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**Outcome of the Fungicide Working Group**

**General Comments**

- 'Loose' and non-detailed, lack of interpretation throughout
- Does not lead the evaluator through the logical decision making process

**Introduction/Background**

- Need more background information
- More cross references to FRAC & FRAG guidelines

**Biological activity**

- Lacking anything on barley and not really required for all pathogens
- Test method used (not detailed enough)
- Details of systemic activity testing not clear (dose rate high compared to EC50 values). Would ask to see report.

**Mode of action of chemzed**

- Not clear that there are 2 distinct MoAs. Not clear where mitochondrial disruption occurs – lack of detail. Effect on hyphal growth could be consequence of first MoA
- Not clear what relevance is of each MoA – particularly in relation to dose.
- Test method not justified (is use of yeast appropriate)

**Baseline monitoring**

- No specific dose range stated
- Sampling in fields – before/after treatment, treated/untreated plots ?
- Need to clarify variation in sensitivity (600 fold range, 0.008 – 4.9)
- Explain lower sensitivity of Italian samples – is it just natural variation or?
- Lacking data from barley? Open to discussion as to its need !
- Lacking interpretation of the baseline data

**Laboratory studies.**

- Sample source should be specified
- Relevance?

**Existing uses**

- Relevance? Apple mildew not cereal.
- Poor documentation

**Pattern of use**

- Important statement for risk assessment

**Resistance Risk Assessment**

*Powdery mildew is known to be an organism with a high risk of developing resistance.*

- Agree

*Although the mode of action has not been fully elucidated chemzed appears to act on at least two different biological pathways and can therefore be considered as a multi-site inhibitor.*

- To be determined

*This, supported by evidence from existing overseas use for several years and the laboratory studies that showed only a small change in sensitivity after 25 applications indicate that chemzed is a low risk chemical to which resistance is unlikely to develop.*

- Wrong interpretation of lab' study and overseas use experience

*Overall there is considered to be a moderate risk of resistance developing and a resistance management strategy is required.*

- In view of uncertainties and chemical being regarded as moderate not low risk, overall risk seen as moderate to high risk.

**Resistance management strategy.**

*A resistance management strategy incorporating the following components is proposed.*

*Based on a maximum of three applications to wheat a maximum of two applications per wheat crop will be permitted. On barley and other cereals, where fungicide use is less intensive only a single application will be permitted.*

Restrict to max 1 on wheat, barley OK

No mention of mixing partners or sequences which may allow 2 treatments on wheat to be acceptable.

*As curative treatments are considered to present a greater risk of encouraging resistance, the label will include advice not to use Product Z once disease has established.*

Needs to be re-worded as several things wrong (curative, established) – 'Use at first sign of infection'.

*No routine monitoring is proposed but the applicant will investigate reports of control failure. It is also an ongoing development programme such that Product Z, and other formulations including chemzed, will be used in trials over the next 3-5 years. Any poor performance in these trials will be fully investigated.*

Request routine monitoring in wheat. Barley?..... (Value discussed extensively between industry and regulators)

**ADDITIONAL DATA REQUIREMENTS**

- Cross resistance not mentioned – studies - particular on strobilurin resistant strains/populations required. (Potential data requirement for approval)
- Curative studies ( may be in efficacy section)
- Baseline for barley

# Appendix 1

# Product Z (fungicide)

**Background information**

Product Z, active substance chemzed, is a protectant fungicide with some curative ability. The mode of action has not been fully elucidated but current information indicates that the active substance has an effect on mitochondrial respiration and appears to disrupt and inhibit hyphal growth. Product Z is initially proposed for the control of powdery mildew (*Erysiphe graminis*) in cereals (wheat, barley, oats rye and triticale). *Erysiphe* species have developed resistance to a range of fungicides including DMIs and strobilurins.

# Dossier for product Z

## 1.1. Introduction

Product Z contains 100 g/l chemzed.

Product Z is being registered initially for the control of powdery mildew in cereals, wheat, barley, oats, rye and triticale. It is intended in the future to submit applications for the control of other powdery mildews including those affecting cucumbers, sugar beet, top and bush fruit and ornamentals crops.

Powdery mildews are generally considered to present a high risk of developing resistance. *Erysiphe graminis* has developed resistance to DMIs, pyrimidines, quinoxyfen and strobilurins. In many cases resistance has developed rapidly following the introduction of an active substance. Wheat powdery mildew (*E. graminis* F.sp. *tritici*) appears to be more prone to developing resistance than strains adapted to other cereals. This may be due to the greater tendency to produce sexually reproducing forms.

