Project title:	Control of white rust in commercial chrysanthemum production
Project number:	PC 231
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Location:	Central Science Laboratory
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Grower summary

Headline

No evidence of resistance was detected in *P. horiana* isolates when two industry standard fungicides were applied as protectants although curative activity was not effective for one product suggesting a possible shift in sensitivity. A comparable protectant and more effective curative fungicide was identified.

Background and expected deliverables

This project aims to develop effective approaches to control chrysanthemum white rust (CWR), which recognise the importance of minimising 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 dependant 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 will:

1. Determine 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. Quantify 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. Develop clear treatment guidelines that enshrine best practice for product stewardship, so that an armoury of effective fungicides will be available to support 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 fungicides azoxystrobin or propiconazole.
- Fungicide treatment is likely to be most effective when used in protectant situations.
- Curative activity is relatively short even for the best products. Curative activity from propiconazole was not found to be effective which may reflect a shift in the sensitivity of the population to triazole fungicides.
- Signum, (BASF, pyraclostrobin + boscalid) was shown to provide 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.

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 varieties where possible

Financial benefits

In the short-term

Improved control of chrysanthemum white rust as the recommendations provided at the 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 spray schedules that they are implementing against the mode of action groups. 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. Bumper). Glasshouse screening for fungicide activity has not indicated any detrimental effects to crop health from Signum. However, formalised crop safety tests under near commercial conditions, will test this explicitly at Stockbridge Technology Centre.

The project team is keen to receive additional white rust isolates from growers. This includes samples from sites where acceptable disease control is being achieved without notable difficulty and where management is proving more challenging (and resistance is suspected). Samples should be collected as follows:

1. Select at least five stems showing fresh CWR symptoms from the same production area.
2. Cut the stems into a single 20 cm section containing the infected leaves.
3. Remove any open flower heads.
4. Loosely wrap the leaved stems together in moist paper and place the bunch in a plastic bag. Inflate the plastic bag slightly and seal.

5. Record the date that the sample was made, the variety, the fungicides treatments made to the crop and the dates of application and include this in the package.
6. Send the package to:

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Science Section

Control of white rust in commercial chrysanthemum production

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 the main pillar of control for most commercial crops.

In 1999, UK growers began to report the occurrence of CWR that could not be controlled adequately using propiconazole, which belongs to the DMI 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, which also belongs to the DMI group. Further outbreaks of CWR that were not controlled by either propiconazole or azoxystrobin (from the QoI group) were reported in 2000. Tests at the Central Science Laboratory (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. And since 2001, there has not been any systematic measurement of sensitivity within populations on commercial holdings.

Materials and Methods

Isolate collection

Isolates collected in the mid-1990s that were designated insensitive to either, or both azoxystrobin and propiconazole were available as frozen specimens: but these could not 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: routinely, infrequently and never.

Table 1. Fungicides available for control of Chrysanthemum white rust

		Crops		
Active	**Product**	**Protected**	**Outdoor**	**MOA[1]**
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. See Appendix for more description of the modes of action.

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 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 and their dates of application.

To date, isolates have been collected from holdings where problems controlling disease control have been reported (Table 2).

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^{o}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.

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

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 (Plate 1). 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. This method has been improved throughout the year, with the addition of better photographic images and further enhancements to the programme. The benefits are a substantially quicker time to process the images: which allows many hundreds of images to be measured quickly (Plate 1).

