# FUNGICIDE RESISTANCE IN CROP PATHOGENS: HOW CAN IT BE MANAGED?

2nd, revised edition

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CropLife INTERNATIONAL

FRAC FUNGICIDE RESISTANCE ACTION COMMITTEE

**Cover:**
Scanning electron micrograph of 7-day-old colony of powdery mildew (*Blumeria graminis* f.sp. *tritici*) on a wheat leaf. Insert shows a 2-day-old colony at higher magnification. Although the sensitivity of mildew populations towards certain fungicides has changed considerably over the years, implementation of resistance management strategies has helped to sustain an overall satisfactory degree of control.
(Syngenta)

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# FUNGICIDE RESISTANCE IN CROP PATHOGENS: HOW CAN IT BE MANAGED?

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## SUMMARY

**This publication gives a broad overview of efforts world-wide to combat problems in crop protection that are caused by development of resistance to fungicides. The following major points are emphasised:**

- Fungicide treatments are, and will remain, essential for maintaining healthy crops and reliable, high-quality yields. They form a key component of integrated crop management, and their effectiveness must be sustained as long as possible.
- Pathogen resistance to fungicides is widespread. The performance of many modern fungicides has been affected to some degree.
- Resistance problems could be much worse. All types of fungicide are still effective in many situations. Current countermeasures are by no means perfect, but they have proved to be necessary and beneficial.
- Resistance builds up through the survival and spread of initially rare mutants, during exposure to fungicide treatment. This development can be discrete (resulting from a single gene mutation) or gradual (considered to be polygenic). Resistance mechanisms vary, but mainly involve modification of the primary site of action of the fungicide within the fungal pathogen.
- Resistance risk for a new fungicide can be judged to some degree. High risk indicators include: single site of action in the target fungus; cross-resistance with existing fungicides; facile generation of fit, resistant mutants in the laboratory; use of repetitive or sustained treatments in practice; extensive areas of use; large populations and rapid multiplication of target pathogen; no complementary use of other types of fungicide or non-chemical control measures.
- Monitoring is vital, to determine whether resistance is the cause in cases of lack of disease control, and to check whether resistance management strategies are working. It must start early, to gain valuable base-line data before commercial use begins. Results must be interpreted carefully, to avoid misleading conclusions.

- The main resistance management strategies currently recommended are: avoid repetitive and sole use; mix or alternate with an appropriate partner fungicide; limit number and timing of treatments; avoid eradicant use; maintain recommended dose rate; integrate with non-chemical methods. Wherever feasible, several strategies should be used together. Some are still based largely on theory, and further experimental data are needed on the underlying genetic and epidemiological behaviour of resistant forms, and on effects of different strategies. Lowering dose may not be adverse in all circumstances.
- The industrial body FRAC has been remarkably effective in its essential and difficult role of coordinating strategy design and implementation between different companies that market fungicides with a shared risk of cross-resistance. Education and dissemination of information on resistance have also been valuable activities. New types of fungicide continue to appear, and receive close attention by FRAC.
- Much research and formulation of advice on fungicide resistance have been done by agrochemical companies. Public-sector scientists and advisers also have contributed greatly to resistance management, in research and practice. Their liaison with industry has been generally good, and there are opportunities for further interaction.
- The sustained supply of new and diverse types of chemical and biological disease-control agents, and their careful introduction, are seen as key anti-resistance strategies. This aspect of product development is now increasingly recognised by national and international registration authorities, many of which now require from applicants detailed information on the actual or possible occurrence of resistance, on base-line data, and on proposed monitoring activities and instructions for use.

# INTRODUCTION

**'A mutable and treacherous tribe'** – *this apt description of the fungi was written by Albrecht von Haller in a letter to Carolus Linnaeus, ca. 1745.*

For some 35 years now the agricultural industry has faced problems arising from the development of resistance in fungal pathogens of crops, against the fungicides used to control them. Since the first cases of widespread resistance arose, agrochemical manufacturers, academic and government scientists, and crop advisers, have put a great deal of effort into analysing the phenomenon and establishing countermeasures. In 1994 the Fungicide Resistance Action Committee (FRAC), now affiliated to CropLife International, commissioned a broad review of progress world-wide in dealing with fungicide resistance, and of the outstanding difficulties that need to be overcome.

This was published as FRAC Monograph No 1 (Brent 1995). The key tenets of resistance management have not changed over the intervening years, but there have been many developments in fungicide chemistry, in the incidence of fungicide resistance, in knowledge of resistance mechanisms, and in resistance management projects. As far as possible these have been incorporated into this Second Edition. As before, this publication aims to be an informative article for all who are concerned professionally with crop disease management, including biologists, chemists, agronomists, marketing managers, registration officials, university and college teachers, and students. It is meant to be read, or at least skimmed, as a whole. It is not intended as a detailed work of reference for the specialist, although a limited number of literature citations, out of the several thousand publications on this topic, are provided for those readers with a deeper interest. Earlier reviews concerning fungicide resistance management (Dekker, 1982; Brent, 1987; Schwinn and Morton, 1990; Staub, 1991) were drawn upon freely in the original preparation of this monograph and are still of considerable value. A review paper by Kuck (2005) has provided more recent information and comment. Where appropriate the authors have endeavoured to discuss differing viewpoints, but conclusions are theirs and do not necessarily reflect the views of FRAC.

Two further FRAC Monographs (No 2, Brent and Hollomon 1998; No.3, Russell, 2003), respectively address in more detail two major components of fungicide resistance management: the assessment of risk, and the establishment of sensitivity baselines. A second, revised edition of Monograph No. 2 is available.

## CHEMICAL CONTROL OF CROP DISEASE

Fungicides have been used for over 200 years to protect plants against disease attack by fungi. From small and primitive beginnings, mainly to protect cereal seeds and grape-vines, the number of crops and crop diseases treated, the range of chemicals available, the area and frequency of their use, and the effectiveness of treatments, have increased enormously, especially since the second world war.

Remarkably, two very old-established remedies, copper-based formulations and sulphur, are still used widely and effectively. Several 'middle-aged' fungicides (phthalimides, dithiocarbamates, dinitrophenols, chlorophenyls) have been used steadily for well over 40 years. A large number of more potent fungicides, of novel structure and mostly with systemic activity not found in the earlier products, were introduced in the late 1960s and 1970s. These included 2-amino-pyrimidines, benzimidazoles, carboxanilides, phosphorothiolates, morpholines, dicarboximides, phenylamides, and sterol demethylation inhibitors (DMIs). Introductions in the 1980s mainly were analogues of existing fungicides, particularly DMIs, with generally similar though sometimes improved properties. Over the past decade, however, a number of novel compounds have been introduced commercially or have reached an advanced stage of development – these include phenylpyrroles, anilinopyrimidines, quinone outside inhibitors (QoIs, including strobilurin analogues), benzamides and carboxylic acid amides

Modern spraying of fungicides in cereal fields in Europe. Use of wide spray booms and 'tram-lines' aid timely and precise application, but the continued effectiveness of the fungicides themselves is a more basic requirement. (FRAC).

The more recent fungicides are generally used in relatively small amounts, because of their more potent action against plant pathogens. However, their margins of safety to mammals and other non-target organisms are no smaller and are often greater, when compared weight-for-weight with those of the older materials.

Spraying has always been the principal method of fungicide application, and the conventional hydraulic sprayer still predominates. Reduction in spray volume, and more stable and safer formulation, are probably the most significant advances that

have been made in application technology. The frequency and timing of spraying have not changed a great deal from early recommendations, although the advent of the systemic fungicides has permitted some greater latitude in these parameters and has increased the feasibility of using disease threshold or forecast approaches. Roughly half of the crop diseases treated require treatment only once or twice per season, and the remainder require three or more (in some cases up to 20) applications. Systems of integrated crop management involving minimum necessary chemical and energy inputs, and use of complementary non-chemical protection measures wherever possible, have been widely adopted and to some extent have led to a reduction in spray number and dose in some situations.

At present some 150 different fungicidal compounds, formulated and sold in a several-fold larger number of different proprietary products, are used in world agriculture. The total value of fungicide sales to end-users is approximately 7.4 billion US dollars (source: Phillips McDougall, Industry Overview, 2005). Nearly half of the usage is in Europe, where fungal diseases cause the most economic damage to crops. Most of the recommended treatments generally provide 90% or greater control of the target disease, and give the farmer a benefit: cost ratio of at least 3:1. Some diseases, e.g. wheat bunt caused by *Tilletia* spp. or apple scab caused by *Venturia inaequalis*, require an extremely high level of control for various commercial or biological reasons. For some others, e.g. cereal powdery mildews (*Blumeria graminis*), the risks associated with somewhat lower standards of control are smaller. Some fungicides control a rather wide range of fungal diseases, whereas others have a limited spectrum of activity against one or two specific groups of plant pathogens. Although many fungicides are marketed, any one major crop disease typically is well controlled by only three or four different types of fungicide, so that any fall in effectiveness of a previously reliable fungicide through resistance development can be a very serious matter for the grower.

## DEFINING FUNGICIDE RESISTANCE

A potential new fungicide is identified in laboratory and glasshouse tests on different types of fungal pathogen, and is then tested in field trials against an appropriate range

of crop diseases in different regions and countries. Only if it works uniformly well against important crop diseases in a large number of trials over several seasons is it considered for development and marketing. The pathogens it works against are deemed to be 'sensitive', and those that it does not affect or hardly affects are regarded as 'naturally' or 'inherently resistant'. This pre-existing type of resistance is of no further practical interest once it has been identified as a limitation to the range of use of the fungicide. Reasons for natural resistance are seldom investigated, although sometimes they can be deduced from mode of action studies.

The 'fungicide resistance' we are considering here is a different phenomenon, sometimes called 'acquired resistance'. Sooner or later during the years of commercial use of a fungicide, populations of the target pathogen can arise that are no longer sufficiently sensitive to be controlled adequately. They generally appear as a response to repeated use of the fungicide, or to repeated use of another fungicide which is related to it chemically and/or biochemically through a common mechanism of antifungal action. This emergence of resistant populations of target organisms, which were formerly well controlled, has been widely known for antibacterial drugs (e.g. sulphonamides, penicillin, streptomycin) and for agricultural and public health insecticides (e.g. DDT) for almost sixty years.

Some people prefer to call this phenomenon 'insensitivity' or 'tolerance'. The former term is preferred by some plant pathologists, because they believe that fungicide resistance is easily confused with host-plant resistance to certain species or pathotypes of fungi. Some agrochemical companies have also tended to use 'insensitivity', 'loss of sensitivity' or 'tolerance', because these sound less alarming than 'resistance'. On the other hand, two studies on terminology recommended that 'resistance' should be the preferred term (Anon, 1979; Delp and Dekker, 1985). Also 'resistance' has been in use for many years to describe precisely the same phenomenon in bacteriology and entomology, and it is now very widely used with reference to fungicides also.

Workers within the agrochemical industry have objected from time to time to the use of 'resistance' to describe shifts in fungicide sensitivity occurring either in non-crop situations such as the laboratory or experimental glasshouse, or in the field but to a degree which is too small to affect disease control. They recommend that 'resistance' should denote only situations where failure or diminution of crop disease control is known to have resulted from a change in sensitivity. It is true that observations of 'resistance' generated in the laboratory, and detection of rare or weakly resistant

variants in the field, have on occasions been misinterpreted by scientific authors, or by commercial competitors, as indicating actual or impending failure of a product to perform in practice, when in fact good control was still secured.

However, attempts to restrict in this way the meaning of such a broadly used term as 'resistance' are bound to fail and to create more confusion. It is better to qualify the term when necessary. 'Field resistance' (in contrast to 'laboratory resistance') has been used sometimes to denote specifically a crop disease control problem caused by resistance. However, detection of some signs of resistance in the field can still be a far cry from having a control failure. It seems preferable to use 'field resistance' to indicate merely the presence of resistant variants in field populations (at whatever frequency or severity), and 'practical resistance' to indicate consequent, observable loss of disease control, whenever such precise terminology is necessary. 'Laboratory resistance' or 'artificially induced resistance' also are useful, precise terms which are self-explanatory. Some authors have claimed to find 'field resistance' in studies where the resistant variants actually were detected only after the field samples were subjected to subsequent selection by exposure to the fungicide in the laboratory. This is a borderline case, which is hard to categorise.

