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Evolution of resistance to fungicides in populations of *Mycosphaerella graminicola*: emergence of new phenotypes highly resistant to DMIs

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EPPO Workshop on Azole fungicides and Septoria leaf blotch control

Conference Centre, Rothamsted Research,
Harpenden (GB), 2010-12-07/09

PROGRAMME
SUMMARIES OF PRESENTATIONS AND POSTERS
LIST OF PARTICIPANTS



The Organizing Committee:

Paul Ashby, CRD
Bart Fraaije, Rothamsted Research
Jean-Luc Genet, FRAC
Lisa Nistrup-Jorgensen, Aarhus University
John Lucas, Rothamsted Research
Louise Plumer, Rothamsted Research
Anne-Sophie Walker, INRA
Robert Sunley and Vlasta Zlof, EPPO Secretariat



EPPO Workshop on Azole fungicides and Septoria leaf blotch control

PROGRAMME

Conference Centre, Rothamsted Research, Harpenden (GB), 2010-12-07/09

Tuesday, 07 December 2010

11:00 Pre-workshop planning meeting for organizing committee, chair persons and rapporteurs
(Pirie Room, Conference Centre)

12:30-14:00 Registration and poster display

14:00 Welcome by EPPO, **Robert Sunley** and **Vlasta Zlof**
Aims and objectives of the workshop **Fiona Burnett**, SAC

Session I 'Introduction to Septoria leaf blotch control' [Chair: Bernd Rodemann]

14:15 Septoria management across the EPPO region - feedback from the questionnaire
Paul Ashby, CRD

14:45 Target *Mycosphaerella graminicola*; genetic variation and potential for change
Gert Kema, Plant Research International

15:15 The contribution of plant breeding to controlling *Septoria tritici*
James Brown, John Innes Centre

15:45 – 16:15 Coffee break

16:15 Chemical control of Septoria leaf blotch: history, biological performance and molecular mode of action of DMI fungicides
Klaus Stenzel, Bayer CropScience

16:45 Impact of the new EU regulations on the management of Septoria leaf blotch and rust diseases in cereals
Paul Leonard, BASF

17:15 Introduction of new fungicides for Septoria leaf blotch control
Andy Leadbeater, Syngenta

18:00 Poster presentations



18:30 Welcome buffet-reception kindly offered by FRAC

Wednesday, 08 December 2010

Session II 'Azole sensitivity monitoring of Septoria populations' [Chair: Anne-Sophie Walker]

- 09:00 DMI sensitivity in European populations of *Mycosphaerella graminicola*
Helge Sierotzki, Syngenta
- 09:30 Sensitivity of *Mycosphaerella graminicola* to DMI fungicides across Europe and impact on field performance
Gerd Stammler and **Martin Semar**, BASF
- 10:00 Outliers or shifts? Sensitivity of the Irish *Mycosphaerella graminicola* population to triazole fungicides
Steven Kildea, Teagasc
- 10:30 Monitoring *Mycosphaerella graminicola*: relevance of *in vitro* testing and *CYP51* mutations
Andreas Mehl, Bayer CropScience
- 11:00 – 11:15 Coffee break

Session III 'Scientific basis of azole resistance development' [Chair: John Lucas]

- 11:15 Overview. Azole resistance mechanisms in agriculture
John Lucas, Rothamsted Research
- 11:45 Selection of *CYP51* variants in UK field populations of *Mycosphaerella graminicola*: a historic perspective
Bart Fraaije, Rothamsted Research
- 12:15 Evolution of resistance to fungicides in populations of *Mycosphaerella graminicola*: emergence of new phenotypes highly resistant to DMIs
Anne-Sophie Walker, INRA
- 12:45 Functional characterization of *Mycosphaerella graminicola* sterol 14 α -demethylase (*CYP51*) variants resistant to azole fungicides
Hans Cools, Rothamsted Research
- 13:15 – 14:00 Lunch

Session IV 'Fungicide resistance management strategies and practical use of azoles in European agriculture' [Chair: Andy Leadbeater]

- 14:00 Fungicide resistance risk
Neil Paveley, ADAS
- 14:30 The evaluation of fungicide resistance management strategies using a modelling approach
Peter Hobbelen, Rothamsted Research
- 15:00 DMI use in France: efficacy and future recommendations
Claude Maumene, Arvalis
- 15:30 Spray programmes and advice to farmers in the UK
Bill Clark, Brooms Barn
- 16:00 – 16:30 Coffee break
- 16:30 Recent experiences with triazole fungicide use for Septoria leaf blotch control on winter wheat in Ireland
Tom McCabe, University College Dublin
- 17:00 Optimizing fungicide use in Denmark for control of *Mycosphaerella graminicola*
Lise Nistrup-Jørgensen, Aarhus University
- 18:00 Poster presentations



- 19:00 Workshop Dinner (Conference Centre)

Thursday, 09 December 2010 Working Group Programme

[Chair: John Lucas]

09:00 Current and future regulatory considerations for azole use
Bernd Rodemann, JKI

09:30 Assembling of working groups
Paul Ashby, CRD

Working Group I. Understanding and assessing changes in sensitivity

[Chair: John Lucas; Rapporteur: Gert Kema]

Working Group II. Strategies for management and communication of risk

[Chair: Lise Nistrup-Jørgensen; Rapporteur: Paul Ashby]

10:00-12:00 **Morning Session ‘Working Groups I and II’**

12:00-13:00 Lunch

13:00-15:00 **Afternoon Session ‘Working Groups I and II’**

15:00-17:00 **Outcome of Working Groups I and II; Conclusions and Recommendations**

[Chair: Fiona Burnett]

- Representative of each WG presents results of the session
- Plenary discussion
- Conclusions



17:00 Closure of workshop

Septoria management across the EPPO region - feedback from the questionnaire

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A questionnaire was sent to all EPPO Member Countries to gain some background information on *Mycosphaerella graminicola*. Information was sought on the importance of this disease, the main strategies used to control it (main active substances, spray strategies, varietal tolerance) and the field performance of azoles. A summary of the main findings of the questionnaire are presented. In total 15 countries responded of these 11 rated *Mycosphaerella graminicola* as a major disease. Information presented shows the range of azoles and other modes of action used, the importance of varietal tolerance and doses recommended.