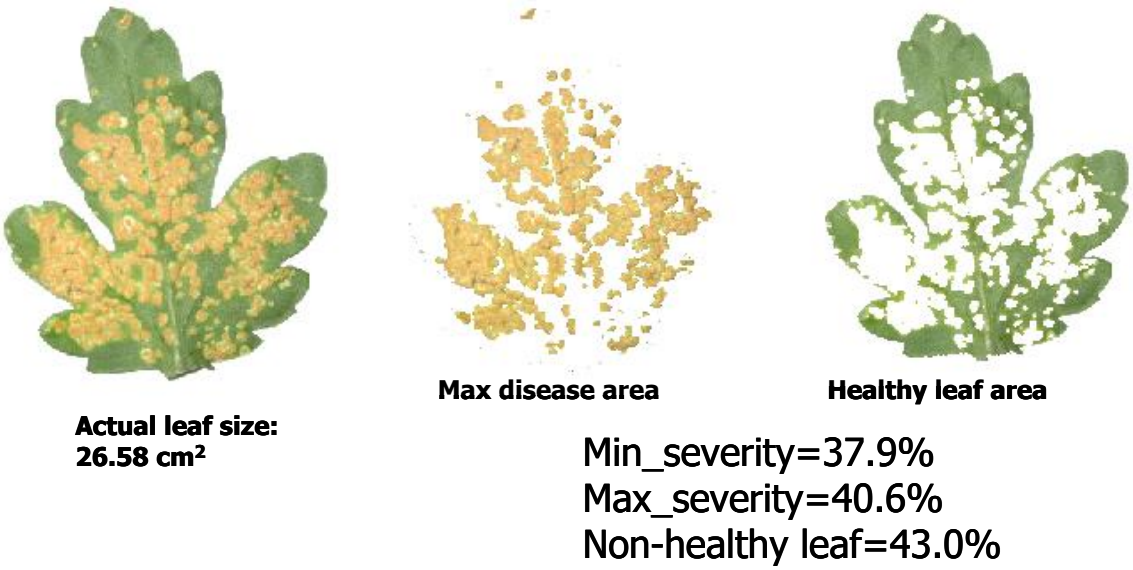

Plate 1. Photographs of leaves treated in experiments are decomposed to diseased and healthy areas and measured by images analysis

Quantitative sensitivity and efficacy testing

Following from the initial primary screening (2005-06), more detailed replicated experiments were completed. 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

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

	fungicide applied	
T+7	Inoculation 7 days before fungicide applied	Curative

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-7 and T-4 ;Table 3) and protectant (T0, T+4 and T+7; 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. Eighteen replicated experiments have been completed using different white rust isolates (Table 5).

Table 4. Fungicides tested for efficacy and their respective Modes of Action

Product	Active ingredient	Mode of action	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	epoxicanazole/pyraclostrobin	3	BASF
Opus	epoxicanazole	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

Table 5. Fungicides tested against different white rust isolates (refer to table 2 for further detail of the isolates tested)

Run Number	Treatment	Isolate	Dose
1	Control	3	
	Twist		2.0l/ha
	Signum		1.0l/ha
	Opera		1.5l/ha
	Proline		0.8l/ha
2	Control	3	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Citrox		0.3l/ha
3	Control	3	
	Bumper		0.4l/ha
	Plover		0.3l/ha
	Signum		1.0l/ha
	Rocket		0.1l/ha
4	Control	3	
	Signum		1.0l/ha
	Filan		0.5kg/ha
	Comet low rate		0.33l/ha
	Comet high rate		1.25l/ha
5	Control	3	
	Rhino		1.0l/ha
	Torch Extra		0.9l/ha
	Comet low rate		0.3l/ha
	Comet very low rate		0.05l/ha
6	Control	3	
	Comet high		1.0l/ha
	Comet medium		0.5l/ha
	Comet low rate		0.1l/ha
	Comet very low rate		0.01l/ha
7	Control	7	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Comet low rate		0.33l/ha
8	Control	5	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Comet low rate		0.33l/ha
9	Control	3	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Comet low rate		0.33l/ha
10	Control	6	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Comet low rate		0.33l/ha
11	Control	2	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Comet low rate		0.33l/ha
12	Control	4	
	Amistar		0.1l/ha
	Bumper		0.4l/ha
	Signum		1.0l/ha
	Comet low rate		0.33l/ha
13	Control	3	
	Shirlan		0.3l/ha
	Systhane		0.225l/ha
	Opus		1.0l/ha
	Bravo 500		0.22l/ha
14	Control	5	
	Proline 0.4		0.4l/ha
	Proline 0.2		0.2l/ha
	Proline 0.1		0.1l/ha
	Proline 0.05		0.05l/ha
15	Control	5	
	Comet high		1.0l/ha
	Comet medium		0.5l/ha
	Comet low rate		0.1l/ha
	Comet very low rate		0.01l/ha
16	Control	6	
	Comet high		1.0l/ha
	Comet medium		0.5l/ha
	Comet low rate		0.1l/ha
	Comet very low rate		0.01l/ha
17	Control	7	
	Comet high		1.0l/ha
	Comet medium		0.5l/ha
	Comet low rate		0.1l/ha
	Comet very low rate		0.01l/ha
18	Control	2	
	Comet high		1.0l/ha
	Comet medium		0.5l/ha
	Comet low rate		0.1l/ha
	Comet very low rate		0.01l/ha

Measurement of control

Crop safety

Laboratory experiments

In addition to the direct measures of disease control made in the laboratory experiments 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, 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	Bud colour show

