Project title	Protected Chrysanthemum: Development of a fungicide strategy for the control of White rust.
Project number:	PC 292
Project leader:	Cathryn Lambourne, Stockbridge Technology Centre
Report:	Final report, January 2009
Previous report	None – 1 year project (Previous project PC 231)
Key staff:	Deborah Liddell Iwona Burdon
Location of project:	Stockbridge Technology Centre Cawood, Selby, N. Yorks YO8 3TZ
Project coordinator:	Colin Frampton, Donaldson's Flowers Vinnetrow Rd Chichester, West Sussex, PO20 1QD
Date project commenced:	July 2008
Date project completed (or expected completion date):	November 2008
Key words:	Chrysanthemum, *Dendranthemum morifolium*, protection, curative, efficacy, spray programmes, Reagan, Ruby Red, White rust, *Puccinia horiana*, fungicides, control, resistance, pesticides, disease.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Cathryn Lambourne
Project Leader
Stockbridge Technology Centre Ltd

Signature ..... Date .....

Report authorised by:

Dr Martin McPherson MBPR (Hort)
Science Director
Stockbridge Technology Centre Ltd

Signature ..... Date .....

CONTENTS

Grower Summary

Headline

- A glasshouse raised crop of a commercial cut flower Chrysanthemum species, cultivar Ruby Red Reagan, was established and successfully artificially infected with *Puccinia horiana* (white rust).

- The efficacy and crop safety of a total of 11 fungicide programmes was investigated during 1 growing season.

- The standard programme resulted in a 50% reduction in infection compared to the untreated control.

- Several alternative programmes were superior providing up to 94% control.

- The addition of a wetting agent should be approached with caution due to an increased risk of phytotoxicity.

- Caution is also needed with some of the products evaluated as they may leave visible spray deposits if applied close to harvest.

Background and expected deliverables

Chrysanthemum White Rust (CWR), caused by *Puccinia horiana*, is a major disease of Chrysanthemum, affecting both protected and outdoor crops. Disease pressure can be reduced by good crop hygiene and effective environmental management; however fungicides continue to provide the main pillar of control in commercial crops during periods of adverse weather.

Work carried out under the HDC funded project PC 231 investigated the reported claims of loss of sensitivity in CWR isolates to propiconazole (an industry standard). Small scale laboratory studies at the Central Science Laboratory (CSL) and glasshouse trials at STC resulted in the identification of alternative fungicides that could potentially be used commercially to control CWR.

This current project was designed to build on the knowledge gained during PC 231 to develop effective spray programmes to improve white rust control. It was hoped that this would increase the number of products available and reduce the risk of further resistance problems developing. All products selected for evaluation had operator safety data packages for use under protection so, in theory, it should be possible to use these products in protected chrysanthemums.[1]

[1] It must be noted that at the time of the project the Long Term Arrangements for Extensions of Use were still in place. However, these arrangements are currently being transferred to specific off-label approvals and these changes will need to be watched carefully.

Summary of the project and main conclusions

A crop of Chrysanthemum cv. Ruby Red Reagan was raised to a commercial standard in a 150m^2 glasshouse at STC. Plants were bought in as unrooted cuttings and propagated at STC prior to planting in mid-August 2008. Pots containing CWR infected plants of the same variety were provided by colleagues at CSL and were placed centrally in each plot approximately 2 weeks post-planting. Following a latent (symptomless) period, very high levels of CWR developed and the spray programmes commenced. A total of 6 spray applications were carried out (2 more than originally planned) prior to the final disease assessment being carried out in early November.

Treatment Programmes employed during the 2008 investigation

Treatment no.	Spray Number					
	1	2	3	4	5	6
1	Untreated (water control)	Untreated (water control)	Untreated (water control)	Untreated (water control)	Untreated (water control)	Untreated (water control)
2$^+$	Amistar	Bumper	Amistar	Bumper	Amistar	Bumper
3	Amistar + wetter	Bumper + wetter	Amistar + wetter	Bumper + wetter	Amistar + wetter	Bumper + wetter
4	Signum	Bumper	Signum	Bumper	Signum	Bumper
5	Nativo	Signum	Plover	Amistar	Nativo	Signum
6	Karamate	Karamate	Nativo	Nativo	Karamate+ Nativo*	Karamate+ Nativo*
7	Karamate	Systhane	Signum	Nativo	Systhane + Signum*	Systhane + Nativo*
8	Karamate	Switch	Signum	Nativo	Switch + Signum*	Switch + Nativo*
9	Guru	Signum	Nativo	Signum	Nativo + Signum*	Nativo + Signum*
10	Guru	Guru	Signum	Signum	Guru + Signum*	Guru + Signum*
11	Bravo	Signum	Nativo	Signum	Bravo + Nativo*	Bravo + Signum*
12	Bravo	Bravo	Signum	Signum	Bravo + Signum*	Bravo + Signum*

* tank mix at full rate + commercial programme (standard)

Three detailed disease assessments were carried out during the trial period. Disease pressure was very high across the trial providing a very stern test for the programmes under investigation. Good differences in the level of infection of the cut stems (60cm length) were observed. All of the spray programmes under investigation significantly reduced the level of CWR infection compared to the untreated control (Chart 1 & Fig 1). The standard commercial programme provided relatively poor control and resulted in only 57% control. Surprisingly, substitution of Amistar with Nativo and Plover (T5) did not provide any improvement in control of white rust. In contrast, the introduction of Signum into the programme improved rust control significantly (T4).

