

## NEW MODES OF ACTION OF FUNGICIDES

Geoff Hewitt of the School of Plant Sciences at the University of Reading discusses the continuing search for new fungicidal modes of action

### Introduction

History is littered with instances serious food shortage caused by plant disease. Yield losses due to disease vary between crops and regions and are often 10–20%. However, under favourable conditions for fungal growth, crop yield may be more severely reduced, threatening food security and the livelihoods of farming communities. The importance of disease control has been accepted at least since the times of the Greek and Roman Empires and the various ways, some more spiritual than practical, that were then developed to control crop loss laid the foundations of plant protection technology and the search for new and more effective fungicides. The Ancient World knew, for example, that sulfur, when sprayed onto cereals would protect against foliar disease, but did not understand either the causes of disease or the reasons behind the success of the treatment. It is a sobering thought that the empiricism inherent in that philosophy remains a significant part of fungicide discovery today. The last 40 years, however, have witnessed a revolution in pesticide discovery that is particularly evident in the quest for new modes of fungicide action.

### Mode of action and performance

The discovery of new modes of action that provide improved activity of fungicides against commercially important targets, combined with assured environmental and public safety, is a critical step in safeguarding food security. Such products must also contribute to our ability to manage the development of fungicide resistance and thereby extend the utility of established fungicides. However, mode of action alone is not the key to success. Crop protection companies sell biology not chemistry, and ultimately farmers buy products because they give cost-effective control of diseases like powdery mildew and not because they have new modes of action, or are based on exciting new chemistry. The objective of industry is to provide its customers with products that give acceptable levels of performance and not new modes of action. Of course, understanding how fungicides work is a vital part of their utility, particularly for resistance management, but it is overall efficacy at a competitive cost-benefit determines whether products succeed and others fail, and not innovative discovery. The discovery and development by DowElanco (now Dow AgroSciences) of guanidines and amidines as competitors in the morpholine market was based upon a clear understanding of the role of the isomerase carbocation intermediate as a target site in the

ergosterol biosynthesis pathway (Arnold *et al.*, 1995) and upon elegant molecular modelling. The compounds failed to progress beyond early field trials because the excellent levels of efficacy demonstrated under glasshouse conditions and high volume applications did not transfer to a field performance comparable with the standards. In contrast, compounds active against ubiquitous biochemical processes may, nevertheless, prove useful under field conditions if other factors such as their physico-chemical properties provide a sufficient performance disparity between pathogens and non-target organisms. Mancozeb is an old but prime example of a general cell toxicant with the capability to be phytotoxic, but which in practice achieves selectivity through its inability to be absorbed into the plant. Hence the product is confined to leaf surfaces where it operates as a protectant against a wide range of fungi. Mancozeb is widely used and as an established and cheap commodity it will be hard to dislodge on efficacy grounds alone. The underlying message is that mode of action studies are always qualified by field performance.

New fungicides will have to comply with the increasing regulatory pressure to reduce application rates, to lower fungicide toxicity to non-target organisms and to ensure environmental safety. Added to this is the clear trend towards low-input farming and the need to develop new fungicides to control the development of resistance. These are interrelated and comprise the key criteria in the validation of new modes of action.

Fungicides with a specific developmental activity may comply more easily with the requirements of environmental safety and low resistance risk than those having a more general effect on fungal growth. Two examples show how a targeted approach to specificity can relate mode of action to performance characteristics:

#### *Fungal growth stage*

Different stages of fungal developmental are characterised by particular biochemical events. Different specific modes of action may separate fungicides into those that control pathogens before entry into their host, those that are effective during colonisation, and those that will operate only in the final reproductive stages of development. Modes of action that target more general processes have potential for activity against all growth stages but may also lack crop selectivity or have adverse toxicology. Many fungicides fall into the first category, and it is interesting that in the last six years, 17 new fungicides have been announced at the Brighton Crop Protection Conference, and the majority of

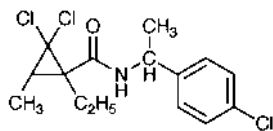


Figure 1. Carpropamid

these had specific activity against pre-penetrative development stages.