Treatment rates and water volume

Each fungicide treatment was applied at the rates detailed by Table 7, which in most cases have been extrapolated from other protected crops where available, primarily lettuce and strawberry. The water rate did vary at each spray timing e.g. during the first application the water rate 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 to maintain the product concentration. 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 Programme:	azoxystrobin	1l/1000l water	Chrysanthemum
Amistar	azoxystrobin	1l/1000l water	
Amistar Bumper	propiconazole	0.4l/1000l water	
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 (pers comm. to GMM)

Although primarily a crop safety trial, attempts to establish white rust were be made to investigate the feasibility of establishing infection within the glasshouse for experiment planned later in the project. Plants infected with Isolate 3 (Table 2), 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 7-10 days after each application. If treatment differences are observed these will be recorded and appropriate measures of severity and incidence will be made along with photographic records. At the final assessment a more detailed assessment of any crop damage will be carried out, involving destructive sampling if necessary along with further quantitative and photographic records. A measurement of plant height was also made across treatments.

Results

Sensitivity and efficacy testing using replicated runs

A preliminary analysis to identify the most promising fungicides combined data from across 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 8 & 9). 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 benchmarks, Citrox, Filan, Plover and Rocket did not provide improved protectant efficacy (Tables 8 & 9). Under curative situations efficacy of Amistar and Bumper were similar (Table 10 & 11).

For the more powerful balanced ANOVA, testing 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 for the isolates tested.

Table 8. Protectant efficacy of fungicides tested against Bumper (BASF; a. i. propiconazole).

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

[1]symbol in parenthesis 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 (p > 0.067) is adjusted according to the Bonferroni correction to allow multiple comparisons.

Table 9. 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]symbol in parenthesis 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 (p > 0.067) is adjusted according to the Bonferroni correction to allow multiple comparisons.

Table 10. 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]symbol in parenthesis 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 (p > 0.067) is adjusted according to the Bonferroni correction to allow multiple comparisons.

Table 11. 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]symbol in parenthesis 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 (p > 0.067) 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)

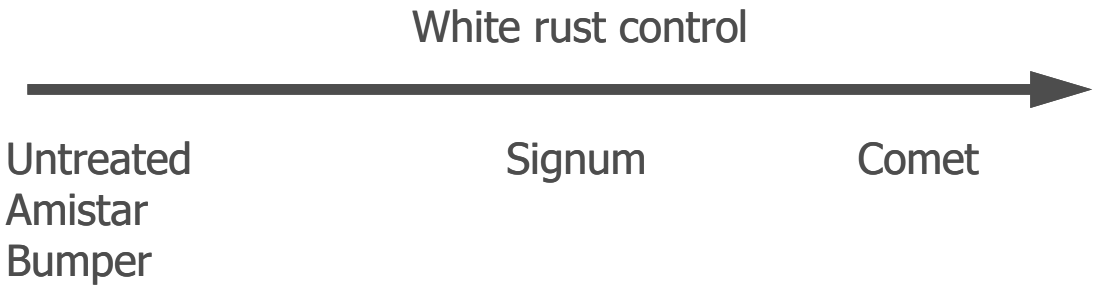

Figure 2. Curative performance of fungicides against white rust. Differences are measured using a balanced ANOVA design and are significant for position along the arrow (P<0.05)

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 1-4).