Chart 1. Mean rust infection/plant based on mean infection of 5 leaves taken along the marketable stem at the final disease assessment – 11th November 2008

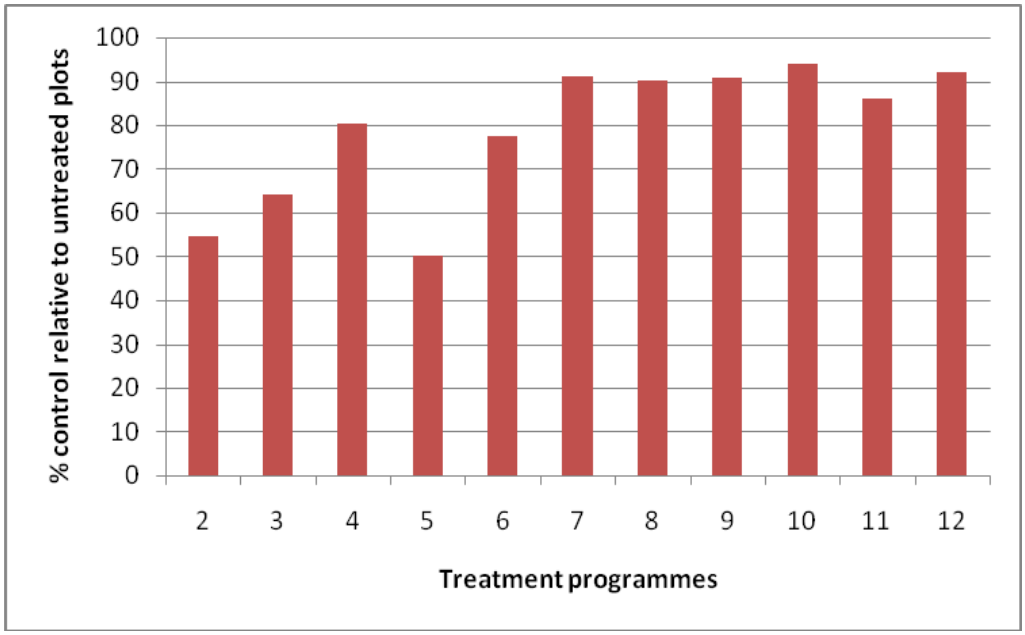


Figure 1. Comparison of white rust infection severity. Untreated plants in the foreground, treated plants in the background.

Spray programmes which included a combination of multi-site protectant fungicides and single site products (T7-T12) resulted in the lowest levels of CWR infection. T10 provided the best overall control (94%) during this trial.

The addition of the wetter to the standard fungicide programme (T3) improved control slightly though resulted in leaf distortion. It is not clear whether this was caused by the wetter itself, or simply by a reduction in leaf wax which subsequently made the plants more susceptible to later products.

Following application of the tank mixed products (for the last 2 spray applications), spray residue (just visible in Fig 1) was observed on T6, T8, T9, T10, T11 and T12. Application of the products at a lower rate may potentially provide a similar level of control without the resulting residue issue. Current advice would be to apply such products early in the spray programme reducing the rate the closer the crop is to harvest.

Financial benefits

It is anticipated that implementation of the spray programmes which showed the most promise in this year's trials would result in excellent financial benefits for Chrysanthemum growers in the UK. Products with both preventative and curative action have been chosen to ensure that growers can treat crops and produce a high quality disease free crop with excellent marketability and reduced losses.

Action points for growers

- Continue to implement strict crop hygiene regimes and manage the glasshouse environment to reduce the risk of CWR infection.
- Ensure young plants are free from white rust at delivery.[2]
- Monitor crops for early signs of infection e.g. leaf chlorosis and loss of plant vigour and individual pustules.
- Treat any outbreaks of CWR immediately.
- Develop a spray programme which starts with multi-site protective products reserving single-site curative fungicides for later in the programme.
- Aim to use a range of products with different modes of action (see FRAC codes) to try and reduce the risk of fungicide resistance in the pathogen.
- It must be noted that at the time of the project the Long Term Arrangements for Extensions of Use were still in place. However, these arrangements are currently being transferred to specific off-label approvals and these changes will need to be watched carefully.

[2] It might be worth considering the development of a molecular (PCR) test to ensure young plants do not have a latent infection.

Science Section

Introduction

Chrysanthemum white rust was first seen in Britain in 1963. During the following 25 years MAFF instigated a statutory eradication policy and this helped to slow the spread and severity of infections in the UK. However, the pathogen (*Puccinia horiana)* is now endemic and regular outbreaks on protected and outdoor Chrysanthemum (*Dendranthemum morifolium*) are common and can result in heavy losses if not adequately controlled. Commercial growers rely on good nursery hygiene, careful environmental management and a range of fungicides to control outbreaks and minimise losses.

White rust infections are characterised by the development of pale green indentations on the upper surface of the leaf, with corresponding raised buff or pink coloured pustules (teleutosori) on the under surface. The pustules are packed with large numbers of teleutospores which usually germinate *in situ* producing between 1 and 3 minute sporidia. Mature sporidia can be blown on wind currents or mechanically spread to infect other leaves, stems or flower bracts. The spores require leaf wetness, an RH of around 96% and temperatures of approximately 17°C to germinate and produce a new infection. Spore survival is greatly reduced by lower relative humidities. Survival time is reduced from approximately 1 hour to just 5 minutes at RH of 80%. Under optimum conditions a germinating spore can infect and release spores in as little as 7 to 10 days.

There are significant differences in varietal susceptibility to white rust whilst the fungus produces different 'races' with variable virulence. We now know that genetic changes in the fungus have led to reduced sensitivity to some fungicides, resulting in a lack of disease control for commercial growers. In 2001, the first report of strains of *P. horiana* tolerant to triazole and/or strobilurin fungicides was made (Cook 2001). It was also reported that in Europe there were strains resistant to carboxamide fungicides (Dirkse et al*, 1982*).

