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IDENTIFICATION OF EFFECTIVE STRATEGIES FOR MIXING NOVEL AND CONVENTIONAL FUNGICIDE GROUPS TO CONTROL POWDERY MILDEW IN WHEAT

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IDENTIFICATION OF EFFECTIVE STRATEGIES FOR MIXING NOVEL AND CONVENTIONAL FUNGICIDE GROUPS TO CONTROL POWDERY MILDEW IN WHEAT

by

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1. ABSTRACT

This was a six month project the aim of which was to test a limited number of wheat powdery mildew isolates that had clearly defined differences in sensitivity to conventional fungicides for their sensitivity to novel fungicide groups. Six isolates were tested using a detached leaf method to determine the sensitivity of the isolates to the conventional fungicides tebuconazole, fenpropimorph, fenpropidin, and to the novel fungicides, azoxystrobin, kresoxim-methyl, spiroxamine, cyprodinil and quinoxyfen. EC_{50} values were determined by fitting dose response curves for each of the isolates. Based on the patterns of fungicide resistance determined, mildew control strategies were developed to minimise the risk of fungicide resistance.

The results showed that there was a wide range in sensitivities within the wheat mildew population to the morpholine fungicides, to the triazole fungicides and to spiroxamine. The isolates tested were all found to be highly sensitive to the fungicides azoxystrobin, kresoxim-methyl, cyprodinil and quinoxyfen.

A cross resistance pattern between the morpholines and spiroxamine was determined in the work. This means that these fungicides should be regarded as belonging to the same resistance group and would not form an affective anti-resistance strategy if used in mixture or in alternation. There were no other cross resistance patterns determined in the work but the number of isolates tested was small, and did not include the newly identified resistant isolates to the strobilurins or isolates resistant to quinoxyfen. More work would be needed to see if there is any cross resistance between the strobilurins and other chemistry or quinoxyfen and other chemistry. There are no reports of resistant strains of mildew to cyprodinil.

In conclusion, the use of mixtures would appear to be one of the most effective anti-resistance strategies in wheat powdery mildew. The mixing partner should come from a different fungicide group and should be used at a rate where it gives effective control. Particular care should be taken when treating crops that are already heavily infected with mildew when a high dose of an eradicant fungicide should be applied in a mixture.

There is no evidence that the use of low doses increases the risk of resistance to conventional chemistry such as the morpholines or the triazoles, unless they are used as part of multiple split applications. There may be a risk, however, associated with the use of low doses of strobilurins and until this is further investigated they should be used at an effective dose. The best evidence available at the moment would also point to them always being used in mixtures.

There is evidence that multiple applications of morpholines and triazoles increases the risk of resistance, and the number of applications of strobilurins is restricted to two per cereal crop. Quinoxyfen is restricted to use in the early part of the season and this is a particularly effective approach to managing resistance to this product.

2. SUMMARY

After three decades of reliance on the morpholines and the DMIs for mildew control several new fungicide groups have been introduced to control mildew. They include cyprodinil, quinoxyfen, spiroxamine and the strobilurin fungicides. These novel fungicide groups represent a very significant opportunity for improved disease control and increased flexibility in disease control programmes. Inevitably, because of the increased costs of these chemicals, growers utilise them by including them in disease control programmes that also include the cheaper conventional fungicides, an approach that also broadens the spectrum of activity. The impact of using these novel fungicides both alone, in sequence or in mixtures on the sensitivity of the mildew population is not known.

This was a six month project the aim of which was to test a limited number of wheat powdery mildew isolates that had clearly defined differences in sensitivity to conventional fungicides for their sensitivity to novel fungicide groups. Six isolates were tested using a detached leaf method to determine the sensitivity of the isolates to the conventional fungicides tebuconazole, fenpropimorph, fenpropidin, and to the novel fungicides, azoxystrobin, kresoxim-methyl, spriroxamine, quinoxyfen and cyprodinil. EC_{50} values were determined by fitting dose response curves for each of the isolates. Based on the patterns of fungicide resistance determined, mildew control strategies were developed to minimise the risk of fungicide resistance.

The objective was to provide an early indication of the presence or absence of cross resistance patterns between the novel and conventional fungicide groups. For example if testing showed that isolates that were less sensitive to morpholines were also less sensitive to one of the novel groups then this would imply a cross resistance pattern. This would mean that using a morpholine would select for less sensitive mildew to the novel product and that that product should not therefore be used in the same programme as a morpholine. A negative cross resistance pattern would mean that using one product would select for more sensitive isolates to the second product and that these two products would therefore work well together in a disease control programme as each would increase the efficacy of the other.

The work reported here, although based on a limited number of mildew isolates showed that there was a wide range in sensitivities within the wheat mildew population to the morpholine fungicides, to the triazole fungicides and to spiroxamine. The isolates tested were all found to be sensitive to the fungicides azoxystrobin, kresoxim-methyl, cyprodinil and quinoxyfen.

A cross resistance pattern between the morpholines and spiroxamine was determined in the work. This means that these fungicides should be regarded as belonging to the same resistance group and would not form an affective anti-resistance strategy if used in mixture or in alternation. They are the only two fungicide groups with strong eradicant activity against mildew but this means that in practice they would be most effectively mixed with fungicides that are more protectant in nature and with which there is no evidence of cross resistance.

There were no other cross resistance patterns determined in the work but it should be remembered that the number of isolates tested was small, and did not include the newly identified resistant isolates to the strobilurins, or isolates resistant to quinoxyfen. More work would be needed to see if there is any cross resistance between the strobilurins and other chemistry or quinoxyfen and other chemistry. There are no reports of resistant strains of mildew to cyprodinil.

On the basis of this report the use of mixtures would appear to be one of the most effective anti-resistance strategies in wheat powdery mildew. The mixing partner should come from a different fungicide group, as described above, and should be used at a dose rate where it gives effective control. Due regard for the characteristics of the fungicide should be made so that fungicides that are largely protectant in nature are not applied where mildew infection is already established. Particular care should be taken when treating crops that are already heavily infected with mildew when a high dose of an eradicant fungicide should be applied in a mixture. In practice this means applying either a morpholine or spriroxamine. For wheat powdery mildew the most effective morpholine is fenpropidin. Spiroxamine and the morpholines should not be mixed in the same programme as part of an anti-resistance strategy.

The issue of dose rates is controversial. There is no evidence that the use of low doses increases the risk of resistance to conventional chemistry such as the morpholines or the triazoles, unless they are used as part of multiple split applications. There may be a risk however associated with the use of low doses of strobilurins and until this is further investigated they should be used at an effective dose. They also show the best yield responses and cost effectiveness when applied at higher rates. The best evidence available at the moment would also point to them always being used in mixtures.

Application number should be carefully determined. There is evidence that multiple applications of the conventional fungicides, the morpholines and triazoles, increases the risk of resistance, and the number of applications of strobilurins will be restricted to two per cereal crop after the 1999 season. This reduces the selection pressure in favour of resistant strains of mildew and should prolong the useful activity of the fungicides. Quinoxyfen is restricted to use in the early part of the season and this is a particularly effective approach to managing resistance to this product. Strains of mildew have been identified which are resistant to quinoxyfen but there is a fitness cost associated with this, and where the fungicide is not present they will die out, leaving only sensitive isolates.

It is critical that anti-resistance strategies are followed with powdery mildew as it is a disease at high risk of developing resistance to fungicides. It has already evolved strains resistant to the DMIs and morpholines with the result that these fungicides are less effective than they were, and in Germany in 1998 strains were identified that were resistant to strobilurins. It is essential that, to maintain the cost effectiveness and yield benefits of the fungicides with activity against mildew, anti-resistance measures are carefully followed by growers and their advisors.

3. INTRODUCTION

While fungicide resistance is still with us 30 years after it was first identified as a problem, much has been learned about managing resistance and reducing it's impact. Pesticide manufactures have long had to submit packages of data to show the safety and efficacy of new fungicides. They now also have to a submit a resistance risk analysis for each new product. This means that the risks associated with products are at least partially understood before launch and that products with a very high risk of resistance occurring may not even reach the market, or may be launched only in mixtures or with other restrictions applied.