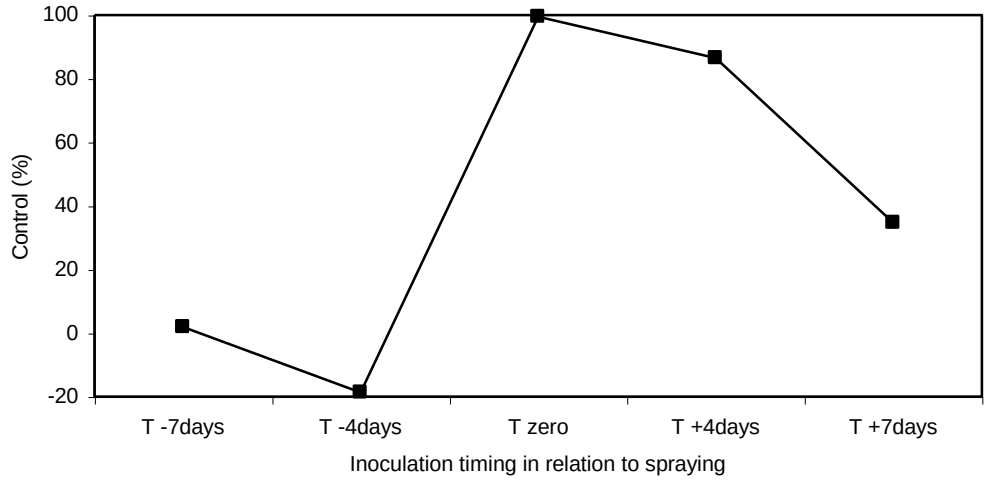

Figure 1. Percentage control measured from the mean of the first 3 leaves below the tag for isolate 3 treated with Amistar. Refer to Table 3 for explanation of spray timings

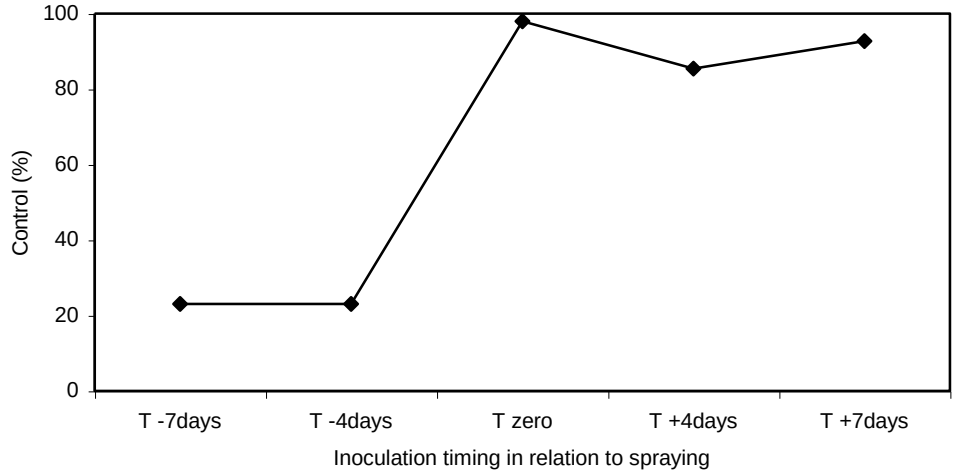

Figure 2. Percentage control measured from the mean of the first 3 leaves below the tag for isolate 6 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

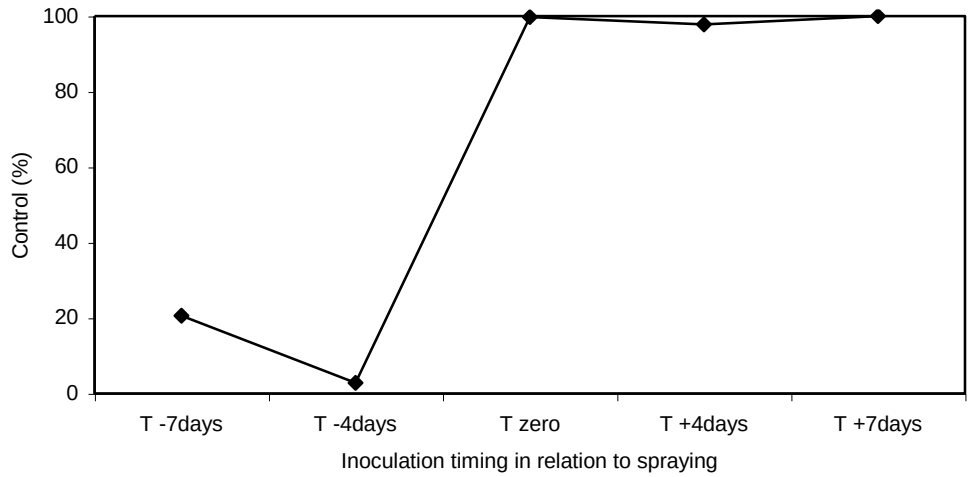

Figure 3. Percentage control measured from the mean of the first 3 leaves below the tag for isolate 2 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