A previously funded HDC project (PC 231) was carried out by the Central Science Laboratory and Stockbridge Technology Centre to try and investigate more recent claims of tolerance to propiconazole (Bumper) and a general lack of control experienced by commercial growers. Small scale laboratory trials followed by larger glasshouse trials to investigate the crop safety and efficacy of a range of novel products were carried out during the 3 year investigation. A number of products were found to be significantly more effective at controlling white rust in preliminary screening and in glasshouse trials. However some of the products initially evaluated were experimental and not approved for use on protected crops and had only been trialled as single products. The emphasis of the work in this additional project was to only evaluate products with existing Operator Safety Data Packages and which by default could therefore be used in protected crops. The aim was also to develop effective commercial spray programmes utilising several different products and which could also help to reduce the risk of resistance developing.

Materials and methods

Unrooted cuttings of the Chrysanthemum variety Ruby Red Reagan were propagated at STC in July 2008. Once rooted the plants were planted through 10cm wire mesh into beds in a 150m^2 glasshouse (see Appendix 1 for trial plan). A total of 11 spray programmes, each representing a 'treatment' were included with 4 replicate plots of each treatment in a fully randomised design. Trickle-tape irrigation was used and the crop was subjected to additional lighting for 6 hours during the night for the first 3 weeks to encourage stem elongation. The crop was maintained at 18°C day and night, venting at 23°C. Two applications of the growth regulator daminozide (B-Nine) were made, the 1st 12 days after the lights were turned off, and the 2nd 7 days later.

Figure 1. General shot of glasshouse crop in 2008

Pathogen Inoculation

Two weeks post-planting, pots containing CWR infected cuttings of the same variety were placed in the centre of each plot. These plants were infected by staff at the Central Science Laboratory using the white rust isolate collected from Hampshire (Redhill) in 2006/7 which had been retained from work carried out for PC 231. Following 'inoculation' the crop was maintained and monitored daily. Overhead watering using a hand lance was carried out at the end of each day to ensure leaf wetness. Clear polythene was used to cover the crop each night (for approximately 10 days) to raise humidity and encourage CWR infection.

Treatments

The original spray programmes were developed to include only 4 spray applications at 10-14 day intervals, however due to high disease levels which necessitated reducing the interval between the spray applications to 6-7 days it became necessary to carry out an additional 2 applications to maintain cover throughout the crop duration (Table 1).

Table 1. Details of the spray programmes.

Treatment no.	Spray Number					
	1	2	3	4	5	6
1	Untreated (water control)	Untreated (water control)	Untreated (water control)	Untreated (water control)	Untreated (water control)	Untreated (water control)
2+	Amistar	Bumper	Amistar	Bumper	Amistar	Bumper
3	Amistar + wetter	Bumper + wetter	Amistar + wetter	Bumper + wetter	Amistar + wetter	Bumper + wetter
4	Signum	Bumper	Signum	Bumper	Signum	Bumper
5	Nativo	Signum	Plover	Amistar	Nativo	Signum
6	Karamate	Karamate	Nativo	Nativo	Karamate+ Nativo*	Karamate+ Nativo*
7	Karamate	Systhane	Signum	Nativo	Systhane + Signum*	Systhane + Nativo*
8	Karamate	Switch	Signum	Nativo	Switch + Signum*	Switch + Nativo*
9	Guru	Signum	Nativo	Signum	Nativo + Signum*	Nativo + Signum*
10	Guru	Guru	Signum	Signum	Guru + Signum*	Guru + Signum*
11	Bravo	Signum	Nativo	Signum	Bravo + Nativo*	Bravo + Signum*
12	Bravo	Bravo	Signum	Signum	Bravo + Signum*	Bravo + Signum*

* tank mix at full rate + industry standard programme

The products chosen were aimed at broadening the choice of both protectant and eradicant products, and are not all necessarily new to CWR control. It was hoped that this approach would reduce the risk of resistance. Where possible products were included which already had Operator Safety Data Packages (OSDP) which, in theory at least, permit use of the product under protection. Possible exceptions to this are found in mixed formulation products where individual components are approved for use under protection, but where the product itself is not. PSD have advised us that whilst it cannot be automatically assumed that the mixed product may be used under protection, applications for approval of such products would be evaluated on a case-by-case basis.

Treatment 3 which utilised the standard programme of alternating azoxystrobin (Amistar) and propiconazole (Bumper) also included the addition of a wetter (Activator 90) to investigate possible effects on the crop and disease.

Details of the active ingredients and application rates used are shown in Table 2.

Table 2. Details of the active ingredients and application rates used.

Product P= protectant C=curative	Active ingredient	Chemical group	FRAC code	Application rate (per litre)
Amistar (P)	azoxystrobin	strobilurin (QoI)	11	1ml
Bumper (P&C)	propiconazole	triazole	3	0.4ml
Signum (P)	pyraclostrobin & boscalid	strobilurin & carboxamide	11 & 7	1.5g
Nativo (P)	trifloxystrobin & tebuconazole	triazole & strobilurin	3 & 11	0.4g
Plover (P&C)	difenoconazole	triazole	3	1ml
Karamate (P)	mancozeb	dithiocarbamate	M3	1.8g
Systhane (P&C)	myclobutanil	triazole	3	0.3ml
Switch (P)	cyprodinil & fludioxinil	anilo-pyrimidine & phenylpyrrole	9 & 12	0.8ml
Guru (P)	chlorothalonil & mancozeb	chloronitrile & dithiocarbamate	M5 & M3	3.3ml
Bravo (P)	chlorothalonil	chloronitrile	M5	2.2ml
Activator 90	alcohol ethoxylates & fatty acids	non-ionic wetter	-	1ml

The fungicides were applied using an Oxford Precision Knapsack sprayer operating at a constant 2 bar pressure and using a 3 nozzle boom. The water volume was increased during the trial period being 1000 L/ha for the 1st application, when the plants were smaller, increasing to 1500 L/ha for the 2nd application, and again to 2000 L/ha for the 3rd spray, and 2500 L/ha for the 4th, 5th and 6th spray applications. However, the product concentration remained constant and this provided a 'worst case' scenario from a phytotoxicity perspective. The majority of the spray applications were carried out during the morning.

