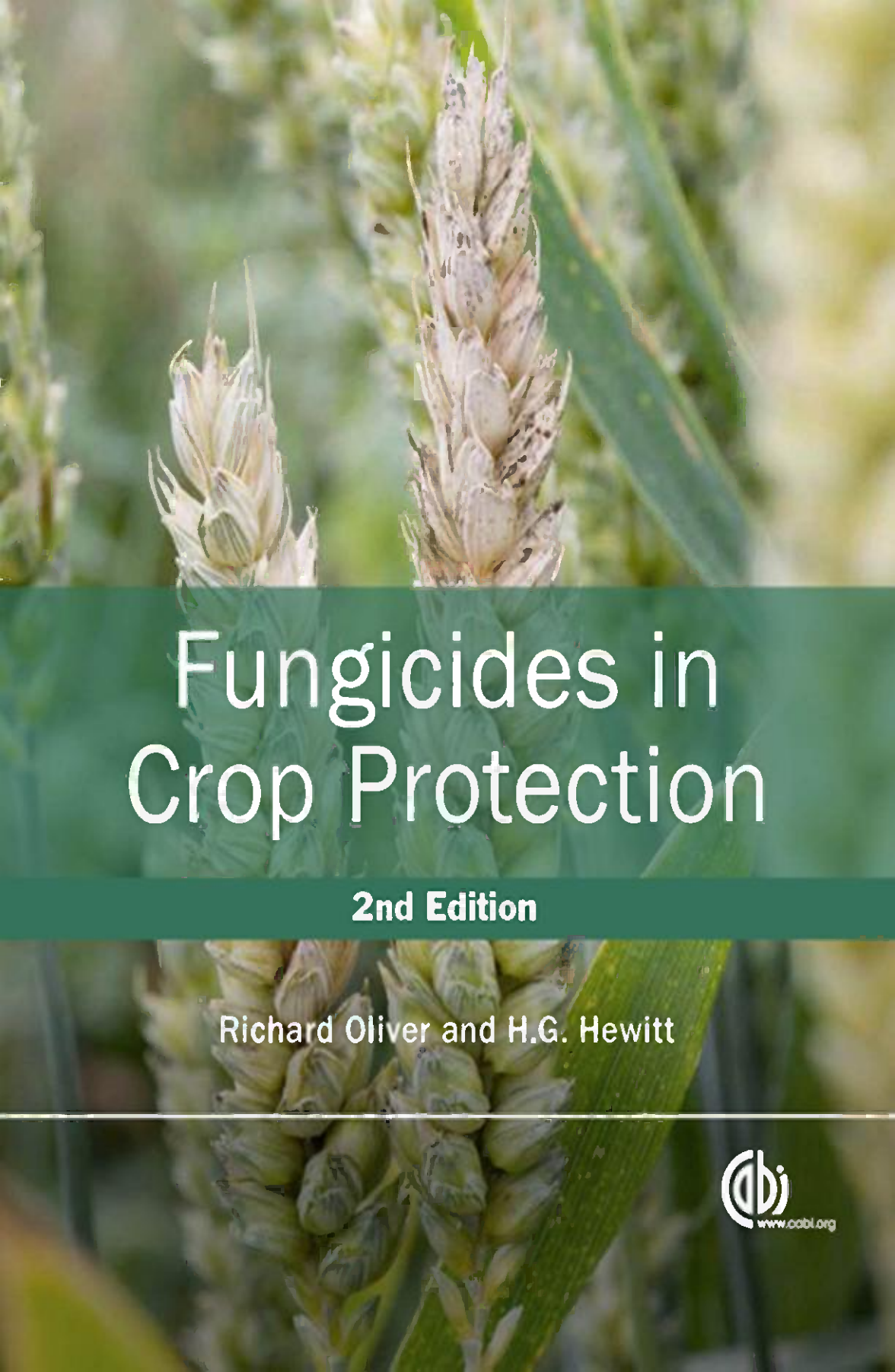
Fungicides in
Crop Protection
2nd Edition
Richard Oliver and H.G. Hewitt
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Preface to the First Edition

Fungal diseases of crops limit our ability to produce food safely in sufficient quantity and of an acceptable quality to satisfy a rapidly expanding and discerning world population. The discovery and development of effective chemical control emerged only in the mid-19th century and did not become a significant part of crop production until comparatively recently. Current methods of agriculture and horticulture rely heavily upon the use of fungicides to the extent that some crops cannot be grown in their absence.

All crops are host to a range of fungal pathogens, many of which cause severe economic damage under suitable conditions. However, fungicide development is driven not by the occasional or regional fungal problems of crops, but by their global value to the manufacturing industry. The need to return sufficient profit from a research investment is becoming more difficult to fulfil under ever increasing legislative stringency and spiralling costs of product development. More and more, the potential benefits of fungicides to growers are being challenged as the levels of economic return to the industry hasten their withdrawal from low-value crops.

This book approaches the subject of fungicide use from an economic standpoint. Discovery and development are shown to be dependent firstly upon the capacity of new products to support further research investment, and secondly upon biology. Much of the text describes the chemistry and biochemical mode of action of a wide range of fungicides, but the emphasis is predominantly biological and demonstrates that growers do not purchase clever chemistry but practical performance.

Other important features are described which highlight the continuing diversification of an industry seeking to integrate the opportunities available in the use of natural products and their derivatives with biological control systems and in the application of biotechnology to crop protection. Because of the weakening reliance on traditional fungicide use, the industry is now more correctly called a crop protection business. Inevitably there have been casualties in the number of companies trading in chemical control. The drive to continue to fund the discovery and development of new products urges companies to acquire or form partnerships with others in order to gain market size and hence to fund research and registration expenditure.

It is from this background of proven benefit, economic constraint, industry change and new technical opportunity that the text launches a description of fungicide use in crop protection. Little weight is placed on application technology or on those aspects of the fungicide industry that are common to herbicides and insecticides, although comparisons are made between the *value* of each agrochemical sector.

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I gratefully acknowledge the encouragement and guidance given to me by colleagues from the agrochemicals industry and academia, especially Dr Mike Smith, Research

and Development Manager, Novartis (UK) and Professor Peter Ayres, Department of Biological Sciences, Lancaster University. It is also appropriate to thank everyone who, over the last 20 years, has played a key role in showing me that plant science and its application to crop protection is exciting and worthwhile. In that respect, I single out Dr Len Copping for his unfailing support and wit.

There were times when I was tempted to abandon the effort and if, in reading the book, you find it useful in any way, then your thanks must go to my wife and family for persuading me to do otherwise.

The contribution of Zeneca Agrochemicals in providing the cover illustration of Azoxystrobin (© ZENECA Limited) is gratefully acknowledged.

H. Geoffrey Hewitt

Preface to the Second Edition

It is 15 years since the first edition of *Fungicides in Crop Protection* was published. These 15 years have seen very significant changes in the world of crop protection in general and in fungicides in particular that more than justify the need for an update of this book. The most significant of these changes is the growth in demand for food crops. The world's population has risen from about 6 billion to 7 billion in that period. Many people eat more meat than before and hence the demand for grain is growing even faster than the population. In clear contrast to the 1990s, we no longer hear about food surpluses. There is an undoubted and urgent need to grow more food, on less land, using less water, fertilizer and other resources and it is clear that fungicides have a major role to play in this.

Fungicide utilization has grown significantly in the last decades. In 1998, fungicide use was dominated by Europe and Japan but is now much more widespread. Fungicides are widely used in Asia, Australia, New Zealand and the Americas. Use is particularly heavy in regions producing vulnerable crops such as grape vines and bananas.

In some countries, regulations limiting the use of fungicides are becoming ever more rigorous. This is particularly true of the European Union. As a result, about 50% of the pesticides available in 1990 have now been withdrawn.

While many of the fungicide classes in use in 1998 are still providing good value, several new classes, especially the quinone outside inhibitor (QoI) and succinate dehydrogenase inhibitor (SDHI) groups, have been introduced. There is a strong pipeline of new compounds especially to combat *Oomycota* and powdery mildews.

The underlying sciences have advanced in important ways. We now have a firm understanding of the evolution of the major groups of target organisms. *Oomycota* have been clearly differentiated from the true *Fungi* and we no longer talk about *Deuteromycota* or the *Fungi Imperfecti*.

Fifteen years ago, it was widely predicted that many crop cultivars would carry transgenes conveying disease resistance. While the area grown to genetically modified (GM) crops has expanded rapidly, these crops generally carry only two GM traits, herbicide resistance and insect tolerance. The failure to develop and release GM disease resistance traits is partly due to the inherent difficulty of developing useful genes, but also due to the widespread public antipathy to GM technology. As a result the regulations are very stringent and so the costs associated with developing GM traits are very high. It remains to be seen whether the next 15 years will witness the widespread introduction of GM disease resistance.

The major challenges for the fungicide industry in 2014 are interlinked. It is increasingly more difficult to discover new fungicides and especially new modes of action and to bring such compounds to market. Resistance to fungicides is now a major concern. Genomics is now central to the discovery of new fungicides, determining modes of action and in resistance management. This new edition is designed to introduce this exciting and critical world of fungicide use in crop protection.

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I gratefully acknowledge the cooperation of Geoff Hewitt, the author of the first edition. This edition has been prepared with the help of many people in the fungicide industry who have provided me with insight into the world of fungicides over the last 15 years. These include Andy Leadbeater, Derek Hollomon, Craig White, Craig Ruchs, Craig Pensini, Jenny Davidson, Doug Wilson, Fran Lopez, Frank van den Bosch, Gavin Heard, Geoff Robertson, Gerd Stammler, Hans Cools, Ian Dry, Kithsiri Jayasena, Ken McKee, Kevin Bodnaruk, Lise Nystrup Jørgensen, Michael Csukai, Naomi Pain, Neil Paveley, Nick Poole, Paul Chatfield, Peter Hobbelen, Rick Horbury, Scott Paton, Geoff Thomas, John Lucas, Bart Fraaije, Andy Corran, James Brown and many others. I am grateful to Rothamsted Research for help in selecting images for the front cover. Thanks also go to Kasia Rybak, Wesley Mair and Fran Lopez for supplying photographs and tables. Research in the author's laboratory is funded mainly by the Australian Grains Research and Development Corporation and the Grape and Wine Research and Development Corporation.

Richard P. Oliver

1 Introduction

Fungicides are agents, of natural or synthetic origin, which act to protect plants against invasion by fungi and/or to eradicate established fungal infection. With herbicides, insecticides and plant growth regulators, they form the battery of agrochemicals (also known as pesticides) that is available to protect crops and maintain their yield potential, measured as the quantity or quality of produce. Diseases of crops are caused by a vast range of organisms that include the true fungi (e.g. *Ascomycota* and *Basidiomycota*), fungal-like but unrelated *Oomycota* (e.g. *Phytophthora* and *Pythium*), *Plasmodiophora*, bacteria, viruses and nematodes. The term fungicide is conventionally taken to mean compounds that control the true fungi, *Plasmodiophora* and the *Oomycota*. It does not include chemicals that control bacteria (these compounds are conventionally called antibiotics), viruses (mainly controlled by insecticides) or nematodes (mainly controlled by genetic and cultural methods).

Since the discovery of the various types of pesticide, several factors have ensured their continued use and the growth of the pesticide businesses. They include an increasing world population, higher incomes and direct benefits both to the grower, such as lower labour costs, higher yields and greater profit, and to the consumer, such as a higher consistency of food quality, increased variety of produce and lower prices.

Population Growth and Food Production

For most of recorded history, the global population growth rate has been below 0.2% per annum. However, the early 19th century witnessed the beginning of an accelerating advance in the control of human disease and in the consistent ability of growers to produce cheaper, higher quality food and a varied diet, which initiated a reduction in mortality rates. In industrialized countries birth rates remained high initially, resulting in a rapid increase in population growth. What we know as the 'developed' world passed through that initial phase and has a low growth rate once again. However, the population of the 'developing' world is still expanding rapidly.

The world population is currently estimated at 7 billion, having increased from 6 billion just 15 years ago (http://esa.un.org/wpp/unpp/panel_population.htm). There is clearly a need for more food to be produced and delivered to the world's population; currently an estimated 25,000 persons die from malnutrition each day (Skamnioti and Gurr, 2009). Conservative estimates predict a world population of 10 billion by 2060. An increasing proportion of the world's population is demanding a diet that is higher in dairy and meat produce. The animals are increasingly fed on grain. The area of land available to grow all these crops is under threat from urbanization, pollution and climate change. There is a clear and urgent need to produce

more food that is nutritious and safe on less land, using less water and fertilizers. And the evidence is convincing that fungicides have had and will increasingly have a major role to play.

Historically, the world's demand for food has been met largely through an expansion of the area under cropping and by improvements in the food distribution network. The increased food needs of Western Europe in the 19th century, for example, were supplied by the expansion of production in the Americas and Australasia. The 20th century introduced a technological revolution into agriculture which has made possible a rapid rate of growth of food production to feed a historically unprecedented growth of world population. Central to the growth in food production was the development of artificial fertilizers and high-yielding crop varieties – the Green Revolution (Evenson and Gollin, 2003). The high yields increased disease levels. This both increased the need for fungicides and justified their costs.

Agriculture makes a significant impact on global warming (Berry *et al.*, 2010). About a seventh of all greenhouse gas (GHG) emissions can be ascribed to agriculture. These include direct use of fossil fuels for transport and tillage, indirect use of fossil fuels for nitrogen fertilizer production, and GHG emission due to soil microbe release of methane and nitrogen oxides. It is therefore possible to quantify food production not just on a tonne per hectare basis but also on a tonne per GHG emission basis. Such studies consistently show that the disease control and green leaf area duration promoted by appropriate use of fungicides maximizes both food production per hectare and per GHG equivalent (Berry *et al.*, 2008). It is therefore somewhat provocatively argued that fungicide-based agriculture is the most 'ecological'.

Recent studies of disease losses and fungicide use have been made in Australia (Murray and Brennan, 2009, 2010). Australia has a generally low rainfall and poor soils, giving average cereal yields in the range of 1–2 t/ha. These are conditions in which disease levels would be expected to be low by world standards. It is sobering that even under these close-to-ideal conditions, pathogens still cause percentage losses in major, highly researched crops of up to 30% (Table 1.1). Table 1.2 details the absolute actual loss in Australian dollars in comparison to the loss expected if no control methods (genetics, cultural or fungicide) were applied. The difference between the potential loss and the actual has been apportioned to each of the major control methods. It is clear that fungicides have a very significant role in protecting yield. This varies between disease, crop, variety and season, but overall the annual AUS$250 million expenditure on fungicides in Australia generates a return of AUS$2000 million; an 8 to 1 ratio.

Table 1.1. Current estimates of losses due to disease in major crops in Australia. (Modified from Murray and Brennan, 2009, 2010.)

Crop	% yield lost to diseases
Wheat	18.0
Barley	13.5
Field pea	29.6

Table 1.2. Breakdown of losses to disease and gains to genetic, cultural and chemical disease control in selected grain crop diseases in Australia; all figures are in AUS$ million. The 'potential loss' is the loss incurred if no control measures were in place; the 'actual loss' is the current estimate. The difference between potential and actual is assigned to either genetic control, cultural practices or fungicide control. It is clear even in low-input, sustainable agriculture situations like Australia that fungicides contribute heavily to disease control. (From Murray and Brennan, 2009, 2010.)

Disease	Potential loss	Actual loss	Genetic control	Cultural control	Fungicide control
Tan spot	676	212	200	155	108
Stripe rust	868	127	431	78	359
Septoria nodorum	230	108	36	51	35
Barley mildew	103	39	10	3	52

Agricultural Technology and the Impact of Fungicide Use

Crop production is a process governed by a series of limiting factors which interrelate. These are crop variety (i.e. the varying degree of genetic disease resistance), nutrition, water supply and crop management (pest, weed and disease control, cultivation). Each factor may assume a dominant, yield-limiting role, depending upon the crop, husbandry practices and the region. For example, water availability is the major factor governing plant distribution and in crops it is often the determining factor in yield production. Historically, the combined action of early improvements in irrigation and the introduction of new varieties with higher genetic potential for yield resulted in dramatic yield increases. Later, the use of fertilizers relieved the limitations to yield dictated by nutrient deficiency and allowed the inherent yield capacity of the crop to be realized to a point that was limited by weed populations, insect infestation and disease. In the 20th century, intensive breeding programmes have further improved the genetic potential for yield in many crops and their capability to respond to other inputs such as fertilizers and agrochemicals.

One of the consequences of increased fertilizer use is more frequent and damaging attacks by fungi, and in intensively grown crops their control is a significant factor in yield determination. However, to a large extent the development and use of pesticides have permitted an even greater use of fertilizer and further increases in yield.

Since the 1940s, the search for new fungicides has intensified and the total value of the crop protection business, as fungicide sales, now stands at US$13 billion, compared with US$6 billion in 1995 (http://www.amisglobal.com/).

The economics of pesticide use vary from crop to crop, between targets and according to the levels of weed, insect or disease infestation. Recent studies in Australia document the gain of AUS$8 for every AUS$1 spent on fungicides (Murray and Brennan, 2009, 2010). This figure is driven by the sharp reductions in the cost to farmers for some fungicides in the last 10–15 years. The cost of off-patent fungicides has fallen to less than AUS$5/ha and so disease gains need only be small to justify the costs. The value gained from the use of small amounts of fungicide to control seed-borne diseases is very large. More modest but still significant gains are obtained when controlling foliar diseases. The use of cereal fungicides in Western

Europe probably accounts for an extra 2–3 million t of grain annually, equal to US$400–600 million. In some cases the benefit gained through fungicide use is more critical to the extent that certain crops cannot be cultivated in the absence of disease control. By the late 1800s coffee rust epidemics were a serious and frequent problem in India, Sri Lanka and Africa. Eventually, production levels became uneconomic and stimulated a change in cropping from coffee to tea. The recovery of the coffee industry was, and remains, totally dependent on the use of fungicides.

The impact of fungicide use on wheat in the UK is illustrated in Fig. 1.1. The average yield of wheat in the UK increased from about 4 to 8 t/ha from 1960 to 2004. During this period, first methyl benzimidazole carbamate (MBC), then demethylation inhibitor (DMI) and finally quinone outside inhibitor (QoI) fungicides were introduced. Each introduction coincided with a further increase in yield.

The History of Fungicide Use

The devastating social effects of plant disease are a common feature of history, extending into Biblical times and beyond with references to 'blasting and mildew' in the books of Deuteronomy and Amos (Large, 1940/2003). Wheat rusts were known

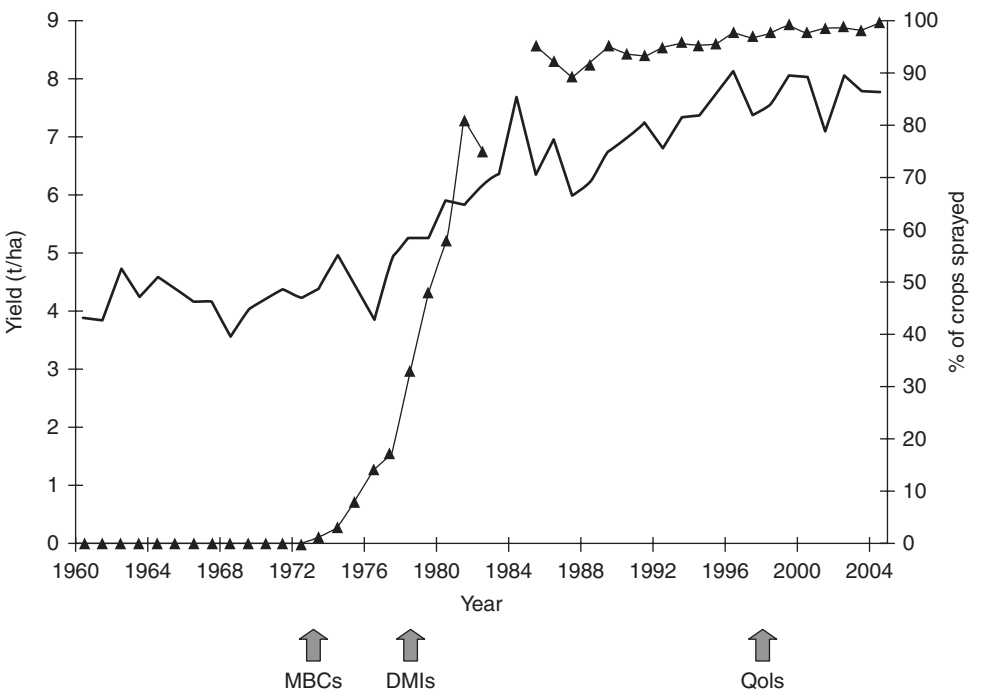

Fig. 1.1. Average wheat yields in the UK, 1960 to 2004 (——; original data source: Cereal Production Surveys, Defra), introduction of the main fungicide groups (arrowed; MBCs, methyl benzimidazole carbamates; DMIs, demethylation inhibitors; QoIs, quinone outside inhibitors) and percentage of crops sprayed with fungicides (—▲—; original data source: Polley and Thomas, 1991; Crop Monitor, Defra/CSL). (From Lucas, 2006 with permission from HGCA.)

at least from Roman times and were considered so important that their occurrence was attributed to divine action. Regular festivals to appease the gods Robigus and Robigo were held in the hope that cereal rust disease could be prevented. However, the gods were clearly not to be trusted and some rudimentary chemical disease control was also practised, the therapeutic but mysterious nature of sulfur being passed down from the ancient Greeks.

Other than crop failure, fungal disease can have a dramatic and direct effect upon human welfare. In 943, a European chronicler described the 'wailing and writhing' of men in the street suffering from a disease which came to be known as 'St Anthony's fire', named after the behaviour of people who, in hope of a cure, visited the shrine of St Anthony in France. The cause is now known to be rye grain contaminated with the alkaloids present in the ergot fungus *Claviceps purpurea*.