## OCCURRENCE OF RESISTANCE

Table 1 gives a much condensed history of the occurrence of practical fungicide resistance world-wide, and lists major fungicide groups for which resistance is well documented. Leading examples are given of the more important diseases affected, and a few key literature references are cited. Up to 1970 there were a few sporadic cases of fungicide resistance, which had occurred many years after the fungicide concerned was introduced. With the introduction of the systemic fungicides, the incidence of resistance increased greatly, and the time taken for resistance to emerge was often relatively short, sometimes within two years of first commercial introduction. Many of the fungicides introduced since the late 1960s have been seriously affected, with the notable exceptions of the amine fungicides ('morpholines'), fosetyl-aluminium, anilinopyrimidines, phenylpyrroles and some of the fungicides used to control rice blast disease (e.g. probenazole, isoprothiolane and tricyclazole), which have retained effectiveness over many years of widespread use. Some recently introduced fungicides

## Table 1
## Occurrence of Practical Fungicide Resistance in Crops

| Date first observed (approx.) | Fungicide or fungicide class | Years of commercial use before resistance observed (approx.) | Main crop diseases and pathogens affected | Ref* |
|---|---|---|---|---|
| 1960 | Aromatic hydrocarbons | 20 | Citrus storage rots, *Penicillium* spp. | 1 |
| 1964 | Organo-mercurials | 40 | Cereal leaf spot and stripe, *Pyrenophora* spp. | 2 |
| 1969 | Dodine | 10 | Apple scab, *Venturia inaequalis* | 3 |
| 1970 | Benzimidazoles | 2 | Many target pathogens, | 4 |
| 1971 | 2-Amino-pyrimidines | 2 | Cucumber and barley, powdery mildews *Sphaerotheca fuliginea & Blumeria graminis* | 5 |
| 1971 | Kasugamycin | 6 | Rice blast, *Magnaporthe grisea* | 6 |
| 1976 | Phosphorothiolates | 9 | Rice blast, *Magnaporthe grisea* | 6 |
| 1977 | Triphenyltins | 13 | Sugar beet leaf spot, *Cercospora betae* | 7 |
| 1980 | Phenylamides | 2 | Potato blight and grape downy mildew, *Phytophthora infestans & Plasmopara viticola* | 8 |
| 1982 | Dicarboximides | 5 | Grape grey mould, *Botrytis cinerea* | 9 |
| 1982 | Sterol Demethylation inhibitors (DMIs) | 7 | Cucurbit and barley powdery mildews, *S. fuliginea & Blumeria graminis* | 10 |
| 1985 | Carboxanilides | 15 | Barley loose smut, *Ustilago nuda* | 11 |
| 1998 | Quinone outside Inhibitors (QoIs; Strobilurins) | 2 | Many target diseases and pathogens | 12 |
| 2002 | Melanin Biosynthesis Inhibitors (Dehydratase) (MBI-D) | 2 | Rice blast, *Magnaporthe grisea* | 13 |

*References: 1. Eckert, 1982; 2. Noble *et al*. 1966; 3. Gilpatrick, 1982; 4. Smith, 1988; 5. Brent, 1982; 6. Kato, 1988; 7 Giannopolitis, 1978; 8 Staub, 1994; 9. Lorenz, 1988; 10. De Waard, 1994: 11. Locke, 1986; 12. Heaney *et al*. 2000; 13. Kaku *et al*. 2003.

such as benzamides and carboxylic acid amides have not yet encountered serious resistance problems, possibly because of the management precautions which have been taken. Most of the older materials such as copper fungicides, sulphur, dithiocarbamates (e.g. mancozeb), phthalimides (e.g. captan) and chlorothalonil, have retained their full effectiveness in all their uses, despite their extensive and sometimes exclusive use over many years.

Often the onset of resistance has been associated with total, or almost total, failure of disease control. Indeed it was growers' observations of obvious and sudden loss of effect that generally gave the first indication of resistance. Of course it was necessary to show that resistance really was the cause, by checking for abnormally low sensitivity of the pathogen in tests under controlled conditions. There was, and to some extent still is, a temptation for growers and advisers to blame resistance for all cases of difficulty of disease control. There are many other possible reasons, such as poor application, deteriorated product, misidentification of the pathogen, unusually heavy disease pressure. However, there remained many examples where no other explanation was found, and where serious loss of control was clearly correlated with greatly decreased sensitivity of the pathogen population as revealed in laboratory tests on representative samples.

Resistance of the kind just described, characterised by a sudden and marked loss of effectiveness, and by the presence of clearcut sensitive and resistant pathogen populations with widely differing responses, is variously referred to as 'qualitative', 'single-step', 'discrete', 'disruptive' or 'discontinuous' resistance (Fig.1). Once developed, it tends to be stable. If the fungicide concerned is withdrawn or used much less, pathogen populations can remain resistant for many years; a well-documented example is the sustained resistance of *Cercospora betae*, the cause of sugar-beet leafspot, to benzimidazole fungicides in Greece (Dovas *et al*., 1976). A gradual recovery of sensitivity can sometimes occur, as in the resistance of *Phytophthora infestans*, the potato late blight pathogen, to phenylamide fungicides (Cooke *et al*., 2006). In such cases, resistance tends to return quickly if unrestricted use of the fungicide is resumed, but re-entry involving also a partner fungicide has proved useful in some instances.

Sometimes, as in the case of the DMI fungicides, and of the 2-amino-pyrimidine fungicide ethirimol, resistance has developed less suddenly. In such cases, both a decline in disease control and a decrease in sensitivity of pathogen populations as

revealed by monitoring tests, manifest themselves gradually, and are partial and variable in degree. This type of resistance is referred to as 'quantitative', 'multi-step', 'continuous', 'directional' or 'progressive' (Fig.1). It reverts rapidly to a more sensitive condition under circumstances where the fungicide concerned becomes less intensively used and alternative fungicides are applied against the same disease.

The first appearance of resistance in a particular fungicide-pathogen combination in one region has almost always been accompanied, or soon followed, by parallel behaviour in other regions where the fungicide is applied at a similar intensity. Whether the fungicide also meets resistance in other of its target pathogens depends on the individual case. Generally it does occur in other target pathogens that have a comparable rate of multiplication, provided that the fungicide is used in an equally

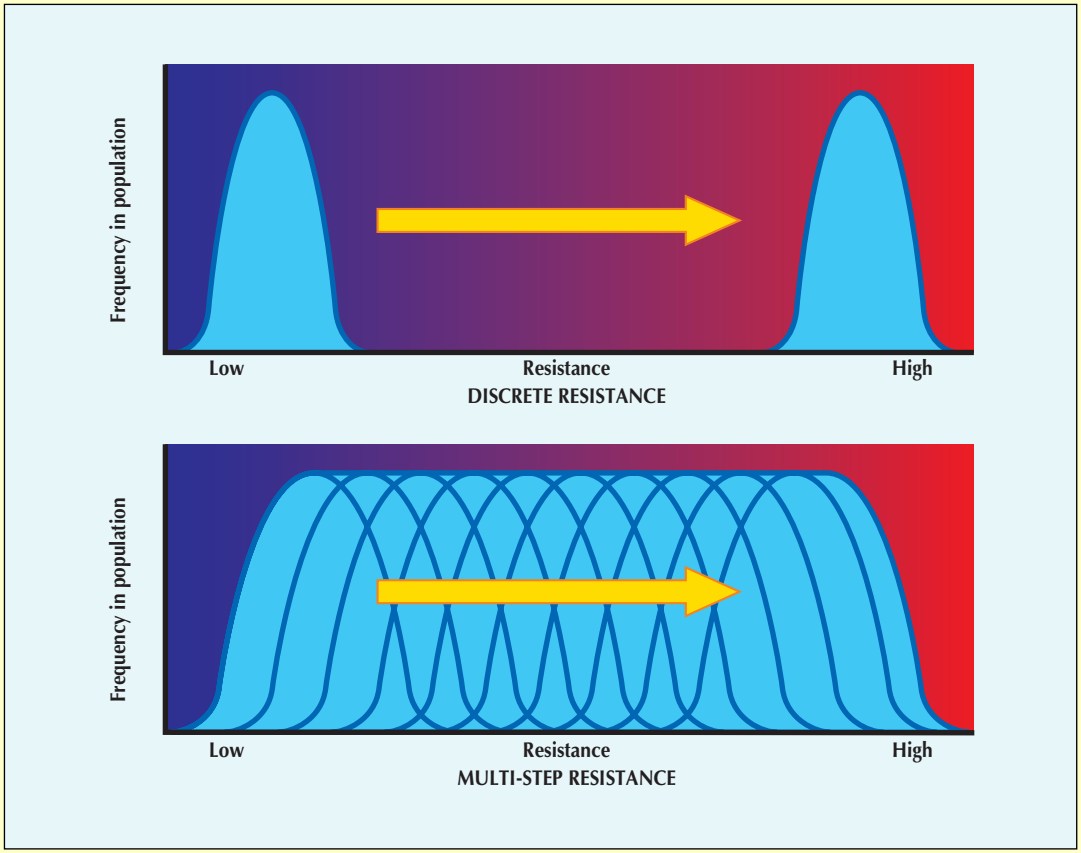



**Fig. 1**
Diagrams showing the bimodal and unimodal distributions of degree of sensitivity which are characteristic of the discrete and multi-step patterns of resistance development. Blue shading indicates original sensitive population, and red shading subsequent resistant population.

intensive way. It is notable that rust fungi, despite their abundant sporulation and rapid spread, appear to be low-risk, seldom producing resistance problems (Grasso *et al*., 2006).

Pathogen populations that develop resistance to one fungicide automatically and simultaneously become resistant to those other fungicides that are affected by the same gene mutation and the same resistance mechanism. Generally these have proved to be fungicides that bear an obvious chemical relationship to the first fungicide, or which have a similar mechanism of fungitoxicity. This is the phenomenon known as 'cross-resistance'. For example, pathogen strains that resist benomyl are almost always highly resistant to other benzimidazole fungicides such as carbendazim, thiophanate-methyl or thiabendazole. Sometimes cross-resistance is partial, even when allowance is made for the greater inherent activity of different members of a fungicide group.

There is a converse phenomenon, 'negative cross-resistance', in which a change to resistance to one fungicide automatically confers a change to sensitivity to another. This is much rarer, but several cases are well characterised; one, involving carbendazim and diethofencarb, has been of practical importance and is discussed later.

Some pathogen strains are found to have developed separate mechanisms of resistance to two or more unrelated fungicides. These arise from independent mutations that are selected by exposure to each of the fungicides concerned. This phenomenon is totally different from cross-resistance in its origin and mechanism, and is usually termed 'multiple resistance'. An example is the common occurrence of strains of *Botrytis cinerea* that have become resistant to both benzimidazole and dicarboximide fungicides.

## ORIGINS OF RESISTANCE

Once it arises, resistance is heritable. It results from one or more changes in the genetic constitution of the pathogen population. There is overwhelming circumstantial evidence that a mutant gene that causes production of a particular resistance mechanism pre-exists in minute amounts in the population. Before the fungicide was

ever used in the field, such a mutation would confer no advantage to the growth or survival of the organism, and could well cause a slight disadvantage. Hence it would remain at a very low frequency, probably dying out and re-appearing spontaneously many times.

Spontaneous mutations of all kinds are continually occurring in all living organisms. The rate of mutation can be increased greatly in the laboratory by exposing the organism to ultra-violet light or chemical mutagenic agents, and thus resistant mutants can be produced artificially. However, it cannot be assumed that such artificial mutants are necessarily identical in resistance mechanism or in other respects to those that arise in the field.

Typically, a resistant mutant might exist at an initial frequency of the order of 1 in 1000 million spores or other propagules of the pathogen. Amongst the survivors of a fungicide treatment, however, the resistant forms will be in much higher proportion ('the survival of the fittest'). It is only when this reaches say 1 in 100 or even 1 in 10 in the population that difficulty of disease control and the presence of resistant individuals will have become readily detectable. Thus the obvious onset of resistance is often sudden, but prior to this the resistance will have been building up insidiously at undetectable levels. If a fungicide treatment is very effective, with few survivors, selection will be very rapid. If the fungicide is only 80% effective, then after each treatment the number of variants will be concentrated only 5-fold and the build-up will be slower.

Several fairly obvious but important deductions, which can influence assessment of risk and design of avoidance strategies, can be made from consideration of this simple process of mutation and selection. Accumulation of resistant mutants will be enhanced by higher frequency of treatment with the fungicide concerned, by a more effective application method or dose, by the presence of larger pathogen populations before treatment, and by greater spore production and shorter generation times in the pathogen.

The selection process outlined above is based on much genetic analysis of sensitive and resistant strains, and on much field experience. However, it represents the simplest form of resistance, the discrete pattern referred to earlier, which is also termed 'major gene' resistance. One point mutation causing a single amino acid change in the target protein is responsible for a high level of resistance, and the sensitive and resistant forms fall into very distinct classes. This pattern is characteristic of resistance to

several major groups of fungicides including benzimidazoles, phenylamides, dicarboximides and QoIs. Other mutations in the target protein may give rise to lower levels of resistance. For example, the F129L mutation in the b-cytochrome target of QoIs causes only low levels of resistance in many pathogens, and hence is of little practical importance, in contrast to the G143A mutation which causes a high degree of resistance, and consequent loss of disease control (Gisi *et al*. 2002).

A somewhat different 'polygenic' process of genetic change is thought to underlie the 'quantitative' or 'multi-step' pattern of resistance. Again resistance results from the selection of mutants, but in this case a number of different genes, each with a partial effect, appear to be involved. The more genes that mutate to resistance-causing forms, the greater the degree of resistance. This would account for the gradual observable development of resistance, and for the continuous range of sensitivity that can be found (Fig.1). Although the theory of polygenic resistance is widely accepted, it must be said that the genetic evidence for polygenic resistance in field isolates is rather thin. The best known and most studied examples of continuous resistance in practice have been in cereal powdery mildews, which are rather hard to study genetically, and some of the data are conflicting (Hollomon, 1981; Hollomon *et al*., 1984; Brown *et al*., 1992). Biochemical evidence for polygenic resistance to azole (DMI) fungicides indicates involvement of at least four resistance mechanisms which are discussed below. However, Sanglard *et al*. (1998) studying the human pathogen *Candida albicans*, found that different mutations in the same target-site gene may accumulate in a single strain, and their individual effects may be additive, or possibly synergistic. In this way polyallelic changes may contribute to multistep development of resistance.