Contrast this with the rapid rise in resistance in potato blight to the phenylamide group of fungicides 20 years before. It is now recognised that resistance was probably naturally present in the population even before launch and problems were therefore inevitable. This is probably also a good example of how resistance can be managed as these fungicides are still widely used, and effective despite the early identification of resistance, with the restrictions that they are never used alone and that they are never used on active blight infection. This is the aim of resistance studies - to prolong the effective life span of useful fungicide groups.

Fungicides based on the active ingredients of the triazole and morpholine groups have been used to control powdery mildew in wheat for over 20 years. After decades of reliance on these two fungicide groups, the major agrochemical manufactures have introduced several new fungicide groups to control mildew. They include cyprodinil, quinoxyfen, spiroxamine and the strobilurins. These novel fungicide groups represent a very significant opportunity for improved disease control and increased flexibility in disease control programmes. Inevitably, because of the increased costs of these chemicals, growers utilise them by including them in disease control programmes that also include the cheaper conventional fungicides. The impact of using these novel fungicides both alone, in sequence or in mixtures on the sensitivity of the mildew population is not known, and experience has shown that effective anti-resistance strategies have to be developed early for maximum effectiveness. It was the aim of this project to identify effective strategies for mixing novel and conventional fungicides that would maximise powdery mildew control but that would minimise the risk of resistance to any of the products.

Fungicide resistance

When a fungicide controls a fungus effectively at the recommended dose, the fungus is classed as 'sensitive' to the fungicide, but less sensitive strains of the fungus may arise which are less sensitive and the pathogen is not adequately controlled. It is a source of some debate at which level of insensitivity pathogens are classed as resistant, but it is generally agreed that fungicide resistance is defined as a measurable loss in sensitivity in the lab, combined with a reduction in effectiveness in the field. Changes in the sensitivity of the pathogen to the fungus can either be non-genetic and therefore of little importance in practice (Dekker, 1972), or they can be genetic in which case the characteristic can be inherited giving rise to resistant strains of the pathogen.

Fungi may become resistant to a fungicide in a number of different ways. The most common is through a change in the target site for the fungicide within the fungal cell, so that the fungal cell has a reduced affinity for the fungicide. Another mechanism of resistance is that changes may occur in the fungal cell which reduce the uptake of the fungicide so that less reaches the target site within the cell. Resistance to triazoles is widely believed to occur in this way as resistant isolates appear to accumulate reduced levels of the fungicide (Klepser *et al.*, 1997).

Resistance can also occur when strains of the pathogen can detoxify the fungicide before the site of action is reached or the pathogen can compensate for the inhibitory effect, perhaps through the increased production of an inhibited enzyme. Finally pathogens can also become resistant where they circumvent the blocked biochemical pathway by the operation of an alternative pathway, although this is rare.

Genetics of resistance

The genetics of resistance are an important aspect of resistance studies as this will affect both the pattern of resistance and the management strategies. Resistance to some fungicides develops in a single step as a result of a mutation in a single gene and such a mutation can achieve the highest level of resistance possible and with this type of genetic control populations of the fungus fall in to two distinct groups - one resistant, the other sensitive, with no overlap between the groups (Georgopoulos and Skylakakis, 1986).

An alternative form of resistance, however, can occur when many mutated genes are required to achieve the highest level of resistance. As these resistant genes will be found in combination with the unmutated genes in various ratios there is a continuous distribution in the measured sensitivity within the population rather than two distinct resistant and sensitive groups. This type of resistance is termed polygenic resistance (Georgopoulos, 1987)

Resistance risk analysis

In order to prepare a resistance risk analysis for a new product, manufacturers must look at both the target pathogen and the novel chemistry. Some pathogens have an inherently lower risk if they reproduce slowly and have relatively stable genomes. Others have a much higher inherent risk if they reproduce rapidly and have a genetic flexibility brought about by either mutation or sexual reproduction so that changes in the genome commonly occur. Mildew is classed as being a pathogen at high risk of resistance as it multiplies rapidly and can have many, many generations within the one season. It is also easily airborne and has a sexual stage allowing for the recombination of genetic material.

The mode of action of the fungicide is also a significant factor in the risk analysis. Up until the 1960s nearly all fungicides were classed as multi-site inhibitors, in other words they acted at many sites within the fungal cell (Henry, 1992). These multi-site fungicides were all protectant in nature. With the introduction of the first of the

systemic fungicides that could be transported within the plant there was a huge step forward in disease control as these products also offered curative disease control. Yield responses were large and they were soon widely used. These systemic products were much more specific in their of mode of action - most are now classed as single site inhibitors - and were therefore relatively easy for the target pathogens to overcome (Henry, 1992).

One major influence on the risk of resistance is the fitness of any resistant isolates. Some resistant forms are not so well adapted for survival and do not compete as well as the sensitive forms of the pathogen so that in the absence of the fungicide they die out and disappear from the population. If resistance is correlated with reduced fitness then it may slow down resistance development significantly (Shaw, 1989).

In a resistance risk analysis the sensitivity of the pathogen population to the novel fungicide is measured before the novel fungicide is introduced to the market. Resistant isolates may be easily generated using mutagenic agents in the laboratory. If resistant mutants are easily generated this can be an indication that resistance may occur easily in the field. Field monitoring will also be carried out to see if naturally occurring resistant isolates already exist. If, and when, resistant isolates are found their fitness and any cross resistance patterns with other fungicide groups will be studied, as will the genetics of resistance to determine if it is monogenic or polygenic and how stable the resistance is (Hilber and Schüepp, 1994). All this will determine the resistance risk associated with the novel product.

The resistance risk with a product is a combination of the inherent risk and the management resistance risk (Staub and Sozzi, 1984). This means that fungicides with a high risk of resistance can still be used successfully if an effective management strategy is developed, and also that low risk fungicides can develop resistance problems if they are used improperly. Several methods have been proposed to manage the resistance risk in pathogen populations. The use of either mixes of fungicides or alternations of fungicides is one key component of such anti-resistance strategies. For these strategies to be effective both components in the mixture or alternation must be effective against the fungus, and there must be no cross-resistance pattern between them. The alternation strategy will only work where there is a fitness cost associated with the resistance characteristic. Alternative strategies can involve limiting the number of applications of the fungicide that can be made, or limiting them to a particular part of the disease control programme. Occasionally an active ingredient can be withdrawn and then re-introduced at a later date.

Powdery mildew *Erysiphe graminis*

Powdery mildew is caused by the fungus *Erysiphe graminis*, which is a major disease of cereals in temperate regions world wide. It is also an important disease of cereals in the UK and can reduce yield by 40% (Gair *et al.*, 1978). Where plants are very severely affected the leaves may be deformed and ear development may be wholly or partially checked. Infections to very young cereals in the autumn can reduce tiller numbers. An early infection of mildew can reduce the frost hardiness of plants and can also reduce the size of the root system. This can have serious implications on the

yield potential of the crop, especially if soil moisture is low later in the season. Later attacks largely affect the size of the grain although the number of grain sites can also be reduced. This causes a direct reduction in yield as well as a reduction in quality through more smaller grains, which can have additional finical implications.

The fungus is found as specialised forms (*formae speciales*) which are restricted to the specific host that they attack so that forms on wheat only attack wheat and those on barley only attack barley etc. Within these specialised forms are different physiological races which can be distinguished by the reaction of certain cultivars of the appropriate host.

The symptoms of powdery mildew are of a white fungal growth on the surface of the plant with a powdery appearance. The upper surface of the leaves is normally affected but it can attack the underside as well as leaf sheaths, stems and inflorescences (Jones and Clifford, 1983). The first symptoms appear as white, fluffy pustules which can eventually coalesce to cover large areas of the leaf surface. As the mycelium ages it forms a thick mat in which can be embedded small, black, globose bodies called cliestothecia. Infections are likely to be worse where crop growth is lush, as when the crop is growing rapidly or in high nitrogen situations.