As the majority of the products used did not have a current label application rate for use on protected chrysanthemum, the rates used were taken from a commercial pesticide database and are based on either the rate for protected ornamentals, lettuce or strawberry.

A range of additional crop protection products (insecticides) were used as required during the trial period to control pests including caterpillar, aphid and thrip. These products were applied separately from the fungicide applications e.g. not tank mixed.

Crop Diary

30.7.08	Cuttings arrived and planted into 104 module trays. Trays placed under milky polythene with a mister system and maintained at approx 20°C.
6.8.08	48 x 30cm pots of cuttings potted (3/pot) and transferred to CSL for inoculation (infector plants). Polythene removed from crop and Rovral spray applied to control early *Botrytis* infection on lower leaves.
15.8.08	Rooted cuttings planted through wire netting into glasshouse FF6. Lights programmed to be on between 2100 and 0300hrs.
27.8.08	Infector plants introduced. 1 pot in the centre of each plot. Crop foliage to be wet each evening and polythene covers pulled over and removed each morning until infection starts.
5.9.08	Additional night break lighting turned off.
11.9.08	Dynamec application to control thrip.
15.9.08	1st Disease assessment carried out. 1st spray application made.
17.9.08	Application of B-nine growth regulator to crop.
24.9.08	2nd application of B-nine carried out.
25.9.08	2nd trial spray application carried out.
30.9.08	Toppel 100 EC applied to crop. Some leaf distortion noticed in certain plots.
6.10.08	3rd trial spray application carried out. Leaf distortion noted in T3 plots.
8.10.08	2nd full disease assessment carried out.
16.10.08	4th spray application carried out.
21.10.08	Toppel 100 EC applied to crop.
22.10.08	5th spray application carried out.
30.10.08	6th spray application carried out.
4.11.08	Chess applied to crop.
6-11.11.08	Final destructive disease assessment carried out.

Disease assessments

Three detailed disease assessments were carried out in the crop. The first was carried out prior to the first fungicide application on 15th September. During this assessment 10 plants/plot were examined and the severity of the white rust infection on a leaf at mid-stem and the 3rd leaf from the top of the plants was recorded using the 0-5 severity scale detailed below. The 2nd disease assessment was carried out on the 8th October when 3 (out of a total of 6) spray applications had been carried out, although the 3rd spray had only been applied 2 days prior to the assessment. The same severity scale was used to measure the level of infection on the 3rd, 5th and 7th leaf from the top of the plant. A total of 20 random plants/plot were assessed.

0-5 White Rust Severity Scale

0 = No infection
1 = 1-10 pustules per leaf
2 – 11-20 pustules per leaf
3 = 21-50 pustules per leaf
4 = 51-100 pustules per leaf
5 = >100 pustules per leaf

The final, destructive disease assessment was carried out between the 6th and 11th November. During the final assessment 20 random stems were cut from each plot. The total plant height was recorded after which time the stems were trimmed to 60cm. The total number of leaves, and the number of healthy (pustule free) leaves were scored. The number of pustules on a bottom, middle and 3 upper leaves (3rd, 5th & 7th leaf from flower) were also recorded. Symptoms of phytotoxicity were also noted.

The assessment data recorded during the 1st and 2nd assessment was converted from a 0-5 scale to a 0-100 index using the following formula:

$$\frac{0(0) + 1(1) + 2(2) + 3(3) + 4(4) + 5(5)}{\text{Total no. of values}} \times \frac{100}{5}$$

Statistical Analysis

Data from the trial was subjected to analysis using ARM trial management software.

Official Recognition

The study was undertaken in accordance with the guidelines for Official Recognition of Efficacy Testing Organisations.

Certificate No.	204
Effective Date of Issue:	1 June 2006
Expiry Date	31 March 2011

Archiving

A copy of the final report and the raw data pertaining to the study will be archived for a minimum period of 5 years in the designated archive at Stockbridge Technology Centre Ltd.

Results

The cuttings responded well during propagation producing a good root ball after 10 days. Low levels of *Botrytis* were observed on the cuttings during the first week in August on the lowest leaves, and this was controlled by fungicide application prior to planting the crop.

The crop was planted on the 15th August and established well. Following introduction of the infector plants the crop was monitored daily for signs of early infection. The first lesions were observed on Friday 12th September. A full disease assessment was carried out on 15th September prior the 1st fungicide application and employed the 0-5 severity scale shown in Materials & methods (Table 3). The visible evidence of CWR infection had increased dramatically over the weekend period suggesting that conditions in the previous 10-14 days had been optimum for spore release and infection of the crop plants.

