6	-	64.1	-

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

A moderate level of white rust was present in the crop at this time, with greatest severity on RRR. Large variability in disease within each plot and across treatment (c.v. 47-64) was evident, which explains the lack of significant differences in disease measurement for either variety. However, applications of Signum, Comet (ZW only) and Opera, resulted in a smaller incidence of pustules compared to the untreated control (Table 17).

Table 17 also shows a comparison of the white rust incidence relative to the untreated control (Abbott's transformation). In both varieties epoxiconazole + pyraclostrobin (Opera) provided a large reduction of disease. Comet (pyraclostrobin) did not provide any reduction of disease compared to the untreated control. Signum (pyraclostrobin + boscalid) resulted in a moderate level of disease control in both varieties.

The third and final assessment of disease was on the 9 November 2007. A large number of parameters were assessed and recorded during this assessment and the data is shown in the following tables and charts.

Plant heights and the number of flowers present were similar within each variety, none of the applied treatments resulted in detrimental effects on plant growth or the number of flowers present (Table 18).

Table 18. Plant Heights and number of flowers present.

Treatment	Ruby Red Reagan		Zembla White	
	Mean plant height (cm)	**Mean no. flowers present**	**Mean plant height (cm)**	**Mean no. flowers present**
1. Untreated	148.6[a]	9.4[a]	144.7[a]	7.5[a]
2. Commercial Programme	145.4[a]	8.9[a]	145.5[a]	7.8[a]
3. Signum	145.7[a]	8.9[a]	141.9[a]	7.3[a]
4. Rhino	146.3[a]	9.6[a]	145.6[a]	7.6[a]
5. Bumper	138.6[a]	8.9[a]	143.4[a]	7.4[a]
6. Comet	147.6[a]	9.4[a]	142.1[a]	6.4[a]
7. Proline	147.4[a]	9.3[a]	142.6[a]	7.2[a]
8. Opera	146.1[a]	8.5[a]	144.6[a]	7.0[a]
LSD (P=0.05)	6.2	1.6	7.1	1.0
Standard Deviation	3.5	0.9	4.1	0.5
Coefficient of Variance	2.4	9.9	2.8	7.5

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

Table 19 shows the incidence of white rust pustules on leaves 1m from the stem base and also a mean value for the number of pustules on the top 5 leaves of the randomly chosen plants.

Table 19. The incidence and severity of CWR on 9 November 2007.

Treatment	Ruby Red Reagan		Zembla White	
	Mean no. pustules on leaf at 1m from stem base	Mean no. pustules on top 5 leaves below the flower	Mean no. pustules on leaf at 1m from stem base	Mean no. pustules on top 5 leaves below the flower
1. Untreated	7.7[a] (0)[h]	23.2[a] (0)[h]	3.1[a] (0.0)[h]	10.2[a] (0.0)[h]
2. Commercial Programme	3.2[a] (34.5)[f]	3.9[b] (61.6)[f]	2.0[ab] (40.9)[f]	3.6[b] (50.2)[g]
3. Signum	5.3[a] (47.7)[d]	0.3[b] (96.7)[a]	0.2[b] (86.6)[c]	0.0[b] (99.5)[a]
4. Rhino	3.8[a] (46.6)[e]	2.0[b] (78.6)[e]	0.8[ab] (53.6)[e]	0.2[b] (94.8)[e]
5. Bumper	4.6[a] (33.4)[g]	9.1[b] (43.7)[g]	1.3[ab] (28.7)[g]	2.6[b] (58.5)[f]
6. Comet	2.1[a] (60.0)[b]	1.8[b] (87.2)[d]	0.1[b] (91.6)[a]	0.1[b] (98.3)[c]
7. Proline	1.8[a] (59.6)[c]	0.5[b] (91.9)[c]	1.0[ab] (71.4)[d]	0.3[b] (95.1)[d]
8. Opera	1.0[a] (75.5)[a]	0.6[b] (93.3)[b]	0.2[b] (87.8)[b]	0.0[b] (99.1)[b]
LSD (P=0.05)	7.5 (0.0)	11.9 (0.0)	1.7 (0.0)	5.4 (0.1)
Standard Deviation	4.3 (0.0)	6.8 (0.02)	0.9 (0.0)	3.1 (0.0)
Coefficient of Variance	116.2 (0.0)	131.7 (0.0)	86.7 (0.0)	144.4 (0.0)

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)
Nos. shown in brackets show % control relative to untreated (Abbott's transformation).