Target *Mycosphaerella graminicola*; genetic variation and potential for change

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Mycosphaerella graminicola, the causal agent of septoria leaf blotch, is currently the most important Western European wheat disease. Despite the recent identification of 15 resistance genes and their potential application in plant breeding, disease control is largely achieved by fungicide applications. However, fungicide resistance development in natural *M. graminicola* populations frequently occurs and is a serious concern. Depending on the fungicides this may develop gradually, such as with resistance to azoles, or much more rapidly as was observed for strobilurin fungicides. To understand the (rapid) evolution of resistance we have performed a range of crossing experiments, which clearly show that fungicides can stop or slow down disease progress but cannot prevent sexual development. As *M. graminicola* is a highly sexual pathogen, understanding the influence of sexual reproduction and genetic recombination on the evolution of fungicide resistance is very important. We use an *in planta* crossing protocol that reliably enables the isolation of segregating populations and hence the unbiased mapping of genomic regions that are involved in fungicide resistance. Ongoing programs and results will be discussed.

The contribution of plant breeding to controlling *Septoria tritici*

James KM Brown

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When *Septoria tritici* blotch (*Mycosphaerella graminicola*) first became a serious disease of wheat in the UK around 1980, many varieties were susceptible to it. The situation has changed substantially since then because most wheat varieties released in the last five years have at least moderate resistance to *Septoria* while a few have very good resistance. I will review what is currently known about the genetics of *Septoria* resistance in wheat, discuss how improvements in resistance have been made, describe constraints on resistance breeding, and outline prospects for the future, as controlling *Septoria* with fungicides will become increasingly difficult.

Research over the last 15 years has shown that, in common with other plant diseases, there are broadly speaking two types of resistance to *Septoria*. Major genes which follow the gene-for-gene relationship confer strong resistance to avirulent pathogen isolates. This type of resistance has little or no value beyond the short term, however, because *M. graminicola* genotypes lacking avirulence evolve rapidly in response to the use of major genes. The second type of resistance is partially effective against all pathogen genotypes. While it is much more durable than major gene resistance, partial resistance is typically controlled by several genes with varying levels of effectiveness so it may be more difficult to use in breeding.

Early efforts to improve breeding for resistance to *Septoria* in Europe focussed on the possibility of using exotic wheat varieties, synthetic hexaploid wheats and even other grass species. It was subsequently found that there are in fact many genes for partial resistance in current and recent wheat varieties but progress had been limited because many breeders had inadvertently been relying on a single source of resistance. The substantial improvement in the general level of resistance in wheat varieties in recent years may have resulted from diverse sources of partial resistance being combined in breeding programmes.

Interactions of *Septoria* resistance with other traits may also have caused progress in breeding to be slow during the 1980s and 1990s. It appears that selection for yield limited improvements in *Septoria* resistance, most likely because varieties used as sources of other desirable traits carried genes which increased susceptibility to *Septoria*. Nevertheless, a joint analysis of selection for yield and resistance has indicated that UK wheat breeding programmes contain genes which increase yield but do not increase susceptibility to *Septoria* or vice-versa.

Future wheat production will need to rely ever more heavily on resistant varieties as the mainstay of disease control. A major challenge will be to achieve acceptable disease control while maintaining high yield and quality. This can be done by applying an appropriate level of selection for all three traits separately in breeding programmes. It would be inappropriate to focus too strongly on disease resistance because this would most likely result in selection of genes which improve resistance but depress yield. It would also be inappropriate to use major genes as a short-term fix to control *Septoria* because they would mask more useful, durable, partial resistance. A desirable but demanding goal is to combine adequate resistance to *Septoria* and other important diseases with high yield and excellent quality.

This talk is based on research supported by DEFRA, HGCA, Elsoms Seeds Ltd, Lantmännen SW Seed, Limagrain UK Ltd, Sejet Plant Breeding I/S and Syngenta Seeds Ltd through the Sustainable Arable Production LINK programme.

Chemical control of Septoria leaf blotch: history, biological performance, and molecular mode of action of DMI fungicides

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The DMI-fungicides were introduced in agriculture in 1969 and have experienced a rapid and successful development. The chemical class of triazoles is the most relevant DMI class used on nearly every crop, with major focus on cereals. The reason for the great successes of triazoles in agricultural practice is several folds: favorable biological properties with broad spectrum of activity against asco- and basidiomycetes, systemic activity allowing flexible use for curative and preventative treatments, compound-specific differences in level and spectrum of activity and utility in crops, and, finally, the medium risk of resistance.

The mode of action, the specific inhibition of the sterol biosynthesis (C14-demethylation), was elucidated in the 1970's. The *cyp51* gene, especially of *Mycosphaerella graminicola*, was intensively studied to clarify underlying mechanisms of reduced sensitivity and related mutations. Triazoles differ in their activity and, due to the shifting type of resistance, effective disease control can be ensured also under severe disease pressure by selection of the most active products. Triazoles play an indispensable role in effective disease management in cereals and their activity must be retained by effective resistance management.

Impact of the new EU regulations on the management of Septoria leaf blotch and rust diseases in cereals

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It is estimated that without effective Septoria leaf blotch (*Mycosphaerella graminicola*) and rust control, European cereal production would be reduced by up to 30%. Modern high yielding cereal varieties continue to be heavily dependent on the use of azole fungicides to control these pathogens, with an estimated 80% of cereal fungicides containing azole fungicides.

European reregistration, under Directive 91/414/EEC, has reduced plant protection product (PPP) availability by at least 70%. In addition, the current shift from scientific risk assessment to hazard-based regulation, under Regulation 1107/2009, could further impact PPP availability, by an estimated 12 to 17%. Additional uncertainty, regarding fungicide availability, will be triggered by the incorporation of comparative assessment and substitution in the new regulation (article 50, Annex IV and Annex II point 4).