The disease is favoured by warm and humid conditions that favour spore germination and infection. The spores are air borne and are blown into the host crop. On germination a germ tube grows out along the plant surface and penetrates the host plants cell wall. Once inside the cell a haustorium is formed which is the body used by the fungus to draw nutrients and water from the plant. Secondary haustoria are formed in colonies usually within 4 days of inoculation. The mycelium is entirely superficial and after around 5 days this will give rise to conidiophores, which are short chains of spores called conidia and which may be fully developed 2 days later (Smith and Blair, 1950). The whole infection cycle is normally 7 - 10 days. The conidia represent the asexual form of reproduction but there is also a sexual phase which starts with the formation of cleistothecia in older colonies. These resting bodies form mainly when the host plants are senescing and are important as they not only allow the recombination of genetic material, allowing increased variation in the population, but they are also the main method by which the fungus survives the period before and during harvest when there is little or no green host plant material available.

Mildew can only survive on living plant tissue and therefore survives over the winter on winter wheat and barley as well as on volunteers. During the spring conidia are produced in large numbers and the disease spreads, first to neighbouring plants and then to surrounding crops (Gair *et al.*, 1978). The disease is wind dispersed and although conidia are short lived it is now widely agreed that they can survive and travel for long distances of several hundred kilometres (Hermansen *et al.*, 1978).

The specialised from of mildew that attacks wheat is called *Erysiphe graminis f.sp. tritici*. Visually it differs slightly from the form found on barley, *Erysiphe graminis f.sp. hordei*, in that is tends to form smaller and more discrete pustules on the leaves. It is also more common on the stem and lower leaf sheaths than the barley equivalent.

Control of powdery mildew

The principal methods of controlling mildew are the use of host plant resistance, the use of fungicides and through cultural methods such as reducing the carry-over of inoculum on stubble, volunteers and winter cereals.

Cultivar resistance is an important factor in controlling mildew. For winter barley and wheat resistance there is a combination of minor and major genes in a horizontal, polygenic back ground but the adaptability of the disease means that it is often able to form new physiological races capable of over coming the resistance genes of a resistant cultivar. One of the most durable resistance genes has proved to be the *Mlo* gene in spring barley which was first introduced in 1979. It is now present in many varieties and can therefore be present in up to 70% of the spring barley sown in a season (Newton and Young, 1996). A single gene spread over such a wide area may pose a serious threat, especially as an *Mlo* aggressive race of mildew has been reported in Japan (Lyngkjaer et al., 1995).

Fungicides groups effective against powdery mildew

There are several fungicide groups currently used in the UK which have activity against powdery mildew in cereals. These include active ingredients from the morpholines, the DMIs, the hydroxypyrimidines, the spiroketalamines, the quinolenes, the strobilurins and the anilinopyrimidines.

a. Morpholines

The morpholines are one of just two chemical groups with a strong eradicant activity against mildew. They are fully systemic compounds with a strong vapour phase which also allows redistribution of the compounds. As well as eradicant activity they also offer some protectant activity.

The morpholines work by inhibiting sterol biosynthesis at at least two point in the biosynthetic pathway. Ergosterol is an important constituent of fungal cell walls and inhibition of sterol biosynthesis is therefore detrimental to fungal growth and development. They are therefore very effective at preventing mycelial growth.

One example of this group is the active ingredient fenpropimorph. Products include Mistral, Aura (Novartis Crop Protection UK Ltd.), Corbel (BASF plc) as well as many others. A second morpholine, tridemorph (i.e. Calixin, BASF plc), from 1999, can no longer be used in the UK for reasons of safety. A closely related active ingredient, fenpropidin, is also commonly included in the morpholine group and products include Mallard and Tern (Novartis Crop Protection UK Ltd.) and Patrol (Zeneca Crop Protection).

b. DMIs

A large number of DMI or triazole fungicides have been introduced for use on cereals and they act by blocking ergosterol biosynthesis, at a different point in the biochemical pathway to the morpholines. Ergosterol is an important constituent of

fungal cell walls and inhibition of sterol biosynthesis is therefore detrimental to fungal growth and development.

The DMIs are fully systemic and have eradicant and protectant activity and are the most widely used chemical group on wheat and barley. Active ingredients include epoxiconazole (i.e. Opus or Epic, BASF plc), tebuconazole (Folicur or Halt, Bayer plc), propiconazole (i.e. Tilt, Novartis Crop Protection UK Ltd).

c. Hydroxypyrimidines

Ethirimol is the only commercially available active ingredient in this group of fungicides which act by inhibiting the synthesis of nucleic acid. They therefore interfere with cell division and prevent fungal growth in this manner. Ethirimol is now only available in a mixture with flutriafol and thiabendazole for use as a seed treatment in barley (Ferrax from Bayer plc).

d. Spiroketalamines

Spiroketalamines are a novel group of fungicides that act by inhibiting the production of ergosterol thereby interfering with fungal cell wall structure and inhibiting fungal growth.

The only commercially available active ingredient is spiroxamine i.e. Torch, Neon (Bayer plc). It is the only group, along with the morpholines, to offer a strong eradicant effect on mildew.

e. Quinolenes

The mode of action of the quinolines is unknown, but they are strong inhibitors of the early stages of mildew infection. The only commercially available active ingredient in the UK, quinoxyfen i.e. Fortress or Apres (Dow AgroSciences), has a strong protectant effect on powdery mildew. Quinoxyfen is fully systemic and was introduced in the UK in 1998. It does not have eradicant activity. It has been widely used since it's introduction to control powdery mildew in susceptible cereal varieties.

f. Strobilurins

The strobilurin group of fungicides are based on an antifungal compound found in toadstools. They act by inhibiting electron transport during respiration in the mitochondria, the part of the fungal cell that generates energy. The strobilurins are particularly effective at preventing sporulation in fungi, but will also reduce mycelial growth (Gold and Leinhos, 1995). They are most effective when used in a preventative manner and do not offer eradicant activity. The strobilurins also tend to have a greening effect on the crop and have been linked with significant yield improvements over conventional chemistry. The strobilurin group and related compounds have been given the generic name of 'STAR' products.

Active ingredients from this group that are already on the UK market are azoxystrobin (Amistar, Zeneca Crop Protection) and kresoxim-methyl (in Ensign, Mantra, Landmark, BASF plc). Azoxystrobin is fully systemic and kresoxim-methyl is classed as quasi-systemic as it redistributes itself over the leaf surface in a vapour phase. Most of the other major chemical manufactures also have strobilurin

fungicides in their development systems and these will come on to the market in the coming few seasons.

g. Anilinopyrimidines

This is another of the novel classes of fungicides that have reached the market place in the last couple of seasons. The only commercial example from this group that is approved for use on cereals in the UK is the active ingredient cyprodinil (i.e. Unix, Novartis Crop Protection UK Ltd.). It is fully systemic and widely used on wheat at early stem extension due to it's activity against common eyespot. It is also widely used on barley as it has activity against Rhynchosporium and net blotch. It shows protectant activity against both wheat and barley mildew.

The anilinopyrimidines act by interfering with the biosynthesises of methionine, possibly at a single site, and therefore have the strongest activity against germ tube formation, just as mildew spore are germinating. They are systemic in nature.

Fungicide resistance in powdery mildew

The high risk of resistance with powdery mildew is best demonstrated by looking at the number of fungicides that have been used in the last 30 years, and the speed with which the efficacy of many of them has declined. Up until 1996 three groups of systemic fungicides had been approved for use in the UK for the control of wheat and barley mildew. Ethirimol was the first systemic fungicide to be used to control powdery mildew in the UK when it was introduced as a seed treatment in 1969 (Brooks, 1971). Mildew sensitivity to ethirimol quickly declined and Brown *et al.* (1990) found that resistance to ethirimol in barley powdery mildew was under the control of just a few major genes, which may explain why it failed so rapidly.

Following the decline in sensitivity to ethirimol, from 1978 onwards ethirimol was largely replaced by the broader spectrum triazoles, triadimefon, propiconazole and triadimenol. Intensive use of these active ingredients was soon followed by a decline in their effectiveness so that by the mid 1980s the activity of these DMIs against powdery mildew had fallen to a level where disease control failure was commonly encountered (Clark, 1992). Although newer triazoles are more effective against powdery mildew than older triazoles, there are clear cross-resistance patterns between the various products (Gisi *et al.*, 1986).