By the final assessment a clear difference was evident in the susceptibility of each variety to the CWR isolate introduced to the glasshouse via the inoculator plants. Substantially greater amounts of disease were present on RRR variety than ZW. For both the upper leaves and those from 1m, the mean numbers of pustules recorded on RRR, were approximately double those recorded on ZW (Table 19).

For the most susceptible variety, RRR, all of treatments reduced disease on the uppermost leaves compared to the untreated control. However where a single leaf (at 1m height) was assessed no significant differences were found, probably due to large within plot variability. However, compared to the untreated, the mean number of pustules was reduced by all treatments, Opera, Proline and Comet performing most effectively (Table 19).

Similarly for ZW, Signum, Opera and Comet reduced significantly disease on leaves sampled from 1m, and all the treatments reduced the number of pustules on the uppermost leaves (Table 19). It is particularly important to control the development of pustules on the upper leaves to produce a good quality cut flower crop and Plates 8-23 shows differences across treatments.

Plate 8

Plate 9

Plate 10

Plate 11

Plates 8-11. Comparison of Ruby Red Reagan plants treated with fungicide regimes described by Table 8.

Plate 12

Plate 13

Plate 14

Plate 15

Plates 12-14. Comparison of Ruby Red Reagan plants treated with fungicide regimes described by Table 8. See Plate 8 for untreated appearance of untreated plants.

Plate 16

Plate 17

Plate 18

Plate 19

Plates 16-19. Comparison of Zembla White plants treated with fungicide r egimes described by Table 8.

Plate 20 **Plate 21**

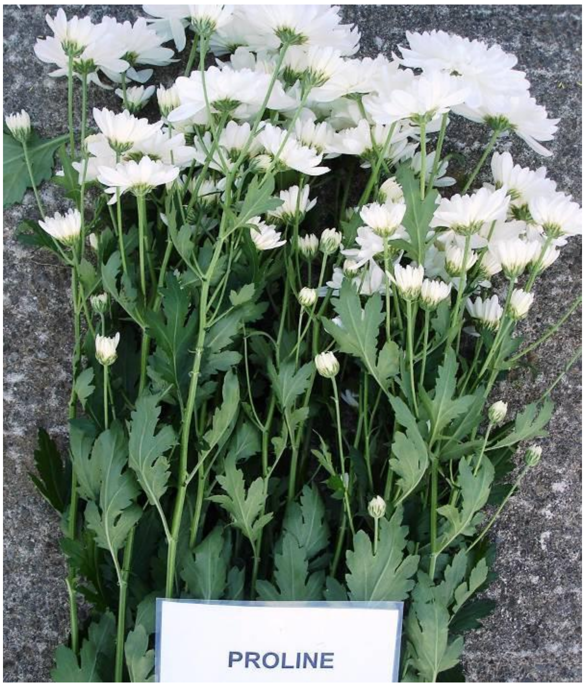

Plate 22 **Plate 23**

Plates 20-23. Comparison of Zembla White plants treated with fungicide regimes described by Table 8. See Plate 16 for untreated appearance of untreated plants.

During the final disease assessment 2 leaves were removed from each of the harvested stems. Two individual leaves were taken from 1m and 1.2m above soil level for disease imaging purposes. Digital images of each of the excised leaves were taken and software was used to accurately measure the leaf area and also the area with CWR symptoms. This provided an objective quantification of the percentage white rust present on the leaves in each treatment. The mean disease severities for each treatment are shown for RRR and ZW in Figures 15 and 16 respectively. These data supports the visual assessments reported in Table 19, and demonstrate that, for both varieties, effective disease control on the upper leaves was provided by Signum, Comet, Opera and Proline.