Under the new Regulation, azole fungicides are at risk of regulatory exclusion, resulting from their association with endocrine activity. However, it continues to be challenging to develop alternative and equally effective Septoria and rust control technologies.

The need for resistance management is recognised in Regulation 1107/2009, which provides the possibility of derogation, under Article 4.7, to ensure sufficient chemical diversity. Member States, which may be concerned by potential loss of strategically important azole fungicides, will therefore need to prepare for this eventuality.

Introduction of new fungicides for Septoria leaf blotch control

Andy Leadbeater

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The introduction of new fungicides is essential for sustainable control of major crop diseases. Over the past 30 years, despite escalating costs and legislation, there has been a constant flow of these, with over 65 novel compounds introduced. New modes of action are rare for cereal disease control, but two significant innovations can be recognized: the triazoles (DMIs) and the strobilurins (QoIs), which have made major contributions to wheat management for many years. There is a heavy reliance today on these two groups of fungicides for wheat production in the EU. Preliminary impact assessments of the new European Regulation and of comparative assessment indicate that key members of the triazole group may be lost from the market. In addition, widespread resistance has occurred to the QoI fungicides in *Mycosphaerella graminicola* (Septoria Leaf blotch). Clearly, new solutions are needed to ensure the future control of this devastating disease.

The pipelines of the agrochemical companies contain several new fungicides for cereals, mainly from the carboxamide chemical family (SDHIs). Assuming these pass successfully through registration they will become major components in future fungicide products for Septoria control. These fungicides and others are discussed and their role in disease- and resistance- management programmes considered.

DMI sensitivity in European populations of *Mycosphaerella graminicola*

Helge Sierotzki, Regula Frey, Carolina Buitrago, Jürg Wullschleger, Ulrich Gisi, Gabriel Scalliet
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Sensitivity studies over the past 10 to 15 years show that populations of *Mycosphaerella graminicola* have adapted to the selection pressure exerted by the use of DMI fungicides. The sensitivity shift affects all DMI's but its dynamics may be compound specific and vary between countries. The data also suggest that the shift has continued. Recently, a lot of information on *cyp51* genotypes and their sensitivity to DMI's has been presented. However, the *cyp51* gene seems to be dynamic, and new genotypes are emerging with altered sensitivity patterns towards DMI's. Despite some differences in sensitivity pattern of certain *cyp51* genotypes to particular DMI fungicides, a general cross resistance can be assumed based on the same mode of action for all DMI's. However, the selection process may favour certain genotypes depending on the major DMI used for disease control. The large variation in sensitivity within each genotype suggests that, in addition to mutations in the *cyp51* gene, other mechanisms must play an important role for sensitivity towards DMI's. Sensitivity shifts within genotypes will be described and discussed.

Sensitivity of *Mycosphaerella graminicola* to DMI fungicides across Europe and impact on field performance

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Since the occurrence and spread of QoI resistance in *Mycosphaerella graminicola* in 2003 in Europe, Demethylation-Inhibitors (DMI) are the backbone for control of Septoria leaf blotch disease (*M. graminicola*). European monitoring studies carried out by various research institutes and DMI manufacturers have shown a shift of the European *M. graminicola* population towards increased ED₅₀ values for DMI fungicides. Populations of *M. graminicola* are very heterogeneous within a region and even within a field regarding DMI sensitivity and *CYP51* haplotype, the target site of DMIs. Different *CYP51* haplotypes are selected by specific DMI treatments, which indicate that the *CYP51* haplotypes specifically influence the sensitivity to different DMIs. This was subsequently confirmed by sensitivity studies with thousands of isolates which showed different sensitivity patterns of *CYP51* haplotypes and either no, weak, or even a negative correlation of the sensitivity to various DMIs. A key for sustainable control of Septoria leaf blotch is therefore the availability of different DMIs. Besides DMIs, only protective fungicides or single site inhibitors with a higher resistance risk are currently available and this is unlikely to change in the near future.

The implementation of the new European regulation 1107/2009 with its new definition of cut-off criteria may decrease the diversity of DMIs and negatively affect effective control of Septoria leaf blotch.

Outliers or Shifts? Sensitivity of the Irish *Mycosphaerella graminicola* population to triazole fungicides

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The profitability of winter wheat production in Ireland is currently dependent on the abilities of fungicides to successfully inhibit *Mycosphaerella graminicola* infection and the subsequent development of septoria tritici blotch. The loss of active ingredients through the development of resistance within this pathogen has dramatically reduced the range of fungicides available to fulfil this role, to a select few of the triazole fungicides and the chloronitrile fungicide, chlorothanil. Due to their importance in Irish wheat production the sensitivity of the Irish *M. graminicola* population to these triazole fungicides has been monitored at Teagasc Oak Park since 2003. Using both agar plate and microtitre plate assays, over 1500 isolates obtained from commercial wheat crops have been assessed for sensitivity to epoxiconazole, prothioconazole and tebuconazole. In 2005 the presence of isolates with reduced sensitivity to tebuconazole was confirmed within the population and more recently (2008 onwards) a shift in sensitivity to both epoxiconazole and prothioconazole has been observed. Analysis of the CYP51 gene in a sub-sample of these isolates has identified within the Irish population, the presence of mutations associated with these changes in sensitivity.

Monitoring *Mycosphaerella graminicola*: relevance of *in vitro* testing and *CYP51* mutations

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Resistance towards site-specific fungicides is mostly based on point mutations of the target, often leading to disruptive resistance patterns and, e.g. in the case of QoI fungicides, partly fast decreasing field efficacy of solo applied products.

Although DMI fungicides also target a single protein and point mutations of the sterol-14 α -demethylase (*CYP51*) target site have been reported in *M. graminicola*, sensitivity changes during the last decade have developed much more slowly, following a 'shifting-type' pattern. Genotype and phenotype groups of the pathogen have been described via *in vitro* sensitivity and *cyp51* studies. With regard to loss of *in vitro* efficacy, the relevance of target mutations in the development of changes in sensitivity to azole has been confirmed, but not all DMIs are equally affected. In practice, modern DMI solutions continued to perform very well on the European scale.

