Project title:	Chrysanthemum White Rust: Estimation of prevalence of strains of *Puccinia horiana* on chrysanthemum tolerant to strobilurin and triazole fungicides and the development of a protocol for identifying tolerant strains.
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Headline

Fungicide tolerance to strobilurin (eg. Amistar) and triazole fungicides (eg. Tilt or Bumper), in *Pucciana horiana*, the cause of chrysanthemum white rust (CWR) disease, has been detected in the UK.

If fungicide tolerance is suspected, growers and crop advisors are recommended to send a sample to the Central Science Laboratory at York, of at least 20-30 leaves containing healthy, white, viable pustules sampled at the first sign of disease outbreak and if possible before fungicide application. Fungicide tolerance testing for CWR will take about 5 weeks to complete. More strategic research is needed before a rapid test can be developed.

Background & objectives

For the past twenty years, chrysanthemum white rust (CWR), caused by the fungus *Pucciana horiana*, has been effectively controlled by triazole fungicides, e.g. propiconazole (as Tilt), and more recently with strobilurin fungicides, e.g. azoxystrobin (as Amistar). However in 1999, some growers reported a loss of fungicidal control, which the Central Science Laboratory (CSL) confirmed was due to the development of fungicide tolerance (resistance). The following year, strains of *P. horiana* tolerant to both propiconazole (as Tilt or as its replacement Bumper) and azoxystrobin (as Amistar) were identified by CSL.

Currently, tests determining fungicide sensitivity (susceptibility/tolerance) take 5 weeks using an *in planta* test on artificially infected plants. This is because the fungus cannot be cultured outside host tissue. A more rapid assay to identify tolerant strains of CWR is highly desirable so that growers can be informed quickly about the classification of their outbreak. This would then enable informed selection of the most appropriate control measures.

Recent research has identified genes responsible for strobilurin and triazole tolerance in other fungi. This project was set up to investigate the prevalence of fungicide tolerance to both strobilurin and triazole fungicides, and whether this tolerance in *P. horiana* is controlled by the same genes responsible for tolerance in other fungi. If this was confirmed, a molecular test for these gene markers could be produced to provide a rapid fungicide tolerance screening service for chrysanthemum white rust for use by the industry.

Specific Objectives

1. Assess the extent of CWR fungicide tolerance in England and Wales and to identify sources of test isolates by way of a grower questionnaire.
2. Investigate whether strobilurin tolerance in CWR is caused by the same gene as in certain other fungi, enabling the development of a molecular diagnostic protocol.
3. Investigate whether triazole tolerance in CWR is caused by the same gene as in other fungi, enabling the development of a molecular diagnostic protocol.
4. If CWR fungicide tolerance has the same genetic basis as described for other fungi, use this to develop diagnostic tests for tolerant strains.

Summary of the project and main conclusions

The prevalence of fungicide tolerant isolates of chrysanthemum white rust (CWR)

A questionnaire was produced and sent to 220 HDC growers in August/September 2001, asking whether they had experienced chrysanthemum white rust (CWR) over the last two seasons and, if so, what control measures were used and how successful they were. Thirty-three questionnaires (15%) were returned. Twenty four of these growers, covering an area of 24 ha, reported CWR outbreaks over the survey period (2000-2001) and 22 were asked to send in samples for *in planta* fungicide testing.

Growers reported the occurrence of CWR infection throughout the year (see Table 1). Three high level outbreaks (>50%) were reported during the period January - August 2000. During the autumn period September - December 2000, more outbreaks were reported with an increase in the total area affected, but only one outbreak affected >50% of production area. Generally, fewer outbreaks occurred during the period January - July 2001 with diminishing % areas affected on each site. From August 2001 onwards, even though there were outbreaks on 12 sites, the total production area affected was much reduced (8,206m^2 v. 27,073m^2 the previous autumn (2000)) and the infection areas on 11 of the sites were 20% or below. Only one site had an increase in the overall area affected (>50%).

Whilst it is difficult to draw detailed conclusions from this data set, the main trends indicate that more severe and extensive CWR outbreaks occurred during 2000 than in 2001, especially in autumn 2000. The majority of growers appeared to achieve reasonable control of CWR, especially those that were able to bring severe infections under control, although others appeared to have persistent problems.

Table 1. Degrees of site infection (% category) relative to survey periods

% area of sites affected	Number of sites infected with CWR out of the 33 sites surveyed			
	Jan-Aug 2000	Sept-Oct 2000	Jan-July 2001	Aug- 2001
1-10%	3	5	4	4
11-20%	4	4	2	5
21-50%	0	3	2	0
>50%	3	1	0	1

Of the 24 sites where CWR was observed, 5 growers suspected poor environmental conditions were responsible for failure to control CWR, 4 believed fungicide tolerance was the reason for poor control and 8 suspected that both factors were involved. Five growers did not suspect either fungicide tolerance or poor environmental control, and 2 growers gave insufficient information for analysis.