For example, in some fungi melanin is important in the expression of pathogenicity and hence melanin synthesis inhibitors have been developed which inhibit melanin biosynthesis in the walls of appressoria, the structures from which penetration of the host is effected. This is an essential and specific requirement for infection; mutants of *Pyricularia oryzae* (teleomorph *Magnapotha grisea*) that lack the ability to produce melanin are incapable of infecting rice leaf tissue. Later fungal development is independent of melanin biosynthesis and fungicides with this mode of action are therefore restricted to use as protectants.

The new rice blast fungicide, carpropamid (Figure 1), is ineffective *in vitro* other than to produce colour changes in the fungal mycelium. The compound inhibits the melanin biosynthesis pathway at two dehydroxylation steps responsible for the conversion of scylatone to 1,3,8-tetrahydroxynaphthalene and vermelone to 1,8-dihydroxynaphthalene (Thieron *et al.*, 1998). This mode of action is distinct from that of established melanin biosynthesis inhibitors which inhibit the formation of scylatone and vermelone.

The regulation of appressorium formation can, however, be prevented in ways others other than the inhibition of melanin biosynthesis. Quinoxifen, with an unknown mode of action, is arguably one of the most intriguing of the new protectant fungicides (Figure 2). It has specific activity against powdery mildews and it too prevents appressorium formation and host penetration. The complete lack of eradicant activity and failure to inhibit mycelial growth *in vitro* of a broad range of fungi suggests that the target site is unique to early developmental stages. In practice the compound is effective at low rates and is mobile in the vapour phase, providing uniform and long-term levels of control. Resistant isolates have been identified but have a severely reduced capacity to sporulate and are unfit. Competition with the small reservoir of established mildew left unaffected by treatment is thought to result in the maintenance of a sufficiently 'wild-type' population to ensure a low resistance risk.

Quinoxifen is also an excellent example of the delay that often occurs between product introduction and the definition of mode of action. A similar condition exists for the anilinopyrimidines and both are evidence to the strength of empiricism within discovery.

Fungicides that inhibit spore germination, germ tube development and/or host penetration may match most of the performance criteria posed for new fungicides. Because of target site specificity they are likely to be highly selective to the crop and to established fungal colonies which will remain unchallenged. This specificity may also offer an

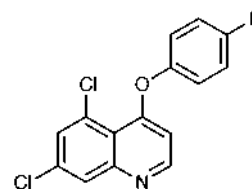


Figure 2. Quinoxifen

attractive route to increased environmental safety and acceptable toxicology. In terms of resistance management, products active against specific steps in the pre-infection stage of fungal challenge may be less likely to encounter short-term resistance than eradicants since an element of the fungal population survives treatment and selection pressure is low. However, the situation is unclear; the strobilurins are effective protectants but have met rapid resistance.

Of course, other modes of action that target different developmental stages or are active against inclusive metabolic events will continue to produce important products. The sterol biosynthesis inhibitors (SBIs) are active against ergosterol biosynthesis, a feature of cell membrane development in all fungi except the Phycmycetes. The activity is the basis of their broad spectrum of use and because many SBIs are mobile within plants they can control many stages of fungal development and have protectant and eradicant uses. At high rates of application some SBIs induce host responses such as growth retardation but the group has the broadest utility of fungicides and 30 years after their discovery still commands 20% of the global market. Similarly, the strobilurins are broad-spectrum products by virtue of their mode of action against the mitochondrial electron transport chain at complex III but in practice their performance segments them largely into the new and growing long-term protectant market.

#### Structural configuration

In nature biochemical processes are sensitive to the configuration of bioactive molecules such as fungicides. Whilst molecular symmetry is not an absolute factor in many reactions, stereoisomerism is important where the applied fungicide exists as a mixture of configuration types arranged around one or more asymmetric atoms or chiral centres. These enantiomers may differ in their biological activities, including their side effects, and in cases where enantiomers have different activities and the least active can be eliminated, there are theoretical opportunities for rate reductions and increased profitability. If the inactive enantiomer has adverse activity in the environment it follows that the pure product will also pose a lower risk, although this remains theoretical.