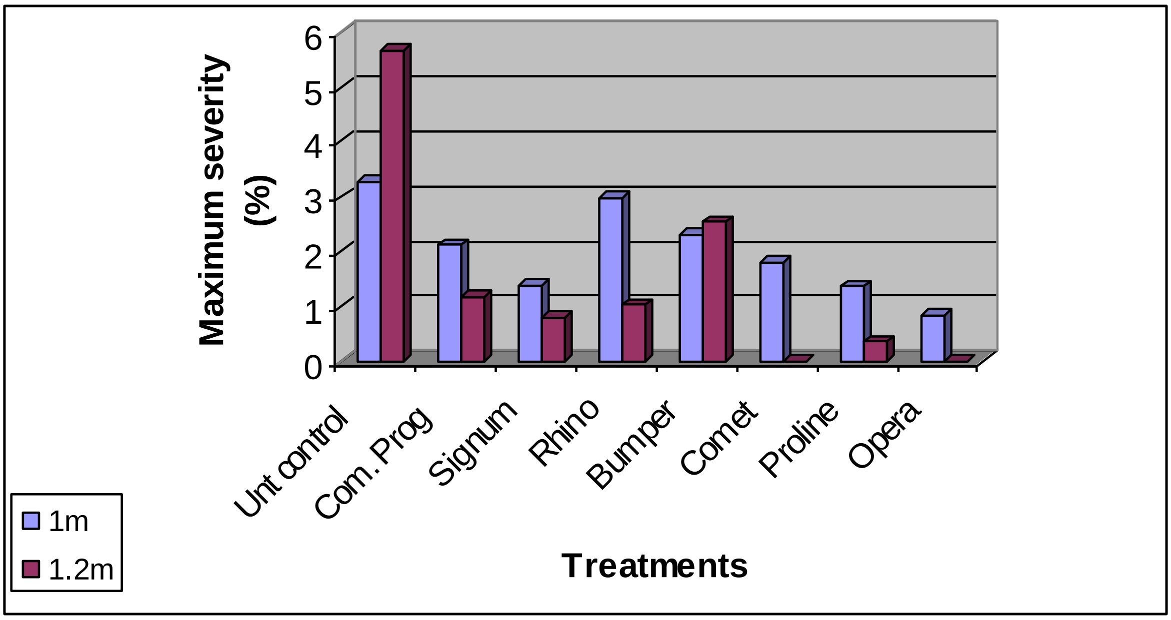

Figure 15. The mean leaf area infected with CWR in Ruby Red Reagan using digital image technology