QoIs (strobilurins) are the first fungicide class to target a protein (cytochrome bc-1) that is encoded by a mitochondrial gene. DNA repair mechanisms are less effective for mitochondrial DNA than for nuclear DNA, and consequently mitochondrially encoded genes are more liable to mutation. The frequency of DNA base changes in mitochondrial DNA is further increased by its close proximity to reactive oxygen species generated during respiration. Depending on the impact of these mutations on fitness, resistance seems likely to develop quickly where target sites are encoded by mitochondrial genes. Onset of resistance to QoIs was in fact rapid in a number of pathogens, although it must be noted that benzimidazole resistance, resulting from a nuclear mutation, developed equally quickly.

## RESISTANCE MECHANISMS

Sampling barley powdery mildew (*Blumeria graminis*). Leaves bearing pustules are removed with scissors, and the spores are used as inoculum for sensitivity tests in the laboratory.
(Bayer CropScience)

Collecting samples of *Botrytis cinerea* from grapes by a battery-driven, portable spore-trapping device. A vacuum deposits spores on a fungicide containing agar plate.
(M.L.Gullino, University of Turin)

A large amount of experimental effort has focussed on this subject, particularly in academic laboratories. A broad outline of current information is given in Table 2. Some of the information is derived from resistant strains generated in the laboratory (e.g. for quinoxyfen) and not from field isolates. We now understand well the most important mechanisms of resistance to the benzimidazole, carboxanilide, phosphorothiolate, dicarboximide, and QoI fungicides. There is extensive information concerning the DMI fungicides, identifying four major resistance mechanisms that may operate. However, there are still many gaps in our knowledge, not only for established fungicide groups (e.g. anilinopyrimidines), but also for new fungicide groups defined by cross-resistance (e.g. carboxylic acid amides, CAAs).

Many types of resistance mechanism are known. These include: alteration of the biochemical target site so that it is no longer sensitive; increased production of the target protein; developing an alternative metabolic pathway that bypasses the target site; metabolic breakdown of the fungicide; exclusion or expulsion of the fungicide through ATP-ase dependent transporter proteins.

By far the commonest mechanism appears to be an alteration to the biochemical target site of the fungicide. This could explain why many of the older products have not encountered resistance problems. Once they have penetrated the fungal cell, the older fungicides act as general enzyme inhibitors, affecting many target sites (hence they are sometimes called ‘multi-site’ inhibitors). They act selectively on fungi, rather than on plants and animals, because they penetrate and accumulate much more readily in fungi. Many sites in the fungus would have to change simultaneously in order to stop the fungicide from working. The chances of the many necessary genetic changes happening are negligible, and in any case an organism with so many alterations would be highly unlikely to be pathogenic or even viable. The occasional cases of resistance to multi-site fungicides presumably have resulted from other types of mechanism, not involving the sites of action.

In contrast, modern fungicides act primarily at single target sites, and are often referred to as ‘single-site’ or ‘site-specific’ fungicides. Thus just a single gene mutation can cause the target site to alter, so as to become much less vulnerable to the fungicide. The rapid development over the past 10 years of PCR-based diagnostic methods for

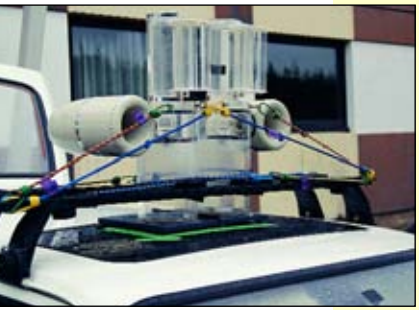
## Table 2
## Mechanisms of Fungicide Resistance

| Fungicide or fungicide class | Mechanism of resistance |
|---|---|
| Aromatic hydrocarbons | Unknown, but show cross-resistance with dicarboximides and phenylpyrroles |
| Organo-mercurials | *Detoxification by binding substances |
| Dodine | Unknown |
| Benzimidazoles | Altered target site (ß-tubulin) |
| 2-Amino-pyrimidines | Unknown |
| Kasugamycin | Altered target site (ribosomes) |
| Phosphorothiolates | Metabolic detoxification |
| Phenylamides | Possibly altered target site (RNA polymerase) |
| Dicarboximides and Phenylpyrroles | *Altered target site (protein kinase involved in osmoregulation) |
| DMIs | Increased efflux; altered target site; decreased demand for target-site product; target-site over-production |
| Carboxanilides | Altered target site (succinate-ubiquinone oxidoreductase) |
| QoIs (strobilurins) | Altered target site (ubiquinol-cytochrome c reductase) |
| Melanin Biosynthesis Inhibitors (Dehydratase) MBI-D | Altered target site (scytalone dehydratase) |

*Some doubt regarding occurrence in field isolates

Reviews by Leroux *et al*., 2002; Yamaguchi and Fujimura, 2005; Brent and Hollomon, 2007; provide further information

detection of point mutations causing resistance has aided the identification of resistance mechanisms, especially those involving target site changes. Several major resistance genes have now been isolated and characterised. In each case a single point mutation causes a change in a single amino acid in the target protein so that the fungicide no longer binds so tightly. Different amino acid changes in a target protein can cause different levels of resistance. For instance, as mentioned earlier, the G143A mutation (causing glycine to be replaced by alanine) at amino acid position 143 in the b-cytochrome of mitochondrial Complex III, causes higher levels of resistance to QoIs



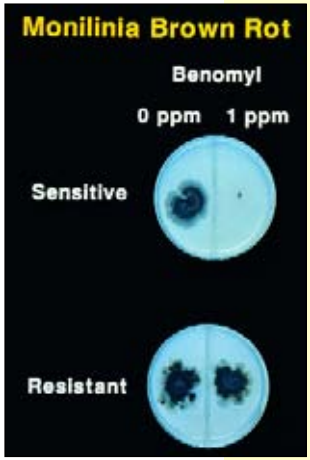
Wind impaction spore trap, mounted on a car roof. This has been used to test for shifts in sensitivity of aerial cereal powdery mildew spores. The trap contains fungicide-treated leaf pieces
(Syngenta)

than the less common F129L mutation (replacing phenylalanine by leucine at position 129) (Sierotzki *et al*., 2005).

The way in which polygenic systems operate to give different degrees of resistance are less clearly understood. The relatively low level of resistance caused by each gene makes the mechanisms of resistance particularly hard to determine. In the case of the DMI fungicides there is some evidence that mutation of different genes may elicit a number of different resistance mechanisms listed in Table 2 (De Waard *et al*., 2006). These are unrelated, but can act simultaneously and possibly in a synergistic way.

It is interesting that those few fungicides that are not directly fungitoxic, but which act indirectly by affecting defence mechanisms in the host plant, e.g. probenazole, have not encountered resistance. Reasons for this are not clear.

## MONITORING: OBTAINING THE FACTS




Radial-growth test on two strains of *Monilinia fructicola* (stone fruit brown rot pathogen) on split agar plates.
(Du Pont)

By 'monitoring for fungicide resistance' we mean testing samples of field populations of target pathogens for their degree of sensitivity to one or more fungicides. This is a crucial area of resistance research, because virtually all our knowledge of the distribution, evolution and impact of resistance in the field has depended on monitoring. It was originally done in the early 1960s to investigate possible resistance in seed-borne diseases of wheat and oats, and in storage mould on citrus fruit. A much larger amount of monitoring is now routinely done world-wide.

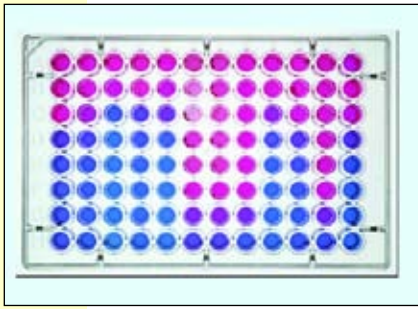
Monitoring can be done to gain early warning of an impending resistance situation. However, as discussed above, single-step resistance only becomes readily detectable in field samples when a relatively high frequency of the resistant variants (>1%) is reached. The next or next-but-one treatment would fail to give normal control. Therefore useful early warning is unlikely to be obtained, unless impractically large numbers of samples are tested (300 samples are needed to give a 95% chance of detecting resistance at 1% frequency).With multi-step resistance, partially resistant strains can exist at high frequency before practical loss of disease control occurs. Detection of these is feasible, so that in this case monitoring can indicate the risk of more severe resistance developing and causing loss of control. If a molecular method has been developed (see below) because a resistance problem has emerged elsewhere,

and the mechanism involved identified, detection of a single step mutation can be achieved at much lower frequencies, allowing earlier warning of the need to implement anti-resistance strategies.

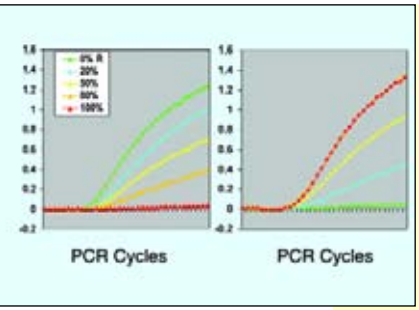
Another important reason for monitoring is to check that management strategies are working. This involves monitoring regularly over large areas of use, an expensive operation but one which has been justified by situations of high commercial risk. Molecular diagnostics have been successfully used to monitor the degree of success of anti-resistance strategies aimed at combating QoI resistance in powdery mildew and septoria diseases of wheat (Fraaije *et al*., 2002; 2005). Monitoring is also done at specific sites in order to investigate complaints from growers of an apparent loss of performance of the fungicide, and/or to give guidance on the selection of future fungicide treatments at the site or in the district.

Many otherwise competent monitoring operations have, in the past, given inconclusive results because one or both of two extremely important steps have been omitted. The first of these is to develop monitoring methods early, and then to use them to obtain base-line data on typical pathogen populations before they are exposed to any widespread use of a new fungicide. This initial assessment of the 'natural' range of sensitivity, which can be considerable, is an enormous help to the interpretation of any later monitoring data in terms of possible shifts in sensitivity. It also ensures that suitable sampling and assay methods have been worked out and tested. Unfortunately, until recent years base-line data were all too rarely obtained. However, largely because of registration requirements, the agrochemical industry is now committing the resources needed to obtain such data prior to commercialisation. FRAC Monograph No. 3 *Sensitivity Baselines in Fungicide Resistance Research and Management* (Russell, 2003) gives a full account of the rationale and methodology of baseline construction.

A second crucial activity to complement resistance monitoring, is to monitor practical performance. Knowledge of the continued degree of effectiveness of field performance is often surprisingly vague and badly recorded, and yet it is a critical indicator of the occurrence of practical resistance. Systematic observations, year by year, must be made on amounts of disease in commercial crops treated and untreated with the at-risk fungicide, and also in any replicated plot trials that are done. In order to confirm that practical resistance has appeared, it is essential to establish a clear correlation, both in time and geographically, between the incidence of resistant



High throughput micro-titre plate assay for sensitivity to azoxystrobin in *Mycosphaerella graminicola*. Twelve isolates were each tested against eight different azoxystrobin concentrations, in the presence of the growth indicator dye, Alamar Blue which turns red where pathogen growth occurs. This test shows four azoxystrobin-resistant isolates.
(B A Fraaije, Rothamsted Research)




Real-time PCR detection of the G143A mutation causing resistance to QoI fungicides. Different labelled probes or primers are used to identify sensitive (left) or resistant (right) alleles. Tests can be formatted to allow the determination of the frequency of resistant alleles in a population.
(B A Fraaije, Rothamsted Research)

biotypes and the deterioration of field performance of the fungicide. Evidence for the latter should be recorded and collated, and not merely anecdotal.