Table 3. Results of the 1st disease assessment on 15th September 2008

Treatment programme	Mean disease severity on mid-stem leaf (0-100 severity index)	Mean disease severity on 3rd leaf from top of plant (0-100 severity index)
1. Untreated	93.5 a	3.0 a
2. Amistar/Bumper/Amistar/Bumper (std)	83.0 a	4.5 a
3. As T2 + wetter (Activator 90)	84.5 a	5.0 a
4. Signum/Bumper/Signum/Bumper	88.0 a	5.5 a
5. Nativo/Signum/Plover/Amistar	91.5 a	1.0 a
6. Karamate/Karamate/Nativo/Nativo	90.5 a	3.5 a
7. Karamate/Systhane/Signum/Nativo	93.0 a	2.5 a
8. Karamate/Switch/Signum/Nativo	86.0 a	5.5 a
9. Guru/Signum/Nativo/Signum	86.5 a	5.5 a
10. Guru/Guru/Signum/Signum	86.5 a	1.0 a
11. Bravo/Signum/Nativo/Signum	91.5 a	4.5 a
12. Bravo/Bravo/Signum/Signum	90.0 a	7.0 a
LSD (P=0.05)	17.8	5.0
Standard Deviation	12.3	3.5
CV	13.89	85.5

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)
Treatment list shows only the 4 initially planned applications due to space restraints.

Levels of infection were very high on the leaves mid-way up the stem. Markedly lower levels of infection were observed on the leaves higher up the plants as might be expected. Disease pressure was similar across all plots in the trial demonstrating an even infection throughout. The first fungicide application was carried out on the same day with the aim of limiting the development of pustules on the upper parts of the stems e.g. the cut stem for marketing.

The 2nd disease assessment was carried out on the 8th October. During this assessment the same 0-5 disease severity scoring scale was used as at the 1st assessment with the 3rd, 5th and 7th leaf from the top of the plant being assessed on 20 plants/plot (Table 4 & Chart 1).

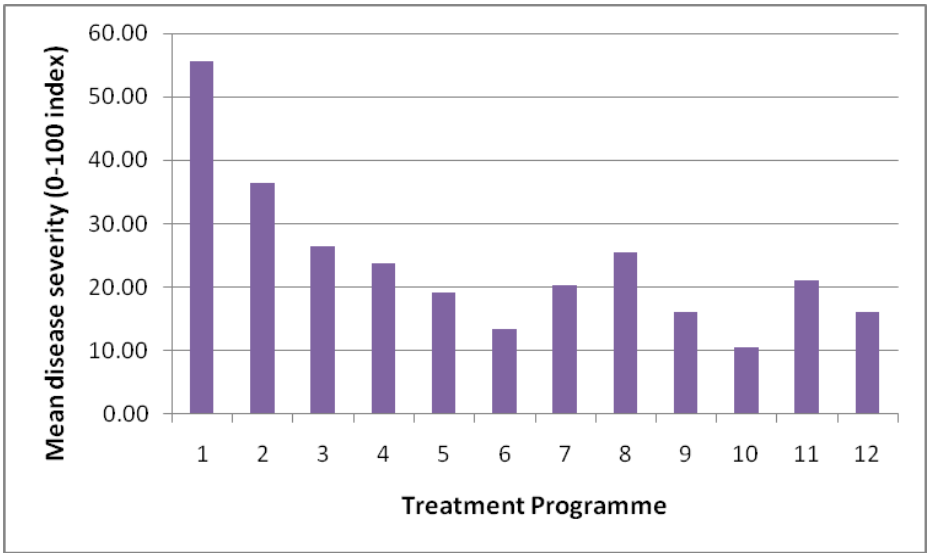
Table 4. Results of the 2nd disease assessment on 8th October 2008

Treatment programme	Mean disease severity on 3rd leaf (0-100 severity index)	Mean disease severity on 5th leaf (0-100 severity index)	Mean disease severity on 7th leaf (0-100 severity index)
1. Untreated	24.5 a	83.0 a	59.2 a
2. Amistar/Bumper/Amistar/Bumper (std)	18.5 ab	58.0 b	33.0 b
3. As T2 + wetter (Activator 90)	2.5 bc	40.7 bc	36.0 b
4. Signum/Bumper/Signum/Bumper	2.2 bc	32.7 bc	36.2 b
5. Nativo/Signum/Plover/Amistar	5.0 bc	39.0 bc	13.7 b
6. Karamate/Karamate/Nativo/Nativo	6.2 bc	18.2 c	15.7 b
7. Karamate/Systhane/Signum/Nativo	3.2 bc	30.2 bc	27.5 b
8. Karamate/Switch/Signum/Nativo	8.5 bc	40.2 bc	27.5 b
9. Guru/Signum/Nativo/Signum	9.2 bc	27.0 c	12.0 b
10. Guru/Guru/Signum/Signum	0.7 c	11.5 c	19.2 b
11. Bravo/Signum/Nativo/Signum	6.5 bc	40.5 bc	16.0 b
12. Bravo/Bravo/Signum/Signum	2.7 bc	17.0 c	28.7b
LSD (P=0.05)	10.3	19.3	16.9
Standard Deviation	7.1	13.4	11.7
CV	94.82	36.6	43.30

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)
Treatment list shows only the 4 initially planned applications due to space restraints.

By the 2nd assessment (following 3 fungicide applications, although 3rd application only carried out 2 days prior to the assessment) clear treatment effects could be seen. The majority of the applied fungicide programmes had significantly reduced the severity of the CWR infection on the leaves assessed when compared to the untreated control plots. The only exceptions to this were the standard programme (T2) where disease severity on the 3rd leaf was only slightly less than that recorded on the untreated plants. Although the standard programme (T2) and the standard programme + wetter (T3) did result in significantly lower levels of disease compared to the control, the mean disease severity was markedly higher in these two treatments than that observed in the other treatments. Treatment 10 (to which Guru/Guru/Signum had been applied at this time) showed the lowest level of CWR infection (Fig 2).