By 1750, cereal diseases had attained such a significant economic status in Europe that the French Academy of Arts and Sciences volunteered a prize for the best treatise describing the cause and control of wheat bunt. The solution was not forthcoming and 10 years later up to half of the French wheat crop failed because of bunt and smut (*Ustilago nuda*) diseases. Mathieu Tillet eventually characterized the causal organism of bunt, which carries his name, *Tilletia tritici*, and went on to describe the life cycle of the fungus. Of equal importance was the work, based on a series of field experiments, which examined the efficacy of various treatments against *T. tritici*. It was demonstrated that crops treated with various materials mixed with lime or putrefied urine could be maintained relatively free from bunt disease and these treatments came to be of major economic importance in France.

The catalogue of incidents of fungal disease during the 19th century is extensive (Table 1.3). However, the social impact of plant disease was at its greatest where potatoes were the staple diet. In those regions threats of famine were common and in Eastern Europe and Ireland reached dramatic reality. In Ireland alone, in the 15 years from 1845, over 1 million people died and 1.5 million were forced to emigrate as a direct result, mainly to the USA.

Table 1.3. Major outbreaks of fungal disease in the 19th century.

Crop	Pathogen	Year reported	Region
Cereals	*Claviceps purpurea* (ergot)	1816	France
Hops	*Sphaerotheca humuli* (powdery mildew)	1840	England
Potatoes	*Phytophthora infestans* (late blight)	1845	Europe
Vines	*Uncinula necator* (powdery mildew)	1845	England
Vines	*U. necator* (powdery mildew)	1848	France
Vines	*U. necator* (powdery mildew)	1851	Europe
Vines	*Plasmopara viticola* (downy mildew)	1865	France
Coffee	*Hemileia vastatrix* (coffee rust)	1869	Sri Lanka
Vines	*Guignardia bidwellii* (black rot)	1880	France
Cereals	*Puccinia* spp. (rust)	1889	Austria
Cereals	*Puccinia* spp. (rust)	1892	Prussia
Cereals	*Puccinia* spp. (rust)	1894	USA
Cereals	*Puccinia* spp. (rust)	1916	Canada, Denmark, Russia, Argentina, South Africa, India

Commercially, plant disease was a critical factor in the survival of some industries. The vine industry, for example, was under continual attack; first from grape powdery mildew (UNCNEC – see Chapter 2 for pathogen abbreviations), initially observed in England in 1845, and then followed in the late 1860s by grape downy mildew (PLASVIT). Out of necessity, this period also witnessed the beginnings of modern fungicide use. Observations by the gardener who first reported UNCNEC in England suggested that applications of sulfur could be used to control the disease. Although his findings were confirmed by Professor Duchartre of the Institut Agronomique, Versailles, the challenge to produce a product that could be applied easily to an extensive area of vineyards was not successful until 1855, when Bequerel produced a fine form of sulfur that could be used to achieve effective plant coverage.

Similar advances were made in 1885 with Millardet's invention of Bordeaux mixture, copper sulfate and lime, for the control of PLASVIT, later shown to be effective against late blight in potatoes (PHYTIN). Several versions of the treatment were explored but the mixture is still in use today for the control of fungal diseases on a wide range of crops.

The technology developed in France in response to the frequency and severity of crop disease, especially in vines, became the stimulus for other international investigations. This led, in 1886, to a large programme of trials in the USA to evaluate all the leading French fungicides against black rot, *Guignardia bidwellii*, on vines; apple scab, VENTIN; gooseberry mildew, *Sphaerotheca fuliginea*; and several vegetable pathogens. This collaboration between the US Department of Agriculture (USDA) and French experts was one of the first to examine the relationship of dose–response, cost of spray per hectare, optimum timing and phytotoxicity.

However, the problem of cereal rust disease that had persisted throughout this period of fungicide development evaded similar attempts at control. Farmers resorted to the use of resistant varieties and altered crop management practices to combat the disease. Little success was achieved and by the turn of the 19th century, world wheat production could be severely limited by rust infection, a situation destined to remain until the advent of systemic fungicides in the mid-1960s. Other crops also suffered from rust diseases. In 1869, coffee rust was reported in what became Sri Lanka, and in 10 years reduced average yields by over 50% to 251 kg/ha. The effective destruction of the coffee industry led to investment in a replacement crop, tea. Henceforth, the cultivation of coffee in India and Sri Lanka was totally dependent on the use of fungicides to control rust disease. An excellent and lively introduction to the social history of plant pathology can be found in Money (2006).

The use of complex organic chemistry began with the introduction of new seed treatments designed for the control of wheat bunt. Studies in the pharmaceutical industry which developed medicinal compounds made from arsenic and various dyestuff intermediates stimulated similar research by German plant pathologists, and resulted in the synthesis of several phenolic fungicides containing metallic elements such as mercury, copper and tin. The discovery by the Bayer Company of a compound containing mercury and chlorinated phenol, active against wheat bunt, prompted the intensive development of organomercury seed treatments; the first, Uspulam, being introduced in 1915 by Bayer, followed by Ceresan from ICI (1929) and Agrosan G, also from ICI (1933). The efficacy of these products ensured their widespread popularity in the farming community and they led the cereal seed-treatment

market until mercury-based products were banned in the 1970s and 1980s on the grounds of adverse toxicology.

The establishment of the commercial organizations that would become the major companies in the agrochemicals industry began in the late 1850s, but significant development did not occur until the late 1940s. During the First World War (1914–1918) in Europe agriculture had responded to the need for self-sufficiency, but after the crisis the incentives were reduced and agriculture retreated into its former uncertainty fuelled by poor wages and fluctuating prices. It was not until after the Second World War (1939–1945) that the potential of fungicide use in crop protection and the maintenance of yield were realized, and it is generally accepted that this marks the real beginning of crop fungicide technology.

The early fungicides business was founded on the control of crop diseases that previously had been unchecked and competition between companies was relatively light. Most of the products that were introduced were in response to clear needs of growers and they created new markets by exploiting latent demand. Later products improved on existing control and were established at the expense of their lesser competitors. This is particularly true of the introduction of fungicides that were able to move within plants and throughout crops, the so-called systemic or mobile materials, which captured a significant part of the market previously held by surface-bound non-systemic (immobile) products such as sulfur and copper-based materials.

Fungi infect plants through wounds or directly via stomata or penetration of the surface layers. In leaves this barrier is further enhanced by the presence of a sometimes thick and waxy cuticle. Before the development of systemics in the late 1960s, all fungicide compounds were non-systemic protectants, effecting disease control only through their activity on the plant surface. Characteristically, after application to foliage these compounds control disease either by killing superficial mycelium, as for example in the powdery mildews that penetrate only the topmost cellular layer, or more commonly by preventing the germination of fungal spores already present on the leaf or impacting on the leaf after application. Non-systemics cannot penetrate the leaf and hence cannot control pathogens already established within the plant tissue. Therefore foliage must be treated before the pathogen has colonized the plant. Subsequent development of the plant exposes new tissues to fungal attack and may rupture protective fungicide deposits. Hence, such products have to be applied frequently during the growing season to maintain acceptable disease control levels. Although the lack of mobility of early fungicides limited their flexibility of use, their inability to penetrate plant tissue allowed them to exploit the control spectrum inherent in their non-specific biochemical mode of action (MOA). This remains a valuable feature in their current uses against minor pathogens and in strategies to control resistance to systemic fungicides.

The introduction of systemic compounds caused a revolution in farmer practice and in fungicide discovery and development. New opportunities for fungicides were immediately identified, as in intensive cereal production in Western Europe. Fungal diseases of wheat and barley had been a disturbing feature of cereal production for at least 2000 years but the use of resistant varieties, stimulated in part by the failure of early products to control pathogens such as mildew and rust, enabled infection to remain at what was considered to be an acceptable level. The associated yield losses were estimated to be insignificant until systemic fungicides were discovered and tested, beginning with ethirimol and tridemorph.

Field trials demonstrated that the yield benefits that could be achieved using the new fungicides were on average about 10%. Yields increased further as the limits of varietal potential were explored using combinations of higher fertilizer inputs and fungicides. European Community legislation encouraged high-output production systems, and inputs such as the use of high levels of fertilizers and pest control chemicals increased to maximize yields. The rate of discovery of new and more effective fungicides also increased and in 20 years the range of foliar and ear diseases for which some control could be claimed had expanded from a few seed-borne pathogens and mildews to include PUCCRT, LEPTNO, SEPTRI, *Fusarium*, *Pyrenophora*, *Pseudocercosporella*, *Cochliobolus* and *Rhynchosporium*.

The new products afforded better levels and duration of control and allowed the grower more flexibility in application. However, even they failed to provide complete disease control, and the search for more effective materials and technology continues.

The appearance of systemic fungicides and the increasing variety of products available to the grower corresponded with the requirement of the fungicides industry to adopt new and higher standards of performance. The most important was, and remains, safety to the manufacturer, the user, the consumer of treated crops and all aspects of the environment. The industry and government registration authorities became responsible for the development of only those materials proven to be safe and environmentally acceptable. In addition, in order to compete successfully, product attributes other than biological activity assumed major roles (Table 1.4).

The number of products and mixtures grew to meet the new market standards of disease control. In the triazole family alone there are on average about ten products (different formulations of solo active ingredients and mixtures) per compound. Many fungicides appear to increase yield beyond that attributable to the reduction of disease. Late-season treatment with benomyl, an early systemic fungicide, was shown to delay senescence and increase yield by up to 10% through a combination of fungicidal action and plant growth regulator effects. Similar activity is reported for QoI

Table 1.4. General targets for new fungicidal products.

Attribute	Type of product improvement
Safety	Safe to users
	Environmentally acceptable
	Safe to consumers of the treated product
Performance	Broader disease-control spectrum
	Extended control period
	Increased reliability
	Anti-resistance activity
	Improved crop safety
Use	Compatibility with other products
	Easy-to-use formulations
	Safe application
Cost	Lower cost per treatment through the use of:
	cheaper fungicides
	lower use rates
	fewer treatments per season
	lower application costs

and succinate dehydrogenase inhibitor (SDHI) fungicides and is associated with the control of phylloplane organisms and, more likely, a direct effect on the maintenance of photosynthetic ability.

Devastating crop diseases and their social impact can now be avoided by the careful use of fungicides. Yet, as in any living system, the threat posed by fungal disease is dynamic and we cannot afford to be complacent. If any one crop can be identified as having stimulated the growth of the fungicide business and been the subject of intensive fungicide use, then it must grapevine. But it appears that even in vines new problems can emerge. In 1977, *Eutypa armeniacae* was identified in France and by 1996 an estimated 50% of all vines in the Cognac region were infected, causing a total loss of about 10%. Once again, the official advice is to destroy affected vines while waiting for new fungicidal treatments to be developed or for the arrival of genetically engineered resistant varieties.

There is little doubt that the intensive agricultural systems that are needed to provide the growing population with food also encourage fungal disease epidemics, and the removal of fungicides from agriculture does not appear to be a realistic option. The emergence of fungicide resistance and the need for more cost-effective products encourage the search for better remedies, whether they be synthetic products or materials derived from natural sources or through the introduction of genetic modification of target crops.

The Growth of the Agrochemicals Industry

Pesticides, synonymous with agrochemicals or crop protection agents, comprise mainly herbicides, insecticides, fungicides and plant growth regulators. Further definition can be confusing. A pesticide is strictly an agent that kills a pest and it can be either synthetic or natural. However, the definition omits plant growth regulators, which are designed to enhance the growth and development of crops directly. In addition, the term pesticide is often applied only to insecticides. Pesticides are better classified as agents that maintain the yield potential of crops under adverse growing conditions, most commonly caused by the presence of weeds, fungi or insects. In other words, pesticides combat biotic stresses.

Agrochemicals companies developed as a diversification of those chemical industries specializing in the manufacture of organic dyestuffs. Originally including the fertilizer industry, the agrochemicals business is now distinct and comprises a large, high-value, high-technology industry that survives upon innovation and the discovery and development of synthetic and natural pesticidal products. Despite the success of the pesticides business, the industry is shrinking. The conflicting forces of price competition, affecting margins and profitability, and the increasing costs of discovery and development of potential products and the maintenance of established pesticides have resulted in a phase of consolidation. The situation was made more acute through the increased political and social recognition of the environmental issues associated with pesticide use and the subsequent demand for more extensive product examination. This led to spiralling increases in the costs of safety testing, the prolongation of development time and a subsequent reduction in effective patent life. A shorter product lifespan and the need to generate a return on a rapidly increasing research and development investment have stimulated the search for economies of scale such that the

agrochemicals industry is now dominated by a few large international companies. Just 25 years ago there were ten major international fungicide companies. Now there are only three major players active in all phases of fungicide discovery, development, manufacture and sales. These are Syngenta, Bayer CropScience and BASF with sales of US$3142 million, US$2501 million and US$2297 million, respectively.

Fungicides form a vital part of the research effort and product ranges of all major agrochemicals companies, driven by their well-established use in a wide variety of globally important crops. Their markets, discovery and use, and the legislation that governs their development, are presented in the following chapters.

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2 Plant Pathology and Plant Pathogens

Key Points

- A very diverse range of organisms cause diseases.
- Two types of pathogens, the *Oomycota* and the *Fungi*, dominate and are the targets of fungicides.
- *Fungi* are more related to animals and oomycetes are more related to plants than either is to the other. This is reflected in the different fungicides that control each group.
- Pathogens are divided into biotrophs and necrotrophs and an intermediate class, hemibiotrophs.
- Effectors are pathogen molecules that interact with plants and contribute to the disease phenotype.
- Biotrophs and necrotrophs have different types of effectors and induce different plant responses.

Introduction

Fungicides work by inhibiting the infection processes used by pathogens to cause disease. A very large range of organism groups cause disease on plants. However, first we must define 'disease'. We can operationally define disease as the ability of a pathogen to reduce the yield and/or the quality of a crop while growing and reproducing on the host plant. Pathogens are defined as organisms that cause disease and are distinguished from saprobes, species that live off dead material. Pathogens can be further subdivided into obligates and non-obligates or facultative pathogens. Obligate pathogens can only grow and reproduce on living hosts, whereas facultative organisms are also capable of growth and reproduction on dead material including artificial media.

Pathogenic species are found in many groups of organisms and include viruses, phytoplasmas, viroids, nematodes, parasitic plants, algae, trypanosomatids, bacteria, *Fungi*, *Oomycota* and *Plasmodiophora* (Strange, 2003). These groups encompass much of the biological diversity found in life. Unsurprisingly, no one strategy can control diseases caused by each of these groups. An understanding of the diverse ecological and biochemical properties of these groups is needed in order to appreciate the potential for chemical disease control.

The first seven groups, i.e. viruses, phytoplasmas, viroids, nematodes, parasitic plants, algae and trypanosomatids, are not considered further as chemical agents for their control are currently not significant. Some groups are transmitted by insects and so are controlled with insecticides. Bacteria cause major diseases in some situations and they can be controlled both by genetics and by chemicals. The chemicals are typically known as antibiotics, reflecting their origin in animal and human therapeutics, or occasionally as bacteriocides (Sigee, 2005). Being prokaryotic, bacterial antibiotics

rarely have any activity against other types of pathogen. Antibiotics and insecticides are not considered further in this book.

The remaining groups are all microbial eukaryotes – i.e. organisms too small to be seen with the naked eye but which share basic biochemical features with animals and plants and which differ in fundamental ways from the various prokaryotic bacterial groups. The defining feature of eukaryotes is that they contain nuclei and mitochondria.

Until recently, the relationship between the different groups of eukaryotic microbes has been problematic and poorly understood. Difficulties of cultivation and the limited availability of morphologically meaningful features have hindered progress. Knowledge on the evolutionary history of eukaryotic microbes has undergone a revolution in recent years as a direct result of advances in molecular biology, taxonomy and phylogenetics. We now have a good understanding of the deep evolutionary differences between these organisms and can now rationalize differences in activity of fungicides against these species (Adl *et al.*, 2005; Keeling *et al.*, 2005). Although many of the species are not fungi, all compounds that control these species are normally referred to as fungicides and will be covered in this book.

Table 2.1 lists the higher-level taxa in which are found the major groups of pathogens. Although the details of the highest level of taxonomy are still subject to revision, it is clear that fungi and animals are relatively closely related and these are very distantly related to the *Oomycota*, *Plasmodiophora* and their plant hosts. This modern view of taxonomy emphasizes that pathogenicity has arisen in multiple and diverse taxonomic groups. It also emphasizes the difficulty of finding compounds that have good spectrum (i.e. that they control a broad range of pathogens) but do not damage either the host plant (known as phytotoxicity) or non-target organisms, such as insects and the human population.

Characteristics of Plant Pathogens

Fungi

The *Fungi* are by far the most important group of plant pathogens especially in terms of the number of species and their pathogenic lifestyles, but also in incidence

Table 2.1. Taxonomic placement of the major groups of microbial eukaryotic pathogens and key non-target groups, animals and plants. Non-target groups are in bold.

Supergroup	First rank	Examples
Archaeplastida	*Chloroplastida*	**All plants**
Opisthokonta	*Fungi* (*Ascomycota, Basidiomycota, Chytridiomycota*)	*Puccinia* *Blumeria* *Magnaporthe* *Ascochyta* ***Mycorrhiza***
	Metazoa	**All animals including nematodes**
Rhizaria	*Cercozoa*	*Plasmodiophora*
Chromalveolata	*Stramenopiles* *Oomycota*	*Phytophthora*, *Pythium* *Peronospora*

and damage. It is no coincidence that compounds that control plant diseases are called fungicides.

All fungi are heterotrophic – i.e. they consume small-molecular-weight nutrients from the external medium. Most fungal pathogens are filamentous and grow by extending a hyphal tip. The hypha is divided into cells by septa. Other fungi are yeasts, single-celled organisms growing by cell division. All fungi have rigid cell walls with chitin as the major strengthening compound. This distinguishes them from oomycetes that have cellulose-based cell walls, like plants. The cell membranes of fungi contain the sterol ergosterol, in contrast to animals which have cholesterol and plants and oomycetes which have more diverse 'phytosterols' and which are derived from their plant hosts. Unlike oomycetes, fungi lack flagella and are incapable of directional movement except via hyphal growth.

Fungi reproduce by producing spores. These can be either or both asexual and sexual structures. Traditionally the taxonomy of fungi has depended on the discrimination of morphological features of spores. As many species produce both sexual and asexual spores, a single species often had two names; a teleomorph based on the structure of the sexual spores (often called the perfect state) and an anamorphic name based on the asexual spores (called the imperfect state). Fungi that were not known to produce sexual spores used to be called the *Fungi Imperfecti* or *Deuteromycota*; this hid their real evolutionary relationships to 'perfect' fungi. Furthermore, as the taxonomy was based on sparse morphological data that had a degree of subjectivity, different authors would suggest different names. As a result, few fungal pathogens had a single agreed name, resulting in confusion, not only among pathologists and growers but also quarantine authorities. Recently, the official bodies have agreed to a system whereby each species has only one name. Where more than one exists, the oldest published name should be used. This ends the automatic priority of names of teleomorphs over anamorphs (Hawksworth *et al.*, 2011). This change should be greeted with relief by the fungicide community which is traditionally reluctant to adopt new names. Nonetheless, species are still known by several names and Table 2.2 lists some of the most important as well as the abbreviations used in the fungicide industry.

These changes have been brought about very largely because of the ease of acquiring and interpreting molecular data compared with morphological or chemotaxomomic data. The same data sets are being used to create phylogenetic trees. This eliminated the *Deuteromycota* and substantially revised the deeper phylogenetics of the fungi (James *et al.*, 2006; Wang *et al.*, 2009).

The fungi are divided into about six major groups of which the *Ascomycota* and *Basidiomycota* are the most important, although there are important pathogens in the *Chytridiomycota*. Chytrids mainly infect animals but a few infect plant species; in particular, maize-attacking and lucerne-attacking species have been described. *Synchytrium endobioticum* is an important potato pathogen, apparently controlled by the oomycete fungicide metalaxyl. The *Zygomycota* include the symbiotic mycorrhizal fungi (also called *Glomeromycota*) and hence are an important beneficial group that could be deleteriously affected by fungicides.

The *Ascomycota* is the biggest phylum and contains most of the important pathogenic species. It includes six mainly filamentous sub-phyla – the *Pezizomycotina* – and two yeast groups. There are few pathogens among the yeasts. Instead yeasts can be regarded as beneficials especially in the fermentation industries; care must be taken

Table 2.2. Abbreviations and names of major pathogens and diseases.

BASF abbreviation	Disease	Host	Preferred name of pathogen	Synonym(s)	Pathogen subgroup (see Table 5.2)[a]
	Bakanae disease	Rice	*Gibberella fujikuroi*		GFA
MYCFIJ	Black sigatoka	Banana	*Mycosphaerella fijiensis*		GFA
PYRIOR	Blast	Rice and wheat	*Magnaporthe oryzae*	*Magnaporthe grisea*; *Pyricularia grisea*	PY
BOTCIN	Botrytis; grey mould	Many, especially grapevines	*Botrytis cinerea*	*Botrytinia fuckeliana*	BC
PUCCRT	Brown rust	Wheat	*Puccinia recondita*		B
	Bunt	Several	*Tilletia* spp.		B
PLASVIT	Downy mildew	Vine	*Plasmopara viticola*		OO
	Dutch elm disease	Elm	*Ceratocystis ulmi*		GSA
	Ergot	Wheat	*Claviceps purpurea*		GFA
	Eyespot	Wheat	*Oculimacula yallundae*	*Pseudocercosporella herpotrichoides*	GFA
	Head blight	Wheat	*Fusarium graminearum*	*Gibberella zeae*	GFA
PHYTIN	Late blight	Potato	*Phytophthora infestans*		OO
	Net blotch	Barley	*Pyrenophora teres*	*Dreschlera teres*	GFA
UNCNEC	Powdery mildew	Vine	*Uncinula necator*		PM
ERYSGT	Powdery mildew	Wheat	*Blumeria graminis* f. sp. *tritici*	*Erysiphe graminis* f. sp. *tritici*	PM

ERYSGH	Powdery mildew	Barley	*Blumeria graminis* f. sp. *hordei*	*Erysiphe graminis* f. sp. *hordei*	PM
	Powdery mildew	Apple	*Podosphaera leucotricha*		PM
	Rust	Soybean	*Phakospora pachyrhiza*	Asian rust	B
VENTIN	Scab	Apple	*Venturia inaequalis*		GFA
	Scald	Barley	*Rhynchosporium secalis*		GFA
SEPTRI	Septoria tritici blotch	Wheat	*Zymoseptoria tritici*	*Septoria tritici*; *Mycosphaerella graminicola*	GFA
	Sheath blight	Rice	*Rhizoctonia solani*	*Corticium sasakii*	GFA
LEPTNO	Septoria nodorum blotch	Wheat	*Parastagonospora nodorum*	*Phaeosphaeria nodorum*; *Septoria nodorum*; *Leptosphaeria nodorum*	GFA
	Take-all	Wheat	*Gaeumannomyces graminis*		GSA
	Tan spot	Wheat	*Pyrenophora tritici-repentis*	*Dreschlera tritici-repentis*	GSA
	Yellow rust	Wheat	*Puccinia striiformis*	Stripe rust	B

[a]Fungicide spectrum divides pathogens into seven major groups: *Oomycota* (OO), *Basidiomycota* (B), general foliar *Ascomycota* (GFA), general soil or seed *Ascomycota* (GSA), powdery mildew (PM), BOTCIN (BC) and PYRIOR (PY).

that fungicides used to control diseases do not interfere with wine or beer fermentation by wild or inoculated yeasts.