Much experience has now been gained with regard to the reliability, logistics, costs and necessity of monitoring. Timely and representative sampling is vital. It has been found very revealing to obtain some samples of the pathogen early in the season before treatment starts, if sufficient infection exists. The observation of a high resistance level after treatment can actually be a sign of very successful control, the resistant forms being concentrated in the small surviving population. Of course practical problems would follow if the resistant population persisted and formed the inoculum for the following year, but this is not necessarily the case. Experience has also shown that the risk of resistance can vary greatly between regions where disease pressures and fungicide use are high, and neighbouring areas where there is less disease or where yields are too low to support widespread fungicide use. For example, in Northern Europe several key cereal pathogens have developed resistance to a

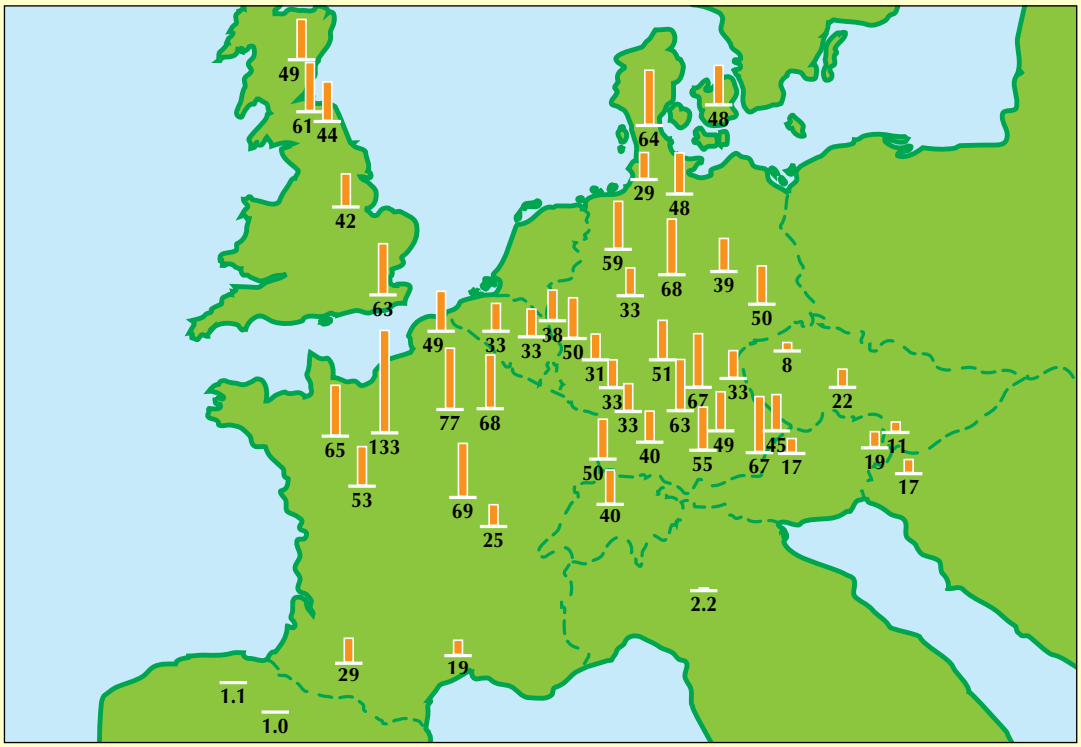


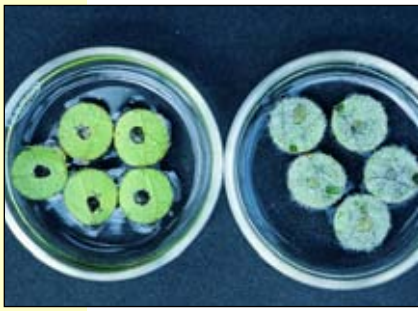

**Fig. 2**
Results of a large-scale monitoring programme for resistance of wheat powdery mildew to triadimenol across Europe. Values are 'resistance factors' for 1993 (or 1992 for Spain), i.e. ratios of the fungicide concentrations required to give 50% inhibition of a field sample and of a standard wild-type strain). Large regional differences were found, with resistance greatest in the north-west where DMI fungicide use had been most intense.
(From Felsenstein, 1994)

number of fungicide groups, whereas in southern Europe the same pathogens have remained sensitive, and the requirement for monitoring is less important (Kuck, 2005).

Sensitivity testing methods must be able to give realistic, quantitative, reproducible and readily understandable results. Standardisation of methods has been an aim of a number of organisations, including EPPO and FRAC. Details of recommended methods were published up to 1992 (Anon, 1991; Anon, 1992), and FRAC is now planning to publish a catalogue of new methods on its webpage at www.frac.info. Standardisation does enable direct comparisons to be made between results obtained by different research centres, especially if an isolate of known sensitivity is tested at each centre. On the other hand, pressure to conform must be applied with caution. If a diversity of methods give similar results, as is generally the case, this actually strengthens confidence in the results. Also it is often hard to judge the advantages and problems of different methods until several years' experience of their use have been gained. Different situations may be best suited by the use of different or modified tests. A few examples of the wide range of methods that have been used are shown in the photographs.

The cornerstone of monitoring remains some form of bioassay, so that a decrease in sensitivity is identified regardless of the underlying mechanism. In recent years tests have been miniaturised where possible. Spore germination assays are done in various multi-well plate formats, permitting larger numbers of samples to be tested. Growth in a liquid medium can be measured for some fungi directly in a spectrophotometer, or by measuring respiration using reduction of a fluorophore (e.g. 4-methylumbelliferyl-N-acetyl-ß-D- glucosaminide) as an indicator (Fraaije *et al*., 2005). But bioassays can be very resource-demanding, especially when applied to obligate parasites such as downy and powdery mildews. Where molecular mechanisms of resistance are known, and point mutations causing them defined, various PCR technologies can be applied to detect Single Nucleotide Polymorphisms (SNPs, McCartney *et al*., 2003; Fraaije *et al*., 2005). The early recognition of the correlation between a single amino acid change (G143A) in the QoI target b-type cytochrome, provided the impetus for large-scale, high-throughput monitoring of QoI resistance using allele-specific real-time PCR (Collina *et al*., 2005; Kianianmomemi *et al*., 2007). Indeed, current monitoring for QoI resistance is almost entirely dependent on real-time PCR diagnostic technologies, which have proved capable of detecting point mutations at frequencies within field populations as low as 1 in $10^8$ .

Whole plant test on sensitive strain of apple powdery mildew (*Podosphaera leucotricha*). Plants untreated (left) or sprayed with 100ppm benzimidazole (right).
(From Anon, 1991)
(DuPont)



Potato leaf disc test on *Phytophthora infestans* (late blight pathogen) with sensitive (left) and resistant (right) spore inocula. Discs are floating on 1 ppm metalaxyl solution.
(Syngenta)

When a point mutation causing resistance is identified in one pathogen, the corresponding sequence can be determined in another pathogen, and a PCR diagnostic assay developed even before practical resistance has been identified in that pathogen (Windass *et al*., 2000). So far, PCR-based monitoring in this way has been restricted to QoI resistance in a large number of pathogens, although application of PCR technology to monitor resistance in other pathogen/fungicide combinations where point mutations causing resistance are well known (e.g. resistance to benzimidazoles, dicarboximides and carpropamid) would be technically feasible.

Interpretation of monitoring results has proved difficult in the past and at times it has resulted in misleading over-prediction of resistance problems. There has been exaggeration of the practical significance of slight variation in sensitivity between field samples, or in the detection of resistant biotypes at low frequency or after a period of artificial selection. This has partly arisen from a lack of rigorous reporting and discussion of results in detailed scientific papers, in favour of verbal reports or brief meeting abstracts. In general, however, careful monitoring, linked to good base-line data and close observation of field performance, has yielded much information of scientific and practical value, and will continue to do so.

Large-scale international programmes of monitoring for insecticide resistance have been organised by FAO and WHO (cited in Brent, 1986). Comparable programmes have not been conducted for fungicides, and it is questionable whether such large schemes are appropriate. To date, the most extensive monitoring programmes for fungicide resistance have been Europe-wide surveys over a number of years of several cereal and grape diseases. Funded by contracts with the agrochemical industry, these surveys were initially carried out by workers at the Technical University of Munich, Fig 2, and more recently by companies specialising in this type of work, such as Epilogic, Biotransfer and Biorizon. More limited surveys within a country may be funded mainly by agrochemical companies or grower organisations, and done either by the agrochemical companies themselves, or by public sector or private research organisations.

## ASSESSING THE RISK

This is a matter of great importance to the chemical manufacturer who is about to develop a new product. Knowledge of the risk of resistance will help to determine whether the product should be developed and marketed, and, if so, of what nature and how stringent should be the resistance management strategies and how much further monitoring should be done.

The possibility that strains resistant to existing fungicides may be cross-resistant to the candidate product is readily determined. The chemical structure of the potential product, or its mode of action if known, may resemble those of existing fungicides, and thus indicate a likelihood of cross-resistance. More direct guidance can be obtained by testing the candidate against field isolates of the target pathogen that are known to resist other fungicides, and this is now done as a matter of routine. If cross-resistance is not found in laboratory tests, and if the field trials are uniformly successful, there still remains the risk of selection and build-up of initially rare resistant mutants during commercial use. This risk is impossible to assess with any precision, but some clues can be obtained, which permit a rough but useful estimation of risk at low, moderate or severe levels. FRAC Monograph No. 2 *Fungicide Resistance: The Assessment of Risk* (2nd revised edition, Brent and Hollomon, 2007) addresses this topic in more detail.

Knowledge of the mechanism of action of a fungicide can be informative. For example, a mechanism involving inhibition of tubulin assembly would, by analogy with the benzimidazole fungicides, be considered a high risk indicator, whereas a multi-site action would indicate relatively low risk.

The potential for mutation to resistance is best studied by treating target fungi with mutagenic chemicals or ultra-violet light, exposing the treated cultures to the new fungicide, and isolating and testing the survivors for resistance. It has long been considered that failure to generate resistant mutants, with unimpaired fitness, in the laboratory may indicate stability of performance in the field, as for example with multi-site fungicides (Georgopoulos, 1994). Conversely the ready production of such mutants could indicate a potential for practical resistance problems, as shown with benzimidazoles, phenylamides and QoIs.

However, ease of mutant production has certainly not proved to be a totally reliable indicator. Mutants that resist the amine (morpholine) fungicides are easy to obtain in the laboratory, but serious practical resistance problems have still not occurred over the many years of extensive use of these fungicides. Mutants of several fungi which were resistant to DMI fungicides were readily obtained in the laboratory, but these had reduced growth rate and sporulation and their degree of resistance was inversely proportional to pathogenicity. In view of these indications of decreased fitness in the field it was concluded that practical resistance would be unlikely (Fuchs and Drandarevski, 1976). Subsequently such resistance in fact appeared, although relatively slowly. In a risk evaluation study on the phenylpyrrole fungicide fludioxonil, resistant strains of *Botrytis cinerea* were obtained in the laboratory, and found to be cross-resistant to dicarboximides. However, dicarboximide-resistant field isolates proved to be sensitive to fludioxonil, and the latter did not select for dicarboximide resistance in field experiments (Hilber *et al*., 1994).

Thus the reliability of genetic experimentation in predicting resistance risk is still a matter of debate, although the consensus view is probably that it gives useful indications for consideration along with other evidence. The degree of correlation between the ease of production of resistant mutants in mutagenic and crossing experiments, their fitness and pathogenicity, and the subsequent occurrence of field and practical resistance, is an important and interesting topic which deserves more research.

Repeated exposure of successive generations of a pathogen to sub-lethal concentrations of a fungicide, sometimes called 'training' or forced selection, might be expected to indicate practical resistance risk. This approach was used to study potential resistance of *Phytophthora infestans* to phenylamides. Resistant strains could be selected *in vitro*, but these either were not pathogenic or could not infect phenylamide-treated plants. Selection on potato plants for 11 generations did not yield any resistant strains (Staub *et al*., 1979). In contrast, exposure of a related fungus to a mutagenic chemical (a nitrosoguanidine) yielded many highly phenylamide-resistant, virulent strains which could infect treated plants (Davidse, 1981). These different outcomes suggested that physically or chemically induced mutagenesis may be more revealing than 'training' in resistance risk studies. Probably this is because starting populations in the laboratory are too small to include the range of spontaneous mutants that occur in field populations. When mutagens are used it is important that

precautions are taken to avoid the risk of releasing resistant strains into host crops in the locality. More research studies comparing mutagenesis and 'training' as predictors are warranted, in relation both to discrete and multi-step resistance development in practice.

The potential for selection of resistant mutants has from time to time been studied in field-plot experiments in which a fungicide is applied repeatedly under conditions which favour infection by a target pathogen. However there seem to be no recorded instances of where such experimentation has yielded useful predictions of either future field problems or their absence. If intensive treatments in the field do generate for the first time fit, resistant pathogen strains then there is a danger that they could spread and initiate problems of control, and suitable precautions must be taken.

As discussed earlier, classes of fungicide differ greatly in their basic vulnerability to resistance arising in target pathogens. Indications of the degree of this intrinsic fungicide risk, whether low, medium or high level, can emerge from mutagen treatments or training experiments, or more reliably (although only after first commercial introduction) from performance-checking and monitoring during early years of commercial use, and from cross-resistance studies.

Different classes of pathogen also vary in their ability to become resistant to fungicides. A number of biological factors are involved in pathogen risk, and can be considered to act together in an additive way (Gisi and Staehle-Csech, 1988a, b; Brent *et al*., 1990). Higher pathogen risk is associated with a shorter life cycle, more abundant sporulation of the pathogen, and rapid, long-distance dispersal of spores. For example, resistance to the benzimidazole fungicides was much slower to develop in cereal eyespot disease, where the pathogen (*Oculimacula* spp.) generally has only one generation per year, with limited spore production and dispersal, and only one fungicide application is made per year, than in cucurbit powdery mildew (*Sphaerotheca fuliginea*) which has many short generations, abundant sporulation and widespread dispersal, and requires repeated fungicide treatments. There are some factors underlying the degree of pathogen risk, probably involving pathogen-specific genomic behaviour, which are not fully understood. For example, it is not clear why rust fungi, despite abundant sporulation and short generation times, have caused no major problems of fungicide resistance. The way in which 'fungicide risk' and 'pathogen risk' combine to determine the overall intrinsic risk of resistance problems is illustrated in Fig. 3.