Chart 1. Mean CWR severity* on 8th October 2008



* Mean of 3 leaves/plant on 20 plants/plot

The data shows a higher disease severity on the 5th leaf from the top of the plant than on the leaves either side. The initial white rust infection created a 'band' effect on the plants with alternating bands of heavy and lighter infection. This is thought in part to be due to variable leaf susceptibility and also to spore release and germination events in the crop along with the fungicide effects.

Figure 2. Comparison of disease severity between untreated plants (left) and those treated with 2 applications of chlorothalonil + mancozeb, and 1 application of pyraclostrobin + boscalid (T10) (right).

Following the first 2 spray applications leaf distortion was observed in many of the plants treated with the standard programme + wetter (Activator 90) (Fig 3). It is not entirely clear whether this was caused by application of the wetter itself or indirectly due to the removal of the leaf waxes which made the foliage more sensitive to the other products applied (Amistar & Bumper).

Figure 3. Leaf distortion on plants treated with standard programme + wetter

Figure 4. Chemical residue on leaves

The final disease assessment was carried out between the 6th and 11th November (full treatment blocks were assessed on each date). During the assessment 20 random stems were cut from each plot. The total height of the stems was recorded, prior to cutting the stem to a 60cm length (commercial practice for marketing). The total number of leaves was recorded along with the number of those leaves which were infected with CWR. A number of leaves were then scored for the percentage of the leaf area affected by CWR. These were a leaf close to the base, one in the middle of the stem and the 3rd, 5th and 7th leaf from the top of the plant (Table 5).

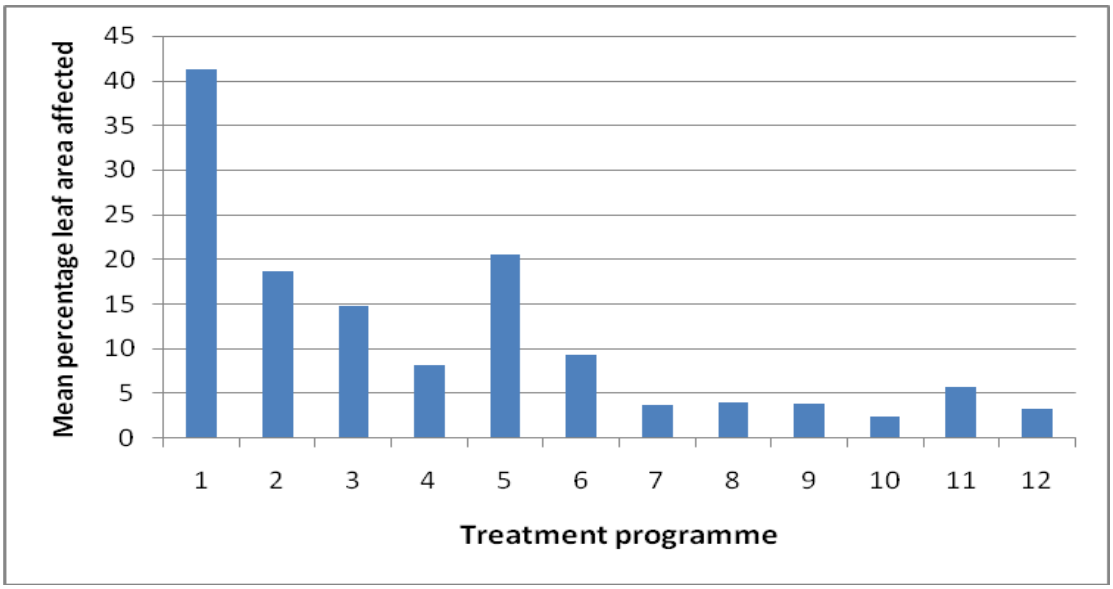
A note was also made of any phytotoxicity symptoms e.g. leaf distortion, scorching etc. and also of any chemical residue present on the leaves. This last parameter was included as residue had been observed on the leaves of plants where tank mixes of fungicide products had been applied as the final additional sprays (Fig 4).
Residue was present on the plants in plots treated with programmes 6, 8, 10, 11 and 12 and to a lesser extent in 9. Plants receiving the Systhane and Signum tank mix (T7) did not show evidence of residue on the leaves. This aspect has potential implications for commercial use, although in a normal commercial cropping situation it is highly unlikely that disease pressure would ever reach the levels which developed on the untreated plots in our trials and the need for additional tank mix sprays is highly unlikely. It is also possible that a reduced rate of each of the tank mix products may have been effective and may not have resulted in spray deposits on the foliage.