The new phylogeny groups together some organisms in a biologically relevant way but it is also clear that fungi from different groups share apparently common features. Within the *Ascomycota* order *Dothideomycetes* is the class *Pleosporales* that includes most of the species known to produce necrotrophic effectors: *Cochliobolus*, *Alternaria*, *Pyrenophora* and *Stagonospora* (Oliver and Solomon, 2010). In contrast, it is surprising that the archetypal host-specific biotrophic pathogens, the powdery mildews (*Blumeria* and *Erysiphe*), and the archetypal non-host-specific necrotrophs, *Botrytis* and *Sclerotinia*, are combined in the class *Leotiomycetes*. Species with a hemibiotrophic lifestyle are found in the other classes of the *Dothideomycetes* (e.g. the major wheat pathogen *Zymoseptoria tritici* and the major apple pathogen *Venturia inaequalis*) as well as the *Sordariomycetes* (e.g. bean anthracnose, *Colletotrichum lindemuthianum*; rice blast, *Magnaporthe oryzae*; and the Fusarium wilt pathogens).

The *Basidiomycota* include just two major groups of pathogens: the *Ustilaginomycotina* and the *Pucciniomycotina*. Both groups figure heavily in histories of plant pathology and continue to cause major losses. The *Ustilago* genus includes the bunts and smuts which include mainly seed-borne and flower pathogens. Many of this group are considered to be biotrophic. The control of seed-borne bunts and smuts by fungicides is one of the great success stories of the chemical industry. Resistance problems are very rare. The main issue with these diseases is that because the chemicals work so well genetic resistance can easily be neglected.

The *Pucciniomycotina* include the infamous rust diseases that have for so long been the scourge of growers that they were mentioned in the Bible. All rusts are archetypal biotrophic pathogens showing a high degree of host specificity and the inability to be cultured on media.

Oomycota

The other major group of pathogens is the *Oomycota*. This group includes several highly destructive and historically significant pathogens. The most famous example is the potato late blight pathogen *Phytophthora infestans* (PHYTIN) responsible for the great 1847 Irish famine; it still causes major losses today and is a major target for fungicide development. The other two groups are *Pythiales* and *Peronosporales*. *Pythium* species are the cause of seedling damping-off diseases, whereas the *Peronospora* cause the downy mildews. Oomycete diseases typically require wetter conditions than fungi; hence their old name the water fungi. The diseases caused by *Oomycota* include many that can be described as biotrophic, such as the downy mildews, as well as hemibiotrophic interactions, such as those caused by the *Phytophthora* group.

At first glance, these three groups share many of the features of fungi. They are eukaryotic, heterotrophic, acquire nutrients only by adsorption and grow by filamentous expansion. They cause diseases with mildew, blight or rot symptoms just like fungi. However there are also obvious differences. They have motile spores that use flagella. They lack chitin and ergosterol and instead have cellulose-reinforced cell walls with phytosterols in their cell membranes. And importantly, most of the fungicides that work against oomycetes do not control fungi and vice versa. These differences

were resolved once molecular phylogenetic data were applied to eukaryotic taxa (Forster *et al.*, 1990). These data clearly showed that oomycetes were completely unrelated to fungi. Indeed fungi share a common ancestor with animals and if anything, oomycetes share more common features with plants.

Plasmodiophora

A common root disease of brassica crops called clubroot is caused by *Plasmodiophora brassicae*. This organism has been placed into a distant taxon, the *Rhizaria*. It was previously known as a slime mould, and placed with the 'protists'. Fungicides are generally ineffective, not least because it is a soil pathogen (Humpherson-Jones, 1993).

Biotrophs, necrotrophs and hemibiotrophs

Plant pathologists have traditionally divided pathogens into two broad classes: biotrophs and necrotrophs. The definition of biotrophy is that the pathogen requires living host cells to acquire nutrients; in contrast, necrotrophs can complete their live cycle on dead or dying material. The two classes are associated with several other characteristics. Biotrophs tend to be obligate; i.e. they cannot be grown in culture. It is still accepted that all obligates are biotrophs but the reverse has exceptions. Biotrophs tend to be host-specific and to be well controlled by major resistance genes unless the resistance breaks down. This tendency to overcome resistance genes – the boom and bust cycle – was conceptualized by Flor into the gene-for-gene hypothesis (Flor, 1956; Catanzariti *et al.*, 2007). The feeding of biotrophic fungi is always associated with a specific feeding structure, a haustorium. Resistance is linked to the salicylic acid pathway. In contrast, necrotrophs can always be grown in culture, often have a broad host range and genetic resistance tends to be partial. Necrotrophs often produce copious cell-wall-degrading enzymes in culture and toxic compounds that promote disease. Resistance is more likely to be associated with accumulation of jasmonic acid and to resemble defence against insects and wounding (Oliver and Ipcho, 2004).

Many pathogens do not fit neatly into either class and some are formally classified as hemibiotrophs – pathogens that exhibit both biotrophic and necrotrophic characteristics. These phases can be differentiated in time (first biotroph and then necrotroph) or space (initial penetration of establishment is biotrophic but once a deeper tissue is reached, the fungus becomes necrotrophic).

Fungicide sensitivity is not correlated with whether a fungus/oomycete is described as a biotroph, necrotroph or hemibiotroph. Instead, taxonomic placement has turned out to be a much better predictor.

Avirulence genes, host-specific toxins, PAMPs and effectors

This confusing picture has largely been resolved in the last 10–20 years following the application of molecular genetics tools to plant pathology (Zipfel *et al.*, 2004; Chrisholm *et al.*, 2006; Dodds *et al.*, 2009; Oliver and Solomon, 2010). The new

paradigm revolves around the concept of the pathogen 'effector' and the nature of the plant's response. Effectors are defined as molecules produced by the pathogen that interact in a specific way with the plant so as to produce a reaction that has a bearing on the outcome of the disease; i.e. effectors affect the plant and effect disease. Effectors are produced by all classes of pathogen and can be divided into four major classes. The first class is called PAMPs (or MAMPs) (pathogen- or microbial-associated molecular patterns). These are molecules produced uniformly by multiple classes of microbe and are detected by the plant using specific receptors. Recognition induces the plant to produce an immune response. The second class of effectors is found only in biotrophic pathogens. Their role is to prevent the recognition of PAMPs or at least to damp down the response. Now known as biotrophic effectors, they were previously called avirulence (avr) genes (see Fig. 2.1). This name derived from the finding that resistant plants evolved the ability to recognize the effector and induce an effective defence response. Loss of the recognition by loss or alteration of the effector allowed the pathogen to once again cause disease. Hence, in formal genetic terms, the effector operated as a molecule that prevented disease; an avirulence gene. Recognition of the avr gene product by the plant was done by resistance genes. Hence resistance was dominant. The third and fourth classes are only associated with necrotrophy. Necrotrophic effectors (NEs) include both host-specific and non-host-specific types (see Fig. 2.1). Host-specific NEs interact with a specific host gene

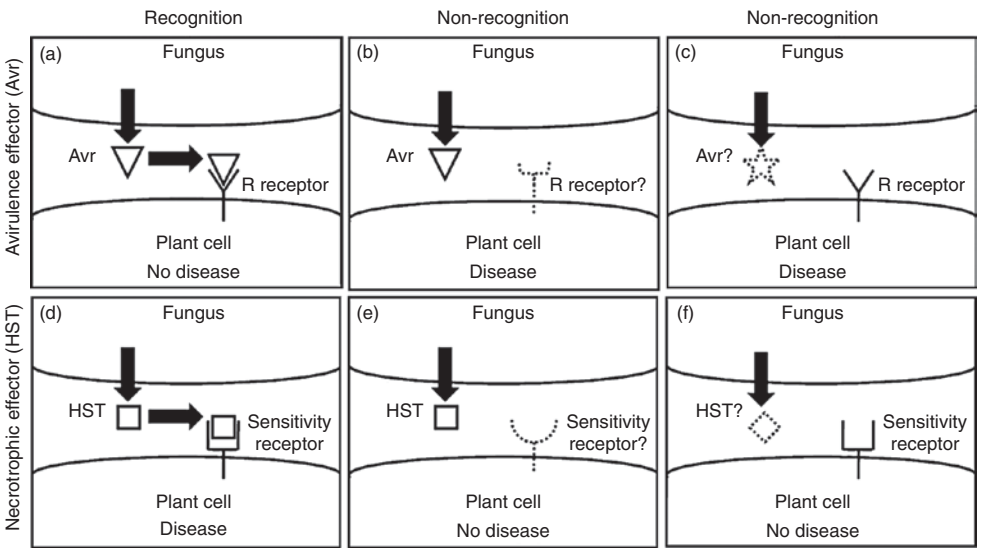

Fig. 2.1. Schematic diagram contrasting the role of pathogen avirulence gene products (now called biotrophic effectors) and necrotrophic effectors (previously called host-specific toxins) in disease. Panels (a) to (c) illustrate effectors in biotrophic interactions. In (a), the specific plant protein recognizes the fungal avirulence gene product (Avr) and this induces a successful defence response. Absence of the plant protein (b) or absence of the Avr product (c) leads to disease. Panels (d) to (f) illustrate effectors in necrotrophic interactions. In (d), the effector (called a host-specific toxin; HST) is recognized by the specific plant protein, inducing a response that leads to disease. Absence of the plant protein (e) or absence of the HST (f) leads to resistance.

and induce a defence response. However, unlike the case of the biotrophs, where the death of the host cell spells failure for the pathogens, the defence response to NEs allows the pathogen to enter deeper into the plant and to acquire nutrients from dead and dying tissues. The final class is non-host-specific NEs. These are molecules that non-specifically induce damage in the host. Examples would be oxalate, toxins such as solanopyrone and the many cell-wall-degrading enzymes.

The Impact of the New Paradigms on Fungicide Research

The resolution of the previous confusion in pathogen names, pathogen types and pathogenicity mechanisms has explained many previous inconsistencies in fungicide performance. The clarification of the gulf between fungi and oomycetes has helped explain fungicide spectrum. Spectrum is the term used to describe the range of pathogens controlled by a particular fungicide. The resolution of the confusion between obligates and non-obligates versus biotrophs has impacted on the way fungicides are discovered and developed (see Chapter 4 for details). In one case, the fungicide Bion (acibenzolar-*S*-methyl; ASM) that operates by potentiating the salicylic acid defence response, its efficacy mainly against biotrophic pathogens is now understandable.

Nomenclature in the Literature and Practice

Plant pathology is beset with a confusing set of nomenclature rules. Each pathogen that causes an important disease can have a variety of names. As we have seen, the fungal nomenclature rules have changed substantially in the last few years but several different names persist for most if not all fungi. In addition the disease can have several names; e.g. tan spot is known as yellow spot in some countries, yellow rust is also known as stripe rust. The fungicide industry has adopted a 6- or 7-letter abbreviation for some of the more important diseases and pathogens (Table 2.2). The abbreviation is based on a binomial that existed at one point in history; what we now call *Blumeria graminis* f. sp. *tritici*, but used to call *Erysiphe graminis* f. sp. *tritici*, has the abbreviation ERYSGT. In this book, we shall use the 6/7-letter code when it exists and the preferred pathogen and disease names when it does not.

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3 The Fungicides Market

Key Points

- Fungicides are discovered and marketed mainly by large, international, private businesses.
- The discovery and development of a new fungicide is very expensive and risky.
- Sales of major fungicides need to amount to around US$1000 million to recoup costs.
- Fungicides are sold to nearly all countries; sales in middle-income countries are rising sharply.
- Cereals, fruit and vegetable crops, grapevines, soybean, rice and pome fruits make up 85% of fungicide use.

Introduction

The discovery, development and marketing of fungicides is (and always has been) almost exclusively performed within the private sector, by large, independent, multinational companies. In contrast, plant breeding and extension activities, which support genetic and cultural disease control methods, were until recently mainly in the domain of state agencies and universities. Fungicide development has received only very limited public-sector support, mainly through co-investment in upstream research. Thus, to stay in business, a company producing fungicides needs to provide a satisfactory rate of return for its investors and to generate resources essential to company growth and development.

The agrochemicals business is risky and the companies continually review their commercial objectives and tend to attack only those markets that are large enough to support additional products, or are dominated by product(s) that are vulnerable and are under-developed or new. Fungicide targets and their priorities in the discovery process are defined not by their biology, but by their economics. The exercise of target definition is straightforward and common to all companies, the only differences between companies being the level of return or risk deemed to be acceptable in the pursuit of a particular market goal. For example, the control of oilseed rape (canola) pathogens may appear an important target to farmers or to regional sales managers wishing to extend their influence in the market, but it may not be large enough to support a dedicated fungicide research programme. Similarly, the control of *Gaeumannomyces graminis* in cereals is estimated to hold very large financial benefits for both the farmer and the fungicide manufacturer, but to commit resources to a discovery effort directed towards a market that has not been proven through the successful introduction of a product is risky, as the commercial size of the problem is difficult to quantify.

What level of return is required by industry in order for the control of a particular disease problem to become an acceptable commercial target? To answer that question it is necessary to understand the costs involved in the discovery and development process, and to appreciate the effects of financial thresholds that companies impose upon the sale of products.

Candidate fungicides enter the process of biological evaluation and commercialization from various sources and range in cost from several hundred to many thousands of dollars each. Passage through the screening and development system eliminates most candidates, with approximately one commercial product emerging for every 140,000 compounds screened (Sozzi *et al.*, 2010). This industry-wide measure of success worsens annually as new materials that meet increasing demands of performance, competition and legislative restrictions become more difficult to discover.

The current industry average cost for the development of a new fungicide is approximately US$256 million, committed over a period of about 10 years, prior to product launch (Walter, 2010). Two-thirds of the total cost is attributed to biological efficacy trials and, in particular, exhaustive toxicological and environmental safety tests which alone may account for 60% of the investment. The primary discovery research, including chemical synthesis, biological testing and toxicology, accounts for only US$85 million. The remaining US$171 million is taken up in production chemistry, field trials, compliance toxicology and registration. The current total compares with US$80 million in 1976 and highlights the contribution of compliance with increasingly stringent regulatory requirements.

Companies normally take out a patent (which in most countries last 20 years; see Box 3.1) near the beginning of the 10-year development period. A new product may not show an operating profit for at least 2 years after commercialization. Thereafter, there may be only 8 years of patent protection in which to recoup the research and development investment costs on all compounds tested, including those that failed at some point in the development process. Companies can expect a few years of maximum profit, before having to contend with direct competition after patent expiry. Clearly, company philosophy must embrace a responsibility to the shareholders, employees and the growth of the enterprise itself, and develop only those products that will achieve the status of a profit maker. Therefore, the projected value of a fungicide at maturity is a critical issue in making discovery and development decisions.

Although companies are reluctant to publicize their economic thresholds, a projected return on investment of up to US$200 million of sales per annum at product maturity may be required to support the development of a pesticide. Furthermore, using that as a measure of commercial acceptability, together with the assumption that even exceptionally good new products will capture only 25–33% of an existing market, it is possible to identify specific disease and crop targets for fungicides. On the basis of a threshold of US$200 million sales annually, and accepting that the industry aim is to produce market leaders, targets would have to possess a current or projected value of between US$800 million and US$1000 million of sales to merit inclusion, not only in the development process for a new product, but also at the level of research. Of course, targets of lesser value may be considered, depending upon the evaluation of investment risk. For example, the development of a biological fungicide may be cheaper than that of a synthetic, and in that case smaller markets may become commercially attractive. However, it is important to note that despite

Box 3.1. Patents and intellectual property

The patenting system has a 'bad press' among the general public, but without it, it is hard to see how we could have access to any of the technological advances, from pharmaceuticals to transport to communications, that make up our modern world. The patenting system is central to the operation of the fungicide companies and an understanding of the basic principles helps explain the nature of the industry.

The purpose of the patenting system is to encourage innovation in all manner of products and industries. It does this in three main ways; first it grants an inventor time to exploit his/her invention during which only the inventor can make and sell the product. Secondly, it forces the inventor to disclose full details of the invention so that competitors can benefit from the underlying knowledge – patent means 'open'; an alternative would be secrecy. Thirdly, it forces an inventor to use a patented invention; failure to do so can result in the granting of licences (permission) to other parties to develop the idea.

The patenting system operates via government agencies called Patent Offices. The European Union has a single office while most other countries have their own. Many countries are signatories to patent treaties that bind themselves to abide by the common principle of respecting the patent system and the free trade of products.

The process of patenting starts when an inventor submits a 'Provisional Application' to the local patent office. The inventor may be the fungicide company, a university or a private individual. This is typically a short document describing the invention and is cheap to file and process. The main purpose of the Provisional from the inventor's perspective is to establish a date from which the eventual Patent, if granted, will be dated. Provisionals are typically filed prior to the full development of the invention. The document is not made public but the inventor can disclose it to organizations to try and secure the financial backing to develop the invention; these might be fungicide companies or venture capitalists, research agencies or charities. If such an organization were interested, the organization might buy the invention and fund the research, granting the inventor a royalty or some other recompense.

The patent office will in due course examine the patent and determine whether the invention satisfies the criteria of patentability; these are novelty, non-obviousness and utility. Novelty is determined by reference to published material, whether other patents, academic papers or the general literature. These are collectively called the 'prior art' and lie in the 'public domain'. The non-obviousness criterion is designed to disallow trivial improvements. Utility is defined as conforming to natural laws (i.e. perpetual motion machines are not patentable) and being capable of commercial exploitation.

The patent office does not examine provisional patents at first. Provisional patents last only 1 or 2 years. If the inventor (or the new owner) wishes to pursue the patent, increasingly large fees need to be paid to the patent office and patent attorneys along with full descriptions of the patent. Furthermore, the inventor must file the patent in all countries in which s/he would like protection. New treaties are making this international filing more straightforward.

The key element of the description is the section called the 'Claims'. Key fungicide patents are typically descriptions of chemicals that can be marketed safely and economically as fungicides. It is likely that, at first, only a single compound is known to the inventor and described in detail. However, nearly all fungicides fall into classes of similar compounds that share a common structural feature and a common MOA. It would be futile to patent just a single compound. All a competitor would have to do,

Continued

Box 3.1. Continued.

following disclosure of the patent, is alter the compound in a variety of ways, find a variant with activity and patent that. The competitor would have saved the huge costs of chemical discovery and the inventor would find its market diminished. Hence the inventor will tend to inflate its discovery and claim the use of all related compounds, including many that may not even have been synthesized. In contrast, the Patent Office, encouraged by competitors, will insist that only tried and tested compounds are included. This tension is central to the day-to-day life of fungicide companies as they seek to outflank each others' patents.

Eventually the patent office may grant the 'Letters Patent'. The owner of the invention now has a specified period, typically 16 or 20 years from the time of the Provisional, for exploitation. In practice however, development of the patent may have taken 5–8 years so the effective period may be only 10 years or less. During this period the inventor not only needs to recoup the cost of manufacture and distribution, but also of research and development. After this period the compound(s) go 'off patent' and anyone can legally make and sell the product. They will have the benefit of full details of the manufacturing process upon which to base their version of the product. The price will inevitably drop. Some companies avoid the process of discovery altogether and choose to specialize in the manufacture of so-called 'generic' products. Furthermore, some countries do not operate a patent system and thus feel free to manufacture any product at any time. They are prevented from selling their products in countries that operate within the patent system by fear of sanctions from the World Trade Organization.

The patent system has many critics. Many complain that companies exploit the system by filing minor improvements as separate patents, thereby extending the effective length of the protection. The system is certainly slow and expensive. However the alternatives would be for companies to rely on secrecy, like Coca-Cola does with its recipes, or to rely on state research organizations to discover and develop the compounds.

the advances in unravelling the biochemical, physical and biological bases of fungicide activity, the discovery process is still serendipitous and it is more likely that products are made on the basis of 'develop what you discover' rather than through a strictly targeted approach.

The Global Fungicides Market

At about 23% of the total agrochemicals market, global fungicide sales are estimated to be US$13.3 billion, including seed treatments (2011 figure) (http://www.amis-outlook.org/). Figures from the USA indicate that 78% of fungicide use is in agriculture, with 18% in industry, commerce and government and 5% used in the home and garden market (http://www.epa.gov/pesticides/pestsales/07pestsales/market_estimates2007.pdf).

In the early phase of the development and use of modern fungicides (1960–1970), the growth of the fungicide market was slow compared with that of the more established herbicide and insecticide sectors. From about 1970, the potential use of fungicides as agents to protect the quantitative and qualitative aspects of yield became widely recognized

and demand increased, stimulating an annual sales growth rate of 3–5% (Fig. 3.1). The increasing potency of the fungicides is illustrated by the slow decline in the weight of fungicides being made and used.

The increase in efficacy has been due the development of systemic fungicides which typically are active in the parts per million range. The increasing pace of new fungicide introductions is shown in Fig. 3.2 and Table 3.1.