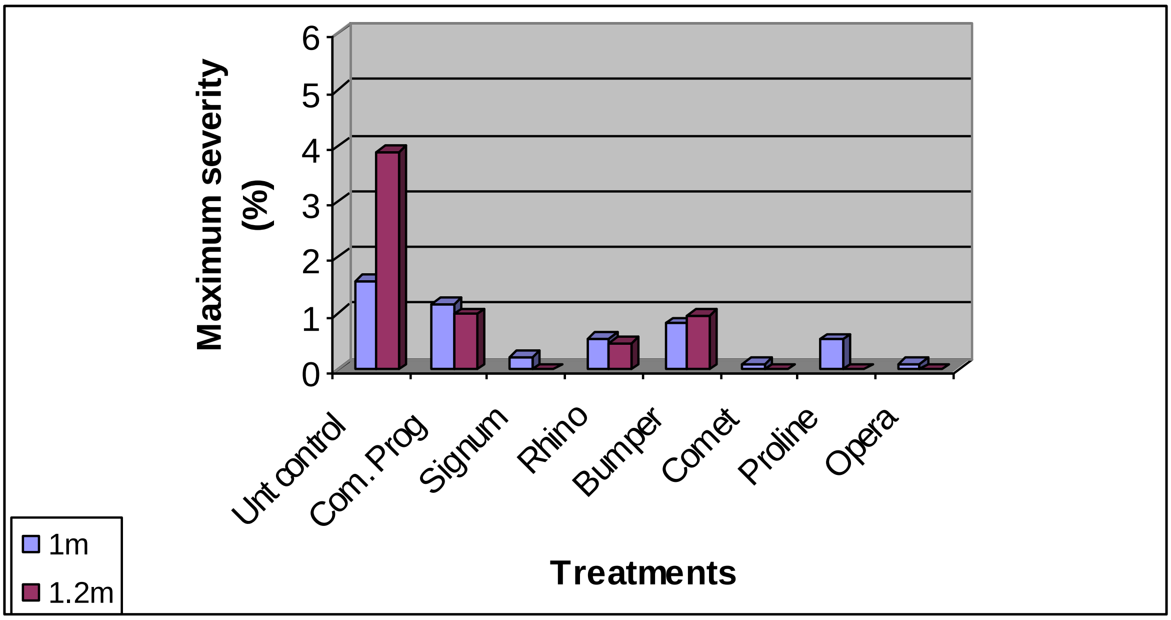

Figure 16. The mean leaf area infected with CWR in Zembla White using digital image technology.

In summary, products containing pyraclostrobin, *i.e.* Signum, Comet, and Opera, were the most effective. Small differences in the level of control were found between these treatments. These are almost certainly attributable to differences in the dose of pyraclostrobin applied and, in the case of Opera, the presence of epoxiconazole, which proved effective against CWR in glasshouse screening work (but caused stunting). Specifically, the weight of pyraclostrobin applied as Comet (0.08 l/ha) was smaller than for Signum (0.105 kg/ha) and substantially less than for Opera (0.1275 l/ha).

Signum, Comet, Opera and Proline were all more effective in controlling disease than the standard commercial programme. Rhino and Bumper were the least effective of the products tested.

Discussion

Two effects; protectant and curative performance, quantify disease control from fungicides. Protectant activity is measured where the fungicide is applied before the pathogen has arrived on the plant, i.e. prior to infection. The curative activity describes the ability of the fungicide to control established infections, *i.e.* situations where the fungicide is applied after the plant is infected. Often however, this distinction is not made explicit in reporting screens of product performance. For example, previous work testing isolates of *P. horiana* detected fungicide insensitivity in protectant performance of both azoxystrobin and pyraclostrobin (Cook, 2001). These fungicides are the benchmarks for fungicide efficacy against *P.horiana.*

In commercial production, curative control of chrysanthemum white rust is most commonly dependent upon the use of propiconazole. The same active ingredient has also delivered protectant control, but in addition azoxystrobin is also used to protect crops at risk from the disease. Recently, disease control failures in commercial crops have been attributed to resistance, or at else reduced insensitivity to both of these active ingredients. This is especially plausible given the observations of Cook (2001).

In year one of this work, unreplicated screening of potential replacements for two benchmarks (propiconazole and azoxystrobin) indicated that some fungicides provided improved control under both protectant and curative situations. Work during the subsequent two years of the study confirmed these preliminary conclusions, provided more comprehensive quantification of candidate fungicides (Tables 10-13, Figures 1-9) and tested the most promising candidates under near-commercial conditions.

In fully replicated experiments (at CSL), propiconazole and azoxystrobin were shown to provide protectant control of all the *P. horiana* isolates tested. Against the same isolates however, the curative efficacy of propiconazole is now very poor. This observation probably explains the large proportion of the disease control failures seen in commercial production. Dependence on protectant control diminishes the flexibility for effective crop and disease management, because timing fungicide application ahead of infection becomes critical: established infections remain active, damage leaves and sporulate to release new inoculum for further infection in the crop.

Disease control from Signum was substantially better under both curative and protectant conditions, than achieved by either Bumper or Amistar. The study has provided the basis for understanding the improved control apparent with use of Signum (BASF; pyraclostrobin + boscalid) under protectant and curative situations. This product is a propriety formulation of two active ingredients. Testing these active ingredients separately as Comet (BASF; pyraclostrobin) and Filan (BASF; boscalid) showed that the disease control benefits above present benchmarks was due, primarily to pyraclostrobin. These tests also indicated that this fungicide was effective even at very small doses.

Fungicides that gave improved control in either protectant or curative were tested more completely in replicated efficacy tests. Observations of treatment effects from these products in small-scale *in-vivo* tests suggest that some of these have associated crop effects; either stunting, scorching or the deposit of visible residues. These negative attributes potentially preclude their use commercially due to the implications for crop safety and quality particularly for later applications which are more likely to impact on crop quality. Therefore products that were identified to have particularly severe crop safety risks were not investigated further, even when disease control appeared to be effective.