Metaxyl, an acylalanine for specific use against the Peronosporales, was launched in 1977 as a 50:50 mixture of two enantiomers. A strategy aimed at risk reduction led to the resolution of the components and the introduction in 1996 of metaxyl M, the first enantiomeric form of any fungicide product (Figure 3; Nunninger *et al.*, 1996). Metaxyl M is at least as active as the original mixture but at half the application rate and the overall safety profile

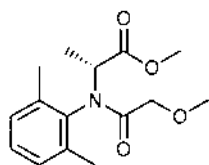


Figure 3. **Metalaxyl M**

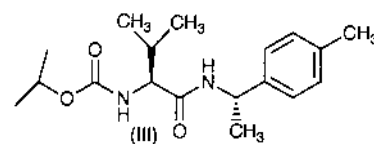
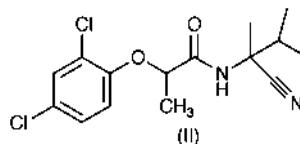
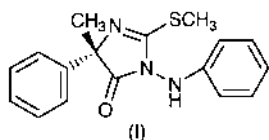


Figure 4. **Recent chiral fungicides: RPA 407213 (left); AC 382042 (centre); and iprovalicarb (right)**

benefits through the subsequent reduction in environmental loading and over double the rate of degradation in soil. In addition to the biological advantages that arise from resolving enantiomeric mixtures, pure products may be viewed by registration authorities as having ‘reduced risk’ and speed official approval.

Only 7% of all agrochemicals are sold as the active enantiomer(s) but development of the large-scale preparation of stereo-pure products will become a major factor in the commercialisation of chiral fungicides. Recent examples of chiral fungicides include an imidazolone (RPA 407213), an inhibitor of mitochondrial electron transport at the enzyme ubihydroquinone:cytochrome c oxidoreductase with activity confined to the S-enantiomer, a phenoxyamide (AC 382042) with activity against the dehydratase enzymes of melanin biosynthesis and an amino acid amide carbamate (iprovalicarb), a possible inhibitor of amino acid metabolism in oomycetes, both of which have two chiral centres and comprise mixtures of four and two isomers respectively (Figure 4; Mercer *et al.*, 1998; Sieverding *et al.*, 1998).

### The search for new modes of action

Our level of understanding of the disease syndrome in terms of biochemical knowledge of fungal action and plant response, does not yet permit the design of specific fungicides. For many years industry’s hope has been that synthesis from rational design will short-cut the discovery and development process. Whilst, however, design drives the optimisation of empirically-discovered activity it cannot claim to have been responsible for any product discovery. The objective is not only to discover a new mode of action but also to translate the activity into favourable field performance and regulatory acceptance at a competitive cost. Whilst a holistic approach may not be realistic, novel sources of chemistry and techniques are becoming available that will help in the separation of probable from possible solutions.

### Bioactive organisms

The control of plant disease by whole organisms continues to be an attractive alternative to the use of synthetic chemistry, the most lucrative sources being from the genera *Pseudomonas*, *Burkholderia* and *Bacillus* (e.g. *Bacillus subtilis*). At least nine fungi (e.g. *Ampelomyces quisqualis*) are known to have potentially useful activity. The number of biologically active agents is increasing and the technology is particularly suited to niche markets that are unable to support strong financial investment in research and to those crops where repeated applications are acceptable, for example, fruit. In some situations, as in the control of soil-

borne diseases, conventional chemistry is not effective and biological antagonists may offer a possible solution. In addition, the growing use of integrated methods of crop protection may encourage greater use of whole organisms to control disease, especially in combination with synthetic fungicides to reduce resistance risk, but as yet few products have had any commercially significant impact.

### Natural products

Over the last 40 years only 24 fungicidal natural products have been commercialised (8 chemistries, 16 microorganisms). Commercial products include the polyoxins, blastidicin S, kasugamycin, mildiomicin and validamycin and most are produced by fermentation of *Streptomyces* spp. All have specific modes of action and since their introduction in the 1960s and 1970s resistance has become widespread. A departure from the trend is soraphen A which is isolated from *Sorangium cellulosum* and has activity against Oomycetes through the inhibition of acetyl-CoA carboxylase.