The Western European temperate cereal and vine industry was traditionally the largest fungicide market but other countries and regions are fast catching up. Europe has 40% of world sales compared with 28% in the Americas. In Asia and the New World, fungicide sales were restricted due to low crop values or to the presence of yield-limiting factors other than disease, such as water deficiency. Even so, the early 1990s witnessed a fungicide sales growth of over 5% per annum in those regions, in response to increased usage in South-east Asia on rice and in South America on high-value crops such as bananas. Table 3.2 lists some of the incomplete data compiled by the Food and Agriculture Organization of the United Nations (FAO). Several major countries such as China do not report to the FAO. The numbers show that the traditional users of fungicides especially in Europe are reducing the quantity of active ingredient being applied. In contrast, many middle-income tropical countries are fast increasing their use of fungicides (Schreinemachers and Tipraqsa, 2012); see Table 3.2.

Fungicide sales by mode of action

Two fungicide classes dominate global sales (Table 3.3), with DMI and QoI making up over 50% of sales. The DMI group has been the mainstay of foliar disease protection

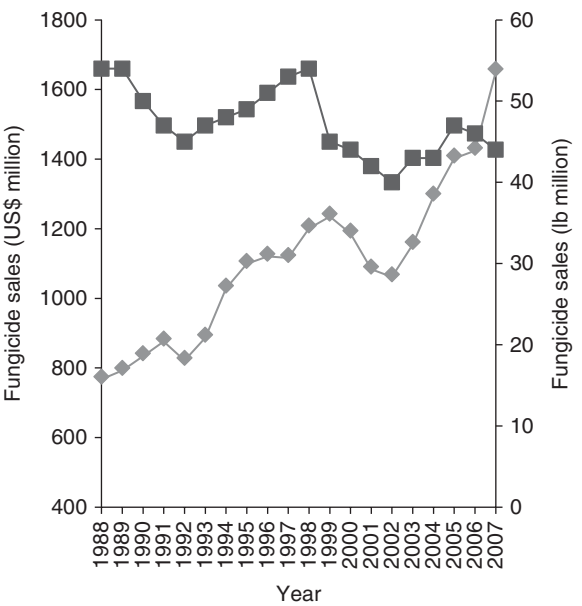

Fig. 3.1. The US fungicides market: increasing sales (—◆—) but declining weight (—■—).

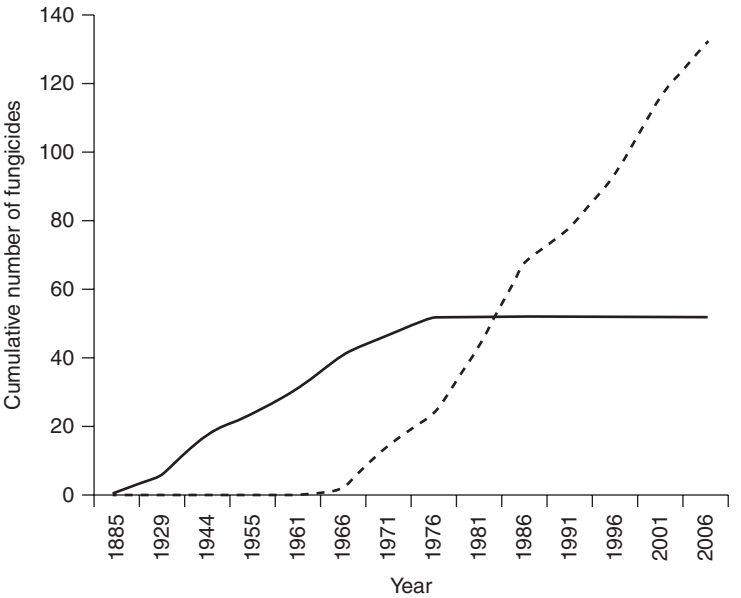

Fig. 3.2. Development of non-systemic (——) and systemic (– – –) fungicides.

Table 3.1. Fungicides introduced 1960–2005.

Date	Fungicides introduced
1940–1960	Thiram, zineb, nabam, biphenyl, oxine copper, tecnazene, captan, folpet, fentinacetate, fentinhydroxide, anilazine, blasticidinS, maneb, dodine, dicloran
1960–1970	Mancozeb, captafol, dithianon, propineb, thiabendazole, chlorothalonil, dichlofluanid, dodemorph, kasugamycin, polyoxins, pyrazophos, ditalimfos, carboxin, oxycarboxin, drazoxolon, tolyfluanide, difenphos, benomyl, fuberidazole, guazatine, dimethirimol, ethirimol, triforine, tridemorph
1970–1980	Iprobenfos, thiophanate, thiophanate-methyl, validamycin, benodanil, triadimefon, imazalil, iprodione, bupirimate, fenarimol, nuarimol, buthiobate, vinclozolin, carbendazim, procymidone, cymoxanil, fosetyl-A1, metalaxyl, furalaxyl, triadimenol, prochloraz, ofurace, propamocarb, bitertanoldiclobutrazol, etaconazole, propiconazole, tolclofos-methyl, fenpropimorph
1980–2000	Benalaxyl, flutolanil, mepronil, pencycuron, cyprofuram, triflumizole, flutriafol, penconazole, flusilazole, diniconazole, oxadixyl, fenpropidin, hexaconazole, cyproconazole, myclobutanil, tebuconazole, pyrifenox, difenoconazole, tetraconazole, fenbuconazole, dimethomorph, fenpiclonil, fludioxonil, epoxyconazole, bromuconazole, pyrimethanil, metconazole, fluquinconazole, triticonazole, fluazinam, azoxystrobin, kresoxim-methyl, metaminostrobin, cyprodinil, mepanipyrim, famoxadone, mefenoxam, quinoxyfen, fenhexamid, fenamidone, trifloxystrobin, cyazofamid, acibenzolar-*S*-methyl
2000–present	Picoxystrobin, pyraclostrobin, prothioconazole, ethaboxam, zoxamide, fluopicolide, flumorph, benthiavalicarb, iprovalicarb, mandipropamid, boscalid, silthiofam, meptyldinocap, amisulbrom, orysastrobin, metrafenone, ipconazole, proquinazid, penthiopyrad, isopyrazam, ametoctradin

Table 3.2. Major fungicide users. (From http://faostat.fao.org/site/424/default.aspx#ancor.)

Country	Year of data	Tonnes
Italy	1990	106,121
Australia	1992	94,193
France	1997	64,050
Mexico	2008	50,845
Colombia	2004	44,370
Japan	2000	40,612
USSR	1990	26,000
USA	1998	24,493
Turkey	2008	17,862
Ecuador	2004	15,505
India	2006	13,367
Portugal	2002	13,320
Spain	1990	12,312
Thailand	2004	12,292

Table 3.3. Market share of different fungicide groups. (From Krämer *et al.*, 2012.)

Fungicide group	Code	Market share (%)
Demethylation inhibitors (DMIs)	G1	29.2
Quinone outside inhibitors (QoIs)	C3	22.1
Dithiocarbamates	M3	6.8
Copper and sulfur	M1/M2	4.7
Phthalimides	M4	4.2
Methyl benzimidazole carbamates (MBCs)	B1	4.1
Succinate dehydrogenase inhibitors (SDHIs)	C2	3.5
Chloronitriles	M5	3.2
Phenylamides (PAs)	A1	2.5
Morpholines	G2	2.5
Melanin biosynthesis inhibitors (MBIs)	I1 and I2	2.4
Carboxylic acid amides (CAAs)	H5	2.1
Dicarboximides	E3	1.9
Anilinopyrimidines (APs)	D1	1.9
Others		8.1

for 30 years, whereas the QoI have established their market position only in the last decade. Many older contact fungicides with multi-site MOAs retain large market shares after many decades of use. This is a testament to the efficacy of their action, their safety record and the economic benefit they give to the grower. The strong sales of the sole chloronitrile, chlorothalonil, can be attributed to its value as a mixing partner with QoI, DMI and SDHI fungicides. One would expect a gradual decline in sales of MBCs and a corresponding rise in the sales of SDHI fungicides.

Global fungicides market by crop

Fungicide manufacturers focus resources on the research and development of new products that fit the most valuable markets. In terms of crops, vegetables, temperate cereals, rice, grapevine, soybean and pome fruit dominate the global fungicides market, representing nearly 85% of the global sales value in 2005 (Fig. 3.3). These ratios are fairly constant but there has been a large increase of value of the soybean market which has increased from 1.1% in 1990 to 8.3% in 2005.

Large fungicide markets are attractive not only because of their size, but also because they utilize long-established and well-understood technologies and present clear challenges for new-generation compounds. Absolute value, however, has to be balanced against the diversity of targets within a particular market, an assessment of current and potential competition, the level of technology required to succeed in that market and a view of future commercial and technical trends.

With a target validation threshold of US$800 million of fungicide sales, only vegetables (US$1.72 billion), temperate cereals (US$1.20 billion), rice (US$740 million), grapevine (US$700 million) and pome fruit (US$320 million) can be considered as potentially viable commercial targets for investment in the discovery and development of new fungicidal products.

The vegetable market is highly segmented, comprising many crops and a broad spectrum of pathogens. Accordingly, the registration of new products into this market is expensive and as a general target, vegetables do not offer a viable return on investment. Hence, fungicides sold into the vegetable market are always well established for use against pathogens in commercially more important sectors such as cereals. An exception is potatoes where fungicide use has become very intense in Europe. The inadvertent introduction of the *Phytophthora infestans* second mating type into Europe in the 1980s allowed the organism to circumvent numerous resistance genes that were previously effective (Haas *et al.*, 2009). As a result the fungicide companies have introduced ametocotradin and fluazinam to complement the established metalaxyl family of fungicides.

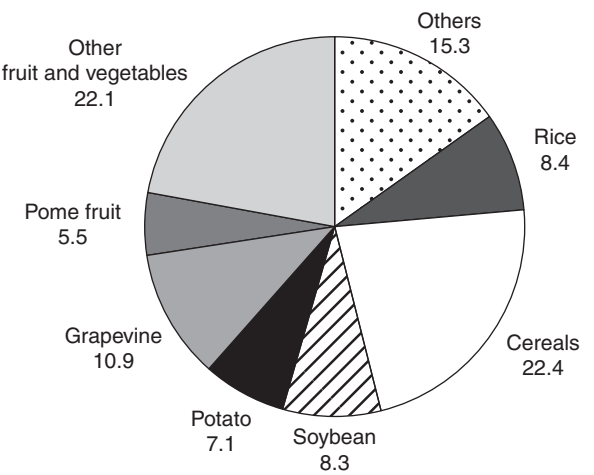

Fig. 3.3. Global fungicides market share (%) for the major crop groups in 2005 (total fungicides market in 2005 = US$7491 million). (Copyright: Phillips McDougall, 2006.)

Cereals

The cereals – wheat, rice, maize and many minor crops – are the mainstays of agricultural production worldwide. In 2011, annual production of rice was 722 million Mt, of maize was 883 million Mt, of wheat was 704 million Mt and of barley was 134 million Mt (http://faostat.fao.org/site/339/default.aspx).

Rice is grown in Africa, the Americas and Europe, but over 75% of the world's production is based in Asia. Average yields range from 1.4 t/ha in Brazil to 4.57 t/ha in Japan, with the most effective producers being Japan, South Korea, USA, China, Europe and Taiwan. Although rice cultivation in Japan accounts for only 1.5% of the global rice area, it commands 67% of the total rice fungicide sales market.

Following the Second World War, Japan began a period of intensive food production. Fertilizers, the use of high-yielding rice varieties and mechanization were encouraged in a bid to increase rice production. It is likely that these measures were also conducive to the incidence and severity of PYRIOR, rice blast, together with a range of other pathogens including *Rhizoctonia solani*, sheath blight and bakanae disease.

Disease control involves the use of cultural methods, resistant varieties and fungicides, usually employed in combination. The use of resistant varieties is a principal method in the control of PYRIOR. However, in Japan fungicide application is the main method of general disease control and this is reflected in the size of the market, currently estimated to be US$647.9 million, although the high price of rice and hence the level of fungicide sales would fall if the Japanese market was opened to wider competition.

Most rice fungicide products are of Japanese origin. Early rice blast control measures based on the use of organomercury products were abandoned with the removal of mercury compounds from Japanese agriculture in 1968. Since then a variety of products have been launched and the character of the market is now highly diverse and fragmented. Rice farmers tend to own small farms that are managed with few inputs or, commonly in Japan, act as an adjunct to another profession. Fungicide use reflects this situation, with products being sold in small packs of easily applied formulations.

Although some products, notably tricyclazole and probenazole, are equivalent in sales value to some successful temperate cereal fungicides, few achieve the high values of the leaders in that market. Tricyclazole is also unusual in that it originated from a non-Japanese company, Eli Lilly (now DowElanco), and has risen in popularity to become the market leader in rice blast control. Most companies will acknowledge the geographic and economic advantages of Japanese manufacturers in developing rice fungicides for Japan and South-east Asia and it is interesting that sources in the industry, including DowElanco, consider the current rice market to be difficult to exploit without a Japanese partner and to be economically risky, given the high return on investment that is required to support appropriate research and development programmes.

PYRIOR is found wherever rice is grown and in Japan is the most serious of all the rice pathogens. However, the climatic conditions in southern China, Sri Lanka, Taiwan, Indonesia, Vietnam and the Philippines favour sheath blight rather than rice blast. Sheath blight also occurs in South America and Africa. Disease control is through the use of fungicides, although in many tropical regions yields are too low to justify fungicide application, and protection from disease depends upon the use of less

susceptible varieties and cultural control. Cropping patterns also differ between areas, with some rice being direct-seeded, some transplanted. Rice may be paddy- or upland-cultivated and, in areas of northern India, deep-water rice is common. The differences in cultivation impact directly on fungicide usage but also reflect the value of the crop, which in turn governs fungicide inputs.

The temperate cereals, i.e. wheat, barley, sorghum, oats and rye, are widely grown outside the tropics, throughout the world, with a total production of about 900 million Mt. Yields range from an average of 10 t/ha in Europe to less than 1–2 t/ha in the Former Soviet Union (FSU), China, India and Australia. However, yields vary between crops and regions, and within regions. In Western Europe wheat yields in excess of 10 t/ha are not uncommon, but yields in parts of Africa may not exceed 500 kg/ha.

Fungicide use in cereals is equivalent to 34% of the total input, or US$1700 million in sales, mainly in winter wheat but with significant usage in winter and spring barley. Because of the dominance of Asia and the FSU in cereal-growing area, it is to be expected that fungicide use and area under cultivation are not balanced. Europe, which supports less than 10% of the total cereals area, provides nearly 20% of the total production and is the primary cereal fungicide market, with an estimated value of US$1500 million.

In North America and Australia, cereal yields are limited more by water shortage than by the lack of disease control and hence the fungicide market is relatively small and localized. Generally, in order to be justified economically, foliar fungicide applications are restricted to areas where yields of over 2 t/ha can be achieved. Most treatments include a triazole, and QoI fungicides are becoming more popular. Specific mildewcides may be justified especially where malting barley production is threatened by triazole resistance as in Western Australia (Tucker *et al.*, 2014). Seed treatments are very widespread; these include triazoles such as fluquinconazole and SDHIs such as carboxin.

The development in Western Europe of techniques of intensive cereal production, in particular the use of fertilizers in continuous cultures of wheat and barley, while allowing for potential yields of over 10 t/ha to be achieved, was accompanied by increased disease levels. The use of fungicides in cereals was stimulated by the need to control disease and permit new levels of production, and profit, to be reached. The main target in Europe is now SEPTRI. Several fungicide groups are used and a remarkably large number of products are available to growers.

Triazoles are highly effective broad-spectrum products. Used as seed treatments and foliar fungicides, they form the most valuable segment of the cereal fungicide market. Their introduction into cereals heralded a revolution in disease control, providing the farmer with the means to control several pathogens for up to 4 weeks and, because of their ability to redistribute in the crop, to achieve a high level of reliability. The earlier appearance of morpholines into the cereal market was not as successful, probably because of their limited spectrum. However, the onset of resistance that eventually reduced the utility of triazoles against ERYSGT and ERYSGH acted to promote a re-emergence in the use of morpholines, which are now usually applied in combination products or tank mixtures with triazoles. Similarly, the failure of benzimidazole fungicides to control wheat eyespot led to the commercial success of the imidazole DMI fungicide prochloraz.

The cereal market is receptive to new product introductions, demonstrated by the rapid rise of the newer DMIs such as epoxiconazole, cyproconazole and prothioconazole (Tables 3.4 and 3.5). Their strength lies in their high activities and their reliability against other major cereal pathogens, particularly SEPTRI. The need to be aware of

Table 3.4. Cereal seed products. (From http://www.hgca.com/crop-management/disease-management.aspx.)

Fungicidal ingredient (products often include an insecticide)	Mode of action	Pathogen groups controlled
Carboxin	C2	B, GSA
Difenconazole	G1	GSA
Fludioxonil	G1	B, GSA
Fluquinconazole	G1	GSA, some control of GFA
Prochloraz	G1	GSA
Flutriafol	G1	GSA
Fuberidazole	G1	GSA
Ipconazole	G1	GSA
Triticonazole	G1	GSA
Prothioconazole	G1	GSA
Silthiofam	C7	Take-all
Thiram	M3	B, GSA

B, *Basidiomycota*; GSA, general soil or seed *Ascomycota*; GFA, general foliar *Ascomycota*.

potential resistance by employing fungicides with different biochemical MOAs encourages the use of a variety of fungicides. Farmers are being encouraged to use reduced frequencies or rates of fungicide application and to use appropriate mixtures to provide broad-spectrum control. There is a re-emergence of the use of non-systemic materials such as chlorothalonil which, although lacking the performance of systemics, have non-specific MOAs and are low-risk compounds with respect to resistance development. The QoIs introduced from 2000 have broad-spectrum activity and complement the triazoles. Many pathogens quickly developed resistance but with the judicious use of mixtures and alternations the QoI have achieved excellent sales.

Specific mildewcides, often developed initially for the grape industry, are also used on cereals. Examples include quinoxyfen, spiroxamine and metrafenone. Their MOAs differ from QoI and triazoles and hence they assist in resistance management strategies.

Many products are sold as mixtures. This is for two main purposes. One reason is to extend the spectrum of the product – i.e. the range of pathogens controlled. Selling products as formulated mixtures has obvious advantages for growers. It allows a range of pathogens to be controlled without having to make multiple applications across their field or having to make so-called tank mixtures of products that might not be compatible. Secondly it has a role in fungicide resistance management (Chapter 6). In addition to other fungicides, mixtures often contain insecticides or nematicides, again increasing the convenience for growers. Tables 3.4 and 3.5 illustrate the wide range of products with overlapping functions.

Grapevine

The principal vine fungicide market is in Europe but large industries also exist in Australia, New Zealand, South Africa and Chile. In all these places, fungicides are critical components of crop protection.

Table 3.5. Foliar products in use in UK for wheat. (From http://www.hgca.com/crop-management/disease-management.aspx.)

		Activity rating[a]					
Active ingredient(s)	Mode of action	Eyespot	ERYSGT	SEPTRI	Yellow rust	PUCCRT	Head blight
Cyflufenamid	U6		4				
Cyprodinil	D1	4	2				
Epoxiconazole + boscalid	G1 + C2	4	2	4	4	5	
Epoxiconazole + isopyrazam	G1 + C2	2	2	4	4	5	
Fluxapyroxad + epoxiconazole	G1 + C2	3	2	5	5	5	2
Prothioconazole + bixafen	G1 + C2	4	3	5	4	5	3
Metrafenone	U8	3	4	1			
Chlorothalonil	M5		1	3	1	1	
Mancozeb	M3		1	2	1	1	
Folpet	M4			2			
Prochloraz	G1	3	1	3	1	1	
Carbendazim	B1	1	1	1	1	1	2
Thiophanate-methyl	B1						2
Fenpropidin	G2		3	1	2	2	
Fenpropimorph	G2		2	1	2	3	
Quinoxyfen	E1		3				
Proquinazid	E1		4				
Spiroxamine	G2		2		2	2	
Azoxystrobin	C3		1	1	3	3	
Picoxystrobin	C3	1	1	1	4	3	
Pyraclostrobin	C3	1	1	1	4	4	
Trifloxystrobin	C3		1	1	2	2	

Dimoxystrobin + epoxiconazole	C3 + G1			3		5	3
Fluoxastrobin + prothioconazole	C3 + G1	4	2	4	4	5	3
Kresoxim-methyl + epoxiconazole	C3 + G1	2	1	4	4	4	
Kresoxim-methyl + fenpropimorph	C3 + G2		2	1	2	2	
Cyproconazole	G1	1	2	2	4	3	
Difenoconazole	G1		1	3	1	3	
Epoxiconazole	G1	2	2	4	5	4	2
Fluquinconazole	G1		2	3	3	3	
Flusilazole	G1	3	2	2	2	2	
Flutriafol	G1		1	2	2	2	
Metconazole	G1		2	3	3	3	3
Propiconazole	G1	1	1	2	2	2	
Prothioconazole	G1	4	3	4	4	2	3
Tebuconazole	G1		2	2	4	4	3
Tetraconazole	G1		2	2	2	2	

[a]From http://www.hgca.com/media/253724/wheat-fungicide-performance-2012-13-1-.pdf.
1, low activity; 5, highest activity.

The market is divided into the control of PLASVIT and UNCNEC, the causes of downy and powdery mildews. Other targets, particularly BOTCIN, grey mould, are economically significant but of secondary value compared with the two major pathogens in many markets. The grapevine is a particular challenge and opportunity for the fungicide industry. The value of a hectare of vintage grapes can exceed several thousand dollars so growers are very keen to ensure adequate protection. The three main pathogens are very diverse organisms (PLASVIT is from the *Oomycota*; powdery mildew and botrytis are from the *Ascomycota*) responding to different classes of fungicides. The crop is a perennial and thus subject to disease build-up in the environment of the vineyard. Being long-lived, the introduction of genetic resistance will always be very difficult to combine with quality.