In order to discuss this discrepancy between reduced *in vitro* sensitivity and/or genotype structure of *M. graminicola* populations and field performance of DMI fungicides, the latest monitoring data and corresponding *cyp51* alterations in single strains were correlated with DMI efficacy results at selected sites. The studies lead to the assumption that with DMIs, in contrast to QoIs, *in vitro* sensitivity data or molecular biological characterizations of target site mutations alone cannot sufficiently predict field performance of the respective product in the cereal growing area in question. Varying correlations between *in vitro*, molecular and *in planta* study results point to the likely presence of other resistance mechanisms, which may have, therefore, a different relevance on DMI *in vitro* efficacy than on *in vivo* efficacy.

Overview. Azole resistance mechanisms in agriculture

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Azole fungicides have been used in agriculture in Europe for at least 30 years, for the most part without the development of serious resistance problems. Unlike other single-site inhibitors, such as the methyl benzimidazoles (MBCs) and the Quinone-outside Inhibitors (QoIs), efficacy has not been compromised by the rapid emergence of target site mutations conferring high levels of resistance without apparent fitness costs. Instead, changes in sensitivity have occurred more gradually, so that higher doses of fungicide may be required to maintain the original degree of disease control. Early examples concerned powdery mildews on cereals, with shifts to more resistant phenotypes, some correlated with amino acid substitutions in the CYP51 target protein. The stepwise emergence of resistance in Septoria leaf blotch, *Mycosphaerella graminicola*, has been well-documented. The first shifts in sensitivity to azoles were reported at least 10 years after introduction of the chemistry in fungicide programmes for wheat crops. The gradual erosion of efficacy has continued to the present day, in regions of high disease pressure and regular azole use. Three features of this evolutionary process are of interest. Firstly, resistance has not affected all agricultural azoles equally, showing that cross-resistance to this chemical class varies in degree. Secondly, shifts in sensitivity are associated with the accumulation of multiple changes in the CYP51 protein, with more complex *CYP51* genotypes emerging in field populations over time. Thirdly, sensitivity tests *in vitro* sometimes correlate poorly with fungicide performance *in planta*. This discrepancy might be due to the operation of resistance mechanisms in lab bioassays that are less important in the complex and dynamic host environment. The advent of complete genome sequences for *Mycosphaerella* species and other fungal pathogens, along with whole genome arrays and transcript sequencing, should assist in the identification of such alternative mechanisms, and provide new insights into the adaptation of fungi to azole fungicides.

Selection of CYP51 variants in UK field populations of *Mycosphaerella graminicola*: a historic perspective

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Septoria leaf blotch caused by the fungus *Mycosphaerella graminicola* (anamorph *Septoria tritici*) has been the most important foliar disease of winter wheat crops in the UK since the mid-1980s. Most cultivars are susceptible and disease control is primarily dependent on fungicide use. The most effective agents are systemic fungicides, but resistance to MBC and QoI fungicides has developed rapidly, within 5 years after market introduction. Resistance development to DMI fungicides has been a much slower process and not all azoles are equally affected. A key resistance factor is the alteration of the target protein, sterol 14 α -demethylase (*CYP51*), with changes to more than 15 different amino acid residues already being reported. By combining DNA testing of archived wheat samples from the long-term winter wheat experiment at Rothamsted (from 1844 onwards) and screening of recent UK *M. graminicola* populations for azole sensitivity pheno- and genotypes, we are now able to identify and measure the dynamics of different *CYP51* variants over time. This information is crucial to obtain a better understanding of the evolution of azole-*CYP51* interactions and can also aid the prediction of the future *CYP51* evolution in *M. graminicola*.

Evolution of resistance to fungicides in populations of *Mycosphaerella graminicola*: emergence of new phenotypes highly resistant to DMIs

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Sterol 14 α -demethylation inhibitors (DMIs) have been widely used for a long time to control *Mycosphaerella graminicola* (anamorph *Septoria tritici*) and erosion of efficacy, correlated with significant shifts in sensitivity of *M. graminicola* populations, has been recorded for most of them over the past few years. More recently, our monitoring, based upon phenotype analysis of bulk populations, allowed the detection of strains highly resistant to some or all DMIs from French, English and Irish populations. These isolates were first observed at very low frequency in 2008 and were present in 30% of the French populations tested in 2010, with a wide range of frequencies.

In field isolates weakly or moderately resistant *in vitro* to DMIs, one or several mutations were recorded in the target encoding-gene *CYP51*. In some field isolates highly resistant to DMIs, cross-resistance with QoIs and SDHIs was also recorded. A combination of alterations in *CYP51* and overexpression of drug efflux transporters is probably involved in these multidrug resistant (MDR) phenotypes. Lastly, some isolates moderately or highly resistant to DMIs harbour an insertion in the *Cyp51* promoter and/or new combinations of already known mutations in the target gene.

This work gives an updated overview of the *M. graminicola* field strains resistant to DMIs. Since some of them are emerging in France and other European countries, population data will be presented as well. These recent findings should be taken into account to adjust resistance and efficacy management strategies in this context of evolving resistance.