The future for natural products is promising given that their agricultural importance as organisms and derived chemistry is estimated to be in excess of \$400m in 2000 (Lisansky and Coombs, 1994), that the bulk of the microbial world is uncharacterised and that less familiar sources of novelty, such as the mud plains of the abyssal oceanic depths, are yet to be explored.

### Derived chemistry

Bioactive organisms operate through competition with other microorganisms in the rhizosphere and phylloplane. The basis for competitive advantage arises from the production of potent antifungal metabolites, many of which are characterised and some of which have inspired the synthesis of highly successful fungicides.

The strobilurins mimic the compounds strobilurin A and oudemansin A, natural products with weak and transient activity against a wide range of fungi. Synthetic programmes to stabilise the molecule and increase activity led to kresoxim-methyl and azoxystrobin, the first examples of this new group (Figure 5). Strobilurins inhibit electron transfer in complex III of the mitochondrial electron transport chain.

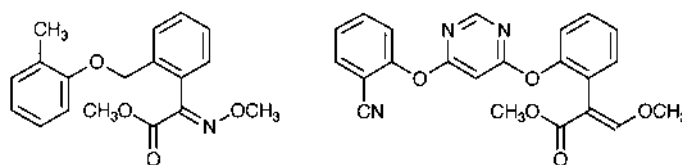


Figure 5. **Strobilurin fungicides: kresoxim-methyl (left); azoxystrobin (right)**

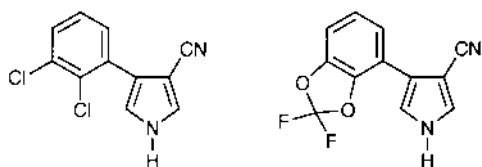


Figure 6. Pyrrolnitrin-derived fungicides: fenpiclonil (left); fludioxonil (right)

The field performance is predominantly protectant with activity against the pre-penetrative developmental stages of most commercially important fungi and, importantly, incorporates a strong element of long-term efficacy. Redistribution in the crop is via systemic movement and a continuous mechanism of absorption and desorption from the waxy cuticular leaf surfaces mediated by the vapour phase. There is intensive competition within the class and it appears set to occupy a leading position in the fungicide market.

The secondary metabolite pyrrolnitrin, obtained from cultures of *Pseudomonas pyrocinia* is UV-unstable and although fungicidal is unsuitable as a product. Directed synthesis produced fenpiclonil (poorly systemic) and fludioxonil (non-systemic) which have broad spectrum and systemic activity with the exception of the Phycomycetes (Figure 6), used as seed treatments.

#### Induced resistance

Induced resistance or systemic acquired resistance (SAR) describes the non-phytotoxic and non-specific defence responses in plant tissue that are triggered at a distance from the site of infection. The process is distinct from the production of phytoalexins which is closely associated with the infection site. The phenomenon is well documented but poorly understood, and is based upon the induction of antifungal proteins by long distance signal molecules such as salicylic acid. Use of this phenomenon in disease control has the potential advantages of providing long-lasting and very broad spectrum protection with low resistance risk that is intrinsically less challenging to the environment than conventional technology. Characteristically, inducers of SAR will be inactive *in vitro* but possess broad protectant activity *in vivo*. In practice, acibenzolar (Figure 7; Kessmann *et al*, 1996), a highly mobile benzothiadiazole and a known inducer of SAR, triggers activity against fungi, bacteria and viruses and in many cases provides useful control but phytotoxicity, most likely in the form of reduced yield potential may also be produced.

The practical potential of induced responses is not yet clear. The only commercially significant product in the class

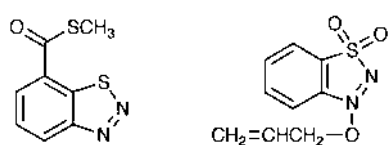


Figure 7. Compounds producing induced resistance responses: acibenzolar (left); probenazole (right)

is probenazole which operates through the induction of fungitoxins such as  $\alpha$ -linoleic acid and several enzymes including phenylalanine ammonia lyase and peroxidase, and has specific use against *P. oryzae*.