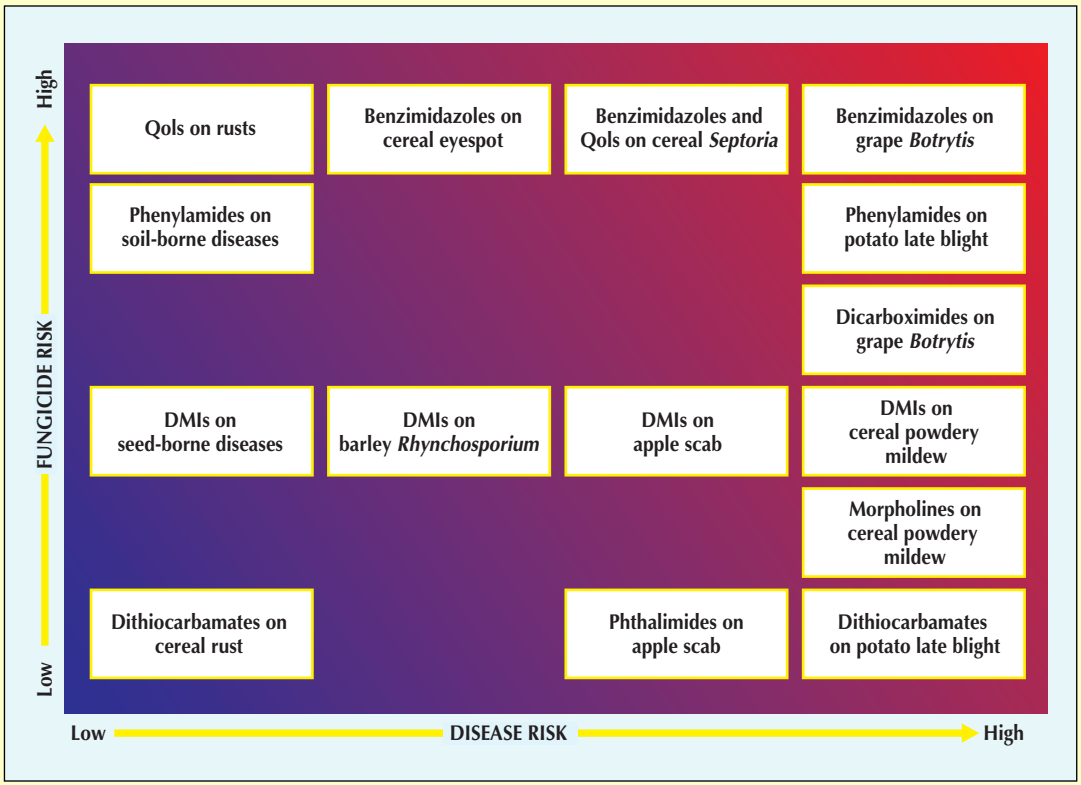



**Fig. 3**
Matrix diagram to exemplify how separate and sometimes differing degrees of resistance risk are associated with the use of a particular fungicide class, and with the control of a particular target pathogen. Estimates are approximate and based on experience to date. Blue shading indicates lower risk, and red shading higher risk.

Overall risks of resistance development in crop disease situations depend not only on these intrinsic or inherent risks attached to particular types of fungicide or pathogen, but also on the conditions of fungicide use. Unlike the intrinsic risks, the conditions of use can vary much between regions and from farm to farm. They comprise environmental factors, especially climatic and topographic conditions that affect the severity and spread of crop disease, and a range of farmer-determined agronomic factors. The latter include fungicide selection, application frequency and dose, use of glass-houses or polythene tunnels (these tend to isolate pathogen populations and prevent ingress of sensitive strains), pattern of crop rotation, choice of cultivar and its degree of susceptibility to infection, and the extent of use of hygienic practices. If the regional environment and farm practices tend not to favour disease development and

spread, and hence reduce the need for intensive fungicide use, and if the exclusive use of the at-risk fungicide is restricted or avoided, then the overall risk of resistance problems will be smaller.

Assessment of degree of risk of resistance development for a particular location must take into account and integrate as far as possible all influential factors including the intrinsic risk for each fungicide-pathogen combination, the environmental conditions and their likely effects on disease incidence, and relevant agronomic practices which should incorporate any specific fungicide use strategies recommended by the fungicide manufacturer. Inevitably, such risk assessment can only be an approximate estimate, at best indicating low, medium or high level, because many factors are involved, and with our present state of knowledge their effects cannot be measured precisely or given accurate weightings for relative importance.

## MANAGEMENT STRATEGIES

Theoretical argument, experimental evidence and practical experience all indicate that the build-up of resistance is greatly favoured by the sustained, sole use of fungicides with specific mechanisms of action. Conversely, their occasional use, interspersed by the use of other, unrelated products is unlikely to lead to resistance problems. In practice, however, resistance management strategies must combine the long-term conservation of fungicide effectiveness with an amount and pattern of use that are sufficient both to satisfy the needs of the farmer and to provide a reasonable pay-back to the manufacturer. It is not an easy task to design and implement such well-balanced programmes.

Strategies must be applied uniformly over large areas in order to obtain their full biological benefit, and also to ensure that any short-term commercial disadvantage and long-term advantage are shared amongst all manufacturers of the same group of fungicides. Thus to have a chance of success any strategy must be reached by agreement and depend upon a commitment to implementation from all supply companies involved. It must also be understandable and acceptable to the farmer. To achieve all this, on the basis of limited data and understanding of the phenomenon, is the difficult but important major aim of FRAC.

The approaches taken for different groups of fungicides will be discussed later, but first let us consider briefly the range of use strategies for resistance management that are available. Although they are discussed individually, the integrated use of combinations of different strategies is feasible, beneficial, and often implemented.

**1. Do not use the product exclusively**

Apply it as a mixture with one or more fungicides of a different type, or as one component in a rotation or alternation of different fungicide treatments.

The 'companion' or 'partner' compounds applied in either of these ways will dilute the selection pressure exerted by the at-risk fungicide and inhibit the growth of any resistant biotypes that arise. The companion compound can be a multi-site compound known to have a low risk of inducing resistance. Alternatively, it can be a single-site fungicide that is known not to be related to its partner by cross-resistance or (in the absence of known resistance) by a similar mode of action. Use of a mixture of two single-site fungicides must carry some element of risk of selecting dual-resistant strains. However, the chances of two mutations occurring simultaneously will be very small compared to that of a single mutation (e.g. $10^{-18}$ instead of $10^{-9}$). Consecutive development of double resistance could occur, but would seem much less likely to develop than if the two components were used separately and repeatedly.

This type of strategy is widely recommended by industry and also by advisory bodies. The use of formulated ('pre-packed') mixtures of two different fungicides has often been favoured by manufacturers. If an at-risk fungicide is not sold alone, then use of the mixture is the only use option open to the farmer and implementation of the strategy is ensured. Also the control of many pathogens only requires one or two treatments per annum so that the rotational approach is not appropriate. Mixtures are of course also marketed for other purposes, such as broadening the range of pathogens which can be controlled or enhancing control by increasing the duration of protection. Questions of what application rate is appropriate for each mixture component are difficult and have been debated many times. Some reduction relative to the full recommended separate rates has often been made, to keep down costs. This may reduce selection pressure for the 'at risk' fungicide, but clearly it is vitally important to maintain the companion compound at a level where it can still exert an effective independent action against the target pathogens

Numerous mathematical models predicting rate of development of resistance in relation to different regimes of fungicide use have been published, and are discussed

by Brent *et al*. (1990), Birch and Shaw (1997), and Brent and Hollomon (2007). They reveal that two basic principles underlying resistance management are to reduce the growth rates of both sensitive and resistant types, and to reduce the growth rate of the resistant type relative to the sensitive type (Fry and Milgroom, 1990). Most of the strategies that are used involve one or both of these effects. The models all indicate that use of both mixtures and rotations can delay, but not prevent, the build-up of resistant variants. They favour one or other of these two approaches to different degrees depending on the various assumptions that are incorporated. Experimental data relating to the effectiveness of mixture and rotation strategies are limited. Growth-room and plastic-tunnel studies on *Phytophthora infestans*, showed that applications of mixtures of a phenylamide fungicide with mancozeb or mancozeb plus cymoxanil decreased the build-up of phenylamide resistance, compared with phenylamide alone (Staub and Sozzi, 1984; Samoucha and Gisi, 1987). Selection for QoI resistance in *Plasmopara viticola* was delayed by a mixture with folpet, fosetyl-aluminium or mancozeb (Genet *et al*., 2006). Whilst small-scale studies such as these, done under controlled conditions and with prepared inocula, can give clear and reproducible results, there is also a need to test strategies against the much larger and more diverse populations that occur in the field.


Leaf segment test on barley powdery mildew. Spores from one field isolate are dusted on segments from plants grown from seed treated with different ethirimol concentrations. (Syngenta).

A recent modelling study (Parnell *et al*., 2006) has predicted that the regional spread of single gene resistance over large distances will depend on the proportion of fields of a particular crop that are sprayed, and not only on within-field use strategies. The extent of any loss in fitness caused by the resistant mutation, and the effectiveness of the fungicide against the wild-type sensitive pathogen, also influence the speed that resistance will spread. It is suggested that some fields should be left untreated, or treated with different, non-cross-resistant fungicides. Both verification of the model and systematic commercialisation of such a 'patchwork' strategy will probably be difficult to achieve, although the authors point out that analogous non-Bt-treated refugia for Bt-sensitive insect populations have been established in Arizona through legislation.

Field experimentation on resistance management strategies is always a difficult task, requiring large, replicated plots, and sustained cropping, treatments and assessments for several successive years. Variation in infection conditions and disease pressure from year to year, irregular availability of adequate samples of the pathogen, movement of inoculum between plots, ingress of external inoculum into the

Cucumber powdery mildew caused by *Sphaerotheca fuliginea*. The healthy leaves were on a plant treated with dimethirimol via the roots. In this instance the mildew population was sensitive to dimethirimol, but resistant populations soon became common in some countries.
(Syngenta)

experimental area, and other difficulties often render such work inconclusive. An early field experiment on *Cercospora beticola* showed that alternation of benomyl and a tin fungicide delayed the development of benomyl resistance (Dovas *et al*., 1976). In several studies on cereal powdery mildews (*Blumeria graminis* f. sp. *tritici* and *hordei*), field application of mixtures of triazoles with morpholine or aminopyrimidine fungicides was found to hinder the development of resistance to one or to both of the fungicides applied, which did occur after sequential applications of each fungicide alone (Heaney *et al*., 1988; Brent *et al*., 1989; Lorenz *et al*., 1992). Effects of fungicide alternation were less regular, giving either a similar or a smaller benefit according to the particular study.

Development of resistance of *Botrytis cinerea* on tunnel-grown strawberries to dicarboximides, and of *Polyscytalum pustulans* and *Helminthosporium solani* on potatoes to thiabendazole, was shown to be delayed by the application of certain fungicide mixtures (Hunter *et al*., 1987; Carnegie *et al*., 1994). In experiments on grape powdery mildew (*Uncinula necator*) a mixture of triadimenol with sulphur or dinocap at roughly half normal rates did not slow down the evolution of triadimenol resistance; however, alternations, at full rates, did decrease resistance development (Steva, 1994). Build-up of QoI resistance in *Mycosphaerella graminicola* in field plots of wheat was much reduced by application of an azoxystrobin/epoxiconazole mixture, compared with a solo azoxystrobin treatment (Gisi *et al*., 2005). Overall, field experimentation does appear to support the adoption of mixture and rotation strategies, but since there are some inconsistencies and the range of diseases and fungicides worked on is rather limited, further work should be encouraged.

Practical experience also suggests that both mixture and rotation strategies have delayed resistance development, and examples are discussed later. However, fully conclusive evaluations of commercial-scale strategies are difficult to make because comparable ‘non-strategy’ areas have seldom existed.

**2. Restrict the number of treatments applied per season, and apply only when strictly necessary. Use other fungicides both beforehand and subsequently**

This approach, like rotation, reduces the total number of applications of the at-risk fungicide and therefore must slow down selection to some extent. It can also favour decline of resistant strains that have a fitness deficit. However, the treatments, which are still applied consecutively, generally coincide with the most active stages of epidemics when selection pressures are highest.

Thus any delay in resistance may not be proportional to the reduction in spray number. On the other hand a substantial break in use at a time when the pathogen is still multiplying can allow a beneficial resurgence of more sensitive forms. Examples are considered later.

**3. Maintain manufacturers' recommended dose**

For many years farmers have often used reduced rates of application of fungicides, mainly to reduce costs, especially in conditions where disease pressures are usually low, or where the risk of financial loss from reduced performance was not great. Also, advisory services in pursuing lower-input approaches for economic and environmental reasons, have recommended use of smaller doses for certain situations. On the other hand it is the view of FRAC that recommended doses must be maintained, not only because they will retain the built-in safety factor and secure the claimed levels of performance under a wide range of conditions, but more particularly because it is possible that reducing the dose could enhance the development of resistance.