Functional characterization of *Mycosphaerella graminicola* sterol 14 α -demethylase (*CYP51*) variants resistant to azole fungicides

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The recent decline in the effectiveness of azole fungicides against *Mycosphaerella graminicola* has been accompanied by the accumulation of mutations in the *MgCYP51* gene encoding amino acid alterations in the target enzyme, sterol 14 α -demethylase. Analogous to the development of azole resistance in other fungi, for example the opportunistic human pathogen *Candida albicans*, *MgCYP51* alterations in *M. graminicola* are most often found in combination, with isolates most resistant to azoles carrying multiple amino acid substitutions compared to the wild type. To study the impact of both individual and combinations of *MgCYP51* alterations on azole sensitivity, we have introduced mutations by site directed mutagenesis and expressed mutated *MgCYP51* proteins in a *Saccharomyces cerevisiae* strain carrying a regulatable promoter controlling native CYP51 expression. We have shown the wild type *MgCYP51* gene complements the function of the orthologous gene in *S. cerevisiae* and that introduction of some mutations, for example those encoding amino acid alterations between Y459-Y461, substantially reduce azole fungicide sensitivity. Some substitutions, including I381V, destroy *MgCYP51* function in the *S. cerevisiae* mutant when introduced alone. However, this can be partially rescued by combining I381V with alterations between Y459-Y461. Therefore, these studies provide functional evidence underlying the sequence in which *MgCYP51* alterations in the Western European *M. graminicola* population emerged. In addition, these data may also have value in predicting *MgCYP51* changes that will be important in the future.

Fungicide resistance risk

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Assessments of resistance risk are used to guide the extent of anti-resistance strategies for plant protection products. Recently, it has been proposed that resistance risk should also be taken into account in the implementation of substitution and comparative assessment (EC 1107/2009) – to ensure that more modes of action are retained where there is a high risk of resistance. Risk is usually assessed, following guidance in EPPO standard PP 1/213(3), by combining risk values for the fungicide, pathogen and agronomic system. This paper reports results from an objective test of the predictive value of such an assessment scheme. The risk variable to be predicted was taken as the number of years between the introduction of a new mode of action to control a particular pathogen and the first detection of resistance (termed ‘FDR time’). Across all European crops, 84 mode of action by pathogen combinations were identified where FDR time could be quantified. Risk values could be calculated, using a risk assessment table, for 54 of these cases. Risk values explained approximately 70% of the variation in FDR time. Constraining the analysis to single-site modes of action reduced the variation accounted for to approximately 30%.

The evaluation of fungicide resistance management strategies using a modeling approach

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Mathematical modeling could help identify promising fungicide resistance management strategies which justify experimental testing. The authors derived a model to predict the usefulness of resistance management strategies for fungal pathogens of cereal crops. The model describes the seasonal growth and senescence of the crop in order to account for competition between resistant and sensitive strains for leaf area. The pathogen population is divided into latent and infectious stages to account for differences between fungicides in the developmental stages in the life cycle of a pathogen that they constrain. The model was successfully tested (and peer reviewed) by comparing model predictions with an independent dataset on selection for resistance in powdery mildew on spring barley in response to different doses and number of applications of QoI-fungicide. The model can therefore be used with some confidence to evaluate the usefulness of fungicide resistance management strategies. The criterion used to quantify the success of a strategy is the number of years that disease-induced loss of green leaf area can be kept to a commercially acceptable level (the ‘effective life’ of a product). The effects of mixtures, dose, number of treatments and alternation of treatments on effective life are being assessed, and results will be presented.

Utilisation des IDM en France : efficacité et future recommandations

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Depuis 2008, en France, des souches de *Septoria tritici* TriHR, hautement résistantes aux IDM, ont été identifiées à une faible fréquence. Si les facteurs de résistances sont élevés pour ces souches, leur impact est considéré aujourd'hui comme négligeable eu égard à leur faible fréquence dans les populations naturelles. Un essai, sous inoculation artificielle a permis en 2010, d'observer en plein champ le comportement du prothioconazole, de l'époxiconazole seul ou en association avec du prochloraz, du chlorothalonil, et d'un représentant de la nouvelle classe des carboxamides, en présence de 3 souches représentant les catégories TriMR6, et 2 souches qualifiées respectivement de TriHR9 (résistance de type mutation de cible) et TriHR6 (résistance de type Multi Drug Resistant, MDR). Les souches TriHR présentent des dynamiques de développement plus rapide que la souche de référence. Les résultats d'efficacité des matières actives testées sont en interaction avec le type de souches considéré et peuvent parfois être sévèrement affectés. Seul le chlorothalonil présente des résultats régulièrement élevés sur les 3 types. Ces résultats invitent à maintenir la plus grande diversité de molécules sur le marché, à renforcer les messages de prudence à l'égard des risques de résistance, et à inciter les producteurs à diversifier les molécules et les modes d'action utilisés.

DMI use in France: efficacy and future recommendations

Since 2008, in France, highly resistant strains of *Septoria tritici*, TriHR, have been identified at a low frequency. If the resistant factors are very high for these strains, their impact is considered today as negligible taking into account their low frequency in the natural populations.

A field trial in 2010 using artificial inoculation, has allowed, the effects of prothioconazol, epoxiconazol solo or mixed with prochloraz, chlorothalonil, and a representative of the new class of carboxamid, to be observed on three strains representing the categories TriMR6 and two strains qualified respectively as TriHR9 (resistance type target mutation) and TriHR6 (resistance type Multi Drug Resistant, MDR). TriHR strains presented development dynamics faster than the reference strain. Efficacy results of the active substances tested is strongly dependant on the type of strain considered and the results of trials can sometimes be severely affected. Only chlorothalonil is systematically effective against the three types of strains. These results suggest it is necessary to maintain the highest possible diversity of products on the market, to reinforce the safety messages to take into account the risk of resistance, and to encourage the producers to diversify the products and the mode of action used.

Spray programmes and advice to farmers in the UK

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Wheat crops in the UK receive on average 3 fungicide treatments during the season, usually at the T1, T2 and T3 timings. About one third of crops receive 4 fungicide sprays. Sprays are usually applied in a prophylactic strategy. The fine-tuning of timing and fungicide dose is determined by geographic disease pressure, disease thresholds and varietal resistance to disease. Programmes are dominated by triazole (DMI) fungicides, strobilurin (QOI) fungicides and chlorothalonil. SDHI fungicides are increasing in use. Most fungicides are applied in mixtures, either tank mixed or ready formulated. Many fungicides are typically applied at half the label recommended dose. Current UK varieties are very responsive to fungicide use. Current UK wheat varieties (2010) give an average yield response of 2.1 t/ha to fungicide use. The most responsive varieties give yield responses of up to 2.8 t/ha. This level of yield response (with a value of over £450/ha) makes any decisions that result in sub-optimal outcomes very costly. Fungicide resistance is not a major factor in fungicide choice for growers, but in practice most fungicides are applied in mixtures. As a consequence of this, fungicide activity is probably being inadvertently protected against resistance development.