The first of the morpholines to be introduced was tridemorph in the 1960s. Reduced sensitivity to this product was first reported in 1979 (Walmsley-Woodward et al. 1979). Fenpropimorph and fenpropidin were introduced in the 1970s and reduced sensitivity to fenpropimorph was detected in barley mildew in 1986 (Wolfe *et al.* 1986). The decline seen up until 1992 was not thought to be large enough to affect field performance (Brown and Evans, 1992). Since then there have been further declines in sensitivity with reports that more frequent applications were required for field control (Readshaw and Heaney, 1994). Within the UK work has shown that barley sampled from Scotland was significantly less sensitive to morpholines that that sampled in England (Burnett and Zziwa, 1998). The double block effect on the

synthesis of ergosterol that the morpholines have may be one reason why resistance has been so slow to evolve despite their intensive use. Resistance to the morpholines in wheat powdery mildew has been linked to reduced fitness (Engles and de Waard, 1996) which may also have delayed the onset of resistance problems.

By the mid 1990s several new active ingredients were approaching the UK market place (Godwin *et al.* 1992, Heyes *et al.* 1994). One important group was the strobilurins of which azoxystrobin (Amstar) and kresoxim-methyl (in Ensign) are now widely used in the UK. These products are now commonly known as the STAR compounds (strobilurins and related chemistry).

These compounds were thought to represent a low risk of resistance but in the summer of 1998, as this project was drawing to a close, isolates of wheat mildew that were resistant to strobilurins were identified in Germany. There was an associated loss of control in the field and this has been tentatively linked to the way the strobilurins had been applied in Northern Germany. Multiple low dose applications had been made of the product Juwel, containing kresoxim-methyl and epoxiconazole, in an area where mildew pressure was very high (S. Heaney. pers.comm.). Circumstantial evidence therefore points to repeated applications of strobilurin products representing an increased risk of resistance, particularly when used on active mildew infections. The Fungicide Resistance Action Committee - the body that represents the agrochemical production companies therefore issued a statement in 1999 recommending that STAR products be used as follows:-

1. Use no more than 2 STAR containing sprays per crop
2. Use manufacturers recommended rate
3. Use effective doses of effective mixture partners

FRAG-UK, the group that represents independent scientists broadly endorsed this strategy, but it remains unclear what the precise influence of dose and number of applications has on STAR resistance.

Resistant isolates have not yet been identified in the UK and it is hoped that by putting in place anti resistance strategies the problem can still be avoided in this country. Clearly, however, the development of resistant isolates poses a very real danger of these compounds failing and makes the development of effective and validated strategies for use more critical.

Studies have suggested that the resistance risk for quinoxyfen, from the novel group the quinolenes, is low (Hollomon *et al.*, 1997). During the determination of base line sensitivity data for this fungicide, however, resistant isolates were found in the field and in the lab, after treatment with the fungicide. Theses strains were always defective in some way and all required the presence of quinoxyfen to survive. The recommended anti-resistance strategy avoids its use in seed treatments and late season applications after GS 49. It is recommended for use as a protectant and if mildew is present at spraying an effective eradicant fungicide should be mixed.

Objectives

After decades of reliance on the morpholines and the DMIs for mildew control several new fungicide groups have been introduced to control mildew. They include cyprodinil, quinoxyfen, spiroxamine and the strobilurin fungicides. These novel fungicide groups represent a very significant opportunity for improved disease control and increased flexibility in disease control programmes. Inevitable, because of the increased costs of these chemicals, growers utilise them by including them in disease control programmes that also include the cheaper conventional fungicides, an approach that also broadens the spectrum of activity. The impact of using these novel fungicides both alone, in sequence or in mixtures on the sensitivity of the mildew population is not known.

The programme of research reported here used a limited number of mildew isolates already categorised for sensitivity to the morpholines and triazoles fungicides, which have been traditionally used by growers, and tested them for their sensitivity to the novel groups now entering the market.

The aim was to provide an early indication of the presence or absence of cross resistance patterns between the novel and conventional fungicide groups. For example if testing showed that isolates that are less sensitive to morpholines were also less sensitive to one of the novel groups then this would imply a cross resistance pattern. This would mean that using a morpholine would select for less sensitive mildew to the novel product and that that product should not therefore be used in the same programme as a morpholine. A negative cross resistance pattern would mean that using one product would select for more sensitive isolates to the second product and that these two products would therefore work well together in a disease control programme as each would increase the efficacy of the other.

The aim of the project was therefore to provide an early indication of which fungicide mixtures and sequences would offer effective control, and would also represent a rational strategy for managing fungicide resistance. This careful management of novel and conventional chemistry is essential in order to provide growers with more flexibility to use new fungicides and reduce costs without shortening the life of novel or existing fungicides by increasing the risk of disease resistance.

4. MATERIALS AND METHODS

The method used to test the sensitivity of wheat powdery mildew isolates was a standard leaf segment method that had been used at SAC for many years in sensitivity monitoring work.

The isolates used were taken from the set of standard isolates held at SAC and chosen to represent isolates that were known to differ in their sensitivity to conventional chemistry. The isolates used are listed in Table 1.

Table 1. *Erisiphe graminis* f.sp. *tritici* strains used in tests

Isolate name	characteristics
WC/3	triazole sensitive, morpholine sensitive
AB/96/A	triazole sensitive, morpholine sensitive
96/183/1	triazole sensitive, morpholine resistant
R156	morpholine resistant triazole resistant
95/307/C	morpholine resistant triazole resistant
ATH/94/B	morpholine sensitive, triazole resistant

Maintenance of mildew isolates

The wheat cultivar used to maintain isolates and to carry out the tests was the variety 'Cerco' which carries no known resistance genes to powdery mildew. The wheat isolates were maintained on this cultivar on isolation propagators, using whole plants so that sufficient inoculum could be multiplied up for the tests. For the multiplication of inoculum around 20 seedlings were used per pot. The isolation propagator uses 12.5 cm pots covered in clear perspex covers and operates by blowing a continuous stream of air up through the centre of each pot and cover to avoid contamination from airborne inoculum and cross contamination from other mildew isolates. Plants are watered from a reservoir underneath through wicks.

Mildew isolates were sub cultured twice, taking six weeks, on whole plants on the isolation propagator to generate sufficient inoculum (22 pots inoculum required per isolate).

Testing of isolates

To carry out the tests, wheat seedlings were grown as above with around 10 plants per pot. Test plants were grown on an isolation propagator until GS 13 - 14. These were then transferred to a dedicated spray cabinet and sprayed with the test fungicides using a Humbrol spray gun for 10 seconds. The compounds and dose rates used are listed in Table 2.

Control plants were sprayed with water. Each spray treatment was replicated using the same spray cabinet. Treated sets of plants were kept apart for 24 hours before the

preparation of leaf segments. To carry out the tests 16 segments (2 cm long) were cut from the second true leaf of the treated plants from each concentration and spray cabinet combination and then plated, eight per petri dish, on Davis minimal medium containing 80 mg l^{-1} benzimidazole and inoculated with the experimental isolates. Inoculation was carried out by transferring the mildew inoculum from the heavily infected plants used for maintenance and distributing this evenly over the surface of the leaf segment using a fine, sterile paint brush. The inoculum used was 14 days old. Dishes were placed in an 18°C incubator and kept in a 12 hour photoperiod.

After 14 days the surface area of the leaf segments affected was assessed and recorded. This was then analysed using a Genstat 5 programme that calculated the median percentage mildew cover and fitted symmetrical logistic curves to the data. From this EC_{50} values can be calculated. The EC_{50} is estimated from the dose-response curve as the median effective concentration of the pesticide which produces half the maximum effect on the isolate (Brown, 1991). This gives a measure of how sensitive isolates are to the pesticide, a low value representing a sensitive isolate and a high value representing a resistant or insensitive isolate.

Table 2. Test chemicals and dose rates used in tests.