The grapevine fungicide market is accordingly well established and supports many products (Table 3.6). The use of multi-site, surface-active protectants has always had a crucial role in disease management. Initially, control of PLASVIT was achieved solely through the use of Bordeaux mixture, with sulfur being employed to control UNCNEC. More recently, protectants such as mancozeb became widely used and now have an important technical and economic role within the market. Their immobility is a disadvantage as they cannot be used to protect the foliage or fruit that is not impacted during treatment or the extension growth that is subsequently produced. In addition, surface-bound protectants are subjected to the vagaries of the weather and are susceptible to loss through the action of rain. Characteristically, repeat applications of protectants are employed, with an interval between treatments as short as 10 days during periods conducive to disease or of economic importance, e.g. during fruit development.

Use of fungicides in grapes for wine production is constrained especially by the needs of the wine maker and the customer. The fermentation of wine is undertaken

Table 3.6. Fungicides used in Australian wine production. (Modified from Essling and Francis, 2012.)

Product active ingredient(s)	Mode of action	Pathogen targeted
Penconazole, tetraconazole, fenarimol, myclobutanil, tebuconazole, hexaconazole, triadimenol	G1	UNCNEC, BOTCIN
Spiroxamine	G2	UNCNEC
Fenhexamid	G3	BOTCIN
Metrafenone	U8	UNCNEC
Quinoxyfen	E1	UNCNEC
Boscalid	C2	UNCNEC, BOTCIN
Trifloxystrobin, azoxystrobin, pyraclostrobin	C3	UNCNEC, BOTCIN, PLASVIT
Dimethomorph	H5	PLASVIT
Benalaxyl, metalaxyl	A1	PLASVIT
Pyrimethanil, cyprodinil	D1	BOTCIN
Fludioxinil, iprodione	E2	BOTCIN
Chlorothalonil	M5	BOTCIN, PLASVIT
Captan	M4	BOTCIN, PLASVIT
Metiram, mancozeb	M3	PLASVIT

by yeast species that are susceptible to inhibition by fungicides that might persist in the must (pulped grapes). Furthermore, the large supermarket chains demand extremely stringent residues levels. In practice this limits the use of fungicides in two ways. First, many compounds can only be used early in the growth of the berries so as to allow time for the concentration to decline below that detectable in the bottled wine. Secondly, because limits are placed on the number of detectable compounds (including herbicides and insecticides and regardless of hazard) growers tend to use only one fungicide, to the detriment of resistance management strategies (Essling and Francis, 2012).

Pome (top) fruit

The main fungicide targets are in apples, comprising VENTIN (apple scab), *Podosphaera leucotricha* (apple powdery mildew), with the addition of *Alternaria mali* as a specific target in the Japanese fruit market. The control of VENTIN accounts for 50% and *P. leuchotricha* for 25% of the total sales value. Conditions favourable to infection are pathogen-specific and usually the pathogens do not occur simultaneously on the same host. For this reason different regions may be associated with particular disease problems, as in the Po Valley of northern Italy, which, because of its generally high humidity, is noted for severe outbreaks of apple scab. However, to be competitive, the most popular pome fruit fungicides are active against both major pathogens.

Several products make up the pome fruit fungicide market (Table 3.7). Early control measures relied on multi-site protectants, but the advantages of curative activity afforded by newer products were quickly adopted by growers and the major market share is now attributed to systemic materials such as the triazoles. Compounds under development include the broad-spectrum strobilurins. Resistance to the systemics was recorded soon after their introduction and a system of resistance management using mixtures or alternative applications of products with different MOAs is now a characteristic of the market and a feature of any development programme for new materials.

The objective of fungicide applications in pome fruit is to protect yield quality. The maintenance of leaf integrity, while essential to yield quantity, is of secondary value to the production of unblemished fruit. The dominance of apple scab control reflects the demands of the retailer and consumer for clean fruit, even though apples infected by VENTIN are not considered to be harmful and its eradication is purely cosmetic.

Table 3.7. Fungicides used for pome fruit.

Compound	Mode of action	Disease
Bupirimate	A2	*Podosphaera leucotricha*
Captan	M4	VENTIN
Copper	M1	*Alternaria mali*, VENTIN
Fenarimol	G1	VENTIN, *P. leuchotricha*
Fusilazole	G1	VENTIN, *P. leuchotricha*
Hexaconazole	G1	VENTIN, *P. leuchotricha*
Myclobutanil	G1	VENTIN, *P. leuchotricha*
Penconazole	G1	VENTIN, *P. leuchotricha*
Triforine	G1	*P. leuchotricha*

Leading Fungicide Manufacturers

In the past, companies focused upon their national markets but this is now unsustainable. The rising costs of the development of new fungicides and the maintenance of existing products due to increased regulatory pressures have encouraged the industry to consolidate. Consequently, companies have become increasingly international and, through merger, acquisition and considerable good luck in the discovery and development of key products, a few have emerged to dominate the market. Currently there are six major companies in the crop protection area: Monsanto, Syngenta, Bayer CropScience, DuPont, BASF and Dow. However, only three can be considered full-scale fungicide discovery and production companies: Syngenta (sales 2008: US$3142 million), Bayer CropScience (sales 2008: US$2501 million) and BASF (sales 2008: US$2297 million). Dow and DuPont retain niche activity in fungicide discovery.

Only 20 years ago there were ten large fungicide discovery companies. Of the current big three, Syngenta derives from Zeneca and Novartis; Sandoz and CIBA were previously acquired by Novartis; Bayer acquired AgrEvo and Rhone-Poulenc. BASF is unique is remaining a broad-based chemical company whereas Bayer and Syngenta are focused on crops and include seed businesses as well as chemicals.

Another group of companies specialize in manufacturing and distributing off-patent (or 'generic') compounds. They thus avoid the huge cost and risk of fungicide discovery and development. They do incur the costs of registration in smaller markets. On the other hand, they will only survive if they undercut the original patent holder so their profit margins will always be limited. The biggest generics company in the fungicide area is MAI (sales 2008: US$415 million) followed by Nufarm (sales 2012: US$200 million).

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4 Fungicide Discovery

Key Points

- Strategies to discover new fungicides focus on:
  - diseases with the greatest market potential;
  - new and emergent diseases;
  - novel MOAs; and
  - experimentally tractable pathogens.
- 'Leads' are active compounds with the potential to be modified to optimize field performance.
- Screens for leads use *in planta*, *in vivo* and high-throughput strategies.
- Sources of fungicide leads include random compound libraries, natural products, combinatorial chemistry, compounds designed to inhibit specific enzymes and compounds with optimized physicochemical properties.

Target Selection

There are many thousands of species of plant pathogenic fungi. Fortunately, for each crop the array of fungi able to attack, colonize and cause damage is limited. Those that are successful can result in significant economic losses under suitable environmental conditions. In wheat, for example, over 15 pathogens are recognized to cause 19 distinct and severe disease syndromes in Australia alone (Murray and Brennan, 2009). They include biotroph and necrotroph, foliar, root, crown and seed pathogens, and both *Basidiomycota* and *Ascomycota*. In the case of grapevines, the three major pathogens include two ascomycetes and one oomycete (Essling and Francis, 2012).

To a grower, all pathogens that affect yield or quality enough to cause a reduction in profit are important and appear to constitute worthwhile targets for fungicide discovery. However, fungicide manufacturers will invest research and discovery resources only in the control of those pathogens which have the capacity to return an acceptable profit, and the development of fungicides into niche markets is always preceded by their success in major markets.

The questions, then, are how are those major targets distinguished from the plethora of possible crops and their attendant diseases and how are they incorporated into discovery research programmes? The choice of fungicide targets has been driven by a combination of factors. The crop should be widely grown and/or should be of high value. Such crops should also have diseases that are not well controlled by genetic or cultural methods.

Each agrochemicals company has its own commercial strategy with respect to target definition but all adopt the same general process, known as screening, to

identify product candidates. While the discovery of fungicides necessarily includes aspects of biochemistry, synthetic chemistry and formulation, commercial success is founded not upon the ability of a company to deliver novel and clever chemistry, but on the field performance of its products. The driving forces of fungicide discovery, therefore, are the determination of biological activity, novelty of the MOA and its transfer to potentially useful field performance.

The composition of the screen reflects the value placed by the company on the control of the various crop/pathogen combinations and the overall value, in terms of fungicide sales, associated with particular crops.

Definition of target pathogens and crops

Market size

Before planning the development of any new fungicide, manufacturers must be convinced that the product will reach a threshold level of annual sales in order to justify investment, sustain further research, return a profit for the shareholders, maintain a motivated and expert staff and support the future expansion of the company. The threshold varies according to company and target, but may be as high as US$200 million per annum at product maturity. The value of particular crop fungicide markets can be disassembled into the value of control associated with individual pathogens or combinations of pathogens. This process prioritizes existing and commercially proven fungicide targets.

New and re-emergent diseases

The single most critical factor in disease target choice is the economic level of damage caused by the pathogen. Changing market conditions and new knowledge can significantly alter the loss levels. Plant pathogens continually surprise us with their ability to cross borders and to take advantage of new opportunities. These new opportunities arise from a variety of factors. SEPTRI has become the dominant pathogen on cereals in Europe, displacing rusts, mildews and LEPTNO since the 1960s. The reasons for this shift in pathogen is unclear but are linked to reductions in pollution from coal-fired power stations and domestic heating (Bearchell *et al.*, 2005). Hence, SEPTRI has become the number one target for fungicide development. *Fusarium graminearum* causes the disease head blight of cereals (Kazan *et al.*, 2012). The pathogen causes only modest yield losses but contaminates infected grain with mycotoxins. The mycotoxins are extremely toxic to humans and animals and thus make the grain essentially unsellable. Therefore *F. graminearum* has become a more significant target.

One of the most important considerations is the area grown to a crop and the density with which it is planted. Both factors favour the development of diseases. A key example here is soybean, of which 250 million Mt is now grown annually on about 100 Mha. This increase in area has been driven by the need for protein to add to animal feed and human food products. A newly important disease, soybean rust or Asian rust, caused by *Phakopsora pachyrhizi*, emerged in the 2000s in South America (Yorinori *et al.*, 2005).

Soon it spread to North America (Schneider *et al.*, 2005). Fungicides to control the disease were needed urgently and were used on a large area. Over US$500 million was spent. Hence soybean rust emerged as a major target for fungicide discovery.

Potato late blight (PHYTIN) has been a major disease since the 1850s but was well controlled by genetics in Europe until recently. Genetic disease control can be compromised if the pathogen can evolves new virulences faster than plant breeders can breed new resistances. Despite quarantine regulations, the A2 mating type gene emerged in Europe in the 1980s and spread throughout the continent (Fry, 1991; Dyer *et al.*, 1993). This allowed the shuffling of virulence genes and hence the defeat of the resistance genes in the major cultivars. Fungicides then had to be applied in ever-increasing frequency. And hence PHYTIN became a major priority for fungicide discovery.

Fungicide resistance

Fungicide resistance has become one of the dominant factors in target choice. Pathogens differ in their propensity to develop resistance. The pathogens that typically develop resistance first are the powdery mildews followed by BOTCIN (see Chapter 6 for details). For this reason, it is still economic to develop narrow-spectrum compounds that are specific for these pathogens (especially wheat, barley and grapevine). Recent examples include quinoxyfen, metrafenone, bupirimate, proquinazid, spiroxamine, cyflufenamid and, for BOTCIN, fenhexamid and iprodione.

The importance of fungicide resistance has placed a premium on compounds that either would not develop resistance or would protect high-risk compounds from developing resistance. Indeed the design of compounds that would be immune from resistance can be said to be the Holy Grail of the industry. The value of compounds that protect high-risk compounds explains the increased market share of chlorothalonil, used as a mixing partner for QoI fungicides.

New modes of action

The development of resistance in pathogen populations reduces or eliminates the efficacy not only of the fungicide in the test, but also of all others that share its MOA. As only a handful of MOAs are available, resistance is a major threat not just to fungicide company profits but also to global food production. Hence fungicide companies are not merely seeking new fungicides that can be patented and marketed but entirely new MOAs. This realization has altered the way fungicide discovery takes place. Paradoxically, companies are seeking compounds with unknown MOAs. This has placed a premium on the imagination and inventiveness of the researchers. It has reduced, but not entirely eliminated, the practice of seeking derivatives of existing compounds; witness the case of prothioconazole, the first new triazole to be released for 15 years.

Market deconvolution

In crops that are host to many pathogens, as in cereals, the actual value that a grower places on the control of specific pathogens is more difficult to unravel because established products are either broad-spectrum, including primary and secondary targets, or are

specific to the major targets. In those cases, it is important to determine the true values attached to the control of the component pathogens and to particular combinations. We can illustrate how a fungicide market is constructed and how disease targets are prioritized by considering the utilization of products in Great Britain in 1994. In 1994 there were 4.75 million ha of arable crops (Anon., 1994) and fungicides were applied to 67% (3.2 million ha), with, on average, two applications using three products and a total of four active ingredients. The frequency of fungicide input varied according to crop. Fungicides were used in almost all potatoes, whereas only 4.5% of the linseed crop was treated. In total, 10.8 million ha received a fungicide treatment ('treated hectares').

In cereals, with a total area of 2.8 million ha, there were 8.6 million treated ha and the percentage of area treated varied from 71.1% in spring barley to 96.3% in winter wheat (Table 4.1).

Areas were treated with fungicide according to the occurrence and severity of particular diseases. These changed from year to year but were generally dominated by the SEPRTI diseases and powdery mildews. In contrast, rust was less damaging, being severe once in about every 7 years, or only locally severe as in the occurrence of *Puccinia striiformis* in the south-eastern part of Britain.

In winter wheat, most fungicides (excluding seed treatments) were applied at two application timings, stem extension/first node (growth stage 30/31) and flag leaf emergence (growth stage 37). Different pathogen combinations were associated with each timing. In general, the first timing targeted stem base and the early foliar pathogens, eyespot, ERYSGT and SEPTRI. The second timing targeted ERYSGT, SEPTRI, *Puccinia* spp. and LEPTNO, and accounted for the bulk of foliar fungicide use. Based on this split in the chronology of disease incidence and control, figures for treated area with respect to each pathogen can be derived for each application.

In winter barley, two fungicide applications were also used but were of equal merit. Here the major pathogens, as seen by the grower, were eyespot, ERYSGH, *Pyrenophora teres*, *Rhynchosporium secalis* and *Puccinia hordei*. In spring barley, it was usual for only a single fungicide application to be made, in this case for the control of ERYSGH and *R. secalis*.

Fungicide applications cost the equivalent of US$10–50/ha. Combining treated hectares, targets and timing shows that SEPTRI control was the most valuable target for UK fungicides (US$260 million), closely followed by a collective value for wheat and barley powdery mildew (US$240 million). Eyespot and *R. secalis* control were approximately equivalent at US$50 million and US$60 million, respectively, with rust and net blotch control accounting respectively for only 3% and 0.7% of the total UK cereal fungicide market (US$750 million).

Table 4.1. Cereal fungicide use in Great Britain, 1994.

	Wheat	Winter barley	Spring barley	Total arable crops
Area planted (ha)	1,802,191	620,132	450,596	4,756,116
Treated hectares[a]	6,525,831	1,497,801	619,420	10,793,721
Percentage of area treated	96.3	93.8	71.1	

[a]Excluding seed treatments.

The value of the control of multiple cereal pathogens can therefore be estimated (Table 4.2).

A similar exercise can be carried out using the existing fungicide sales value attached to the control of pathogens of major crops in East Asia, South-east Asia, Australia, New Zealand, the Pacific Islands and South America (Table 4.3). In this example, the control of PYRIOR, with a value of US$600 million in Asia alone, is enough to qualify it as a suitable target for discovery investment. On a global basis, *R. secalis* may also be included as a primary target for fungicide discovery.

In pome fruit, the major targets arc VENTIN and *P. leucotricha*. Although activity against VENTIN is preferred, the use of programme spraying and the existing availability of broad-spectrum compounds mean that dual activity is an advantage.

This system of target definition does not accommodate those pathogens, such as BOTCIN or *Rhizoctonia*, which attack a range of different crop species. It is probable

Table 4.2. Estimated current value of fungicide targets – cereals.

Target	Potential fungicide sales (US$ million)
SEPTRI	1250
ERYSGH	950
Eyespot	180
Rhynchosporium secalis	110
Rusts	82
Pyrenophora spp.	12.5

Table 4.3. Estimated current market sizes for selected diseases in Asia and Australasia and in South America.

Pathogen	Asian + Australasian market (US$ million)	South American market (US$ million)
Soybean rust	?	500
Alternaria	125	100
BOTCIN	60	12
Cercospora	75	12
Powdery mildews	105	25
Gibberella	75	
Glomerella	125	
MYCFIJ	20	120
Penicillium	45	
Peronospora	25	
PHYTIN	100	100
Plasmodiophora	30	
PLASVIT	33	10
Eyespot	1	
Puccinia	32	18
PYRIOR	600	25
Rhizoctonia	250	0.5
Rhynchosporium	2.5	
Sclerotinia	45	
VENTIN	140	20

that the most prevalent fungal pathogen on that basis is *Cercospora*. However, no company regards the control of *Cercospora* as a primary target for discovery investment because of its fragmented spectrum across comparatively low-value crops. In practice, the definition of discovery fungicide targets is a balance between their potential global value and the number of economically important crops in which they occur. Although vegetables constitute the largest fungicide market, their diversity means they are less significant than cereals, rice, pome fruit or grapevine.

In general, companies share the same major objectives within existing markets, although some may place greater reliance upon research into the control of particular pathogens such as PLASVIT if they consider the risk–benefit balance to be favourable.

Exploratory targets

Markets are created by fungicides which demonstrate that disease control can be profitable to the grower. There are several pathogens for which no effective fungicidal control exists but which are associated with severe crop losses. Examples are *Sclerotinia* in legumes and take-all in cereals. However, it is difficult to assess with much certainty the commercial value of a fungicide that could control new target pathogens. The problem lies in the puzzle of how to compare a healthy crop against a diseased crop when no disease control exists. In addition, the control of root and crown pathogens requires that the fungicide has phloem mobility, a property that has only rarely been observed and which represents a considerable technical problem.

The investment of research funds into the discovery of compounds for new markets is risky and tends to be a second priority to finding materials to fit existing outlets. The justification to pursue some targets is growing, however (see, for example, Case Study 1).

Screening for Fungicide Leads

A screen is a stepwise series of tests that challenge a candidate pesticide with increasingly difficult biochemical and/or biological hurdles. The steps can be aspects of MOA, application rate, spectrum, phytotoxicity or redistribution in the crop, but essentially need only to include those attributes that affect the practical use of the candidate fungicide by farmers and hence its commercial value. In principle, the term 'screening' can encompass all steps in the biology of pesticide discovery and development up to product status, but it is usually understood to describe only laboratory and glasshouse tests.

The design of fungicide screens

Screens used by fungicide companies can be divided into three broad classes referred to as 'high-throughput', *in vitro* and *in planta* (Table 4.4). These types of screen represent the dilemma of choosing between cheap and easy tests on huge numbers of compounds, but which only rarely lead to a useful product, versus slow and expensive tests of only a few compounds that individually have a much better chance of being ultimately useful.

Case Study 1. The control of *Gaeumannomyces graminis* var. *tritici* – an unmet need?

Gaeumannomyces graminis var. *tritici* is the causal organism of take-all disease of cereals, a name first applied to a devastating incidence of the disease in Australia about 150 years ago. It is an ascomycete and one of the four members of the genus *Gaeumannomyces* that are known to infect the roots of grasses, including cereals and sedges. The main commercially important hosts of *G. graminis* var. *tritici* are wheat and barley, although rye is also susceptible. Infected roots are stunted and blackened, with similar symptoms occasionally extending to the stem base. Plants ripen prematurely and produce bleached ears, commonly known as whiteheads, which produce little or no grain. Following harvest, the fungus survives on stubble and the root remains of the infected crop. Volunteer plants are susceptible to attack and serve to carry infection through to the following year. In the absence of a suitable host, *G. graminis* var. *tritici* survives on crop debris in the soil as a weakly competitive saprophyte.

The pathogen is widely distributed and occurs wherever cereals are grown commercially. It is recognized to be an important determinant of yield in Australia, the Pacific north-west of America, South America and Europe. However, losses attributed solely to *G. graminis* var. *tritici* are difficult to assess because of year-to-year and between-site variations in disease incidence and yield response to infection. Disease incidence is determined mainly by:

- the proportion of susceptible crops in rotations;
- soil type; and
- soil moisture content.

However, it can be profoundly affected by other factors, such as:

- sowing date;
- cultivation practice;
- soil nutrient composition; and
- fertilizer application.

Yield losses in the Pacific north-west of America are estimated to be 10–50%. In moderate/high-risk areas of Western Australia, take-all accounts for losses of up to 40%. In England and Wales, recent estimates are for losses between 1 and 4% in second and subsequent wheat crops, although some workers regard this as conservative.

Yield losses cannot be determined accurately but, in the UK, the contribution of take-all to total loss was approximately one-sixth as great as all the other leaf and stem base diseases combined, or up to US$85 million annually (Hornby and Bateman, 1991; Yarham, 1995).

Take-all can be controlled to a degree by altering farming practice:

- lowering the inoculum levels by growing non-susceptible crops as a rotational break;
- the use of more tolerant cultivars of wheat;
- delayed sowing; and
- carefully planned fertilizer use.

More direct control measures are not practical, but the potential for fungicide use or biological control has been explored. The take-all decline syndrome is a demonstration of biological control, albeit a natural corollary to long-term wheat culture. The accumulation of antagonists by growing a suitable preceding crop, for example grass, can

Continued

Case Study 1. Continued.

delay the onset of the disease. Otherwise, bacterial antagonists such as *Bacillus* or pseudomonads can be applied to the rhizosphere directly or through seed treatment. Most claims for the success of biological control come from the USA and Australia, and several patents have been filed. However, improvements in yield have been demonstrated in only 60% of the treated crops and, at that level of success, the practical use of biological agents to control take-all is too unreliable to be commercial. Synthetic fungicides, regarded as a less environmentally friendly alternative to biological control, have had little more success in controlling the incidence of take-all. It is clear that activity against *G. graminis* var. *tritici* is not uncommon or new. The efficacy of the C14-demethylation inhibitors triadimenol and flutriafol is well documented but earlier examples, such as the pyrimidine nuarimol, demonstrate that compounds with very high activity have been available for many years. However, the failure of these materials to be developed as products for take-all control reflects the distinction between activity and performance and the difficulty in transferring *in vitro* or glasshouse *in vivo* efficacy to utility in the field. In all cases, the underlying problem is one of delivery of the active fungicide to the site of infection. Several strategies have been considered.