Recent experiences with triazole fungicide use for Septoria leaf blotch control on winter wheat in Ireland

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Septoria leaf blotch is the most important fungal disease on wheat in Ireland, being favoured by the extended periods of cool and wet summer weather experienced in many years. The high yield, feed wheat varieties grown in Ireland have low-moderate resistance to the pathogen and there is a dependence on intensive fungicide use to manage the disease. Since the emergence of high levels of resistance (G143A mutation) to strobilurin fungicides in 2002 the disease control programmes are based on the repeated use of high-dose triazole fungicides applied in combination with chlorothalonil. Recent monitoring of pathogen populations in Ireland indicate that there is some evidence of ongoing sensitivity shifts to the triazole fungicides with new races of the *Mycosphaerella graminicola* pathogen being documented as new mutations are identified at the *CYP51* target site. The role of these mutations in affecting the field performance of the key triazole fungicides is being closely studied in field trial studies. In field research studies from 2008 to 2010, the key triazole fungicides epoxiconazole and prothioconazole have shown good to very good efficacy for disease control and yield improvement under high disease pressure conditions. Many of the field studies show clear benefit from the use of chlorothalonil as a partner product with the triazoles, giving improved efficacy and greater persistence from the combined treatment. There are also clear benefits from the use of boscalid and other new carboxamide fungicides in combination with the key triazole fungicides, particularly at the key flag leaf timing. In 2009 and 2010 trials, the azole mixtures, specifically epoxiconazole + metconazole and prothioconazole + tebuconazole combinations, have shown significantly higher efficacy of disease control, and grain yield benefits of 0.5 t/ha+, compared to single azole products. The azole mixtures are now widely used on-farm in Ireland, based on better field performance, but the merits of this practice as an anti-resistance strategy is still uncertain.

Optimizing fungicide use in Denmark for control of *Mycosphaerella graminicola*

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Mycosphaerella graminicola (*Septoria tritici*) is seen as the disease which causes the most important yield reductions in wheat in Denmark. As the attacks vary significantly between years, and as fungicide responses are often relatively low, farmers are recommended to adjust their use of fungicides according to the specific risk situation. The average yield increase from fungicide use during the last 7 years has been calculated to be 6.1 dt/ha. A threshold has been developed based on precipitation and is adjusted depending on the susceptibility of the cultivar.

Days with precipitation are calculated from GS 32 in susceptible cultivars and spraying is recommended if more than 4 days with rain have occurred. In resistant cultivars days with precipitation are counted from GS 37 and treatments are recommended following 5 days with more than 1 mm rain. Analysis of historical fungicide trial data with different input for an ear treatment has shown that the economic optimum varies between 0.4 and 0.75 dose unit (TFI) if effective products are used. Many farmers apply a split application of fungicides, using a total of 0.4 to 0.7 standard dose of an effective product. Denmark relies very much on triazoles for control of septoria as neither chlorothalonil nor prochloraze are authorised products. Boscalid is so far the only non-triazole included for septoria control. Several cultivars have shown very good resistance to *Mycosphaerella graminicola* as well as competitive yields. The variation in yield responses from susceptible cultivars and more resistant cultivars has been in the range of 4 dt/ha.



POSTER

Efficacy of triazoles against *Mycosphaerella graminicola* in Denmark

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Mycosphaerella graminicola is seen as the disease which causes the most important reductions in yield in Denmark. A shift in sensitivity to fungicides has been taking place over the years, it is seen as very important to follow the sensitivity and check the efficacy under field conditions.

Over the years a slight shift in sensitivity has been observed based on EC₅₀ values from epoxiconazole as the main reference. Field performance has been found to be stable for the triazoles although a decrease in efficacy has been seen since the epoxiconazole products were introduced in 2004. Microtiter testing has shown equivalent sensitivity of epoxiconazole and prothioconazole. Characterisation of the CYP51 mutations in the populations has shown a dominance of R6 and R7-types in the Danish population. Repeatedly, the efficacy of epoxiconazole has been seen to be slightly superior to that of prothioconazole, although the yield responses have been equal. Introduction of 3 new triazole mixtures (epoxiconazoles + metconazole; difenoconazole + propiconazole; tebucoanzole + prothioconazole) have shown only slight or no increase in efficacy compared to epoxiconazole used as a reference. For the mixture epoxiconazoles + metconazole an improvement has been seen, which relates mainly to a total increase in input. The mixture of epoxiconazole + boscalid has shown slightly improved control and also a slightly increased yield response and is currently seen as the most effective solution. Despite widespread strobilurin resistance a low input of strobilurins mixed with certain triazole products have been found to still give a slight improvement of control and yield.

POSTER

***Mycosphaerella graminicola*, wheat pathogen of increased interest in Finland**

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In 2009, the wheat cultivation area in Finland was 220 000 ha, spring wheat covering 90% of that area. The incidence of the main wheat leaf spot diseases has been studied in a survey carried out in 2009 on 84 spring wheat fields. According to the results of the PCR-test, only one of the studied fields had none of the tested pathogens. *Stagonospora nodorum* was present on 98%, *Pyrenophora tritici-repentis* on 94% and *Mycosphaerella graminicola* on 6% of the investigated fields. The frequency of both *S. nodorum* and *P. tritici-repentis* infected fields has significantly increased over the last 40 years. Previously, *M. graminicola* was rather common in winter wheat tillers in early spring but the disease has rarely developed further in Finnish conditions. However, since 2006, *M. graminicola* has been observed on the upper winter wheat leaves later in the growing season as well. In 2009, *M. graminicola* was identified on spring wheat in Finland for the first time. The results of the fungicide trials in 2006-2010 showed no evidence of the decreased efficacy of the tested fungicide products in Finland.