Twelve surveyed growers submitted 24 samples for *in planta* testing and a further 17 samples were sent in by the PHSI/ADAS from 8 other growers. Multiplying inoculum for *in planta* testing was dependent on the quality of samples received, e.g. samples recently sprayed with fungicides or with old looking (grey/black) pustules could not be multiplied on receptor plants. Twenty one of the samples submitted were successfully typed for tolerance to propiconazole (Bumper) and azoxystrobin (Amistar) (Table 2), but only 6 of these from 4 sites were obtained from the grower survey set with full site details, fungicide patterns of use and fungicide history. The *in planta* protocol has been modified to maximise the success of the tolerance typing. Growers are now recommended to submit at least 20-30 leaves containing healthy, white, viable pustules sampled at first sign of disease outbreak and before fungicide application. Quick determination of sample quality will also be determined with an initial in vitro basidiospore germination test enabling additional material to be obtained quickly if poor germination levels are observed. 6 of the 21 typed samples (29%) were sensitive to both propiconazole (Bumper) and azoxystrobin (Amistar).

Table 2. Results of *in planta* tests showing the number and percentage of samples (in brackets) typed as tolerant to one or both fungicides

Result of in planta typing	Number of samples; % in brackets	Number of infected sites
Tolerant of Bumper only	8 (38)	4
Tolerant of Amistar only	3 (14)	3
Tolerant of Amistar and Bumper	4 (19)	3
Sensitive to Amistar and Bumper	6 (29)	4

Note
Twenty samples could not be typed because the samples could not be successfully multiplied on receptor plants

Is strobilurin tolerance in chrysanthemum white rust (CWR) controlled by the same gene as for certain other fungi?

Work published on the PCR amplification of the cytochrome b gene responsible for strobilurin tolerance in the fungus *Blumeria graminis* (wheat powdery mildew) was reproduced at CSL. It was not possible, however, to determine if the cytochrome b gene was the same gene governing tolerance in *P. horiana* since the published primer set did not amplify *P. horiana* DNA routinely. A second set of cytochrome b primers were also assessed. However despite comprehensive PCR optimisation experiments, conditions could not be identified which allowed amplification of *P. horiana* DNA. The published technology could not be transferred to P. horiana and hence further research is needed to help develop a rapid test for testing tolerance to strobilurin fungicides in CWR.

Is triazole tolerance in chrysanthemum white rust (CWR) controlled by the same gene as for certain other fungi?

Work published on the PCR amplification of the $P450_{14DM}$ gene responsible for governing triazole tolerance in *Uncinular necator* (grape powdery mildew) was reproduced at CSL. It was not possible, however, to confirm if the $P450_{14DM}$ gene was the same gene governing fungicide tolerance in *P. horiana* as the primers designed to *U. nectator* did not amplify DNA from *P. horiana.* The published technology could not be transferred to *P. horiana* and hence further research is needed to help develop a rapid test for testing tolerance to strobilurin fungicides in CWR.

Development of a diagnostic test for fungicide tolerant strains

More strategic research is needed to develop a rapid molecular test for identifying *P. horiana* strains tolerant to strobilurin and triazole fungicides. Further research should concentrate on typing pure DNA directly from basidiospores of this obligate pathogen. This would enable unequivocal sequencing of genes which may be responsible for fungicide tolerance such as the cytochrome b and $P\text{-}450_{14DM}$ genes. This research would then allow isolates, such as those typed in this project, to be compared on a molecular level.

Financial benefits

The work has been able to inform the industry of the levels of fungicide tolerance to *P. horiana*, the fungus responsible for causing chrysanthemum white rust disease. The presence of fungicide tolerant CWR strains in England and Wales was demonstrated, and levels were significant. Of those isolates, which were able to be typed, 8 (38%) from 4 sites were tolerant of propiconazole (Bumper), 3 (14%) from 3 sites were tolerant of azoxystrobin (Amistar) and 4 (19%) from 3 sites were tolerant of both fungicides.

As a result of the project, fungicide tolerance can now be monitored more successfully following improvements to the *in planta* assay. Although, at present no molecular assay for screening CWR for fungicide tolerance has been produced, groundwork for its development has been laid. With the development of such an assay more rapid diagnosis of fungicide tolerance will allow growers to quickly select appropriate control measures to CWR.

Action points for growers

- Avoidance of disease introduction on propagating material, good hygiene, routine crop monitoring and effective environmental regulation to avoid prolonged periods of leaf wetness and high humidity (<97% RH), is critical to the control of chrysanthemum white rust disease.
- Whilst the success of fungicide spray regimes for the control of chrysanthemum white rust (CWR) are dependent primarily on the sensitivity to the chemicals used, spraying technique and correct time intervals between treatments are also critical. If, for example, areas of the crop are ineffectively sprayed, the repeat application interval is too long, or single fungicides are used repeatedly without alternation with those from another chemical group, this may allow the CWR to develop partial tolerance to the fungicides used (as demonstrated in this study). Such 'mal-practice' could result in 'hot spots' of infection, which with time will become more extensive so that eventually chemical control is ineffective as the tolerant CWR population becomes dominant.

- It has not been possible yet to determine if, by switching to alternative fungicide groups, a CWR outbreak tolerant of one particular fungicide can eventually revert so that sensitivity to that original fungicide is restored.
- If fungicide tolerance is suspected, growers and crop advisors are recommended to send a sample to CSL (York), of at least 20-30 leaves containing healthy, white, viable pustules sampled at the first sign of disease outbreak and if possible before fungicide application. Fungicide tolerance testing for CWR will take about 5 weeks to complete. More strategic research is needed before a rapid test can be developed.