Active ingredient	Commercial product	g a.i./ha	Amount of full commercial dose rate applied.
1) Azoxystrobin	Amistar	250	1/32, 1/16,1/8, 1/4 of normal dose
2) Krezoxim-methyl	test substance*	105	1/32, 1/16,1/8, 1/4 of normal dose
3) Fenpropimorph	Corbel	750	1/32, 1/16,1/8, 1/4 of normal dose
4) Tebuconazole	Folicur	105	1/32, 1/16,1/8, 1/4 of normal dose
5) Water control			
6) Quinoxyfen	Fortress	150	1/32, 1/16,1/8, 1/4 of normal dose
7) Cyprodinil	Unix	1000	1/32, 1/16,1/8, 1/4 of normal dose
8) Spiroxamine	Neon	750	1/32, 1/16,1/8, 1/4 of normal dose
9) Fenpropidin	Patrol	750	1/32, 1/16,1/8, 1/4 of normal dose
10) Water control			

Kresoxim methyl is formulated for use in cereals in a mix with fenpropimorph as the product Ensign (kresoxim-methyl, 150g/l, plus fenpropimorph, 300 g/l). It was applied here as straight kresoxim-methyl at an equivalent rate to the full commercial dose in Ensign.

5. RESULTS

Table 3 shows the results of the sensitivity tests for each of the isolates and chemical combinations used. The results are recorded as EC_{50} values which show the estimated median effective concentration of the pesticide which produces half the maximum effect on the isolate. This concentration is quoted as micrograms of active ingredient per millilitre (μg/ml).

Table 3. EC_{50} values for isolates tested (μg/ml)

	Isolate name					
Active ingredient	96/183/1	AB/96/A	WC/3	ATH/94/B	R156	95/307/C
Azoxystrobin	107	104	107	110	156	127
Krezoxim-methyl	28	28	27	29	29	27
Fenpropimorph	341	220	194	227	297	1571
Tebuconazole	77	74	74	82	99	206
Quinoxyfen	43	42	44	43	45	42
Cyprodinil	322	347	390	573	343	351
Spiroxamine	1361	542	612	605	734	1150
Fenpropidin	283	229	259	226	261	328

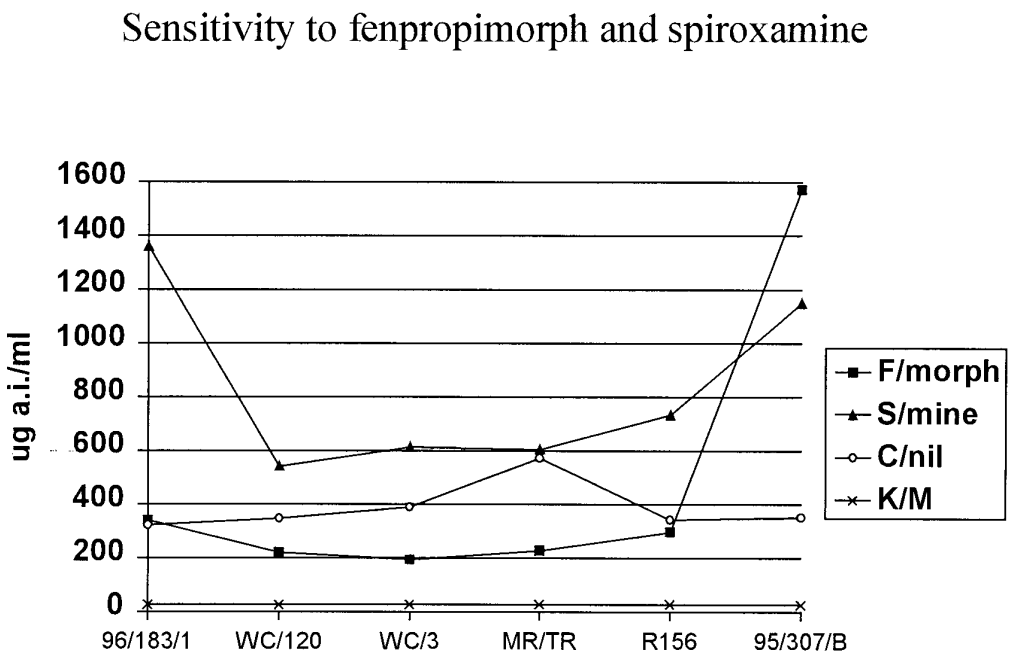
The EC_{50} values of the isolates to the fungicides used showed that for some fungicides the sensitivity of the isolates varied over a wide range of values, while for others the response was quite stable.

The isolates showed a wide range of responses to fenpropimorph indicating the variation in sensitivity to this compound that exists. Some isolates would still be classed as very sensitive, such as WC/3, while others such as 95/307/B would be classed as insensitive.

Sensitivity to fenpropidin, tebuconazole and spiroxamine also varied over a wide range of EC_{50} values. On the other hand the EC_{50} values for the strobilurin fungicides azoxystrobin and kresoxim-methyl were tightly grouped and showed little variation between isolates. Quinoxyfen also showed very little variation in sensitivity between isolates, with EC_{50} values tightly grouped. All isolates were sensitive to cyprodinil, with only small differences between isolates.

Figure 1 shows the different responses of the isolates to the different fungicides used in the sensitivity tests.

Figure 1.



Key

F/morph	= fenpropimorph
S/mine	= spiroxamine
C/nil	= cyprodinil
K/M	= kresoxim-methyl

Figure one shows how the isolates varied widely in their sensitivity to conventional chemistry including the morpholines, fenpropimorph and fenpropidin and the triazole tebuconazole. The was also a wide variation in the responses to spriroxamine indicating that there is a variation in sensitivity to this product.

The responses of the isolates to kresoxim-methyl, quinoxyfen and cyprodinil were very stable, indicating little variation in sensitivity to these products.

Correlation in sensitivity between products.

The EC_{50} values were analysed to see if any cross resistance patterns exist between the active ingredients or groups of fungicides used. Table 4 shows the Pearson correlation coefficients between each group.

Table 4. Correlation coefficients between active ingredients

A.I.	fenprop-idin	fenprop-imorph	quin-oxyfen	tebu-conazole	spirox-amine	kresoxi m-methyl	azoxy-strobin
fenprop-imorph	*0.861						
quin-oxyfen	-0.182	-0.469					
tebu-conazole	*0.823	*0.986	-0.363				
spirox-amine	0.818	0.533	-0.225	0.444			
kresoxim -methyl	-0.591	-0.514	0.383	-0.427	-0.279		
azoxy-strobin	0.289	0.243	0.591	0.375	0.047	0.356	
cyprod-inil	-0.514	-0.237	-0.018	-0.193	-0.460	0.419	-0.239

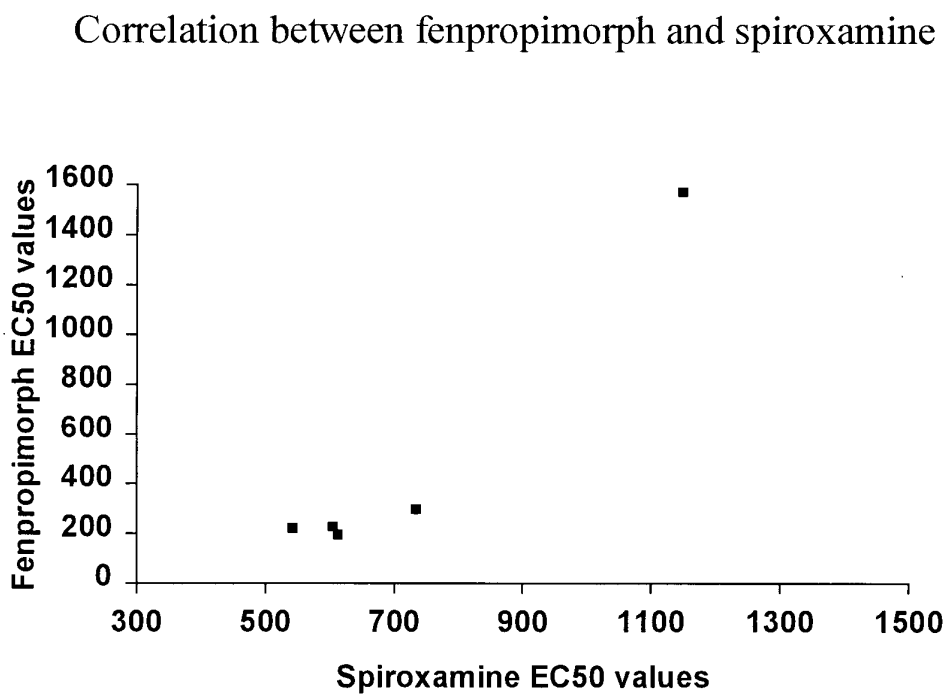
* indicates values that show a significant correlation (P = 0.05)

There was a significant positive correlation between the sensitivity of the isolates to fenpropimorph and fenpropidin. There was also a significant correlation in sensitivity to these to morpholines and tebuconazole.