## 1.2. Biological activity

Product Z shows high levels of activity against powdery mildews. Lower levels of activity are also shown against rusts and yeasts. Little or no activity was recorded against a range of other fungi tested.

| **Fungus** | **Disease** | **EC50 (µg/l)** | **MIC (µg/l)** |
|---|---|---|---|
| *Erysiphe graminis (F. sp tritici)* | Cereal powdery mildew | 0.05 | 0.1 |
| *Podosphaeea leucotrica* | Apple powdery mildew | 0.02 | 0.08 |
| *Sphaerotheca fuliginea* | Cucumber powdery mildew | 0.05 | 0.12 |
| *Puccinia horiana* | Chrysanthemum white rust | 0.1 | 0.3 |
| *Puccinia sorghi* | Maize common rust | 0.15 | 0.4 |
| *Yeast* | - | 0.1 | 1.0 |
| *Botrytis cinerea* | Grey mould | 1.0 | 20.0 |
| *Septoria tritici* | Wheat leaf spot | 1.0 | 25.0 |
| *Pyricularia grisea* | Rice blast | 2.0 | 25.0 |
| *Phytophthora infestans* | Potato late blight | 3.5 | >100 |
| *Aspergillus nidulans* | - | 5.0 | >100 |

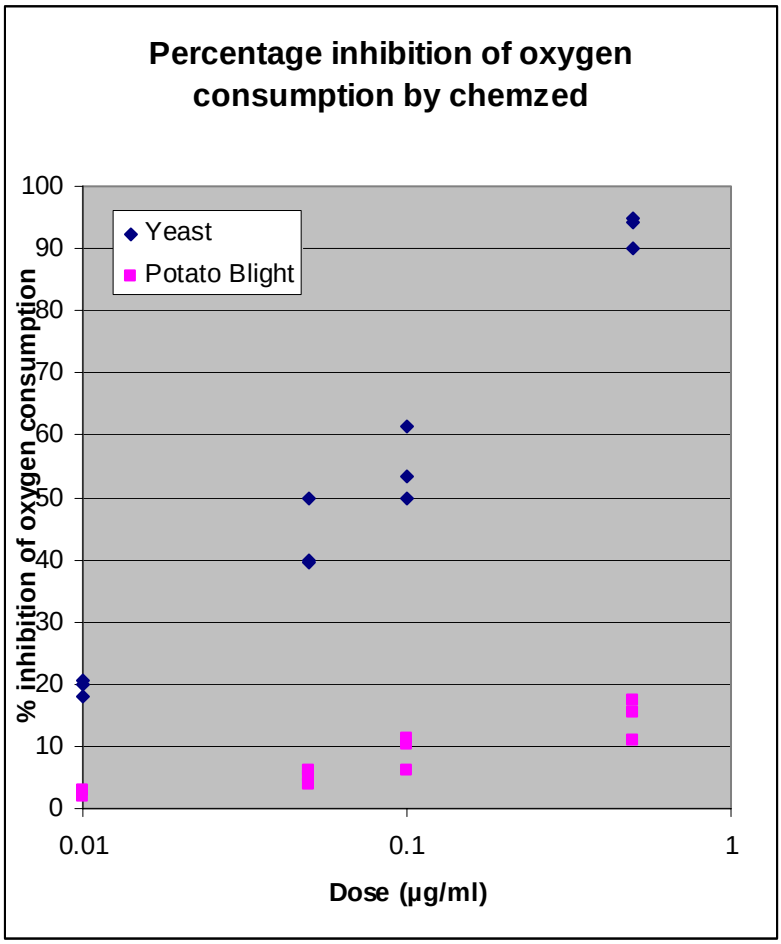
Chemzed has very limited systemic activity. Individual drops of 25 µg/l Chemzed were applied to different positions on barley leaves that were subsequently inoculated with *E. graminis*. Inhibition of fungal growth dropped of rapidly outside the treated area of leaf.

## 1.3. Mode of action of chemzed

Chemzed appears to act by inhibiting mitochondrial respiration and by the disruption and inhibition of hyphal growth caused by disruption of Spitzenkorper, the organelle that attracts vesicles to the growing tips of hyphae.

### 1.3.1. *Effect on mitochondrial respiration*

Powdery mildews and rusts are both obligate parasites making studies on these groups in the laboratory almost impossible. Studies were therefore conducted on yeast which are also sensitive to product Z.




Oxygen consumption in whole cells was reduced in a dose dependent manner by chemzed. Cyanide and azoxystrobin, which both also affect mitochondrial respiration, elicited similar responses. In *Aspergillus nidulans* and the oomycete *Phytophthora infestans* neither of which is significantly affected by chemzed, the response was much weaker.

Using potentiometric dyes to measure the membrane potential showed that chemzed had no effect on mitochondrial membrane potential.

Studies with isolated yeast mitochondria and indicate that chemzed probably affects complex III activity.