Soil fumigation

Soil fumigants are difficult to use on a large scale and are expensive. They have the added disadvantage of being non-selective, raising the potential problem of a subsequent rapid build-up of take-all due to the depletion of natural antagonists.

Soil fungicides

The immediate problem of using soil-incorporated fungicides to control take-all is the dilution effect of the soil on the applied product. Compounds would have to be delivered in large quantity, probably in a granular formulation, or be extremely active against *G. graminis* var. *tritici*. Assuming a recommended rate of fungicide application of 100 g of active ingredient (a.i.) per hectare and complete mixing in the soil, the fungicide concentration would decline to negligible levels by 30 cm.

Most fungicides demonstrate their highest activity *in vitro*, but few are active against their target fungi at levels below 1 ppm. On that basis, the dilution effect of the soil would probably preclude the use of soil-incorporated products. In practice the situation is much worse because of the difficulty in achieving complete ground cover and presentation of the product in the infection court.

Beyond that, the physicochemical characteristics necessary for a fungicide to act via the soil are well understood. The demand is for highly active compounds with moderately low lipophilicity, to avoid adsorption to soil particles and allow redistribution in the soil water, combined with the persistence characteristics that would establish long-term control. For highly mobile compounds, slow-release formulations would provide a means to deliver long-term control. However, the technical targets for persistence and movement are in direct conflict with the registration requirements that govern the use of agrochemicals in soils, effectively removing the development of soil fungicides as an option for take-all control.

Seed treatments

Seed treatments provide the most reliable control; in the USA and Europe the use of triazoles (triadimenol, flutriafol) is known to deliver some protection to roots until early spring. In this case, slow-release formulations would help to provide long-term control.

Continued

Case Study 1. Continued.

Foliar fungicides

Although there is an increasing understanding of the physicochemical parameters that govern fungicide movement in the phloem, there are few fungicide products that can be demonstrated to act in that manner, none of which is active against take-all of wheat. It is likely that until clear technical advances in fungicide delivery and performance are made, the control of take-all will remain a debatable commercial target. However, future developments in the control of this and other soil-borne diseases may focus more on the use of crop biotechnology rather than on the discovery of conventional fungicides.

Table 4.4. Characteristics of different types of fungicide screen.

Type of screen	Amount of test chemical needed	Indicative number of chemicals that can be tested per annum
High-throughput tests	Less than a microgram	100,000
In vitro tests	A few micrograms	10,000
In planta tests		
Detached leaf tests	A few milligrams	1,000
Glasshouse, whole plant sprays	A few grams	100
Outdoor plot trials	A few grams	100

In planta *screens*

In planta screens are the most time-consuming and expensive but also the most predictive of final success. An *in planta* test is one where the pathogen undergoes its full life cycle on plant tissue. The plant tissue may be a seedling or explant grown in soil for several weeks in a glasshouse or growth chamber. At an appropriate stage, the pathogen is inoculated and the plant is incubated so as to promote disease. The test chemicals may be applied before the pathogen to screen for preventive activity or after to screen for curative activity. The amount of disease is scored some days or weeks later and compared with that produced by the pathogen alone. This is a demanding process requiring highly skilled staff and extensive and expensive facilities. It explains the many hectares of glasshouses found around the grounds of all fungicide companies. Such *in planta* tests also require relatively large amounts of the test compounds – at least a few milligrams and possibly several grams (Fig. 4.1).

For all these reasons, primary compound screening tests typically use some sort of detached leaf assay. Leaf discs or short sections as small as 5 mm are cut out, often with specialized machinery but also by hand, and then placed on a special agar or liquid medium. The medium contains a cocktail of compounds proven to maintain the healthy life of the leaf piece, long enough for the pathogen to complete its life cycle. The pathogen is then dusted or pipetted on to the leaf pieces. The test compounds may be sprayed on the leaf pieces or may be incorporated in the bathing medium. In the latter case, the companies would need to be aware of the potential for the compound to translocate into the leaf piece and thus come into contact with the pathogen. Finally, after an appropriate period the degree of infection is assessed either by eye or by some sort of computerized image analysis. The infection level is normally converted to a per cent disease control parameter.

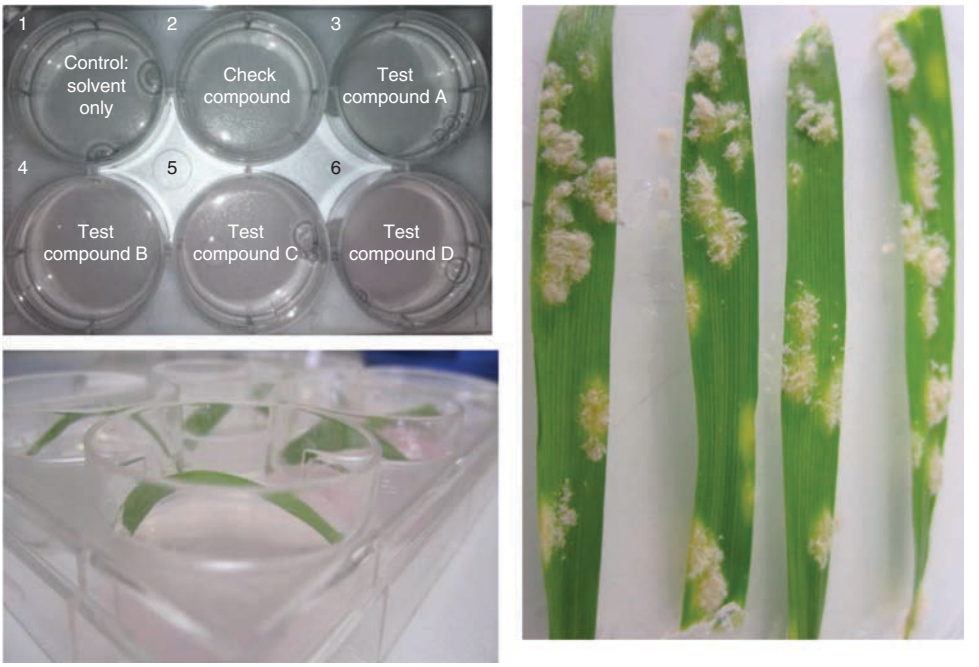


Fig. 4.1. *In planta* test of compounds against ERYSGH. Leaves of a susceptible barley cultivar are excised and placed on an agar suspension containing supplements that inhibit senescence. Each well contains a different compound, but with the same solvent: well 1 has no compound and is a positive control; well 2 has a standard check compound; wells 3–6 have four test compounds (top left). Spores are dropped on to the leaves and the plates are sealed and incubated in moderate light (bottom left). After 1 week the infections are scored (right).

In planta screens have the advantage that they tell the researcher whether the compound is toxic to plants, exhibiting so-called phytotoxicity. But even if a compound is safe to plants and inhibits the disease *in planta*, it may not be suitable as a fungicide. Many will prove to be toxic to non-target organisms or may have insufficient stability or rainfastness to work in the field.

In vivo *screens*

In the fungicide industry, *in vivo* refers to the growth of a fungus away from a plant. It is a conceptually simple matter to grow a fungus in an agar plate or microtitre plate-well and to add aliquots of test compounds. *In vivo* tests use much less compound than *in planta* tests.

If the fungus is inoculated into the centre of an agar plate containing the compound, the reduction in radial growth rates caused by the compound can be easily measured (Fig. 4.2). Multiple compounds can be added to different sectors of a plate to increase the number of tests. Agar plates are large and unwieldy, so companies prefer to use microtitre plates that have 96 wells in an 8 × 12 array. The growth of the fungus can be measured by assaying light scattering in the well using automated equipment. An 8 × 12 plate can be used to test 12 compounds at eight different concentrations, or 24 compounds at four different concentrations.

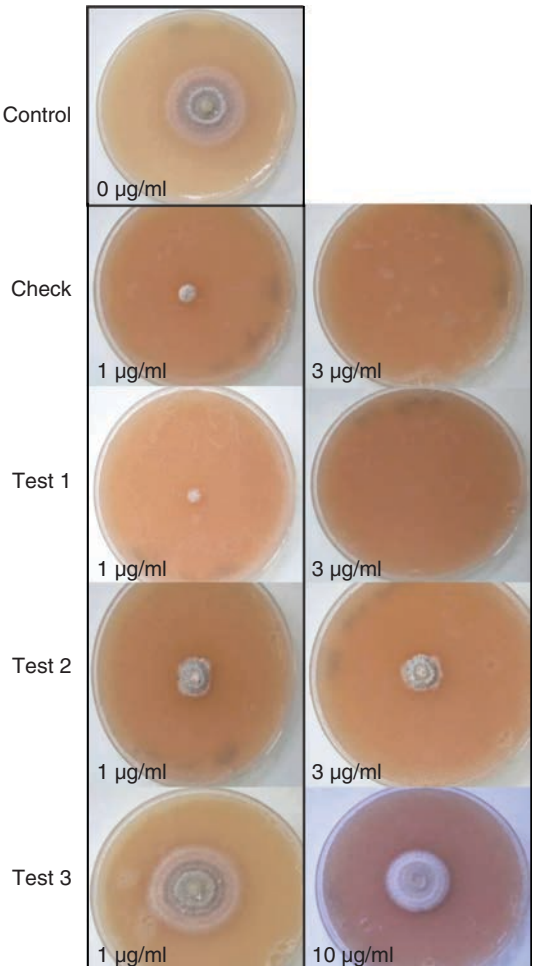


Fig. 4.2. Radial growth assays of LEPTNO. Each plate contains a nutrient agar medium amended at two concentrations with solvent (control), check (current fungicide) or test compounds. The plates are inoculated with spores or a mycelial plug in the centre and allowed to grow for 2–7 days. The average radius of growth is measured.

In vivo tests tend to generate many false positives and even a few false negative results, and hence are treated with some suspicion. The false positive results occur when a compound that inhibits growth in the plate assay fails to inhibit growth in the plant. There are many reasons why this might be the case. The main ones are that the compound may not be translocated in the plant or may be metabolized into an inactive form by the plant. Hence all *in vivo* tests must be followed up with *in planta* studies.

Conversely, there are a few cases where an *in vivo* test would give false negative results. Examples would be compounds such as ASM and probenazole that work by activating plant defence. Discovery of such compounds requires a different and specific strategy.

High-throughput tests

The term 'high-throughput' reflects the frustration of the fungicide industry with the slow pace of fungicide discovery even when using *in vivo* tests. New methods of generating test compounds, such as combinatorial chemistry, led to a backlog of untested

compounds. There was perceived to be a need to develop faster tests. So high-throughput tests encompass a range of tests with the common factor of being faster than an *in vivo* test. The goal was to screen very large numbers of compounds with an assay designed to reflect some essential function of the pathogen. Examples would be an enzyme assay or a bacterial strain with a reporter gene. However, the advantages of high-throughput were soon seen to be outweighed by the disadvantages; very few compounds that were active in the high-throughput test proved to be useful as leads. A compounding paradox was that it was too easy to find compounds that were active in the high-throughput test. Further tests using *in vivo* and *in planta* assays were consuming inordinate amounts of time in company laboratories and leading to few useful leads. Hence this approach has largely been abandoned.

Mode-of-action screens

Assays with the features of high-throughput screens are used to determine the MOA. Fungicide companies are particularly keen to discover compounds with new MOAs as they are very likely to be novel and therefore hold out the promise that the company could develop a dominant position over a whole class of compounds. Furthermore, as there are so many problems with fungicide resistance affecting all major groups of fungicide, a new MOA is likely to have a large market both replacing and protecting fungicides affected by resistance.

Hence companies have developed high-throughput assays that report whether a compound has each of the known MOAs. If an active compound scores negative in each of the tests, the hunt for the new MOA is initiated. The exact methods behind these assays are closely guarded secrets.

Primary target organisms

Fungicide companies have a set of primary target pathogens against which new compounds are screened. The names of the primary targets are commercial secrets but one would guess the list as shown in Table 4.5.

Companies not only focus on the pathogens with the biggest potential market sizes but will also pay attention to taxonomy. A lead compound that had activity against more than one of the major taxonomic groups would attract extra attention. QoI fungicides are exceptional and owe their large market size to having activity against basidiomycete, ascomycete and oomycete pathogens.

Another factor taken into account when choosing primary target organisms is the ease with which they can be tested in a laboratory setting. Pathogens that can be grown in defined artificial media are much more economical to test than ones that must be tested on living plant tissue. Fast-growing fungi such as SEPTRI and BOTCIN are favoured for that reason over VENTIN and MYCFIJ. It is, however, an unfortunate fact that many of the priority targets are obligate pathogens; rusts and mildew, both powdery and downy. Furthermore, history shows that obligate pathogens tend to be sensitive to a greater range of fungicides than the facultative pathogens. Hence companies that screened only facultative pathogens would risk missing out on a lucrative mildewcide. An example would be quinoxyfen.

Table 4.5. Characteristics of major fungicide test organisms.

Code/pathogen name	Disease	Host	Taxonomy	Facultative/ obligate
SEPTRI	Septoria tritici blotch	Wheat	Ascomycete	Facultative
PYRIOR	Blast	Rice	Ascomycete	Facultative
UNCNEC	Powdery mildew	Grapevine	Ascomycete	Obligate
ERYSGT/H	Powdery mildew	Wheat and barley	Ascomycete	Obligate
PUCCRT	Brown rust	Wheat	Basidiomyecte	Obligate
PHYTIN	Late blight	Potato/tomato	Oomycete	Facultative
BOTCIN	Botrytis grey mould	Many but especially grape	Ascomycete	Facultative
PLASVIT	Downy mildew	Vine	Oomycete	Obligate
	Phakospora pachyrhiza	Soybean	Basidiomycete	Obligate
VENTIN	Scab	Apple	Ascomycete	Facultative (but very slow growing)
MYCFIJ	Black sigatoka	Banana	Ascomycete	Facultative (also slow growing)

In addition, some non-pathogenic fungi are widely used in fungicide discovery laboratories. These include the yeast *Saccharomyces cerevisiae* and the filamentous species *Aspergillus nidulans* or *Aspergillus niger*. The use of non-pathogenic species is an obvious consequence of the fact that without very few exceptions, all fungicides that have reached commercial release inhibit the growth of at least some fungi in culture. Furthermore, many fungicides are more potent on plates than on plants and hence are more sensitive for lead detection. The non-pathogenic fungi have been used in fundamental science as model systems. Such model systems were chosen because of their ease of culture and fast life cycles. Generations of fundamental scientists have generated extensive genetic resources such as complete mutant libraries and functional genetic technologies. The first fungal genome sequences to be made publically available were of these model system fungi (Cools and Hammond-Kosack, 2013). Yeast can be regarded as a good model for all fungi but it lacks a filamentous phase and so would fail to detect inhibitors of chitin biosynthesis. The ability to manipulate some model system fungi (and indeed bacteria) means that a specific screen can be designed using engineered yeasts strains.

Sources of fungicide leads

Screening identifies potential products but, more crucially, the lead compounds from which products are developed. The term ‘lead’ is used widely in the industry. It refers to the first compound that shows activity against a target fungus. The chemical structure is then determined and many variants are synthesized. These variants are also tested in the assays until the structural features associated with activity are identified.

Nearly all current products have arisen from the development of leads. The performance of the end product is likely to be considerably different from that of the early lead compound. What constitutes a lead is subject to continual debate, not least because the commitment of resources to lead development can be critical, and one of two philosophies may be applied.

One approach argues that screens should be extensive and that companies should develop any compound with activity, regardless of its initial commercial potential. Thus a chemical with excellent *in vitro* activity against *A. niger* may be investigated further in the hope that the spectrum can be modified to include more important crop pathogens or that it possesses a novel and useful MOA. To some extent, all companies employ this approach, as all new compounds are submitted to be screened as herbicides, insecticides and fungicides and there are many examples of the discovery of activity in one discipline leading to a product in another.

The first tests within the screen proper may be designed to identify or confirm suspected general activity, or may be focused on those attributes that market surveys define as valuable. For example, the inclusion of fungi drawn from as wide a taxonomic range as possible is the most effective method of exploring efficacy. Value is placed on spectrum rather than on commercial targets, and important pathogens such as obligates may be excluded in favour of more easily managed organisms. *In vitro* techniques enable many fungi to be employed, and because the complications inherent in using infected plants are avoided, it is possible to explore the direct effects of compounds upon fungal development. Further, the use of broad-spectrum tests ensures that the company establishes an excellent historic database that can be interrogated to find suitable leads should new commercial targets be found.

The second philosophy demands that only those pathogens identified as commercially useful are used in screening. While this approach has the clear advantage over a non-targeted system that active leads are more likely to produce valuable products, the comparatively narrow spectrum reduces its utility as a historic database. Also if targets change, the screening test must be modified, resulting in the discontinuity of records. Targeted tests are generally carried out *in vivo*, which changes the balance of resources required from the laboratory to glasshouse and controlled-environment facilities. It also means that the fundamental activity of the candidate fungicides may be masked by physicochemical interactions with the environment surrounding the host plant and residing within the host plant.

Fungicide leads arise in five ways:

1. Random chance.
2. Combinatorial chemistry.
3. Analogue synthesis.
4. Biorational design.
5. Chemorational design.

Random screening

Traditionally, fungicide discovery uses serendipity which, at the most fundamental, relies on the laws of chance for success. If enough compounds are supplied and tested, provided a screen is constructed to meet the required commercial targets, a product is guaranteed.

In this system, compounds submitted for screening are chosen in the absence of any prior knowledge of structure–activity relationships or novelty of chemistry. The chemistry of many compounds may be unknown or not divulged, being obtained from third parties under a confidentiality agreement. They may also be purchased or synthesized in-house, either as end products of speculative programmes or as intermediates.

An important source of test compounds is natural products. Academic laboratories and specialized lead discovery companies focus on the identification of various types of organism from which are extracted the products of their secondary metabolisms. Such metabolites will vary depending on the culture condition. A recent success for the natural product route is the strobilurins (see Chapter 5). The original set of compounds was extracted from the fungus *Strobilurus tenacellus* (Anke *et al.*, 1984; Sauter *et al.*, 1999). Over a 20-year period the structure of the compounds was determined and their activity tested. Despite being very active and with a very good spectrum, they proved too unstable for use in the field and were only released after extensive modifications.

Although the chance of finding a compound is vanishingly small, random screening, used as a lead-generating activity rather than a process to identify products, has proven to be the most successful method used in the search for novel pesticides.

Combinatorial chemistry

The improbable partnership of the traditional random approach to pesticide discovery and the novel techniques of combinatorial chemistry was for a period an attractive source of potential leads. The method is based on the generation of a vast but unspecified chemical library, which is then screened. Combinatorial chemistry has found most use in pharmaceutical drug design and its application in the production of peptide libraries is well documented (Nielsen, 1994). The interest within fungicide discovery lies in the production of arrays of easily synthesized, cheap and relatively low-molecular-weight compounds. Compounds are synthesized on the surface of inert materials or bacteriophages. Of course, there is no guarantee that the compounds produced by this method will be novel; nor does the researcher know the relative amounts of each compound residing on the surface of the support medium. The skill is to be able to combine molecules to establish large libraries which can then be screened and, by a series of elimination studies, the active moieties can be defined and re-synthesized in quantity. The advantage of the use of combinatorial chemistry is that huge numbers of chemicals can be screened in specially designed micro-tests at very low cost. Costs rise dramatically only when a particular library is discovered to possess activity.

Analogue synthesis

Analogue synthesis is the practice of synthesizing compounds that retain the important structural core (the pharmacophore) but have different substitutions. Often the identity of the pharmacophore only becomes obvious once a number of analogues have been synthesized and tested. Structural features present in active compounds but absent in inactive compounds are likely to be the pharmacophore.

The goal of analogue synthesis is to optimize the activity of compounds defined as leads in the process of screening and is the most successful form of pesticide discovery. It builds on the random screening described above. The leads may be

company-owned (in-house) or may be based upon known chemistry ('me-too' synthesis). An example of the inventive scope of me-too fungicide discovery is the development by several companies of the triazole series of fungicides into a family of distinct products (Table 4.6).

All triazoles are designed about a common chemical structure, the 1,2,4-triazole ring, but not all 1,2,4-triazoles are fungicides: paclobutrazole and uniconazole are plant growth regulators and fluchlorazole is a herbicide safener (see Box 4.1 for an explanation of chemical nomenclature rules).

In contrast, the relationship of some chemistry to biological activity (structure–activity relationship) is extremely narrow. Tricyclazole, the active component of Beam, a DowElanco product for use against PYRIOR, is the only member of that chemical series found to have significant activity against the target pathogen. In that case, the discovery was made purely by chance.

Analogue synthesis would first be carried out by the company that discovered the original lead and would have preceded the first commercialization. Once announced and patented, other companies have the necessary starting information to begin an analogue synthesis programme of their own. As the lead and the pharmacophore would normally be known, this is likely to lead to the synthesis of many active compounds, compared with random synthesis. On the other hand, the potential market will be less because of the market and patent position established by the first company.

Table 4.6. The triazole family of fungicides.

Compound	Date announced	Company
Triadimefon	1973	Bayer AG
Triadimenol	1978	Bayer AG
Propiconazole	1979	Janssen Pharmaceutica
Bitertanol	1979	Bayer AG
Diclobutrazol	1979	Zeneca Agrochemicals
Flutriafol	1981	Nihon Nohyaku Co. Ltd
Penconazole	1983	Ciba
Azaconazole	1983	Janssen Pharmaceutica
Diniconazole	1983	Sumitomo Chemical Co.
Flusilazole	1984	Du Pont
Imibenconazole	1984	Hokko Chemical Industry Co. Ltd
Tebuconazole	1986	Bayer AG
Cyproconazole	1986	Sandoz AG
Myclobutanil	1986	Rohm and Haas Co.
Tetraconazole	1988	Agrimont SpA
Difenconazole	1988	Ciba
Furconazole	1988	Rhône Poulenc
Epoxiconazole	1990	BASF AG
Hexaconazole	1990	Zeneca Agrochemicals
SSF-109	1990	Shionogi and Co. Ltd
Bromuconazole	1990	Rhône Poulenc
Fluquinconazole	1992	Schering AG
Metconazole	1992	Shell
Triticonazole	1992	BASF AG
Prothioconazole	2002	Bayer AG

Box 4.1. Nomenclature and classification of fungicides.