POSTER

Septoria leaf blotch control in Sweden

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Septoria leaf blotch, caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*) is now the most important foliar disease of wheat in Sweden. As the most widely grown varieties in Sweden only have medium resistance ratings, fungicide treatment is important. Very few azoles are approved in Sweden: prothioconazole, a mixture of difenoconazole and propiconazole, prochloraz and propiconazole. Fungicides with different mode of actions such as chortalonil and boscalid, are not approved. Concerns regarding the issue of declining field performance and results from field trials are presented. Among the approved azoles in Sweden prothioconazole demonstrated the best control of septoria leaf blotch. A mixture of difenoconazole and propiconazole also performed well. Prochloraz performed better than propiconazole, but both showed insufficient control when used as a single substance. To add prochloraz to prothioconazole improved the efficacy in some cases. The efficacy of the fungicides was measured by visual assessment of the Septoria leaf blotch on the wheat leaves, and the pathogen was also detected using quantitative real-time PCR. There was a good correlation between these two methods of assessing the fungus.

POSTER

Assays of mutations of *Septoria tritici* and effects of different active ingredients (triazoles, imidazoles) on these mutations: an overview of Germany

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The efficacy of several fungicides within the class of azoles against *S. tritici* is decreasing. So far the reviewed *S. tritici* isolates could be assigned to different resistance classes based on their resistance pattern. Genetic alterations in the CYP51 gene are one possible explanation for loss in fungicide sensitivity. There are no consolidated findings about the incidence of resistance classes in Schleswig-Holstein and Germany yet. The Institute of Molecular Phytopathology has developed a system supported by Real-time PCR to detect and quantify most important resistance classes of *S. tritici*. This system was initially optimized and validated at the Institute of Phytopathology and at the Institute of Molecular Phytopathology. It enables the relative quantification of single resistance classes in complex field samples. The system is used to determine the effects of different active ingredients on the frequency of single resistance classes in field populations. Finally, the determination of the efficacy of fungicides against every individual resistance class is carried out, *in vivo* tests under controlled conditions. Collectively, these can further the understanding of shifting resistance within populations according to fungicide treatment.

POSTER

Characterization of emergent *Mycosphaerella graminicola* isolates highly resistant to DMIs: efficiency of fungicide strategies and future insights

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Mycosphaerella graminicola (anamorph *Septoria tritici*) is a major pathogen of wheat worldwide, causing septoria leaf blotch disease. In France and Western Europe, sterol 14 α -demethylation inhibitors (DMIs) have been the key components of fungicides used to control this disease in the last 25 years. However, a clear reduction of the sensitivity towards DMIs has been detected in several field isolates. In particular, a number of resistant and cross resistant strains to a wide variety of DMIs and other active substances, have been identified and monitored through the INRA-BIOGER and ARVALIS survey network in France.

The poster presents the latest laboratory findings emphasizing the phenotype of new emergent resistant strains (RFs, patterns of cross-resistance) and the molecular analysis of the DMI targeted gene sequence (*CYP51*) as well as other mechanisms conferring a multidrug resistant pattern (MDR).

In addition, some of the field results from 2010 are shown in regards of the efficacy of the selected fungicides and their subsequent selective pressure in the context of evolving populations of *Mycosphaerella graminicola*.

These results are important prerequisites to the annual spatio-temporal monitoring and integrated pest management of the disease and fungicide treatment in France and other European countries.

POSTER

Azoxystrobin and epoxiconazole sensitivity profiles of *Mycosphaerella graminicola* populations from the Grand-Duchy of Luxembourg in 2007 and 2008

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Mycosphaerella graminicola strains were isolated from symptomatic winter wheat (*Triticum aestivum*) and spelt (*Triticum spelta*) leaves sampled across the Grand-Duchy of Luxembourg. In total, 484 strains were isolated from winter wheat, and 31 from spelt leaves. The sensitivity profiles of these strains towards azoxystrobin (Amistar®, Syngenta Agro GmbH, Germany) and against epoxiconazole (Opus®, BASF Belgium NV/SA, Brussels, Belgium) were assessed in microplates, allowing to test 10 different concentrations for each active substance (0, 0.00316, 0.01, 0.0316, 0.1, 0.316, 1, 3.16, 10 and 31.6µg/mL). 100µg/mL of salicylhydroxamic acid (SHAM) was also added to the medium, in order to prevent the strains from using alternative respiration.

Results of the sensitivity tests for azoxystrobin showed a dose-dependent decrease of *in vitro* growth for all the strains. During both years, strains could be divided into two distinct sub-populations in relation to their azoxystrobin sensitivity, thus forming a bimodal distribution commonly reported to be associated with a single gene-mediated resistance. For wheat, the dominant sub-population was the one showing full resistance to azoxystrobin.

The results of the sensitivity tests to epoxiconazole in liquid media showed a dose-dependent decrease of fungal growth for all the strains tested. The EC50 values followed an unimodal distribution. When the sensitivity distribution of the strains collected in 2008 (n=379) was compared to that observed in 2007, although the populations have remained unimodal, a minor shift towards less sensitive populations was observed.

Compared to strains originating from wheat, the spelt strains were more sensitive towards both fungicides ($p \leq 0.001$).