Spiroxamine also showed a significant positive correlation to fenpropidin. There was no significant correlations between any other combinations of active ingredients.

Figure 2 shows a scatter diagram of the EC_{50} values from the tests involving fenpropimorph and spriroxamine

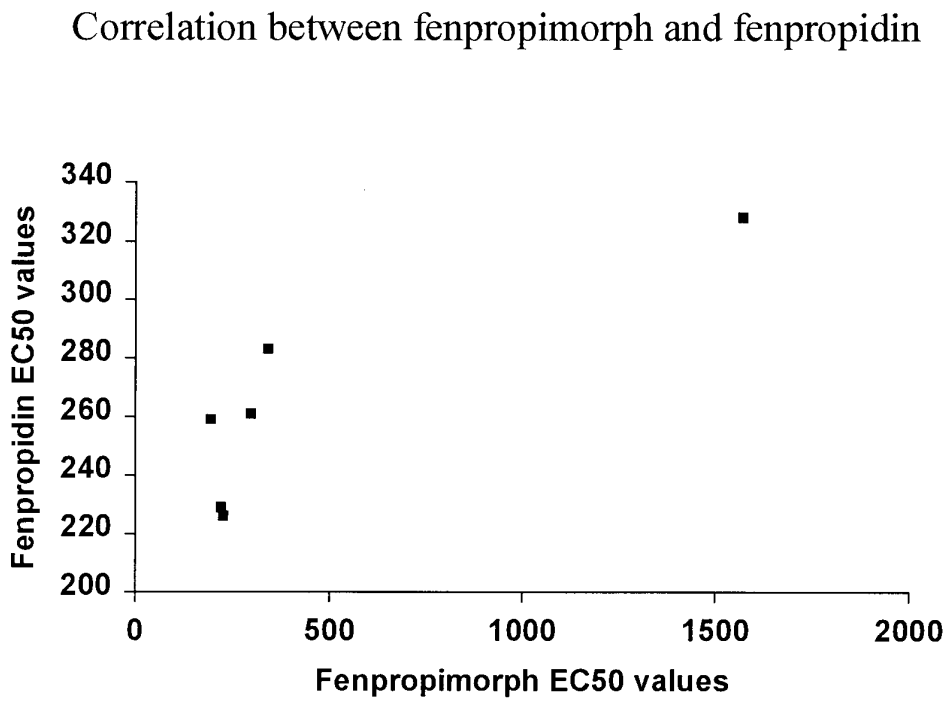
Figure 2.



There was a positive correlation between the sensitivity values for spiroxamine and fenpropimorph, so that isolates that tended to have low EC_{50} values to fenpropimorph also tended to have low EC_{50} values to spriroxamine.

There was a positive correlation in the way the isolates reacted to the two morpholines, fenpropimorph and fenpropidin so that isolates with a high EC_{50} value to one also tended to have a high EC_{50} to the other. This is shown as a scatter diagram in Figure 3.

Figure 3.



There was a positive correlation between fenpropimorph and fenpropidin (r = 0.861).

There was also a positive correlation between fenpropimorph and tebuconazole (r = 0.986) and fenpropidin and tebuconazole (r = 0.823) and this association is shown in Figure 4, overleaf.

There was no association between the sensitivities of the isolates to either of the strobilurins and their sensitivities to the morpholines or tebuconazole. There was also no correlation between the sensitivities of the isolates to these conventional fungicides and that to cyprodinil or quinoxyfen. A scatter diagram showing the absence of a correlation between the EC_{50} values to kresoxim-methyl and fenpropimorph is shown in Figure 5.

Figure 4.

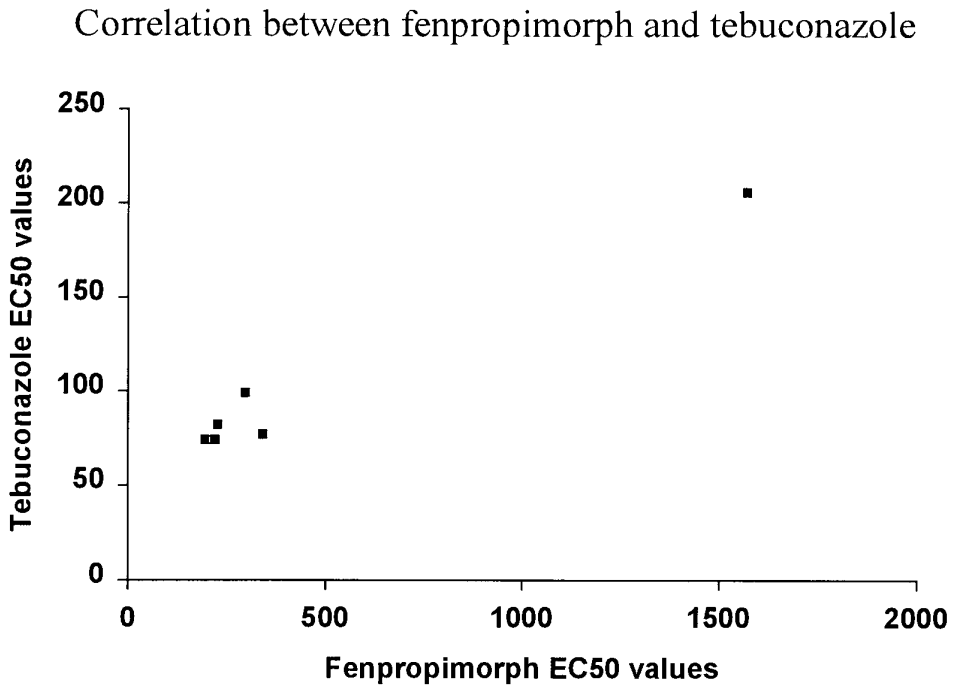
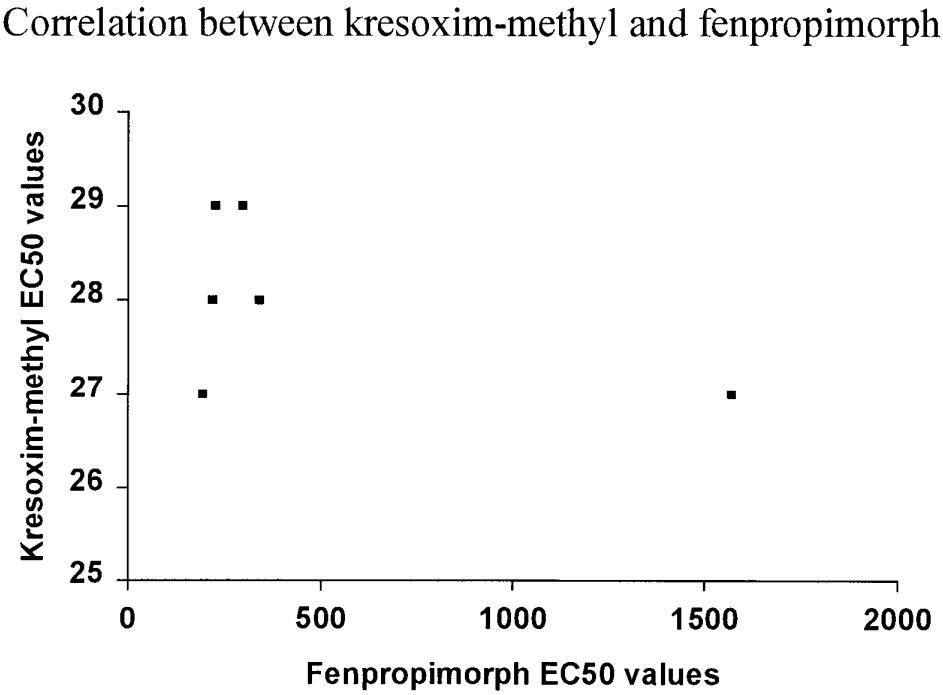


Figure 5.