Fungicides have a complex vocabulary which acts as a significant barrier to understanding. There are multiple nomenclature systems. These include the FRAC (Fungicide Resistance Action Committee) class, the product name(s), the active ingredient name, the formal IUPAC (International Union for Pure and Applied Chemistry) name for the active ingredient, the chemical class (often several levels) and the MOA class. The different names are due in part to the different disciplines of people who work in the industry – chemists prefer chemical names, biologists prefer MOA names, farmers and traders prefer product names. To illustrate one example of the confusing possibilities, consider the case of dimethomorph and fenpropimorph. Both are morpholines but the former is an inhibitor of cellulose synthase and acts against oomycetes whereas the latter is an inhibitor of ergosterol biosynthesis and acts against foliar *Ascomycota*.

Heterocyclic compounds

Most fungicides are heterocyclic organic compounds. That means they are composed of one (and normally several) cyclic moieties that contain not only carbon but also other elements such as phosphorus, nitrogen and sulfur. They may also be saturated (without double bonds) or unsaturated.

The rules for naming heterocyclic compounds are laid down by IUPAC and follow a series of logical steps. The first level is to count the number of atoms in the ring, the second is whether the ring is saturated and the third level follows the identity of the hetero atoms. However, not all of the rules are followed and exceptions are shown below in italics. Furthermore, some linking letters are omitted to improve pronunciation.

Hetero atom	Prefix
O	Oxa-
N	Aza-
S	Thia-
P	Phospha-

	Fully unsaturated compounds		Fully saturated compounds	
Ring size	With N	Without N	With N	Without N
3	-irine	-irene	-iridine	-irane
4	-ete	-ete	-etidine	-etane
5	-ole	-ole	-otodine	-olane
6	-ine	-in		-ane
7	-epine	-epin		-epane
8	-ocine		-ocin	

Continued

Box 4.1. Continued.

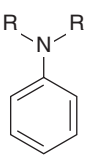
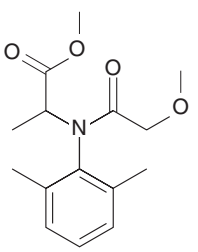
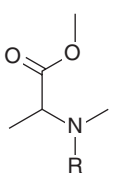
Ring size	Hetero atom	Saturated	FRAC class(es)	Unsaturated	FRAC class(es)
3	N	Aziridine		Azirine	
	N + N			Diazirine	
	N + O	Oxaziridine			
	O + O	Dioxirane			
4	N	Azetidine		Azete	
	O	Oxetane		Oxete	
	N + N	Diazetidine			
	O + O	Dioxetane		Dioxete	
	S + S	Dithietane		Dithiete	
5	N	*Pyrrolidine*		*Pyrrole*	
	O	*Tetrahydrofuran*		*Furan*	C2
	N + N	*Imidazolidine* or *pyrazolidine*		*Imidazole*	G1
	N + N + N			Triazole	G1
6	N	*Piperidine*	G2	Pyridine	G1
	O			Pyran	
	N + N	*Piperazine*	G1	Diazines; *pyrimidine*	A2; G1
	N + O	*Morpholine*	G2	Oxazines	C3
	N + S			Oxathiin	C2
	N + N + N			Triazine	M8

FRAC, Fungicide Resistance Action Committee.

Fused and multiple rings

Many fungicides have fused or multiple rings and an unambiguous systematic naming system would have to be very cumbersome. Instead chemists have tended to focus on natural products and use trivial names.

Irregular pharmacophore classes of the major fungicides are tabulated below.

FRAC class(es)	Chemical group	Example fungicide
A1; RNA polymerase 1	Phenylamide 	Metalaxyl 
	Acylalanine 	

Continued

Box 4.1. Continued.

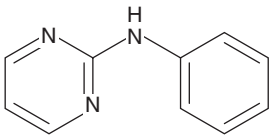
A2; adenosine deaminase	Hydroxy- (2-amino-) pyrimidine	Bupirimate
B1; β-tubulin assembly in mitosis	Benzimidazole	Carbendazim
C2; inhibition of complex II; succinate dehydrogenase	Carboxamide (note: carboxamides are also in C1, C7, I2, P3)	Boscalid
C3; inhibition of complex III; quinone outside inhibitors (QoI)	Methoxyacrylate, etc.	Azoxystrobin

Continued

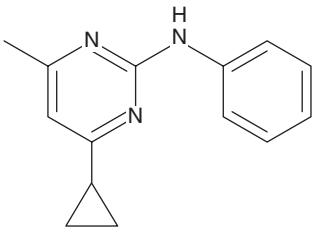
Box 4.1. Continued.

D1; methionine biosynthesis

Anilinopyrimidines

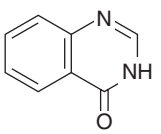


Cyprodinil

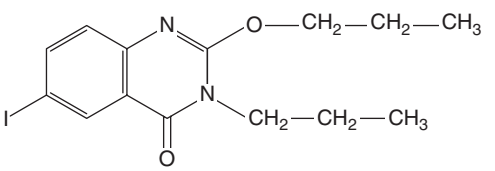


E1; signal transduction (mechanism unknown)

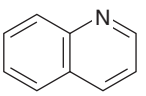
Quinazolinone



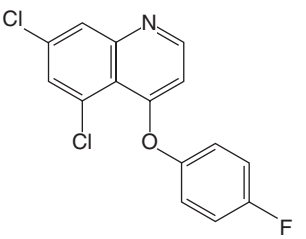
Proquinazid



Quinoline

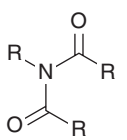


Quinoxyfen

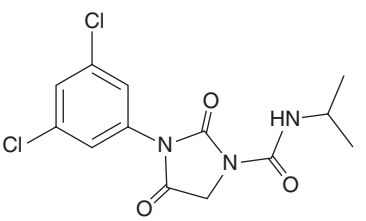


E3; osmotic signal transduction

Dicarboximides

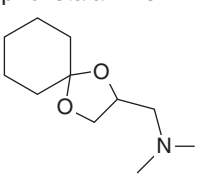


Iprodione

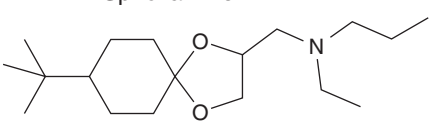


G2; Δ^{14}-reductase and $\Delta^{8}\rightarrow\Delta^{7}$-isomerase in sterol biosynthesis

Spiroketalamine



Spiroxamine

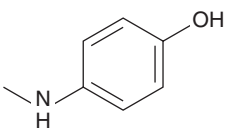


Continued

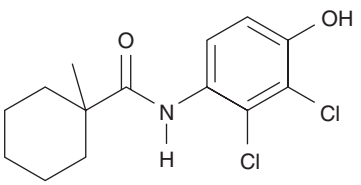
Box 4.1. Continued.

G3; 3-keto-reductase in C4-demethylation (*erg27*)

Hydroxyanilide

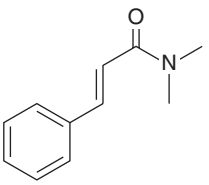


Fenhexamid

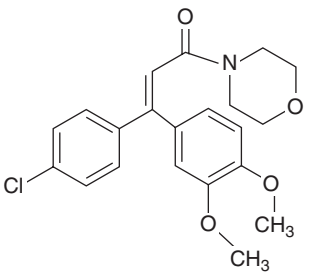


H5; cellulose synthase

Cinnamic acid amides

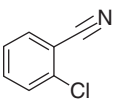


Dimethomorph

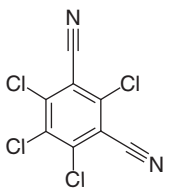


M5; multi-site chloronitriles

Chloronitrile

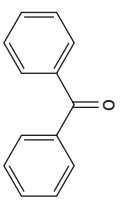


Chlorothalonil

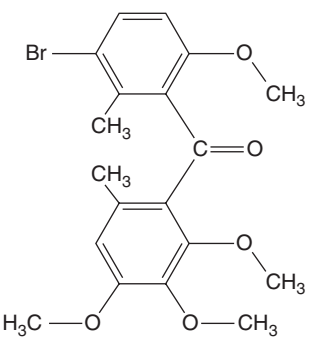


U8; unknowns

Benzophenone



Metrafenone



Biorational design

All the fungicides available today were discovered by empirical and/or analogue synthesis and there is no doubt that these approaches will continue to be successful. However, the success rate is decreasing. Novel compounds are becoming more difficult to discover by conventional means because of increasingly higher standards of performance, toxicology and environmental safety, and this has encouraged the use of more rational approaches to pesticide discovery. The biorational approach to fungicide discovery demands a complete knowledge of specific metabolic processes, including their role in both the pathogen and host, and an ability to use those data in the definition of new target sites. In some cases, computer graphics can be used to construct three-dimensional (3D) models of the active sites of target enzymes. The optimum structural requirements of candidate fungicides can be predicted and synthesis resources directed effectively towards the production of potent inhibitors.

Materials synthesized as part of a rational approach to discovery, and shown to be active against target enzymes in cell-free assays, may lack *in vitro* or, more commonly, *in vivo* activity. Deficiencies in spectrum – poor transport characteristics and problems of metabolism – have limited the development of rationally designed compounds. The complex barriers to acceptable performance exceed simple biochemical activity and, to date, have prevented the advances made in fundamental molecular design from reaching a commercial end point.

The biorational approach is becoming increasingly significant, optimizing lead chemistry with known MOAs. Its first application was with C14-demethylation inhibitors. Members of this class of fungicides are specific inhibitors of the enzyme P450 14α-demethylase. The 3D structure of the enzyme has been partially solved. Using the known physical and chemical properties of existing inhibitors, the structural requirements for their configuration at the active site of the enzyme has been modelled (Fig. 4.3). This led to the directed synthesis of flutriafol and cyproconazole and the determination of the different binding site of prothioconazole (Parker *et al.*, 2011; Kelly and Kelly, 2013).

Many attempts to design novel chemistry to fit known sites of action have failed. An illustration is given in Case Study 2.

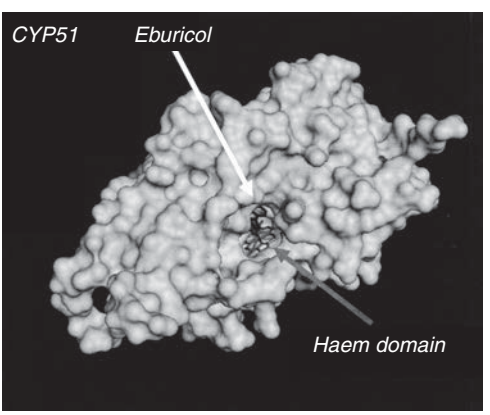


Fig. 4.3. Three-dimensional structure of fungal CYP51 showing the haem active group and the binding site of the substrate eburicol. Such structure allows the *in silico* docking of compounds to predict inhibitory activity prior to the decision whether to synthesize.

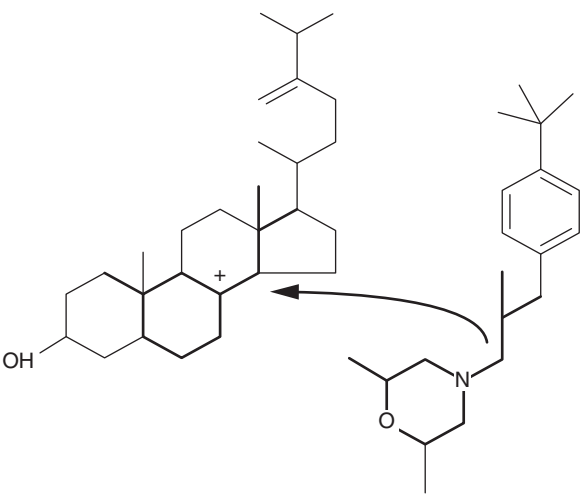
Case Study 2. Carbocation mimicry in amidiniums and guanidiniums.

Morpholine and piperidine fungicides inhibit sterol biosynthesis at the Δ^{14}-reductase and $\Delta^{8}\rightarrow\Delta^{7}$-isomerase enzyme steps, through their action as transition-state analogues of the natural sterol substrate. Using structural mechanisms that stabilize the transition state, they bind strongly to the enzyme and reduce the activation energy of the enzymic reaction.

In sterol biosynthesis, reduction of the Δ^{14} double bond and isomerization of the Δ^{8} double bond are probably mediated by a chemical intermediate known as a carbocation. The protonation of fenpropimorph and piperidine, which occurs at physiological pH, results in the formation of similar intermediates and may explain their activity against powdery mildews, especially ERYSGH. This mechanism was examined by Liebeschuetz and co-workers (Arnold *et al.*, 1995) at DowElanco as a likely target for a directed synthesis programme and work began on the rational design of carbocation mimics. A binding model for fenpropimorph was adopted as a guide for synthesis (Fig. 4.4).

The chemical starting points for the synthesis programme included guanidinium and amidinium structures (Fig. 4.5). The lead compounds conformed with the theoretical fit to the $\Delta^{8}\rightarrow\Delta^{7}$-isomerase and Δ^{14}-reductase carbocationic intermediates (Fig. 4.6). Both compounds were confirmed as active in screening tests against ERYSGH and PUCCRT, at levels equivalent to fenpropimorph. In cell-free enzyme assays derived from *Ustilago maydis*, the lead compounds had activity at the micromolar level which tended to favour interest in the amidinium salt (IC_{50} guanidinium = 30 μM; IC_{50} amidinium = 20 μM). However, in concurrent tests fenpropimorph was superior with an IC_{50} = 0.35 μM.

Subsequent modifications of the lead compounds concentrated in three areas (Fig. 4.7), producing a guanidinium series of 11 compounds and an amidinium series of nine compounds. There was a good correlation between *in vivo* and cell-free assay results for all compounds, but in whole-cell assays the initial activity of the lead compounds and their analogues was drastically reduced, in contrast to the maintenance of high



Fenpropimorph

Fig. 4.4. Binding of fenpropimorph to the isomerase carbocationic intermediate.

Continued

Case Study 2. Continued.

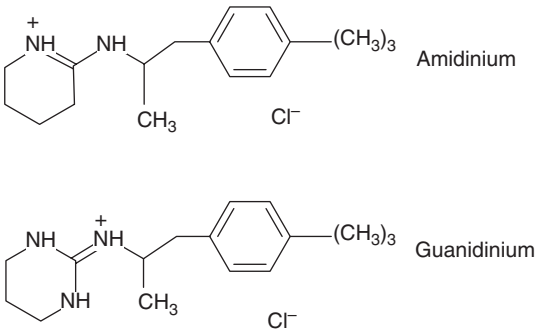


Fig. 4.5. Lead compounds used in the synthesis programme of piperidine and morpholine fungicides.

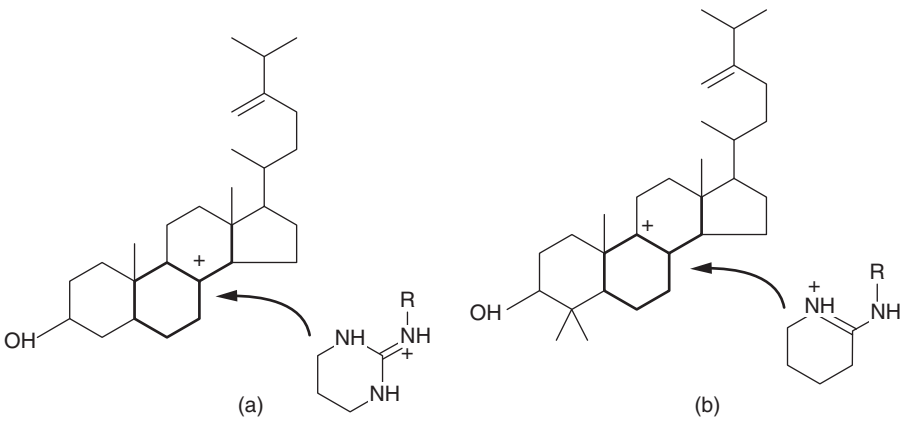


Fig. 4.6. Proposed fit of the cyclic guanidinium (a) and amidinium (b) over the $\Delta^8 \rightarrow \Delta^7$-isomerase and Δ^{14}-reductase carbocationic intermediates.

levels of inhibition by fenpropimorph. Subsequent *in vivo* tests carried out using commercially acceptable application techniques and a spray volume of 300 l/ha demonstrated that neither the guanidinium nor the amidinium series of compounds controlled powdery mildew as effectively as the standard, fenpropimorph. The disappointing levels of disease control were attributed to a lack of redistribution within the crop and a lack of uptake into the target pathogen.

The ability to redistribute is a crucial factor in the success of cereal fungicides. Application volumes high enough to wet leaves or to cover them extensively are used routinely in broad-leaf crops such as grapevine and top fruit in which a component of disease control is the extensive use of immobile surface protectants. These require good coverage in order to operate effectively and volumes as high as 1000 l/ha are not uncommon. In cereals, the major part of disease management is through the use of systemics or compounds such as fenpropimorph that operate in part through the vapour phase. These are less reliant on application volume and treatments are made in spray-tank solution

Continued

Case Study 2. Continued.

(250–300 l/ha). Acceptable efficacy levels of the exploratory guanidiniums and amidiniums appeared to be restricted to high-volume systems, suggesting a mobility problem.

It was also concluded that in whole-cell and *in vivo* tests the barriers to penetration of the polar and highly basic test compounds (pK_a = 10–12) prevented the expression of their intrinsic activity against the target enzymes. Fenpropimorph, however, has a much lower acid strength (pK_a = 7) and *in vivo* is able to cross membranes in an un-ionized form.

The research demonstrates the importance of a holistic approach to discovery which relates biochemical activity to practical performance in a multi-disciplinary fashion. It also clearly shows the advantages of an effective and directed approach to the synthesis of potent inhibitors.

Chemorational design

A further input to the development of a lead is to modify it in ways that are designed to optimize the physicochemical properties of the compound (Fig. 4.7). This process is part science and part art. Chemists use a multitude of inputs to decide how to modify compounds to improve their activity, increase their stability, decrease their toxicity and decrease the costs of synthesis. Also, they must bear in mind the patent situation and seek to make compounds that bypass competitors' patents.

More than 200 compounds have been commercialized as fungicides and many thousands have failed to progress, so there is a good deal of experience of the types of physicochemical properties that are compatible with good fungicidal field performance. Chemists focus on the melting and boiling points and the vapour pressure as these reflect the degree to which the compound will vaporize after application on

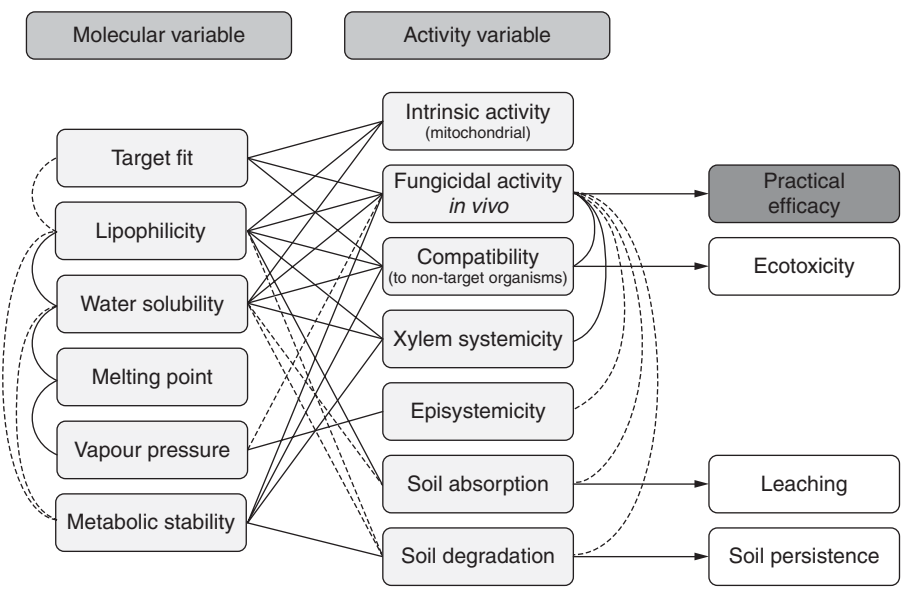


Fig. 4.7. Structure–activity relationships: the complex network between variables. (From Krämer *et al.*, 2012.)

the leaf. They also focus on the log *P*, which is the logarithm of the partition coefficient between n-octanol and water. This parameter measures hydrophobicity/hydrophilicity and predicts whether the compound will be mobile in the leaf and how it will be formulated. They also focus on molecular weight as large compounds will be more unstable. Most fungicides have a molecular mass between 100 and 300 Da.

Screening methodology

Target-orientated screening concentrates resources on those opportunities that are commercially viable and minimizes the risk of developing compounds that will never provide sufficient return to justify the investment in discovery. The testing cascade which forms the screen includes the following activity and performance determinants:

- *Activity* – target pathogens and their hosts.
- *Performance* – persistence, application timing and method, mobility and resistance management.

Company strategy is reflected in the composition of the discovery screen. If the biological targets carry equal commercial merit, the screen may be broad-based in design and include all pathogens and their hosts at the first or primary test level. Subsequent testing then concentrates on the demonstrated efficacy spectrum. Because of the high rates normally employed at the primary level, many submitted compounds demonstrate some fungicidal activity and are elevated to more stringent, secondary examination. This is usually a rate–response study to determine the rate at which the test compound loses efficacy, compared with a suitable standard fungicide. Further tests in the final stages of laboratory and glasshouse screening begin to define the influence of performance attributes on biological efficacy. A screen of this type uses a process of elimination to discover candidate fungicides and can be likened to a series of sieves, with the coarsest sieve being the first test.