However, relationships of fungicide dose to risks of resistance are not yet fully established, and it seems likely that they may vary according to the fungicide in question. Some of the models referred to above indicate that lowering the dose of the at-risk fungicide (but retaining normal spray frequency) can delay build-up of major-gene resistance by decreasing the overall effectiveness, increasing the numbers of sensitive survivors and hence slowing down the selection of resistant forms that can survive the full dose. With regard to multi-step resistance, it has been argued that lowering dose can enhance resistance development by favouring the survival of low-level resistant forms which would be inhibited by the full dose. The low-level resistant forms could then mutate further or recombine sexually to give higher levels of resistance. In practice the doses that actually reach the target organisms vary greatly over space and time, giving very complex mixes of different exposure sequences. Thus it can be argued equally that lowering the dose could hinder multi-step resistance by giving a fore-shortened range of concentrations that would not provide the step-ladder of selection pressure up to the highest levels. Moreover, as the dose rate approaches zero there certainly will be no selection for resistance.

Experimental data regarding effects of different doses are still rather limited and confusing. In a growth chamber experiment, selection for resistance to triazoles in barley powdery mildew was slowed down by lowering fungicide concentrations (Porras *et al*., 1990). Again the work is more difficult to do in the field, partly because

*Apple leaves bearing lesions of scab disease, caused by Venturia inaequalis. Resistance to benzimidazoles and dodine has caused considerable problems in the control of this disease.*
*(K J Brent)*

degrees of effectiveness, which must be critical, vary greatly between and within growing seasons. Decreasing application rates appeared to slow down development of resistance of triadimefon to barley powdery mildew (Hunter *et al*., 1984), but in other experiments on strawberry *Botrytis* and wheat eyespot altering fungicide doses made little difference to resistance build-up (Hunter *et al*., 1987; Hunter *et al*., 1993). When a benomyl-mancozeb mixture was applied to control apple scab, build-up of benomyl resistance was delayed by reducing the benomyl concentration and increasing the mancozeb concentration (Lalancette *et al*., 1987). Halving the rate of triadimenol enhanced development of resistance in grape powdery mildew in France (Steva, 1994), and 'split' (lower dose but more frequent) applications of fenpropimorph and fenpropimorph-propiconazole mixtures led to significant decreases in fenpropimorph sensitivity of wheat powdery mildew in Germany and Holland (Forster *et al*., 1994; Engels and De Waard, 1994 ). However, reducing the dose of fenpropimorph did not affect the sensitivity of barley powdery mildew in the UK (Zziwa and Burnett, 1994). Decreasing the dose of DMI fungicides from one-quarter to one-eighth of the full recommended dose was found to reduce resistance development in *Mycosphaerella graminicola* (Metcalfe *et al*., 2000; Mavroeidi and Shaw, 2006).

It is now widely accepted, on theoretical grounds, limited experimental data and practical experience, that risks of major-gene (single-step) resistance are unlikely to increase, and may well decline as dose is lowered. The situation with regard to polygenic resistance is still not at all clear, and more experimental work is justified in order to obtain a sounder base for recommendations. Some of the published data refer specifically to 'split' schedules, in which dose is lowered but frequency of application is correspondingly increased, to give the same total mount applied each season. It is important to distinguish these from reduced-dose applications made on normally timed schedules so that the total dose per season is decreased. The use of more frequent 'split' applications could increase resistance risk and should be avoided.

**4. Avoid eradicant use**

One of the advantages of systemic fungicides is that they can eradicate or cure existing infections. This property greatly assists their use on a 'threshold' basis, where application is made only when a certain, economically acceptable, amount of disease has already appeared, in order to prevent further spread. However, avoidance of the use of systemic fungicides in this way has been recommended in two different situations as an anti-resistance strategy.

FRAC has recommended that eradicant use of phenylamides should be avoided. This is because they are now always applied for control of foliage diseases as a mixture with a multi-site companion fungicide. The latter does not work as an eradicant, so that the phenylamide is acting alone when the mixture is applied to existing infections.

Avoidance of eradicant use could possibly delay resistance for another, more widely applicable reason. To wait until a threshold population of the pathogen appears, usually means that many sporulating lesions (occupying up to 5% of the foliar area) are exposed to the fungicide. Opportunity for selection could be much greater than if the fungicide had been applied prophylactically to keep populations permanently low. Presumably it is with this risk in mind that FRAC discourages the eradicant use of DMIs in some fruit crops. To the authors' knowledge there is no experimental evidence comparing the resistance risks of prophylactic versus threshold-based schedules, and research on this would be useful.

Storage rot of oranges caused by *Penicillium digitatum*. Resistance to phenols, benzimidazoles and sec-butylamine has caused problems, but the advent of newer fungicides and the adoption of resistance management programmes have enabled satisfactory control to be maintained overall.
(J W Eckert, University of California)

**5. Integrated disease management**

This is a particular aspect of the concept more generally referred to as IPM (Integrated Pest Management). The integrated use of all types of countermeasures against crop disease is not only highly desirable on economic and environmental grounds, but is also a major strategy for avoiding or delaying fungicide resistance. The use of disease-resistant crop varieties, biological control agents, and appropriate hygienic practices, such as crop rotation and removal of diseased parts of perennial crop plants, reduces disease incidence and permits the more sparing use of fungicides, and in both these ways decreases selection of fungicide-resistant forms. Equally of course the application of fungicides reduces the risk of build-up of pathotypes with changed virulence and the consequent 'breakdown' of disease-resistant varieties.

Unfortunately, non-chemical methods of disease control are often weak or not available, so that fungicide application is the predominant or even the sole countermeasure for many diseases (e.g. potato late blight, grape downy mildew, Sigatoka disease of bananas, wheat bunt, stripe (yellow) rust of wheat, to name a few).

**6. Chemical diversity**

The availability of a number of different types of fungicide for the control of each major crop disease is highly beneficial both environmentally and in order to overcome resistance problems. The continued use of one or a very few types of compound over many years presents a much greater risk of side-effects and favours resistance in the

Late blight on potato foliage, caused by *Phytophthora infestans*. Although phenylamide – resistant forms are widespread, phenylamides are still used effectively in mixture or rotation with mancozeb and other fungicides.

target organisms. Thus it is crucial that chemical invention and new product development are sustained. Fortunately, registration authorities now accept the need for diversity, in terms of pesticide chemistry and mechanisms of action, provided that the new compounds maintain safety standards. A new fungicide does not necessarily have to be superior to existing ones in order to be of value. It has to be effective, and, in the resistance context, it should work against strains that are resistant to existing fungicides. This latter property is usually associated with a new mode of action, and ideally there should be more than one site of action to decrease the risk of evolution of resistance to the new fungicide.

However, the development of new, highly active members of an existing fungicide class, which retain the same primary mechanism of action, may also be of some use in resistance management. This is exemplified by the latest triazole fungicide prothioconazole, which is more potent generally and against which smaller resistance factors are exhibited (Kuck and Mehl, 2004). Its introduction has to some extent decreased problems of triazole resistance in cereal powdery mildews.

The withdrawal of fungicides, for example captafol and organo-tin fungicides, for safety reasons has been necessary from time to time, but it has reduced options for resistance avoidance strategies. It must be hoped that further de-registrations do not occur. Restrictions on the use of ethylenebisdithiocarbamates (EBDCs), such as mancozeb, already operate in several countries, and possibly these could become more widespread and severe. This is a worrying prospect with regard to fungicide resistance management. It is notable that in Sweden products based solely on EBDCs have been prohibited, whereas products containing EBDCs together with other fungicides can still be marketed and used.

## IMPLEMENTATION OF MANAGEMENT STRATEGIES

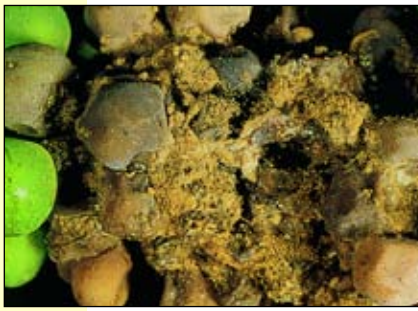
Whilst public-sector research and advisory organisations have contributed greatly to the establishment of countermeasures, the agrochemical industry has had to bear the major responsibility of planning and implementation, and of course the associated financial risks.

When fungicide resistance first emerged as a major problem, the manufacturers concerned had to respond as best they could against an unforeseen situation. Resistance to benzimidazoles arose in 1969-70, only one to two years after first introduction. The companies involved adopted a low-key approach, dealing with complaints on an ad-hoc basis, and placing general warnings of the existence of resistant strains and disclaimer notices on product labels. Results of any monitoring or other studies done by them at this time were not published for 10 years, and no recommendations regarding resistance management were issued.

Resistance to dimethirimol first appeared in Holland in 1970, the second year of use. With hindsight, the year-round, almost universal use of this highly specific, systemic fungicide, in glasshouses, to control the vigorous, abundantly sporulating cucumber powdery mildew, was the ideal scenario for resistance build-up. The manufacturing company mounted quickly a systematic monitoring programme (the first of its kind), obtained clear evidence of practical resistance, withdrew the product from use in affected regions, and published relevant data (Bent, *et al*., 1971).

Signs of resistance of barley mildew to the related compound ethirimol subsequently were found in the UK, and the same manufacturer again published data as did the Plant Breeding Institute (PBI) at Cambridge (Shephard *et al*., 1975; Wolfe and Dinoor, 1973). With advice from PBI, the company introduced a strategy of withdrawal of use from winter barley, to break the year-round cycle of use. The resistance did not worsen and a useful degree of disease control on spring barley was sustained. As more alternative treatments came into use in the late 1970s ethirimol application to winter barley was restored, and the level of resistance actually declined (Heaney *et al*., 1986). Since the company concerned was the sole manufacturer of these two pyrimidine fungicides, it was possible to implement major changes in use strategies uniformly and without reference to other companies.

Carboxanilides and amines ('morpholines'), introduced at about the same time as the benzimidazoles and 2-amino-pyrimidines, did not encounter the rapid onset of major resistance problems. In 1980, however, strong resistance to metalaxyl, a relatively new Oomycete fungicide, occurred in certain countries, and signs of resistance to dicarboximides were also starting to appear. This situation of increasing concern prompted a group of industrial scientists, who were attending a fungicide resistance course at Wageningen in 1980, to propose the formation of an inter-Company Group that would cooperate in investigating resistance problems and establishing



Grapes infested by *Botrytis cinerea*. Resistance to benzimidazole and dicarboximides fungicides has affected control seriously in some regions. New fungicides appear promising.

countermeasures. At a meeting in Brussels in 1981, company representatives agreed a draft constitution and modus operandi for FRAC.

Since then FRAC has been very active in sharing confidential company information on the incidence of resistance, in planning relevant studies with agreed company inputs, and in issuing consensus recommendations for the agrochemical industry and for advisers and farmers (Russell, 2006).

FRAC decided to operate through Working Groups, one for each major class of fungicides to which resistance is known, and which has more than one manufacturer, or potential manufacturer with an announced development product. Currently there are four Working Groups, dealing with SBI (sterol biosynthesis inhibitor) fungicides, anilinopyrimidines, QoI (quinone outside inhibitor) fungicides and CAA (carboxylic acid amide) fungicides. These Groups collect and publish data on resistance status in different crops, pathogens and countries, and issue and review annually resistance management guidelines. Three former Working Groups, concerned with benzimidazoles, dicarboximides and phenylamides, have now converted to Expert Fora, giving relevant information and advice on request. The latest information and guidelines from each Working Group are available on the FRAC website (www.frac.info).

**Benzimidazoles**

Many pathogens adapted very quickly to benzimidazoles, for example *Botrytis* spp. Others took about 10 years before being detected e.g. *Oculimacula* spp., cause of cereal eyespot disease (Locke, 1986) or even 15 years (e.g. *Rhynchosporium secalis*, cause of barley leaf-scald (Kendall *et al*., 1993).

Over the years the use of mixtures or alternations with non-benzimidazole fungicides has been encouraged with varying degrees of vigour by the individual companies concerned and by advisory services. Often this was done too late. When benzimidazole resistance has already become established, it usually persists.

An example of the successful early use of a mixture strategy is the application of benzimidazoles to control *Cercospora* leaf-spots of peanut in the USA. In the southeastern states, where there was sole use of benomyl, practical resistance soon appeared. In Texas, where benzimidazole-mancozeb mixtures were used from the start, no resistance developed over many years except in trial plots where a benzimidazole

alone was applied repeatedly (Smith, 1988). The FRAC Working Group (now an Expert Forum) supported the use of mixtures or alternation in a general way, and the avoidance of eradicant use unless absolutely necessary, but did not make specific recommendations or initiate major monitoring projects.

Use of benzimidazole fungicides worldwide is still substantial, despite the widespread incidence of resistance since the early 1970s. In the absence of data it is hard to say to what extent benzimidazole fungicides are now still effective, and whether use on the present scale is fully justified. Monitoring in 1997-2003 in France revealed the common occurrence at high frequency of benzimidazole-resistant strains of *Mycosphaerella graminicola* and *Oculimacula* spp in wheat (Leroux *et al*., 2003, 2005 a). A comprehensive, up-to-date survey of the situation world-wide regarding the current use and effectiveness of benzimidazole fungicides would certainly be valuable.