6. DISCUSSION

Sensitivity testing

The isolates used in the tests were chosen because they varied in their sensitivity to the conventional fungicides, the triazoles and the morpholines. The results of the sensitivity tests showed a significant positive correlation between the sensitivities of the isolates to the two morpholines used, fenpropimorph and fenpropidin, indicating that isolates that tended to be sensitive to one would also be sensitive to the other but also that isolates resistant to one would also be resistant to the other.

There was a large variation in the range of sensitivities to tebuconazole, fenpropimorph and fenpropidin, indicating the variation in sensitivity that exists within the mildew population to these fungicides. There was also a variation in the response of the isolates to spiroxamine which would tend to show that a natural variation in sensitivity already exists in the mildew population to this novel fungicide. There was a positive correlation between sensitivity to fenpropidin and sensitivity to spiroxamine, with the implication that using one would select for resistant isolates to itself and to the other.

The EC_{50} values of the isolates to the strobilurins, azoxystrobin and kresoxim-methyl, were tightly grouped indicating that there was little variation in sensitivity between isolates to these fungicides. Resistant mildew was identified in Germany in the summer of 1998, and clearly had it been possible to include isolates of this in the work a larger variation would have been noted. This work does set a base line of sensitivity values in the mildew population before the strobilurin fungicides were introduced, and a level to which future isolates can be compared.

The EC_{50} values of the isolates to cyprodinil and quinoxyfen were also very tightly grouped and again showed little variation in their sensitivities to these fungicides.

Cross-resistance patterns

There is often the assumption that because fungicides are from different chemical groups they will also fall into different resistance groups. This need not be the case, as discussed in the introduction, as the genes for resistance to one group may be linked with the genes for resistance to another group so that they tend to segregate together.

The results of the project show a significant positive correlation between the sensitivity of the isolates to fenpropimorph and fenpropidin. Despite the fact that active ingredients are classed within the same group it is still possible for them to have different resistance patterns. Brown and Evans (1992) reported a negative cross resistance pattern in the response of barley powdery mildew to tridemorph compared to its response to fenpropimorph and fenpropidin.

The results also a showed significant correlation in sensitivity between the morpholines and tebuconazole. This result should be taken in the context that the

isolates selected and tested in this project were of a known sensitivity to triazoles and morpholines. They do not represent the proportions of isolates present in the wheat mildew population as a whole and had different isolates been selected this cross resistance pattern would have changed.

Isolate sensitivity to spiroxamine was found to have a significant positive correlation to that to fenpropidin. Since sensitivity to the two morpholines is linked there is an expected link to both morpholines. Spiroketalamines and the morpholines both work by inhibiting the production of ergosterol in the fungi and so the genes for resistance may be linked. These products are now regarded as falling within the same resistance group (FRAG, 1999) and this finding is disappointing in that spiroxamine and the morpholines are the only two fungicide groups with activity against mildew that have a strong eradicant activity against mildew.

There was no indication of any significant correlations between any other combinations of active ingredients in this work. Variations in sensitivity to quinoxyfen, cyprodinil and the strobilurins were very small and it should be born in mind the very limited number of isolates tested in this work. Resistant isolates of mildew to quinoxyfen are known to occur and resistant isolates to the strobilurins have also been identified since this work was completed. Future studies would have to include these isolates to determine if cross resistance patterns occur.

Strategies for fungicide use

Wheat powdery mildew is known to vary in its sensitivity to the morpholines, and isolate from Scotland are less sensitive than those from England (Burnett and Zziwa, 1997), although resistance to this group has been very slow to evolve. Reduced doses do not in themselves increase the risk of resistance to these products (Zziwa and Burnett, 1994), but where repeated split applications are made then shifts in sensitivity have been reported (Engels and de Waard, 1994). The multi site nature of the block that morpholines have on the production of fungal ergosterol may be one reason why resistance has been so slow to evolve, as may be the fact that resistance to the morpholines is under the control of many genes. The morpholines therefore remain a very useful group to cereal growers offering, as they do, good eradicant activity, although advisory experience would seem to indicate some fall off in the persistence of these products in the field.

Sensitivity to the triazoles is also variable in wheat mildew, with many of the older DMIs offering little if anything in the way of mildew control. Some of the newer triazoles such as epoxiconazole or tebuconazole do offer some protectant control of mildew, but there are clear cross-resistance patterns between the different triazoles. Triazoles are believed to block ergosterol synthesis at a single site and resistance is under the control of just a few major genes so that resistance problems with this group were very likely. Reduced doses do not increase the risk of resistance compared to full doses unless they are applied as multiple split applications (Schulz, 1994). Because of their broad spectrum of activity against many diseases of wheat, and in particular against Septoria spp., the triazoles are likely to remain the backbone

of wheat fungicide strategies. This approach of using triazoles in mixtures is likely to slow down the development of resistance to this group.

Ethirimol, the only hydroxypyrimidine fungicide with activity against powdery mildew, is not approved for use on wheat. The risks of resistance to this product are clearly understood and it was only available as a seed treatment for use in barley, in a mix with other active ingredients. This product (Ferrax) will no longer be manufactured or sold from the year 2000.

The resistance risk with quinoxyfen is believed to be low (Hollomon *et al.*, 1997), despite the occurrence of resistant isolates. These isolates are only found where quinoxyfen has been applied and do not seem to occur naturally. They also appear to be less fit than sensitive strains of the fungus and disappear from the population in the absence of the fungicide. No positive correlation between sensitivity to quinoxyfen and sensitivity to triazoles or morpholines was found in this work and this is consistent with Hollomon *et al.* (1997) who also report no positive correlation between quinoxyfen and triadimenol. An analysis of the ED_{50} values determined in their paper for 10 isolates tested for sensitivity to fenpropidin and quinoxyfen gives a correlation coefficient of 0.742 which is significant at the 98% level. Hollomon *et al.* concluded, however, that no positive correlation between sensitivity to fenpropidin and quinoxyfen was found. The basis for this conclusion seems to be that the isolates tested in the reported work did not differ significantly in their sensitivity to either product tested, so that no significantly less sensitive isolates to either quinoxyfen or fenpropidin were included in the work.

Based on the available data the following resistance management strategy has been drawn up. Quinoxyfen will not be used in seed treatments and is not recommended for foliar use after GS 49, in order to restrict the exposure of powdery mildew to the fungicide. This should allow for the less fit, insensitive isolates to die out of the population in the period when quinoxyfen is not used. It is also marketed in a mixture with a triazole (cyproconazole) as the product Divora (Novartis Crop Protection UK Ltd.) and Orka (Dow AgroSciences) and recommended for use in mixtures when bought as the straight product i.e. Fortress, Apres, Erysto (Dow AgroSciences). As discussed further work, with resistant as well as sensitive isolates, needs to be done to confirm the lack of cross resistance pattern with this active ingredient. In addition to these strategies it is also recommended that quinoxyfen is used in alternation with fungicides of a different mode of action when mildew control outside the quinoxyfen window is needed (Hollomon *et al.* 1997). Because of it's protectant nature quinoxyfen should be mixed with an eradicant if mildew is present at the time of application.

The project determined that isolates showed little variation in their sensitivity to cyprodinil. Because of this there was no evidence of cross resistance to other fungicide groups. There have been no reports of resistant mildew isolates to cyprodinil, although the product has only been used in the UK for two seasons. As it is a protectant fungicide is should be mixed with an eradicant fungicide if mildew is present at application. In practice this means a morpholine or spriroxamine.

The isolates tested in this project were all sensitive to the strobilurins as at the time no resistant isolates of mildew to the strobilurin group had been reported. As the work was completed, however, resistant isolates were identified, following reports of poor field control in an area of Northern Germany. The occurrence of resistant isolates has been tentatively linked to the way strobilurins had been applied in this area. The product Juwel (kresoxim-methyl with epoxiconazole, marketed by BASF plc as Landmark in the UK) had been applied as repeated split low dose application in an area of high mildew pressure. The dose used may have been as low as one third of the full commercial dose rate and at this rate epoxiconazole would not have been an effective mixing partner if mildew was already established. These resistant isolates have since been tested in the laboratory as part of an ongoing HGCA project (Project number 1304 (0003/01/98)) and they are resistant to even high doses of either azoxystrobin or kresoxim-methyl. BASF plc have withdrawn Juwel in the area affected and replaced it with the product Juwel Top, a mixture of kresoxim-methyl plus epoxiconazole plus fenpropimorph (marketed as Mantra in the UK).