Crop safety experiments, under near-commercial conditions, support the conclusions about fungicide efficacy from the glasshouse experiments. Specifically that products containing pyraclostrobin were effective in reducing white rust, even under severe (inoculated) infection pressures. These experiments also suggest that doses above 0.1kg /ha (equivalent to 0.1 l/ha) pyraclostrobin active ingredient are necessary to achieve acceptable levels of control under commercial conditions. This dose is just possible at the full label rate for Signum. In contrast with screening studies in the glasshouse, the crop safety experiments did not measure any deleterious crop stunting effect from the triazole fungicides evaluated: epoxiconazole was tested in the formulated product Opera (pyraclostrobin + epoxiconazole) and prothioconazole as Proline. Both of these fungicides provide effective disease control. However they are not available currently in any formulations permissible for use on protected crops. Any future use would need to ensure that application rates were below those likely to cause significant plant growth regulatory effects.

Proposed revisions of EC Directive 91/414/EEC may affect the fungicide armoury available for protection of UK crops. EU Directive 91/414/EEC concerns the placement of plant protection products on the market and currently uses primarily a risk based system to identify cut off criteria for pesticide approvals. Under new proposals from the European Commission for revision of Directive 91/414/EEC, it is planned that cut-off criteria for approvals will become primarily hazard rather than risk based. The commission proposals have identified chemicals that are thought to be of very high concern in terms of their hazard status and have proposed their exclusion. In addition, a list of chemicals for substitution have been identified, which would be approved initially for 10 years beyond the initial exclusion date, but could go through a renewal. Additional amendments have been proposed by the European Parliament which would result in more stringent cut-off criteria for exclusion, and the substitution criteria would require that candidates for substitution were approved once only for a period of five years. These proposals form the basis of four scenarios for revision of the Directive (Commission Exclusion, Commission Substitution, Parliament Exclusion and Parliament Substitution). Fortunately, current interpretations suggest that pyrcaclostrobin will remain available to growers under three of these scenarios, but not the Parliament Substitution, which leads to the loss of all current fungicides.

Conclusions

Curative treatment of chrysanthemum white rust is no longer likely to be effective when propiconazole is the primary active ingredient used. However, successful control of white rust is achievable from the use of Signum (BASF; pyraclostrobin + boscalid) as the studies here have shown that small doses of pyraclostrobin (tested as Comet; BASF) are effective in both curative and protectant situations. It is worth noting that the rate of pyraclostrobin in Signum is relatively small and also, that the contribution of boscalid to CWR control is negligible. Larger label rates of pyraclostrobin are achievable using Comet (pyraclostobin) or Opera (praclostrobin + epoxiconazole), but these currently are **not permissible** for crops grown under protection.

References

Cook RTA, 2001. First report in England of changes in the susceptibility of *Puccinia horiana*, the cause of chrysanthemum white rust, to triazole and strobilurin fungicides. *New Disease Reports* **3.**

Dickens JSW, 1990. Studies on the chemical control of chrysanthemum white rust caused by *Puccinia horiana. Plant Pathology* **39,** 434-442.

Dickens JSW, 1991. Evaluation of some newer fungicides, in comparison with propiconazole, against chrysanthemum white rust (*Puccinia horiana*). Tests of Agrochemicals and Cultivars 12. Annals of Applied Biology 118 Supplement, 32-33.

Dirske FB, Dil M, Linders R, Riestra I, 1982. Resistance in white rust (*Puccinia horiana* P. Hennings) of chrysanthemum to oxycaboxin and benodanil in the Netherlands . *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **47,** 793-800.

Grasso V, Palmero S, Sierotzki H, Garibaldi A and Gisi U. 2006. Cytochrome b gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. *Pest management Science* **62,** 465-472.

Grouet D, Montfort F, Leroux P (1981) Mise en evidence en Frace d'une souche de *Puccinia horiana* resistante a l'oxycarboxine. *Phytiatrie-Phytopharmacie* **30,** 3-12.

Parker, S. R., M. W. Shaw, and D. J. Royle. 1995. The reliability of visual estimates of disease severity on cereal leaves. *Plant Pathology* **44,** 856-864.