POSTER

Distribution of *CYP51* haplotypes in Europe

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Sensitivity of *Mycosphaerella graminicola* to DMIs was monitored over recent years and changes in sensitivity (microtiter test) were observed. Mutations in *CYP51* have been studied as one explanation for this shift. Particularly the impact of the amino acid exchanges V136A, A379G, I381V and mutations/deletions in the YGYG region (at 459-462) on DMI sensitivity have been investigated. In 2009 and 2010, mutations such as V136C, V136A+S524T, V136A+I381V+S524T and D134G+V136A+I381V have been more frequently detected in some European regions. The frequencies of these haplotypes and the frequency of other relatively rare haplotypes containing mutations like D107V, S208T, N284H, A410T and G412A will be followed up. The new haplotypes indicate that the *CYP51* of *M. graminicola* is still an evolving enzyme. However, microtiter assays with DMIs showed wide ranges of ED₅₀ values for all *CYP51* haplotypes indicating that the contribution of the haplotype to the sensitivity is limited and that other mechanisms may be involved. It could be shown that inhibitors of efflux transporters sensitized some isolates to some extent, demonstrating that transporters may influence sensitivity. Studies with heterogeneous collections of isolates from different European regions showed that sensitivities to different DMIs are not strictly correlated. The lack of complete cross resistance suggests that the diversity of DMIs may be an important tool in effective disease control and resistance management.

POSTER

The derivation and validation of a fungicide resistance model

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The evaluation of fungicide resistance management strategies using mathematical models can help select options that are worth testing in the field. However, the usefulness of model predictions depends on their predictive power. To the best of our knowledge, none of the published fungicide resistance models have been validated. In this study, we aimed to derive and validate a mathematical model that predicts the selection for fungicide resistance in foliar pathogens of cereal crops. The model was validated against independent data from four field experiments quantifying selection for a mutation conferring resistance to a quinone outside inhibitor (QoI) fungicide in powdery mildew (*Blumeria graminis* f.sp. *hordei*) on spring barley (*Hordeum vulgare*). Fungicide treatments with azoxystrobin differed in the total applied dose and spray number. For each treatment, we calculated the observed selection ratio as the ratio of the frequency of the resistant strain at the end of the season and its frequency at the start of the season. On a scatter plot of log observed selection ratios on log predicted selection ratios, for all four experiments, the 45° line through the origin explained 89-90% of the variance in the observed selection ratios. We believe this is the first fungicide resistance model for plant pathogens to be rigorously validated. The model can now be used with some degree of confidence to identify potential anti-resistance treatment strategies.

POSTER

Fungicide sensitivity of *Phaeosphaeria nodorum*

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The sensitivity of *Phaeosphaeria nodorum* (the causal agent to stagonospora nodorum blotch) to the fungicides azoxystrobin, propiconazole and prothioconazole was analysed both *in vitro* and through sequencing of the target genes. The *in vitro* sensitivity test of 42 isolates from five Swedish winter wheat fields, collected in 2003–2005, was performed on malt extract agar amended with six concentrations of each fungicide. Four isolates collected during the early 1990s, before azoxystrobin was commercially released for agricultural practice, were used as references. The EC₅₀ values for the sensitivity to azoxystrobin ranged between 0.66 mg L⁻¹ to estimations far above 1000 mg L⁻¹, with an estimated median value of 366 mg L⁻¹. The EC₅₀ values of the reference isolates ranged from 0.02 to 80.72 mg L⁻¹. The *P. nodorum* population is still sensitive to propiconazole and prothioconazole, even though there was some variation in sensitivity for some of the isolates.

Fragments of DNA from 231 isolates, including the reference isolates, were sequenced for the genes of cytochrome *b* and *CYP51* in search for amino acid substitutions known to cause loss of sensitivity to strobilurins and triazoles, respectively. The majority of the *P. nodorum* isolates possessed the amino acid substitution G143A, associated with loss of sensitivity in fungi to strobilurins, except in one field where only half of the isolates had the substitution. The reference isolates were all wild-type. No nonsynonymous substitutions were found in the sequenced part of the *CYP51* gene, associated with triazole sensitivity.

POSTER

Fungicide resistance parameters towards triazoles of the wheat pathogen *Pyrenophora tritici-repentis*

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The pathogenic fungus *Pyrenophora tritici-repentis*, the cause of tan spot in wheat, is an important foliar pathogen worldwide. Strobilurins and DMIs (Triazoles) are today the most widely used fungicides to control *P. tritici-repentis*. In recent years a drift in strobilurin field efficacy has been observed and associated with the development of fungicide resistance, and within the triazoles a major variation in efficacy has been observed. Fungicide resistance parameters towards triazoles of the wheat pathogen *P. tritici-repentis* were investigated. We analyzed the sequence of the two *CYP51A* and *CYP51B* alleles, their upstream sequence, and over expression of the *CYP51* alleles. We selected 14 isolates with a large variation in EC₅₀ values towards prothioconazole, propiconazole, and epoxiconazole. A correlation in sensitivity to these triazoles was observed. Sequencing of the *CYP51A* and *CYP51B* alleles revealed few substitutions. However, it is unclear whether these mutations are linked to fungicide resistance. In the two least sensitive isolates a 127 bp insertion was observed upstream of the *CYP51A* gene, which was the product of transposon movement. However, constitutive overexpression analysis of both alleles did not reveal any overexpression. Further studies are ongoing involving more *P. tritici-repentis* isolates.

POSTER

Eurowheat - Information on *Mycosphaerella graminicola* in an European perspective

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Wheat diseases have a major impact on yield, quality of grain and fungicide requirements. EuroWheat collates data and information on wheat disease management from several countries, analyzing and displaying this information in a European context. It provides significant added value on a European scale to support local advisers, breeders and other partners dealing with disease management in wheat through supporting IPM in the broadest sense.

Fungicides

- Fungicides efficacy ranking – eight wheat diseases including *Mycosphaerella graminicola* ranked by five different countries
- Review of problems related to fungicide resistance
- List of fungicides trade names in seven different countries

Decision support

- Overview and links to wheat decision support systems in Europe
- Disease thresholds for disease control recommended in seven countries
- Overview and documentation of cultural practises reducing specific diseases

Pathogens

- Overview and analysing tool for wheat yellow rust virulence pathotypes in Europe (six countries)
- Fusarium head blight: how to minimise attack and mycotoxins
- Cultivar resistance to Fusarium head blight, including ranking of cultivars

Cultivars and yields

- Yield levels in different countries
- Survey on pesticide use and yield responses to fungicides in EU countries

General information material on disease management

- National documents on disease management

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