There is very little information in the public domain about the nature of strobilurin resistance. The resistance risk to strobilurins was thought to be low but it is now believed that resistance is under the control of a single gene, occurring in the mitochondria where the mutation rate is higher. There also does not seem to be a fitness penalty associated with the resistance gene so that these resistant strains are unlikely to die out where strobilurins are not used. These factors are likely to increase the speed with which resistance problems will occur.

The industry action committee that meets to discuss fungicide resistance (FRAC) issued the following recommendations in 1999 for the use of strobilurin or STAR fungicides in response to the discovery of resistant wheat mildew in Germany.

- They should be applied at the manufacturers recommended rate and at the specific crop growth stage recommended.
- A maximum of two STAR containing sprays should be applied per cereal crop
- In situations where strong powdery mildew attacks are common STAR fungicides should be applied with an effective mixing partner from a different cross resistance group.
- In area where powdery mildew attacks are not common STAR compounds should be applied, solo or in mixture, before powdery mildew is established.
- Where powdery mildew is established at the time of application then STAR compounds should be mixed with an effective dose of an effective curative partner.

This strategy was broadly endorsed by the action group that represents independent scientists on fungicide resistance (FRAG) in 1999 but the influence of many of the parameters is unclear. The issues of dose rate and application number have proved particularly controversial since the statement was issued. Other elements are less contentious. Mixing the strobilurins with other effective active ingredients increases the spectrum of activity as well as decreasing the likelihood of resistance arising and

has been widely recommended by agricultural advisors. The protectant nature of the strobilurins is also widely recognised and an effective eradicant should be mixed if mildew is established at the time of spraying.

There is no evidence of cross resistance between the strobilurins and the DMIs, morpholines, quinoxyfen or cyprodinil, either from this work or from industry (S.Heaney, pers. comm.). Mixtures would therefore seem to be an effective anti-resistance strategy. Alternations would appear to be a less successful approach, if, as is suspected there is no fitness cost associated with strobilurin resistance.

It is unwise to draw parallels between the strobilurins and other chemical groups when trying to draw conclusions about the influence of dose rate. With morpholines and triazoles there is no evidence that reduced rates increase the risk of resistance, although multiple split applications have been linked to a decline in sensitivity of mildew isolates. Much of the evidence against reduced doses is circumstantial, based on the spraying practices in the area in Northern Germany where resistance was identified. What may have been critical here was the fact that mildew pressure was high and the mixing partner included at a rate too low to be effective. Until more work has been done it is impossible to determine the influence of each component.

Strobilurin resistance appears to be carried by a single gene in the mitochondria. The mitochondria are the energy production areas and there are many in a cell. The theory behind the dose rate advice is that a cell can survive with a reduced number of mitochondria so that a low dose may kill the sensitive mitochondria within the cell or spore and allow the resistant mitochondria to survive. If that spore then germinates the mitochondria within the mildew pustule that it forms will all be resistant, with the implication that even high doses will not kill the spores it produces. Even if a few sensitive mitochondria survive a low dose treatment the proportion of resistance genes within the spore will always be increased. In other pathogen / fungicide interactions the counter argument ran that low dose sprays allowed more of the sensitive population to survive and therefore decreased the selection pressure in favour of resistance. This in turn decreased the proportion of resistant genes in the population, the opposite of what may happen with mitochondrial resistance. Much more work is required to determine if this theory is what happens in practice.

A reduction in the number of sprays of strobilurins allowed per cereal crop has also been a controversial recommendation and one that has been widely flouted in the 1999 season. SAC have recommended that the two sprays of azoxystrobin allowed on winter wheat are applied at flag leaf emergence and to the ear, which may favour the longer ripening phase seen in the north of the UK. Zeneca Crop Protection recommend that the two sprays of azoxystrobin are applied at stem extension and to the flag leaf on feed wheats but that the SAC approach is used on crops where quality is important such as milling wheats. A reduction in the number of strobilurin sprays should reduce the selection pressure for resistant isolates, and this recommendation will appear in the statutory use box on the label recommendations, making it enforceable in future seasons (Zeneca Crop Protection, BASF plc, pers. comm.)

8. CONCLUSIONS

The work reported here, although based on a limited number of isolates, showed that there is a wide range in sensitivities within the wheat mildew population to the morpholine fungicides, to the triazole fungicides and to spiroxamine. The isolates tested were all found to be sensitive to the fungicides azoxystrobin, kresoxim-methyl, cyprodinil and quinoxyfen. Their sensitivities to these products were all tightly grouped and showed little variation.

A cross resistance pattern between the morpholines and spiroxamine was determined in the work. This means that these fungicides are should be regarded as belonging to the same resistance group and would not form an effective anti-resistance strategy if used in mixture or in alternation. They are the only two fungicide groups with strong eradicant activity against mildew but this means that in practice they would be most effectively mixed with fungicides that are more protectant in nature with which there is no evidence of cross resistance.

There were no other cross resistance patterns determined in the work but it should be remembered that the number of isolates tested was small, and did not include the newly identified resistant isolates to the strobilurins or isolates resistant to quinoxyfen. More work would be needed to see if there is any cross resistance between the strobilurins and other chemistry or quinoxyfen and other chemistry. There are no reports of resistant strains of mildew to cyprodinil.

On the basis of this report the use of mixtures would appear to be one of the most effective anti-resistance strategies in wheat powdery mildew. The mixing partner should come from a different fungicide group, as described above, and should be used at a dose rate where it gives effective control. Due regard for the characteristics of the fungicide should be made so that fungicides that are largely protectant in nature should not be applied where mildew infection is already established. Particular care should be taken when treating crops that are already heavily infected with mildew and a high dose of an eradicant fungicide should be applied in a mixture. In practice this means applying either a morpholine or spriroxamine. For wheat powdery mildew the most effective morpholine is fenpropidin (Zziwa, 1999). Spiroxamine and the morpholines should not be mixed as part of anti-resistance strategy.

The issue of dose rates is controversial. There is no evidence that they increase the risk of resistance to conventional chemistry such as the morpholines or the triazoles, unless they are used as part of multiple split applications. There may be a risk however associated with the use of low doses of strobilurins and until this is further investigated they should be used at an effective dose. They also show the best yield responses and cost effectiveness when used at higher rates. The best evidence available at the moment would also point to them always being used in mixtures.

Application number should be carefully determined. There is evidence that multiple applications of the conventional fungicides morpholine and triazoles increases the risk of resistance, and the number of applications of strobilurins will be restricted to two per cereal crop. This reduces the selection pressure in favour of resistant strains

mildew and should prolong the useful activity of the fungicides. It should be remembered that where low doses are used , persistence will be reduced and therefore the need for multiple applications increased. Quinoxyfen is restricted to use in the early part of the season and this is a particularly effective approach to managing resistance to this product. Strains of mildew have been identified which are resistant to quinoxyfen but there is a fitness cost associated with this, and where the fungicide is not present they will die out, leaving only sensitive isolates.

Powdery mildew is a fungus which has a high risk of evolving fungicide resistance as past experience shows. It has evolved a degree of resistance to all the systemic fungicides used on cereals to control mildew up until 1996. This has significantly affected the yield benefit and cost effectiveness associated with the products involved. The recent introduction of several novel fungicide groups with activity against mildew represents a very significant opportunity for improved disease control and increased flexibility in mildew control. To maintain the cost effectiveness of these new active ingredients, and to retain the older chemistry, it is essential that anti-resistance strategies are used. The discovery in Germany of resistant isolates of wheat mildew emphasises this point. The threat of resistance in powdery mildew should be taken very seriously by growers and their advisers. The strategies developed, and discussed here represent the most effective ways of avoiding serious problems with mildew control in the UK and should be implemented in disease control programmes.

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