#### Genomics

The identification of genes that govern particular biochemical or physiological processes offers a means to control those processes directly, by genetic engineering, or indirectly through the elucidation of biochemical pathways and the definition of potential sites for inhibition by synthetic fungicides. Selected biochemical processes would be both essential for fungal growth and peculiar to fungi. These are the sought-after targets for the development of new fungicides, whilst natural defence mechanisms are of interest for GM disease resistant crops.

#### ● genomics and plant breeding

Other than in the USA where Bt and herbicide-resistant varieties are already on the market, application of the technology to plant breeding is in the early stages of development and is subject to considerable and often misdirected public scrutiny.

Opportunities follow from work to define the genes responsible for the control of vital function in target fungi and their hosts. The characterisation of the encoded enzymes that govern the underlying biochemistry may indicate targets for a variety of synthetic strategies. The technology can also be used to identify sites of inhibition by fungicides of unknown mode of action. However, the most powerful application of genomics in plant disease management is likely to be in the identification and manipulation of sequences that confer fungal resistance to crops (induced resistance; phytoalexin production) or that allow the overproduction of anti-fungal metabolites by competitive organisms.

#### ● genomics in lead generation

In terms of lead generation, the identification and validation of targets is the priority and genomics looks set to provide the key. The development of combinatorial techniques that enable access to vast libraries of compounds is already a feature of discovery in the pharmaceutical industry and their adoption in pesticide discovery ensures that the bulk of fungicide leads will continue to emerge from empirical screening. The philosophy acknowledges that empirical screening is an exercise in applied statistics; the sample size has to be large enough to include potential leads yet small enough to be screened in a realistic time-frame. The larger the sample, the more leads are discovered. The strength of combinatorial chemistry is its partnership with high-throughput screens that are constructed from a knowledge of vital biochemical or physiological functions in fungal and plant-pathogen interaction, driven by genomics. Armed with information about probable target sites, a suitable screen and combinatorial chemistry, comprehensive searches of different chemistries can be made, raising the screening rate from tens of thousands annually to hundreds of thousands.

Given that the demand for food in quantity and of high quality is increasing and that crop diseases will never disappear, the rejection of technologies that reduce the threat from pathogenic fungi is not an option. Currently, the combination of crop variety management and fungicide use is the main means to control disease in food crops and the situation is likely to continue into the foreseeable future. In controlling disease we participate in the dynamics of trying to stabilise monocultural systems that are continuously at odds with the environment. It is to be expected that fungi will adapt to each and every weapon that the crop protection industry can manufacture and that mankind fights a battle, not to win, but simply to maintain an acceptable level of food security. In practice this means that synthetic fungicides having new modes of action and better performance characteristics will always be required and that those discoveries will have to be managed carefully in integrated systems of cropping that include the best of all technologies. In particular, advances in genomics will grow rapidly, adding a new tier of technology to crop protection, a movement that is matched by the changing structure of research investment in major companies. On the basis of our present state of technology and discovery one has to be optimistic that we shall meet the challenge.

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## Further Reading

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- L. G. Copping and H. G. Hewitt (Eds.). *Chemistry and Mode of Action of Crop Protection Agents* (1998). The Royal Society of Chemistry, Cambridge.
- H. G. Hewitt. *Fungicides in Crop Protection* (1998). CABI Publishing.

*Geoff Hewitt is a plant pathologist with over 25 years experience in the crop protection industry with a wide knowledge of global agriculture and pesticide use but with a specialisation in cereal fungicide discovery. He works as a consultant and freelance lecturer in addition to his role as short courses manager in the School of Plant Sciences, The University of Reading.*

## Disease control in past issues

Interested in disease control and fungicides? – see the following selection of articles published in *Pesticide Outlook*.

- Chemical control of rice diseases in Japan (Inoue) **1**(4), 31
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