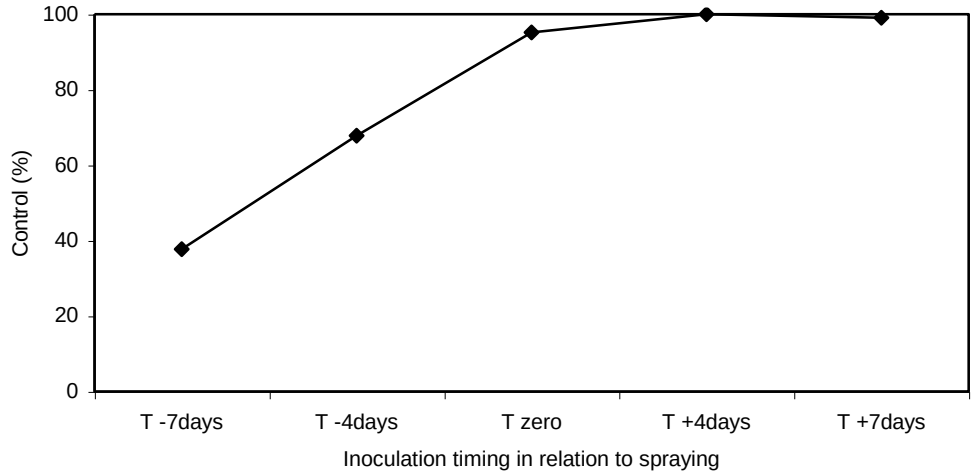

Figure 4. Percentage control measured from the mean of the first 3 leaves below the tag for isolate 4 treated with Amistar. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

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 valuable 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. Product residues were recorded from Signum, but not Comet (Plate 2).

Plate 2. Deposits From use of Signum (left) compared with from Comet (right)

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 before or after inoculation *i.e.,* under the most curative and protectant situations. This trend was repeated when using the very low rate of 0.01l ha^{-1} with disease occurring on the +/-4 day plants as well, highlighting a progressively narrower spray interval with a reduction in dose (Figures 7 & 8).

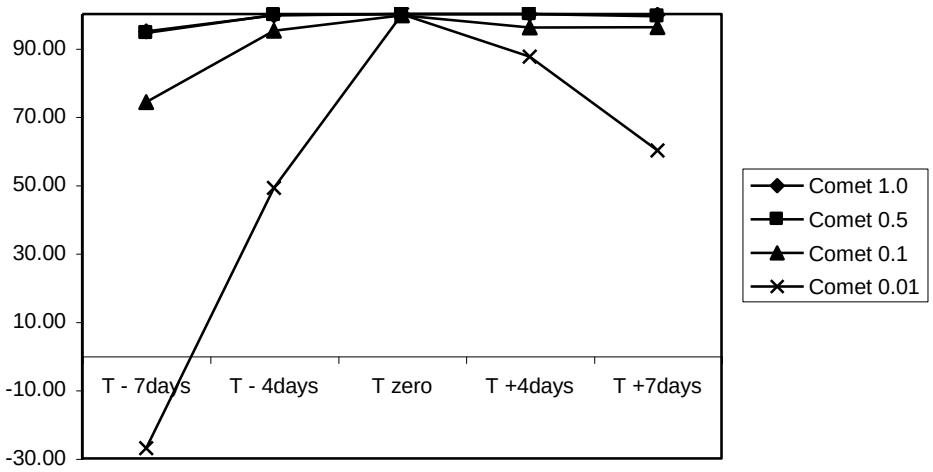

Figure 7. Percentage control measured from the mean of the first 3 leaves below the tag for isolate 3 treated with Comet. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

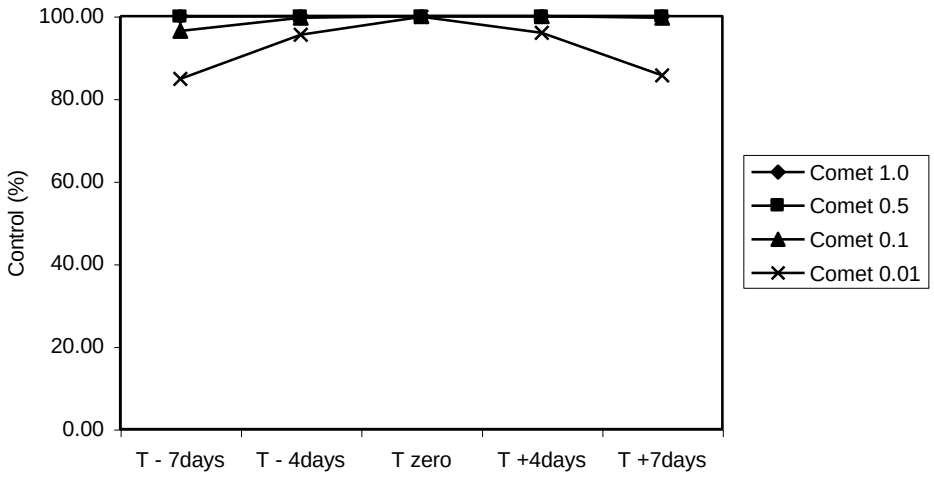

Figure 8. Percentage control measured from the mean of the first 3 leaves below the tag for isolate 4 treated with Comet. Points to the left and right of T zero measure curative and protectant control respectively. Refer to Table 3 for full explanation of spray timings