Table 5. Final disease assessment data during the period 6-11th November 2008

Treatment programme	Mean plant height (cm)	Percentage of infected leaves on 60cm stem	Mean percentage leaf area affected with CWR				
			Basal leaf (60cm stem)	Mid-point leaf (60 cm stem)	7th leaf from flower	5th leaf from flower	3rd leaf from flower
1. Untreated	102.3ab	94.8	5.3a	20.6a	53.2a	59.5a	67.4a
2. Amistar/Bumper/Amistar/Bumper (std)	95.5b	88.1	6.5a	6.1a	36.2b	20.9c	24.1c
3. As above + wetter	98.1ab	85.6	4.4a	12.8a	25.2c	16.2cd	15.3cd
4. Signum/Bumper/Signum/Bumper	99.7ab	77.4	4.5a	3.6a	9.7de	9.1cd	14.0cd
5. Nativo/Signum/Plover/Amistar	103.7ab	74.1	1.4a	8.5a	21.7cd	31.5b	39.8b
6. Karamate/Karamate/Nativo/Nativo	105.4ab	82.8	3.4a	4.0a	14.0cde	12.2cd	12.9cd
7. Karamate/Systhane/Sig/Nativo	104.4ab	76.1	2.6a	3.3a	3.7e	3.9d	5.0d
8. Karamate/Switch/Signum/Nativo	102.7ab	75.7	3.8a	4.8a	3.5e	3.5d	4.5d
9. Guru/Signum/Nativo/Signum	102.4ab	73.1	4.7a	5.5a	5.8e	1.9d	1.3d
10. Guru/Guru/Signum/Signum	107.8a	65.5	1.6a	1.9a	1.2e	3.4d	4.1d
11. Bravo/Signum/Nativo/Signum	102.4ab	75.5	6.8a	5.8a	9.8de	4.0d	2.6d
12. Bravo/Bravo/Signum/Signum	102.8ab	72.2	3.4a	3.0a	3.1e	2.7d	4.1d
LSD (P=0.05)	6.01	-	5.57	11.01	9.81	9.51	11.28
Standard Deviation	4.16	-	3.86	7.63	6.80	6.59	7.82
CV	4.07	-	95.45	114.49	43.56	46.8	48.10

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)
Treatment list shows only the 4 initially planned applications due to space restraints.

Chart 2. Mean percentage leaf area affected* on 11th Nov 08



* Mean leaf area infection based on assessment of 5 leave/plant on 20 plants/plot

No significant differences in plant heights were recorded across the majority of the treatments when compared to the untreated control plants. This suggests that none of the applied products adversely affected the plant growth. The plants in T10 were significantly taller than those treated with the standard treatment (T2) and this is likely to be linked to disease severity in the T2 plants reducing plant vigour.

The number of infected leaves/plant was high throughout (shown as the percentage of the total no. of leaves/60 cm stem in Table 5). However the count did include leaves with only 1-2 pustules. The largest percentage of disease free leaves was recorded on the stems which received spray programme 10.

The leaf area assessment data shown in Table 5 suggests that there were relatively low levels of infection on the basal leaves of the stems assessed in all treatments. This is somewhat misleading as many of the stems assessed had dead leaves at the base where it was not possible to determine what the level of CWR infection had been earlier. The number of dead leaves/stem was recorded and on average 40 leaves were dead/treatment (across 4 replicates). Higher than average (~ 60) leaves were dead on the T2 (std) plants and also in the T9 plants, whilst only 9 leaves were dead in the plants which received spray programme 10. This suggests that, to some extent, leaf death was linked to high rust severity, but that other factors may also have played a part.

High levels of variability within and between plots led to the data for the mid-point leaf being somewhat inconsistent. As a result, whilst there were quite large differences between the average treatment values, there were no significant differences recorded. The recorded values for leaf area affected on the 3rd, 5th and 7th leaf provided a somewhat clearer picture of disease severity in the trial, although it should also be noted that the leaf distortion observed on the plants treated with the standard programme + wetter (T3) did impact on the disease development and severity scoring in a negative way i.e. affected leaves were smaller and so distorted that disease scoring was not easily achieved with the same degree of accuracy as on the non-distorted leaves.

All of the fungicide programmes significantly reduced the severity of infection compared to the untreated control plants (Fig 5 and Chart 2). Examination of the mean disease severity across the assessed leaves showed that treatments 7-12 have resulted in the lowest levels of infection in the trial, with T10 providing the most effective control overall. As discussed earlier, no plants were completely free of CWR in this trial as disease pressure was extremely high from the outset and this provided a severe challenge for any fungicide programme. Figures 6–17 provide images of the harvested plants in each treatment.

Figure 5. Low levels of infection (T8, left) next to untreated plants (right)

Figure 6. Untreated control

Figure 7. Treatment 2 (Am/Bump/Am/Bump/Am/Bump)

Figure 8. Treatment 3 (As T2 + wetter)

Figure 9. Treatment 4 (Sig/Bump/Sig/Bump/Sig/Bump)

Figure 10. Treatment 5 (Nat/Sig/Plov/Am/Nat/Sig

Figure 11. Treatment 6 (Kar/Kar/Nat/Nat/K+T/K+T)

Figure 12. Treatment 7 (Kar/Sys/Sig/Nat/Sys+Sig/Sys+Nat)

Figure 13. Treatment 8 (Kar/Swch/Sig/Nat/Sw+Sig/Sw+Nat)

Figure 14. Treatment 9 (Gu/Sig/Nat/Sig/Nat+Sig/Nat+Sig)

Treatment 15. Treatment 10 (Gu/Gu/Sig/Sig/Gu+Sig/Gu+Sig)

Figure 16. Treatment 11 (Bra/Sig/Nat/Sig/Bra+Nat/Bra+Sig)

Figure 17. Treatment 12 (Bra/Bra/Sig/Sig/Bra+Sig/Bra+Sig)

Discussion

With exceptionally high levels of white rust infection to contend with from the outset this 1 year study certainly provided a very stern test for the products included in the spray programmes. All of the programmes significantly reduced the severity of infection, including the industry standard programme of alternating strobilurin (Amistar) and propiconazole (Bumper). The addition of a wetter (Activator 90) to the standard programme (T3) whilst improving control slightly resulted in distortion of the upper leaves and thus rendered the cut stems unmarketable. Without further testing it is not possible to be certain whether this phytotoxicity effect was caused by the wetter itself, or whether the wetter activity on the leaf wax left the leaves more vulnerable to the effects of the other products which were applied subsequently.