One special and interesting approach to overcoming benzimidazole resistance has been the application of a mixture of the benzimidazole fungicide carbendazim with diethofencarb, to control *Botrytis* in grapes and other crops. Diethofencarb shows negative cross-resistance with respect to benzimidazoles. Remarkably, it inhibits only benzimidazole-resistant strains of the target pathogens and does not affect benzimidazole-sensitive strains. In practice a formulated carbendazim-diethofencarb mixture, introduced in 1987 initially gave good control of *Botrytis*, irrespective of whether pathogen populations were benzimidazole-resistant or not. However, the appearance and spread of strains resistant to both fungicides caused problems (Elad *et al*., 1992; Leroux and Moncomble, 1994) and the product is no longer used.

**Phenylamides**

These fungicides were first introduced in 1977. They act specifically against oomycete pathogens, having no effect on other classes of fungi.

In 1980 the first cases of resistance occurred, suddenly and seriously, against metalaxyl applied to cucumbers for control of downy mildew (*Pseudoperonospora cubensis*) in Israel and applied to potatoes in certain European countries for control of late blight (*Phytophthora infestans*). In the following year resistance appeared also in grape downy mildew (*Plasmopara viticola*) in France and South Africa and in tobacco blue mould (*Peronospora tabacina*) in Central America. These events were unexpected, since results of 'training' experiments done by the manufacturer (Staub *et*

Black Sigatoka disease of bananas caused by *Mycosphaerella fijiensis* var. *difformis*. Strains resistant to benzimidazoles, DMI and QoI fungicides have developed in some countries and have prompted the international adoption of agreed management strategies.
(K.J Brent)

*al*., 1979) had appeared to indicate a low degree of risk. The dramatic occurrence in 1980 of practical resistance problems, in such a promising new fungicide class which was beginning to involve other manufacturers, was perhaps the most compelling influence underlying the formation of FRAC.

Recognising that resistance in *Phytophthora infestans* was associated with the solo use of metalaxyl, and that it had not occurred in those countries where only formulated mixtures with mancozeb were applied, the manufacturer immediately withdrew the single product from use against foliar diseases and recommended that mixtures with multi-site fungicides should be used. Subsequently the FRAC Phenylamides Working Group produced a full set of guide-lines. In abbreviated form, these are:

- Use only as protectants; no curative or eradicant applications.
- For foliar application use only pre-packed mixtures with residual partner fungicide; the latter should be at $^{3}/_{4}$ to full dose, but the phenylamide dosage depends on the intrinsic activity and is defined by the respective company.
- Do not use soil treatments to control foliar disease.
- Limit sprays to 2-4 consecutive applications per crop per year; do not exceed 14 day intervals.
- Use in early season or period of active crop growth only, then switch to a non-phenylamide product.
- Do not use on seed potato crops or in nurseries.

Although not without difficult negotiation, FRAC secured uniform implementation of these guide-lines by all the companies involved, and major use of this class of fungicides continues against all target diseases. Since the problem of phenylamide resistance first arose, several effective new oomycete-active fungicides have been introduced, e.g. QoI fungicides, fluazinam, dimethomorph, cyazofamid and zoxamide, so that many more options for diversified application programmes now exist.

Application of the FRAC recommendations did not in fact delay for long the appearance and spread of resistant variants of *P. infestans*, which have become readily detectable in many crops in most countries of use. Nevertheless there is evidence from field experiments that phenylamide-mancozeb mixtures continue to perform better than mancozeb alone (Staub, 1994), even in re-entry situations where a phenylamide alone was originally used and then withdrawn (Dowley, 1994). The reasons for this are not fully understood. The use of a leaf-disc test with a multiple spore inoculum may

have over-estimated the frequency of resistant mutants within crops. Since the Oomycetes have multinucleate hyphal cells and sporangia, it is possible that the proportion of nuclei with a resistant gene is a critical factor (Cooke *et al*., 2006). The underlying reason for the sustained field activity of metalaxyl in mixtures, which has also been observed in the control of lettuce downy mildew, *Bremia lactucae* (Wicks *et al*., 1994), deserves more detailed study.

Against most Oomycete pathogens, chemical application is the only effective method of control and there is not much scope for the IPM approach. An exception is the downy mildew of lettuce. Metalaxyl-resistant populations of this fungus are composed only of one of a few particular pathotypes. Cultivars carrying genes for resistance specifically against one of these pathotypes have been deployed in combination with phenylamide treatment as a successful integrated control and resistance management strategy (Crute *et al*., 1994). Metalaxyl-resistant strains of a different pathotype do arise from time to time, so that sustained surveillance and modification of recommendations is necessary.

**Dicarboximides**

Fungicides of this class (iprodione, vinclozolin and procymidone) have been used since the mid-1970s mainly to control fungi of the related genera *Botrytis*, *Sclerotinia* and *Monilinia*. They largely replaced benzimidazole fungicides, which in many situations were no longer effective because of resistance. Dicarboximide-resistant variants appear frequently in laboratory cultures, and after about three years of intensive use, resistant strains were detected also in the field. The field isolates have shown differing degrees of resistance, and pathogenicity and other fitness factors tend to decline as the degree of resistance increased. The proportion of resistant strains varies greatly with time of year; they decline after dicarboximide treatment ceases and increase again when it is resumed. Practical control problems, associated with moderately resistant populations occurred, but at first were localised and variable in degree. During the 1980s difficulties gradually increased, especially in grape-vines in the parts of Europe where *Botrytis* is most prevalent, and even where mixtures were used control was sometimes inadequate.

The FRAC Dicarboximide Working Group made the following recommendations:

- Do not apply more than two or three times per crop per season.

- Save applications for times when *Botrytis* infection pressure is high.
- Leave prolonged periods without selection pressure.
- Where resistance is established use mixtures to stabilise *Botrytis* control, using the application rules given for a dicarboximide alone.

Despite extensive use of these guide-lines, practical resistance to different degrees became widespread in grape-vines, especially in parts of France, and a sporadic problem in some other crops. Earlier companion compounds such as captan, thiram, dichlofluanid and chlorothalonil did not give fully adequate control, alone or in mixture with a dicarboximide, but the restricted, once per year use of newer *Botrytis*-active fungicides such as fluazinam, fludioxonil, fenhexamid and the anilinopyrimidines, and also the dicarboximides, is now giving good levels of grape-vine *Botrytis* control in France (Leroux *et al*., 2005 b).

**SBIs (sterol biosynthesis inhibitors)**

This large class of fungicides comprises three distinct groups: the sterol C14-demethylation inhibitors (DMIs, e.g. triazoles, imidazoles, fenarimol, triforine); amines (morpholines e.g. tridemorph, fenpropimorph, piperidines e.g. fenpropidin, spiroketalamines e.g. spiroxamine); hydroxyanilides (e.g. fenhexamid).

DMIs were first used in the 1970s, triforine, triadimefon and imazalil being early representatives. Since then at least 30 more DMIs have been used in agriculture. At the time the FRAC Working Group formed, in 1982, there were very few reports of DMI resistance. They have a site-specific mode of action, and resistant mutants were easily obtained by mutagenic treatment in the laboratory. However, such mutants had reduced pathogenicity and other fitness attributes, so that development of practical resistance was deemed unlikely (Fuchs and Drandarevski, 1976). Practical resistance did in fact develop in several pathogens during the 1980s (e.g. powdery mildews, *Venturia inaequalis*, *Mycosphaerella fijiensis* var *difformis*), but relatively slowly and with fluctuating severity, as is considered to be characteristic of polygenic resistance.

Although amine fungicides have been used extensively for many years, they continue to perform well. Considering the amount of use, their potency, the high multiplication rates of the main target pathogens (e.g. powdery mildews and *Mycosphaerella. fijiensis* var *difformis*), and the ease of generating resistant mutants in the laboratory, the stability of their performance has been remarkable. Some reports of decreased

sensitivity have appeared from time to time. The slightly resistant field isolates were not cross-resistant to the DMI fungicides, which act at a different stage of sterol biosynthesis.

Interestingly, several studies have revealed cross-resistance between isolates of barley and wheat powdery mildews with respect to fenpropimorph and fenpropidin, but little cross-resistance to tridemorph appears to occur (Readshaw and Heaney, 1994). This pattern correlates well with information on mechanisms of action, since fenpropimorph and fenpropidin are considered mainly to inhibit the Δ14-15 reduction step, and tridemorph mainly the Δ8-7 isomerisation step, in sterol biosynthesis (Hollomon, 1994). However, there is evidence for additional sites of action, and a multi-site action, coupled with the flexible, multi-configurational nature of the carbon chain, could account for the durability of action of the morpholine fungicides.

Hydroxyanilide fungicides inhibit yet another step in sterol biosynthesis, catalysed by C3-keto-reductase. Fenhexamid, the sole hydroxyanilide in commercial use is applied specifically for control of *Botrytis* spp. and related pathogens. During eight years of use, no development of resistance to fenhexamid has been detected.

FRAC has made the following general recommendations regarding use of SBI fungicides:

- Do not use repeated applications of SBIs alone on the same crop in one season against a high-risk pathogen in areas of high disease pressure for that pathogen.
- For crop/pathogen situations requiring multiple spray applications, e.g. orchard crops/powdery mildews, use mixtures or alternate (in block sprays or in sequence) with effective non-cross-resistant fungicides.
- If mixture or alternation is not possible, reserve SBI use for the critical part of the season or critical crop growth stage.
- If DMI or amine performance declines and less sensitive forms of the pathogen are detected, SBIs should only be used in mixture or alternation with effective non-cross-resistant fungicides.
- Complementary use of other fungicide classes with different modes of action should be maximised.
- Use as recommended on the label. Do not use reduced doses.
- Use other measures such as resistant varieties, good agronomic practice, plant hygiene.

Recommendations for specific crop sectors have been made, and are published on the FRAC website. In general these confirm and amplify the above general recommendations. Eradicant use is discouraged in apples and grapes.

These recommendations have been widely implemented, and in general the SBI fungicides are continuing to give good control of most target pathogens some 30 years after their introduction. The warning against reduced rates could be open to debate since, as discussed earlier, the relevant experimental data are limited and conflicting. This is clearly an important area for further research. However, it is of course always necessary to use DMIs in amounts sufficient to ensure cost-effective disease control under the particular conditions of use.

**Anilinopyrimidines**

These fungicides, which include cyprodinil, pyrimethanil and mepanipyrim, act against a broad range of fungi. The FRAC Anilinopyrimidines Working Group has focussed mainly on resistance management in *Botrytis cinerea* and *Venturia inaequalis* on apple, which are high-resistance-risk pathogens and also important commercial disease targets for this fungicide class. Resistant strains of both pathogens have been detected in vineyards and apple orchards. These are cross-resistant to all the anilinopyrimidine fungicides, but not to other fungicide classes. They have remained at low frequency, and performance of anilinopyrimidines continues to be very good after twelve years of commercial use.

Guidelines for use have been published by FRAC and implemented throughout this period. These differ according to the crop disease, but the general approach is to restrict the number of anilinopyrimidine treatments to be applied per crop and season.

**QoIs (Quinone outside Inhibitors, "strobilurins")**

The class at present comprises twelve fungicides, from several different, but related chemical groups (e.g. methoxyacrylates, oximino acetates) which have a common mode of anti-fungal mode of action, inhibiting electron transfer at the Qo site in mitochondrial complex III. They were first introduced ten years ago, and have been widely used against a broad range of pathogens.

Within two years after their introduction, marked loss of action against powdery mildew, associated with development of highly resistant populations was observed in

wheat crops in Germany, and soon after throughout north-west Europe (Chin *et al*., 2001). Subsequently, serious resistant problems have been encountered in a range of target pathogens, for example *Mycosphaerella graminicola* (cause of leaf spot of wheat), *Plasmopara viticola* (downy mildew of grapes), *Venturia inaequalis* (cause of apple scab) and *Mycosphaerella fijiensis* var. *difformis* (cause of black Sigatoka disease of bananas). A full list, with literature citations is given by the QoI working group on the FRAC website. In general, resistant forms have shown cross-resistance to all the QoI fungicides. It is notable that resistance has not developed in *Phytophthora infestans* (cause of potato late blight), a major target for some QoIs. As with other fungicide classes, the occurrence of resistant strains, and associated losses of QoI performance, vary greatly between regions of use. For example, resistance of *Plasmopara viticola* is much more prevalent in northern and south-western France, than in Hungary or Spain where disease pressure and QoI use are generally lower.

According to recent FRAC reports, in seventeen pathogens a high level of resistance (resistance factor usually greater than 100) has been shown to be caused by a single mutation (G143A) in the cytochrome bc-1 gene. Another single mutation (F129L), generally causing a much lower degree of resistance, and little or no loss of control provided recommended application rates are adhered to, has been detected in three pathogens. Three further pathogens have produced strains with both these mutations. It is noticeable that QoI resistant oomycete pathogens are sensitive to cyazofamid, a QiI fungicide that blocks electron flow through the second quinone binding site of cytochrome bc-1 which faces the inside of the mitochondrial matrix (Mitani *et al*., 2005). Cyazofamid may be used as a partner to QoIs in resistance management programmes, although it should be recognised that both QoI and QiI fungicides activate alternative oxidase, which causes low levels of resistance to both fungicide groups (Wood and Hollomon, 2003; Hollomon *et al*., 2005; Gisi *et al*., 2005).