Proline (prothioconazole; Run 12) was tested at 4 rates (0.4, 0.2, 0.1, 0.05l/ha) and produced some control at the higher 2 rates although significant stunting in plant growth resulted (Plate 3). This growth regulatory effect was less marked in the low rates but disease severity remained high. No subsequent tests were made with this chemical on different isolates.

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

In run 13 Opus (epoxiconazole) and Bravo (chlorothalonil) provided good protectant control with Shirlan (fluazinam) somewhat less so. Systhane (myclobutanil) showed little difference when compared to the control. Despite good levels of control Opus had a similar stunting effect to that of Proline. Shirlan and Bravo did not produce any stunting, but following spraying after the leaves had dried some residues were seen. Table 12 summarises plant damage observed over the first two years of the study, in the laboratory-based efficacy tests.

Table 12. Damage to plants from chemicals tested during year 1 and 2 of the study

Product	Fungicide activity tested		
	Protectant	Curative	Damage
Proline	✔	✔	Slight stunting
Corbel	✔	✔	Stunting
Fandango	✔	✔	Stunting
Prosaro	✔	✔	Stunting
Sonata	✔	✔	Residues
Elvaron Multi	✔	✘	Residues
Folicur	✔	✘	Stunting

Crop safety

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 13. 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

Phytotoxic effects were recorded as presence/absence (Table 14). Damage to foliage ('scorching') was caused by Torch Extra (Plate 4).

Table 14. Presence/absence score

Treatment	Cultivar			
	Greenbird	Sunny Martin	Sunny Woodpecker	Universe
1.Untreated control	0	0	0	0
2.Comm programme	0	0	0	0
3.Signum 1N	0	0	0	0
4.Signum 2N	0	0	0	0
5.Filan 1N	0	0	0	0
6.Filan 2N	1	0	0	0
7.Comet 1N	0	0	0	0
8.Comet 2N	0	0	0	0
9.Rhino 1N	0	0	0	0
10.Rhino 2N	0	0	0	1
11.Torch Extra ½ N	1	1	1	1
12.Torch Extra 1N	1	1	1	1
13.Nativo 1N	0	0	0	0
14.Nativo 2N	0	0	0	0

Plate 4. Scorch symptoms caused by Torch protectant and curative performance

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) against a range of alternatives indicated that some fungicides provided improved control under both protectant and curative situations. During the period of this report, more complete analysis confirmed these preliminary conclusions and formed the basis for more comprehensive quantification of candidate fungicides (Tables 8-11). More critically, they also provided the basis for understanding the improved control apparent with use of Signum (BASF; pyraclstrobin + 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 to pyraclostrobin. These tests also indicated that this fungicide was effective even at very small doses.

In fully replicated experiments, 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 crop management, because timing fungicides ahead of infection becomes critical: established infections remain active, damage leaves and sporulate to release new inoculum.

Disease control from Signum was substantially better under both curative and protectant conditions, than achieved by either Bumper or Amistar. 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

suggest that some of these have associated crop effects; either stunting, scorching or the deposit of visible residues. These may preclude their use commercially due to the implications for crop safety and quality. Products that were identified to have particularly severe crop safety dangers were not investigated further, even when disease control appeared effective.

Conclusions

Curative treatment of chrysanthemum white rust is unlikely to be effective when propiconazole is the primary active ingredient used. Pyraclostrobin (Comet) was the most effective fungicide tested, providing good protectant, and excellent curative control. This product can be used on **outdoor crops** (under the Long Term Arrangements for Extension of use for non-edible crops from the approvals for edible crops), provided that all of the relevant statutory conditions for approval are observed. However, use of Comet is not permitted on protected crops. Currently pyraclostrobin is only approved for use on protected crops in mixture as product Signum (26.7%:6.7% w/w boscalid + pyraclostrobin). Fortunately, successful control is obtainable from use of Signum, which is consistent with the tests showing that small doses of pyraclostrobin (tested as Comet; BASF) were effective in both curative and protectant situations.

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.