Due to the design of the spray programmes it is possible to measure the changes in efficacy resulting from quite subtle changes to the product selection. Treatment 4 consisted of an alternating programme of pyraclostrobin+boscalid (Signum) with propiconazole (Bumper), and was therefore the same as the standard with a substitution of Signum for Amistar. A comparison of the percentage control relative to the untreated plants (Table 6) indicates that this substitution increased the level of control from 54% to 80% and demonstrates that Signum is a very effective product for CWR control.

Table 6. Mean leaf area affected with CWR during final disease assessment 6-11.11.08

Treatment programme	% control compared to untreated control*
1. Untreated control	-
2. Am/Bump/Am/Bump/Am/Bump	54.5
3. As above + wetter	64.1
4. Sig/Bump/Sig/Bump/Sig/Bump	80.1
5. Nat/Sig/Plov/Am/Nat/Sig	50.0
6. Kar/Kar/Nat/Nat/Kar+Nat/Kar+Nat	77.4
7. Kar/Sys/Sig/Nat/Sys+Sig/Sys+Nat	91.0
8. Kar/Swch/Sig/Nat/Swch+Sig/Swch+Nat	90.2
9. Guru/Sig/Nat/Sig/Nat+Sig/Nat+Sig	90.7
10. Guru/Guru/Sig/Sig/Gu+Sig/Gu+Sig	94.1
11. Bra/Sig/Nat/Sig/Bra+Nat/Bra+Sig	85.9
12. Bra/Bra/Sig/Sig/Bra+Sig/Bra+Sig	92.1

* average of the 5 assessed leaves

Treatment 5 was the least effective programme at controlling CWR in this trial and this may have been due to the use of difenoconazole (Plover) and azoxystrobin (Amistar) as the middle two sprays in the programme, as the remaining products used (Nativo and Signum) appear to have been effective when used in other programmes in this trial e.g. T7, T9 & T11.

Treatment 6 relied on mancozeb (Karamate) and tebuconazole + trifloxystrobin (Nativo). These products have provided a reasonable level of control, however in T7, the 2nd and 3rd spray were substituted with myclobutanil (Systhane) and pyraclostrobin+boscalid (Signum) respectively, and again as tank mixes during the final 2 applications, and this has increased the level of control from 77% to 91%.

Treatment 8 is identical to T7 with the substitution of cyprodinil + fludioxinil (Switch) for myclobutanil (Systhane). The percentage leaf infection was very similar across the two treatments suggesting that Systhane provides a similar level of CWR control as Signum. This programme may provide a good resistance strategy with the products covering 6 different FRAC codings.

Treatments 9-12 can be grouped having just one product substitution. T9 and T10 include chlorothalonil + mancozeb (Guru) whilst T11 and T12 employ a chlorothalonil only product (Bravo). All of the programmes have provided an excellent level of control of the pathogen, with T9 and T10 being slightly more effective than T11 and 12. This suggests that the addition of mancozeb is certainly worthwhile. T10 which consists of only 2 products (Guru & Signum) but which contains 4 active ingredients with different FRAC codes provided the best level of disease control overall in this experiment.

The most effective programmes in the trial are those where a full-rate tank mix of two products were employed at the end of the trial. However, many of these tank mixes did leave a spray residue on the foliage and further work will be required to determine if reduced application rates may be equally effective under conditions of lower disease pressure.

Conclusions

This trial carried out in a single growing season has provided some excellent data for improved control of white rust on protected chrysanthemum crops. The products chosen for the trial were used to build 11 spray programmes which could be compared and potentially used commercially. They should also provide a clear indication of their efficacy both as a programme and to a limited extent as component sprays within each programme. We were also able to make some judgements regarding the crop safety of the products applied alone, or in the case of the later additional sprays, when applied as full rate tank mixes e.g. leaf damage, scorch and effects on plant height.

All of the spray programmes significantly reduced the level of white rust infection in the trial despite very high levels of infection at the trial outset. Due to the high disease pressure the interval between the sprays was reduced and this necessitated 2 additional sprays to ensure full cover until the plants were ready to harvest in November.

A slight improvement in the level of control provided by the standard products (Amistar & Bumper) was seen following the addition of the wetter, however quite severe distortion of the upper leaves was observed on these plants which would make the stems unmarketable in a commercial environment. It is not clear whether the same effect would result with other wetter or adjuvant products.

Spray programmes 7 – 12 resulted in the excellent control of CWR in this trial, with T10 providing the most effective overall control. No plants in the trial were completely free of white rust pustules, but it must be remembered that there were constant high levels of inoculum in the glasshouse due to the need to include untreated control plots and untreated buffer rows between each treated plot. In a commercial setting protectant or eradicant products would be applied over large areas and the inoculum load would be minimised dramatically. We would therefore expect the level of control provided by the most effective treatments to be increased when used commercially.

Finally, it must be remembered that pesticide legislation changes frequently and since the start of this work there have been 2 significant changes. Firstly, the LTAEU are being phased out and replaced by SOLAs and these are likely to be required for some of the products evaluated here. Secondly, the recent decision in the EU to switch from scientific risk assessment to hazard triggers for pesticide authorisation will put some active substances here under considerable threat of revocation e.g. mancozeb and this will need to be considered as soon as possible, once the criteria for the various hazard triggers has been clearly defined.

Technology transfer

The trial was visited by the project co-ordinator on the 7th October 2008.

References

Cook R T A, 2001. First report in England of changes in the susceptibility of Puccinia horiana, the cause of chrysanthemum white rust, to triazole and strobilurin fungicides. BSPP, July 2001.

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

Appendix 1 – Trial Plan