General FRAC guidelines for use of QoI fungicides include the following key instructions:

- Apply QoI fungicides at effective rates and intervals, according to manufacturers' recommendations.
- Limit the total number of applications within a total disease management programme, whether applied solo or in mixture with other fungicides.
- Alternate QoI applications, whether solo or in mixture, or whether single or block treatments, with applications of effective fungicides from other cross-

resistance groups. Specific recommendation on size of blocks are given for specific crops. Block applications of QoIs must only be made in mixture with a non-cross-resistant fungicide.

Specific recommendations for the use of QoIs in cereal crops, grapes and bananas are published on the FRAC website, In cereals and bananas, QoIs should always be used in mixtures with non-cross-resistant fungicides.

**CAAs (carboxylic acid amides)**

A FRAC Working Group has been established recently to promote and co-ordinate resistance management for the carboxylic acid amide (CAA) fungicides. At present those used commercially are dimethomorph, flumorph, benthiavalicarb, iprovalicarb and mandipropamid. They specifically act against oomycete pathogens, and probably have a common mode of action.

Shortly after the first CAA (dimethomorph) was introduced in 1993, and despite recommendations to always use in combination with multi-site fungicides, less sensitive populations of *Plasmopara viticola* were observed in a number of vineyards in France and Germany. Since then the frequency of less sensitive populations, and the degree of loss of sensitivity have fluctuated, with no clear progressive build-up of resistance in these or other regions. CAA resistance in *P. viticola* has been shown to be inherited in a recessive way (Gisi *et al*., 2007). This could limit its spread since oomycete fungi are diploid, or even polyploid, during much of their life cycle. Control appears to remain good, with no complaints received from growers, although it cannot be excluded that use of partner fungicides could in some situations mask a degree of loss of performance. No instances of reduced sensitivity have been shown in other oomycete pathogens, including *Phytophthora infestans* which has received extensive monitoring.

Thus CAAs are regarded by FRAC as moderate-risk fungicides, which should continue to perform well against all target diseases provided guidelines are followed. Key recommendations made by the Working Group for use against *Plasmopara viticola* are:

- Apply no more than four CAA sprays per season.
- Apply always in mixture with effective multi-site or other non-cross-resistant fungicides.

No specific recommendations have yet been made for use against *Phytophthora infestans* or other oomycete pathogens and users are encouraged to follow the manufacturers' recommendations.

**Resistance management in banana production**

The crucial role of frequent fungicide applications in banana plantations, the serious problems caused by benzimidazole resistance in the main pathogen, *Mycosphaerella fijiensis* var. *difformis*, and the importance of securing agreement regarding use strategies between major production companies in different countries, were all considerations that led to the formation of a special Working Group of FRAC concerned with fungicide use and resistance management in bananas. The Group includes a number of growers as well as agrochemical manufacturer members.

## Table 3
## Summary of FRAC recommendations for use of fungicides on Banana to control black sigatoka

Updated during the FRAC working group meeting (Orlando, Florida, USA, 1-2. Feb.2006)

| Chemical class | Solo or mixtures | Alternation or blocks | Maximum number of applications | Spray timing |
|---|---|---|---|---|
| Demethylation inhibitors (DMI) | Both, mixtures preferred | Only in full alternation | 8; not more than **50%** of total number of sprays | * |
| Amine fungicides | Both, mixtures preferred | Block of maximum 2 consecutive sprays, full alternation preferred | 15; not more than **50%** of total number of sprays | No restrictions |
| Qo inhibitors (QoI) | Only in mixtures | Only in full alternation | 3; not more than **33%** of total number of sprays | ** |
| Anilinopyrimidines (AP) | Both, mixtures preferred | Only in full alternation | 6; not more than **50%** of total number of sprays | No restrictions |
| Benzimidazoles (BCM) | Only in mixtures | Only in full alternation | 3; not more than **33%** of total number of sprays | ** |

* Applications starting preferably at onset of annual disease progression curve
** Preferably at lower disease pressure; sprays must be separated by at least 3 months

Over the past twenty years the Group's guidelines have changed considerably, in response to the introduction of new fungicide classes and to the development of resistance to some classes of fungicides in certain countries, as shown by sensitivity monitoring and performance checks. Monitoring is mainly done by germination tests, performed locally, and for QoIs additionally by PCR tests for the G143A mutation. Resistance problems have arisen with benzimidazoles, in all regions, and to some extent with DMI and QoI fungicides, mostly in Costa Rica and Panama. No problems have arisen so far with amines and anilinopyrimidines.

Specific guidelines vary according to the fungicide class, and key recommendations are given in Table 3. General guidelines, applicable to all groups, emphasise well-established points of good resistance management discussed above, but one distinct recommendation is that site-specific fungicides must be applied in oil or oil-water emulsions. These enhance fungicidal action and also exert an independent effect on black Sigatoka disease.

## THE FUTURE

Whilst by no means fully successful, fungicide resistance management has undoubtedly prevented or delayed potentially more serious losses of disease control than those which have actually occurred. When practical resistance develops, it is now recognised and acted upon promptly, so that the wasteful use of ineffective treatments is avoided. Both FRAC and public-sector workers have had major roles to play in developing and implementing resistance management and will continue to do so.

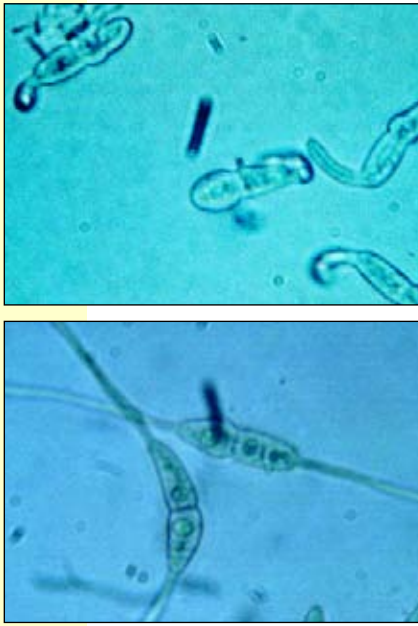
Important new fungicide groups continue to emerge from the industrial laboratories, and of course it is vital to conserve their badly needed activities. It is also important that resources are made available to support the search for new modes of action, which will remain a cornerstone in resistance management. As fundamental research in genetics, biochemistry and epidemiology increase understanding of factors that influence risk, it should be possible to target the search for new modes of action involving inhibition of metabolic processes that offer low risk of resistance developing.

It is heartening to know that baseline studies, and other appraisal and strategy-making activities, are now firmly embedded in the evaluation and development of new fungicides within the individual companies concerned. It is vital that FRAC Working Groups for new fungicide classes should be formed at an early stage for all classes where more than one company is involved. The formation of the anilinopyrimidine and CAA Working Groups, before any practical resistance problems have arisen in these classes of fungicides, are encouraging examples.

In Europe many registration authorities now require protocols for sensitivity test methods, base-line data on the original range of sensitivity, and a statement on resistance risk assessment and management strategy, as part of the registration 'package' (Heimbach *et al*., 2002). Since such information should now be available, and since evidence for efficacy is already a registration requirement, these requirements seem quite reasonable. EU Directive 91-414 sets out the appropriate data requirements and FRAC (and other RACs) have worked closely with EPPO to produce a set of Guidelines (EPPO, 2002) to help in the gathering and interpretation of the necessary data. Also, submission of protocols regarding resistance management is a useful discipline for a company to undergo, and leads to an increased understanding amongst authorities of the problems of resistance management and their avoidance.

There remains a danger, however, of inflexibility through over-emphasis on rigid registration requirements. As experience of use of a new product grows, it may be necessary to change the accepted strategy quickly, and it is essential that this is not inhibited by bureaucratic delays. Any official categorisation of fungicide application to crops, as low-risk, high-risk etc., should be avoided, in view of the present uncertainty of knowledge regarding prediction of resistance development and effectiveness of management strategies, and the known variations in resistance development according to conditions of use in different regions. Of great help, as discussed earlier, would be a more rapid and positive response of registration authorities to new types of fungicides, which will increase diversity. A more positive response of some authorities to applications for registration of pre-packed mixtures will also help resistance management.

From time to time individual companies sponsor research projects concerning fungicide resistance. However, there is scope for a stronger and more sustained interaction between FRAC and public-sector researchers and advisers, and for industrial funding of research projects. A difficulty regarding research projects is that



Germ-tube growth test on sensitive strain of *Mycosphaerella fijiensis* var. *difformis* (banana black Sigatoka disease pathogen). Spores in the top photograph exposed to 5 ppm benomyl. (FRAC)

one company may not wish to fund jointly research in which the model compound belongs to another company. Also a company may not wish its compound to be the subject of an investigation in case undesirable results are obtained. These difficulties may prove hard to overcome in some situations.

There are also opportunities for funding of resistance research by growers through levy-funded organisations, which is very appropriate and should be encouraged world-wide. But national grower organisations can be wary of supporting fungicide research that may also aid production in other countries. It may be possible to obtain funds for resistance management projects in developing countries through the international aid agencies, provided that deserving proposals can be formulated.

Further research is still badly needed on the field behaviour or 'epidemiology' of resistant biotypes, on the biochemical and genetic basis of resistance, and on their interaction with different use strategies. This will provide a sounder basis for effective resistance management, which still depends too much on opinion. Effects of altering dose, both on normal and 'split' schedules particularly require more study, with respect to discrete and multi-step resistance. Genetic evidence for the important concepts of major-gene and polygenic resistance is based largely on studies of laboratory mutants, and more work on field isolates remains a priority.

In the past much monitoring work, particularly that done by industry, has not been fully published. Such information, including base-line data, is of long-term value and is now more often published in scientific journals, or summarised on the FRAC web site (www.frac.info/publ) where status reports and recommendations are also published regularly. A Resistant Pest Management Newsletter is published by Michigan State University (www.whalonlab.msu.com/rpmnews), but the emphasis is strongly on insecticide resistance. Communication and discussion of results and recommendations through occasional symposia, workshops and training courses on fungicide resistance and its management must continue. The role of FRAC in this has been important and one hopes that it will be sustained. Use of the internet to transmit information rapidly to users world-wide, has quickly become a key component keeping growers and users up-to-date with resistance management approaches.

The provision of crop varieties with improved disease resistance, and the development of biological control agents will surely advance, and will strengthen the IPM approach. Care will be needed to maintain the effectiveness of these biological components of IPM, with use of similar strategies to those used for chemicals. The ability of

pathogens to overcome varietal resistance is well recognised, and the development of resistance of a fungal pathogen (*Botrytis cinerea*) to a biological control agent (*Bacillus subtilis* CL27) has been observed (Li and Leifert, 1994).

Where resistance can be shown to result from specific DNA changes in resistant isolates, various PCR diagnostic methods become the choice way to monitor resistance. Management of QoI anti-resistance strategies relies almost entirely on PCR diagnostics, and similar methods could be used to monitor resistance to benzimidazoles, dicarboximides, DMIs, and MBI-D fungicides. It is not only important that researchers keep abreast of advances in real-time PCR and array technologies, but sufficient resources must be made available for laboratories involved in routine monitoring to keep their instrumentation up-to-date in order to obtain the benefits of these developments, such as the greatly increased sample throughput, and rapid delivery of results. However, bioassay protocols, which can also be improved (Fraaije *et al*., 2005), must remain a component of monitoring programmes, since resistance may emerge through selection of different target site mutations, or completely different mechanisms.

There is no doubt at all that chemical control methods will always be required to maintain reliable crop yields of good quality. To conserve the fine fungicides we already have, and to protect new arrivals, attention to resistance management, and work to further improve it, must continue. Increased research effort, increased interaction between industry, public-sector research and advisory services, and registration authorities, and increased publication of information, will all be beneficial. However, moderation should be the keynote, since the lion's share of tight R & D budgets must go to new invention in chemical and biological crop protection.

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## Keith J Brent OBE PhD FIBiol FRAgS

After graduating at London University in Botany and Microbiology, Keith Brent worked for over twenty years in ICI, now Syngenta. At first he studied the biochemistry of filamentous fungi, at the Akers Research Laboratories, Welwyn.

In 1964 he moved to Jealott's Hill Research Station where he led research on fungicides discovery and development and tackled some of the initial problems of fungicide resistance. In 1979 he was appointed Head of the Crop Protection Division at Long Ashton Research Station, University of Bristol, where he also became Deputy Director.

During this period he continued to be involved in fungicide research, and also taught in international courses on fungicide resistance in seven countries world-wide.

Since 1992 he has worked as an international consultant in crop protection and agricultural research management and in 1995 he authored the first FRAC Monograph.

## Derek W Hollomon PhD

Having gained a degree in Agricultural Botany at Reading University, Derek Hollomon started his plant pathology research at Hull University and was awarded a doctorate in 1965. After several years of post-doctoral research in Canada, Australia and the USA, he returned to the UK to initiate research at Rothamsted on the mode of action of the systemic fungicides that were then beginning to be used for cereal disease control. His research soon extended to resistance problems, and these have continued to be a major interest since he moved to the Long Ashton Research Station in 1985.

His work has involved much collaboration with the agrochemical industry, and also has kept him in close contact with the growers. He was awarded the British Crop Protection Council Medal in 1995.

Since 2002, he has been a visiting fellow in the Biochemistry department of the University of Bristol researching the pathway of respiration in pathogenic fungi. He is technical editor of the journal Pest Management Science.