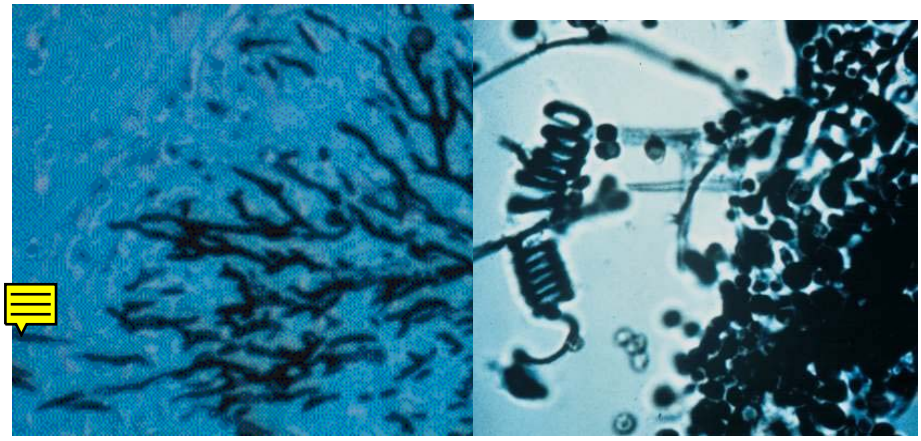
### 1.3.2. *Effect on hyphal growth*

Chemzed also affects hyphal growth in sensitive organisms. Observations of *Erysiphe graminis* colonies on barley showed that the growth of colonies on leaves treated with a low dose of chemzed was reduced compared to those on untreated. In addition, the tips of hyphae showed distortions, growing spirally and with increased branching.

Ultrastructural examination showed that the Spitzenkorper in treated hyphae was smaller and less distinct compared to untreated ones. If the structure and the transport of vesicles to the hyphal tip is disrupted this could account for the disruption in growth seen in treated hyphae.

It is also likely to reduce the growth of hyphae, although it is impossible to determine the relative importance of this mechanism compared to the inhibition of respiration.

**Figure 1, Normal, left and spiral hyphae right**



As well as reducing the rate of growth the type of distortions of the hyphae seen following treatment are likely to prevent the entry of pathogen into the leaf through stomata.

## 1.4. Baseline monitoring

Samples of wheat powdery mildew have been taken from trial sites across Europe (UK, France, Germany, Italy) over four years.

A bioassay has been developed to assess the level of sensitivity of *E. graminis* to chemzed. In 1999 to 2002 samples of spores were taken in treated field using suction samplers. In 2002 samples were taken across wider regions using a spore sampler attached to a car.

In the laboratory, the sampled spores are grown up to single colony isolates that are subsequently transferred onto fresh leaf segments for storage and multiplication before testing. The sensitivity of each single colony progeny was determined on a test set of leaf segments cut from the first leaf of ten-day old seedlings. One day before cutting and inoculation, seedlings were sprayed with different fungicide solutions. Between five and ten doses were used in each bioassay. Each dose was replicate between 4 and 6 times. After incubating for 10 days the percentage coverage of leaf segments was scored relative to the untreated control.

### 1.4.1. *Results*

| Year | Country | No. of samples | EC50 | | |
|---|---|---|---|---|---|
| | | | Average | Min | Max |
| 1999 | Germany | 3 | 0.13 | 0.01 | 0.7 |
| | France | 3 | 0.13 | 0.01 | 0.9 |
| 2000 | Germany | 8 | 0.1 | 0.011 | 1.8 |
| | France | 8 | 0.1 | 0.009 | 1.5 |
| | UK | 3 | 0.15 | 0.01 | 1.1 |
| | Italy | 5 | 0.18 | 0.008 | 1.4 |
| 2001 | Germany | 8 | 0.13 | 0.008 | 1.5 |
| | France | 8 | 0.19 | 0.01 | 1.6 |
| | UK | 8 | 0.07 | 0.012 | 1.5 |
| | Italy | 5 | 0.17 | 0.011 | 3.3 |
| 2002 | Germany | 20 | 0.14 | 0.01 | 2.2 |
| | France | 8 | 0.16 | 0.009 | 1.8 |
| | UK | 20 | 0.13 | 0.008 | 2.1 |
| | Italy | 5 | 0.17 | 0.009 | 4.9 |

## 1.5. Laboratory studies.

Wheat powdery mildew was cultured on wheat plants grown in pots in glasshouses in conditions favouring development of the disease. New plants, treated with sub-lethal dose giving around 50% control, were inoculated every three weeks. This was repeated 25 times. The sensitivity of the population was assessed after every 5 inoculations. At the end of the experiment the average sensitivity of the pathogen had decreased to around half that at the beginning.

## 1.6. Existing uses

Products based on chemzed have been registered for use on powdery mildew in apples in China and Australia/New Zealand for the last four years. Market penetration has been up to 50% of growers in Australia/New Zealand where three sprays per season are permitted. Resistance monitoring has shown no shifts in sensitivity over the period of use. There are no restrictions on use in China and no statistics are available on the extent of use but no reports of lack of effectiveness linked to a reduction in sensitivity have been received.

## 1.7. Pattern of use

UK growers may apply up to three fungicide treatments to control mildew in a bad year, although 1 or 2 are more normal.

## 1.8. Resistance Risk Assessment

Powdery mildew is know to be an organism with a high risk of developing resistance.

Although the mode of action has not been fully elucidated chemzed appears to act on at least two different biological pathways and can therefore be considered as a multi-site inhibitor. This, supported by evidence from existing overseas use for several years and the laboratory studies that showed only a small change in sensitivity after 25 applications indicate that chemzed is a low risk chemical to which resistance is unlikely to develop.

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## 1.9. Resistance management strategy.

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