However, where resources are limited, the system may be rationalized to include only those targets that are deemed essential, either as commercial targets or as models on which to base further work. Subsequent testing is always directed towards the evaluation of commercially important attributes, defined by marketing and by the financial return required by the individual company. A screen of this type has a comparatively narrow primary level base but then expands, based upon initial activity, to include taxonomically related targets and performance attributes before focusing on the most active and/or commercially acceptable candidate. Given that user, consumer and environmental safety are absolute requirements and subjects for later study, screening concentrates on the definition of those characteristics that will make an effective, reliable and flexible product. These are usually arranged in the screen in order of decreasing priority and increasing complexity.

Different target crop/pathogen combinations require particular tests to be carried out to assess the potential value of a candidate fungicide. However, the first steps within the screening process test for activity that can be regarded as essential to further development (see Case Study 3).

Some measure of activity spectrum is implied from the tests. Here the priority is to evaluate the strength of efficacy against target pathogens, compared with the

Case Study 3. A rationalized fungicide screen.

This case study describes an extreme but not unrealistic scenario which is resource-limited and driven entirely by primary targets that individually represent markets of sufficient size to support a product. Thus ERYSGT, PYRIOR, PLASVIT and SEPTRI alone merit inclusion. Activity against any one triggers a cascade of tests designed to establish a possible commercial fit with a marketing objective (Table 4.7).

Specific activity against any single powdery mildew is uncommon and so ERYSGT serves as a general model for activity against powdery mildew fungi. It also activates tests against secondary pathogens which together with ERYSGT form part of a commercial target. Thus, tests against PUCCRT and *Pseudocercosporella herpotrichoides* follow successful primary level activity against ERYSGT. Similarly, VENTIN is included at the secondary level as in top fruit the target market is for a combined apple scab and powdery mildew fungicide.

In grapevine, PLASVIT represents both a commercial target and a model for oomycete fungicides. Secondary tests with compounds showing primary level activity against PLASVIT trigger tests against PHYTIN, a pathogen of secondary commercial importance.

Tests against *R. solani* in rice are prompted by activity against PUCCRT (both are basidiomycetes) and by good control of PYRIOR, the major target for rice fungicides.

At the tertiary level, activity against SEPTRI triggers studies against MYCFIJ, black sigatoka disease of banana.

Table 4.7. Rationalized fungicide screening cascade.

Primary level	Secondary level	Tertiary level a	Tertiary level b
ERYSGT	ERYSGT	ERYSGT	ERYSGT
	UNCNEC	UNCNEC	UNCNEC
	Apple powdery mildew	Apple powdery mildew	Apple powdery mildew
	VENTIN	VENTIN	VENTIN
	PUCCRT	PUCCRT	PUCCRT
	Eyespot	Eyespot	Eyespot
			Barley net blotch
			Barley scald
			Barley leaf rust
			Wheat yellow rust
SEPTRI	SEPTRI	SEPTRI	SEPTRI
	LEPTNO	LEPTNO	LEPTNO
	PUCCRT	PUCCRT	PUCCRT
	Eyespot	Eyespot	Eyespot
			MYCFIJ
			Barley leaf rust
PLASVIT	PLASVIT	PLASVIT	PLASVIT
		PHYTIN	PHYTIN
			Pythium
PYRIOR	PYRIOR	PYRIOR	PYRIOR
		Rhizoctonia solani	*Rhizoctonia solani*

activity of known compounds or standards. At this stage, technical material is used, in a simple formulation such as aqueous acetone, and some weight is given to the fact that this is the lead generation phase of testing: failures to perform to an equivalent level to the standards do not necessarily imply that no further studies should be carried out. However, depending upon the target, high efficacy must be maintained to between 10 and 25 ppm to merit elevation to the next stage of the screen.

The curative properties of compounds are explored early in the selection process. The absence of curative activity is a disadvantage unless some systemicity or the potential to redistribute in the crop is demonstrated. Immobile protectant activity alone limits the use of a candidate to the multi-site-of-action market, dominated by cheap and effective materials such as mancozeb. Further development of such compounds is unlikely.

In some crops, especially cereals, it is important that products are effective when applied at volume rates of approximately 250 l/ha. Commonly, screening for cereal fungicides involves a low-volume test that may also present the test compound in an experimental emulsifiable concentrate formulation.

Later tests develop the notion of activity into that of field performance and include formulated material, comparative tests with finished standard products, further spectrum studies and phytotoxicity trials. The failure of a candidate fungicide may result from the absence of a commercially important attribute, such as inadequate mobility, as much as from poor efficacy.

Formulation

Formulations are vehicles which enable the active material to be applied to the crop under a variety of conditions without loss in performance. They should be:

- safe to the crop;
- easy to handle;
- compatible with other major products;
- straightforward to apply;
- acceptable to registration authorities; and
- suitable for large-scale manufacture.

Logically, the formulation of fungicides should match the complexity of the many interacting factors that affect their performance in controlling disease. These include the host plant, the pathogen, the target stages of fungal development, the biochemical target and the delivery system. However, the fungicidal activity of compounds submitted for laboratory and glasshouse screening tests is usually determined using simple formulations, for example aqueous acetone solutions, and such rudimentary systems may favour those characteristics. Laboratory formulations used in screening are not suitable for use in commercial situations and further work is required to present the active ingredient in a practical form.

Formulated products contain the active component alone or in combination with other actives in a stable form under a wide range of environmental conditions. They should be straightforward to use and should deliver the fungicide in a manner that maintains its intrinsic activity or increases its performance through enhanced redistribution or mobility. In some cases, inventive formulation may enhance performance, as in the case of the microencapsulation of surface-acting fungicides, which serves to

reduce losses through volatile action while increasing the persistence of the product and hence lengthening the period of acceptable control.

Formulation strategies have to be designed for each new active material. Preventing losses through volatility will disadvantage a product that is redistributed in the crop through the vapour phase. Conversely, losses of product must be minimized. Similarly, surface-acting fungicides may be held on the leaf in a variety of ways, but formulation components that (for example) prevent wash-off in rainstorms by increasing uptake of the fungicide into the plant remove the active material from the site of disease control. The instability of the strobilurin analogue kresoxim-methyl in plants has established the need to minimize penetration (Gold *et al.*, 1994).

The addition of adjuvants can profoundly affect the performance of fungicides and they are routinely screened in combination with new materials. For example, it has been shown that small amounts of some alcohol ethoxylate surfactants benefit the curative activity of dimethomorph (Grayson *et al.*, 1996). Similarly, adjuvants may increase the initial penetrative properties of fluquinconazole, thus enhancing redistribution and hence performance (Stock, 1996). The addition of Synperonic A5, a lipophilic alcohol ethoxylate, to prochloraz promotes the foliar penetration of the fungicide to a point that effectively removes most of the applied product from the leaf surface (Fig. 4.8; Stock, 1996). Such modifications may be advantageous or disadvantageous depending upon the proposed treatment timing and the growth pattern of the target pathogen. In some cases, formulation may inhibit fungicide action, as in the removal of activity of prochloraz in wettable powder formulations. Fungicides are formulated in several ways, depending on their physical characteristics and on the needs of the market.

Wettable powders are solid formulations suitable for compounds that have low aqueous solubility. They are produced by crushing a mixture of the active and a solid, inorganic diluent such as clay in a ball mill to a particle size of <25 μm. Wetting agents and dispersion agents are added to assist in particle suspension during application. Other adjuvants may be included to improve persistence (stickers) and photolytic stability (ultraviolet filters). Wettable powders are by their nature dusty and are potentially hazardous to handle. However, many immobile fungicides are formulated as wettable powders.

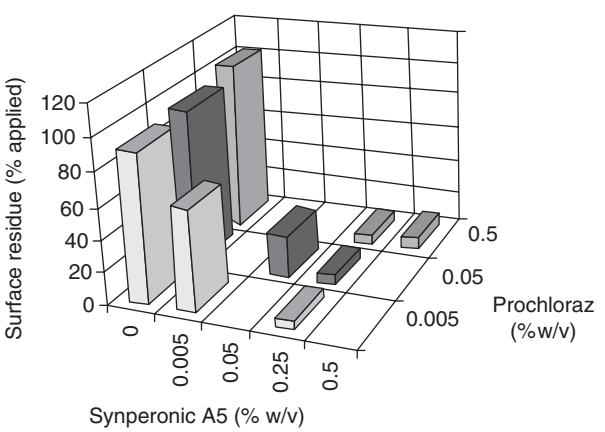


Fig. 4.8. Effect of Synperonic A5 on uptake of prochloraz into wheat leaves. (From Stock, 1996.)

Dust formulations are similar to wettable powders in that they are manufactured by grinding the fungicide, together with a solid diluent, in a ball mill. Particle size is maintained at about 20 μm diameter. The size is a controlled balance between the avoidance of particle coagulation (diameter too small) and an unacceptable reduction in activity (diameter too large). Dusts are difficult to use and tend to be the least effective of fungicide formulations because of losses during application due to drift.

Granule formulations are produced by the adsorption of fungicide on to the surface of porous clay pellets, ranging from 0.5 to 1.5 mm in diameter. A modification driven by new spray technology is the microgranule (100–300 μm diameter) that is designed for use in spinning disc applicators. Granule formulations are easy to apply, are stable in high wind and, being a relatively heavy solid, have good crop penetration characteristics. Granules are frequently used in rice paddies where quick and effective applications can be made by hand.

Suspension concentrates are formulations formed from fungicides that have been ground to a fine powder (<5 μm), suspended in either water or an organic liquid and then blended with a solid inert plus suitable adjuvants. As in wettable powders and dusts, particle size is critical to the performance of the fungicide: too large a particle size may reduce performance. In addition, the choice of adjuvant profoundly affects the utility of the formulation. Suspension concentrates with wetter often give corresponding activity to emulsifiable concentrates. Without wetter, the performance may be reduced or, in extreme cases, removed. Such effects can frequently be related to a lower level of penetration into the leaf by the fungicide. Fungicide phytotoxicity, usually most apparent in emulsifiable concentrates, may be reduced to an acceptable level without loss in performance by formulation as a suspension concentrate, with the addition of the appropriate type and amount of adjuvant.

A modification of the suspension concentrate is microencapsulation. Here the fungicide is incorporated into a small, polymer-based sphere (~15 μm diameter) which is permeable to enable the controlled release of the active material. They are available as microencapsulated flowable concentrates comprising the capsules and suitable wetting agents.

Unlike wettable powders, suspension concentrates do not present dust hazards. They can be easily dispensed and are more convenient to use.

Commercial fungicides are generally not phloem-mobile and are relatively insoluble in water, being more soluble in lipophilic, organic solvents such as xylene or cyclohexane. It may be that the barriers to uptake, translocation and movement to the sites of action restrict what is possible in terms of physicochemical properties. Lipophilic solvents, commonly used in formulations, are insoluble in water and mixtures of the two rapidly separate into layers. A fungicide dissolved in the lipophilic solvent would under these conditions be largely absent in the aqueous fraction and, in the spray tank, would not be delivered during part of the application process. The addition of surface-active agents (surfactants), or emulsifiers, to the organic solvent–fungicide solution enables the formation of an emulsion comprising small spheres (<10 μm diameter) of organic solvent–fungicide in the sprayer. This type of formulation is the emulsifiable concentrate. Emulsions of fungicides formulated as emulsifiable concentrates should remain stable in the spray tank for at least 24 h to facilitate delivery.

Emulsifying agents can be anionic, cationic or non-ionic. Non-ionic agents, for example polyethylene ethers, improve fungicide coverage on the often waxy surfaces

of target crops by reducing surface tension. Such spreaders have a greater solubility in organics than ionic surfactants, and are favoured components of formulations where high water salinity in the spray solution can cause incompatibility problems with polar compounds. However, most formulations contain a mixture of non-polar and anionic emulsifiers. Some fungicides have inherent surfactant (cationic) properties and in these cases the addition of anionic surfactants is avoided.

Because of their toxicity and fire hazard, organic solvents are being replaced by alternatives; for example, microemulsions. Where the active fungicide is soluble in water, the material may be formulated as a water-miscible liquid.

Application

Fungicides may be applied to crops as seed treatments, in foliar sprays or smokes or as fruit dips. Most application methods are universally used for all pesticides and an overview of only the major types is presented.

Seed treatments

Fungicide seed treatments are common. Fungicides designed to be used as seed treatments are of increasing importance. For example, all commercially important cereal seed is treated. Seed treatments (as distinct from seed dressing which refers to a cleaning process, as in the removal of lint from cotton seed) include adhesive dusts, the use of slurries and solutions applied as sprays to seed batches or by immersion. Historically, the use of seed treatments was confined to immobile fungicides such as the organomercurials, but they are now employed routinely to apply systemic materials in a convenient and economic manner. There is considerable interest in the use of slow-release seed treatments of systemics to provide long-term control of crop disease.

Foliar treatments

Most fungicides are diluted in water before application. The mixture is delivered through atomizing nozzles operating under high pressure and designed to disperse fine droplets of the product evenly throughout the crop. Volumes of application vary according to the crop and the activity of the product. Traditionally, immobile protectants are applied in high volumes (>600 l/ha) to ensure good coverage. However, the performance of systemics is less affected by poor coverage and they are applied at lower volumes (100–250 l/ha). There is an increasing trend towards a reduction in volume rates (<100 l/ha) through the use of air-assisted sprayers and higher-ground-speed vehicles. This applies especially to areas like Australia where water is at a premium.

Fungicides used in fruit are a mixture of immobile protectants and systemics, and programmed or repeat spraying is required to achieve acceptable disease control. Spray volumes in fruit tend to be high. In cereals, most compounds are systemic or are redistributed via the vapour phase. Spray frequency is lower than in fruit and application volumes tend to be in the 200–300 l/ha range. Handheld or tractor-mounted

spraying is difficult in some crops and aerial applications are used. In bananas, for example, black sigatoka disease is controlled by the programmed use of fungicides applied in ultra-low volume. In this technique, which uses volumes of about 20 l/ha, oil rather than water is used as the diluent.

Droplet size, nozzle type, operating pressure and formulation are interdependent variables in the application of fungicides. Tractor-mounted, conventional spraying produces a wide range of droplet sizes and can result in the loss of product via drift or due to low retention on the target leaf surfaces. The optimization of droplet size ensures more effective plant coverage, and several systems have been introduced. Spinning disc applicators rely on the delivery of the fungicide spray solution on to a rotating disc. The speed of delivery to the disc, the rotational speed of the disc and its diameter control droplet size. A further development of this technique is electrodynamic spraying. In this technique, a positive charge is imparted to the fine droplets as they leave the surface of the disc. The particles are attracted to the negatively charged crop and little spray is lost. In high-density plantings or when the target pathogen lies deep within the crop canopy, electrodynamic spraying fails to deliver the fungicide in an acceptable manner, most of the product being retained by the upper leaves. In practice, neither of the systems based on spinning discs has found acceptance within the farming community other than in small areas of crops or in protected crops.

Fungicides may also be applied in smokes, where the active ingredient is delivered during burning of the formulated product. This technique is commonly used in glasshouses.

Applications of fungicides in granular form direct to the roots are used in glasshouses, but have a major application in rice nursery beds where they provide the farmer with a convenient and effective method to deliver rice blast and sheath blight fungicides.

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5 Fungicide Performance

Key Points

- Fungicides can be classified according to crop protection performance, mode of action or chemical structure.
- FRAC has established a standardized classification system.
- Each class of fungicide has advantages and disadvantages in terms of activity, spectrum and resistance risk.
- Fungicide redistribution affects persistence and performance.

Introduction

This chapter describes the various classes of fungicides, their origin, mode of action and use. There are currently about 200 chemical compounds that are marketed as fungicides and these compounds can be classified according to either their MOA or their chemical structure. There are several levels to both these classification methods. MOA can refer to whether the product is a protectant, curative or eradicant, the broad biological function that is inhibited or the specific molecular target (if one exists or is identified).

Alternatively, fungicides can be classified by their chemical structure. Box 4.1 gives a summary of the rules for naming heterocyclic compounds as applied to fungicides. Most fungicides are complex organic molecules with several functional groups. As more than one fungicide is developed with similar MOAs, it sometimes becomes clear whether a particular structural feature is of biological significance. This structural feature is called the pharmacophore. Older names such as toxophore are also in use. The pharmacophore for many of the compounds that inhibit the *Cyp51* gene in ergosterol biosynthesis is the triazole group and this has become the most frequently used name for the group. Indeed, the triazole moiety has been shown to be important in binding to the target enzyme. Nonetheless, not all triazoles, not even all the ones used in agrochemistry, have this activity. In other cases, several chemically distinct classes can have the same MOA. Thus many unrelated compounds act as QoIs and SDHIs. It is also sometimes the case that the same chemical structure (e.g. morpholines or carboxamides) has different MOAs as a fungicide. Therefore in some cases, the biochemical function (QoI, SDHI) determines how the group is known. It is therefore important to be aware of the multiplicity of names used to describe groups of fungicides and to appreciate the relative biochemical, chemical and agricultural significance of the naming conventions.

Protectant, Curative or Eradicant; Systemicity

The flexibility of product use and the persistence of control reflect the presence of critical characteristics, such as mobility within the plant, ability to redistribute throughout the crop and compatibility with other pesticides. Fungicide systemicity refers to the movement of fungicide within the target crop, not the pathogen. Systemic fungicides are generally more reliable than non-systemics. Because most have curative or eradicant activity they have a wider window of application. They provide higher levels of efficacy by virtue of their mobility and afford a longer period of control than non-systemics. Depending on their biochemical MOA and pattern of use, systemics also carry the real or potential disadvantage of resistance development.

Systemic fungicides

Systemic fungicides exhibit either apoplastic mobility (movement within the free intercellular space, cell walls and xylem elements) governed by diffusion and the rate of transpiration or symplastic mobility (movement through plasmodesmata from cell to cell) involving uptake and distribution via the phloem. Some systemics may exhibit both apoplastic and symplastic movement, although the balance is determined by their physicochemical characteristics which, in all but very rare examples, favour the former. Most fungicides referred to in the literature as systemic do not comply with the accepted physiological definition, being restricted to acropetal movement; that is, redistribution towards the plant apex or leaf margins. Systemic fungicides act at specific biochemical sites (site-specific). For example, the triazole class of fungicides inhibits sterol biosynthesis at the C14-demethylation step. Systemic fungicides can have protectant, curative or eradicant activity.

Non-systemic fungicides

Non-systemic fungicides do not penetrate the plant. On application, they reside on the surfaces of foliage and fruits. Redistribution in the crop (and loss) occurs through the vapour phase or through the action of rainfall. In many cases, non-systemics are not redistributed and their action is limited to treated foliage. A disadvantage of non-systemic materials is their dependence on complete spray coverage of the target crop being achieved. Non-systemic fungicides are generally multi-site inhibitors, eliciting a response through the disruption of several biochemical processes. This is achieved through their ability to bind with chemical groups, such as thiol moieties, common to many enzymes.

Protectant fungicides

A protectant fungicide is one that is applied prophylactically (i.e. before the infection has been observed) to the target crop. Because of their activity against one or more of the early stages of fungal infection, from spore germination to the preliminary penetration of host tissue, no symptoms of disease develop. Immobile protectant

fungicides are usually only slightly soluble in water. On the leaf surface, uptake into the target fungus from a dilute solution of fungicide permits more solid fungicide residue to dissolve. Further uptake into the fungus results in an accumulation of toxicant until a lethal level is attained. Non-systemic fungicides are, by definition, protectants. Systemic fungicides may also possess strong protectant characteristics.

Curative and eradicant fungicides

Curative and eradicant activities are characteristic of most systemic fungicides. Curative activity is confined to the post-infection, pre-symptomatic phase of fungal infection, and the visible effects are the same as for protectant materials. Eradicant activity describes the effects of fungicides on the post-symptomatic stage of host colonization, for example action against mycelial growth of powdery mildew.

Systemic fungicides that are solely protectants are unusual, and in some cases their MOA is not well understood. Quinoxyfen, a fungicide with long-term activity against powdery mildews, especially ERYSGT, inhibits appressorium formation. However, the mechanism of its movement from the site of application to leaves not developed at the time of treatment, and then to the germinating conidia on the surface of those leaves, has still to be elucidated. Other recently discovered materials act through induction of the host's natural defence mechanisms.

Mobile fungicides

Mobile fungicides may be systemic or non-systemic, and are redistributed from their sites of application to tissue that has not been affected by treatment or that is not present at the time of treatment. This can occur within individual plants (systemics) or within the crop via the vapour phase (systemics and non-systemics).

Modes of Action

Full details of fungicide classes are found in texts such as *Modern Crop Protection Compounds* (Krämer *et al.*, 2012) and *The Pesticide Manual* (Tomlin, 2009). The following is intended as a summary of the most important and interesting classes. The biological and biochemical MOA is described for most fungicides. This is an area of intense research and an up-to-date classification is maintained by the Fungicide Resistance Action Committee (FRAC) whose website (http://www.frac.info/) is a mine of information.

Several fungicides have unknown or poorly defined MOAs, but a dozen broad MOAs and 60 detailed MOAs are described (see Table 5.1). The poorly defined group include the multi-sites, which are believed to simultaneously inhibit several fungal functions.

The broad classes are inhibition of:

A. Nucleic acid synthesis.
B. Mitosis and cell division.
C. Respiration.

Table 5.1. Fungicide classification.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
A; nucleic acid synthesis	A1; RNA polymerase	Phenylamides (PAs)	Acylalanines	Benalaxyl Furalaxyl Metalaxyl	4
			Oxazolidinones	Oxadixyl	
			Butyrolactones	Ofurace	
	A2; adenosine deaminase	Hydroxy-(2-amino-) pyrimidines	Hydroxy-(2-amino-) pyrimidines	Bupirimate Dimethirimol Ethirimol	8
	A3; DNA/RNA synthesis (proposed)	Heteroaromatics	Isoxazoles	Hymexazole	32
			Isothiazolones	Octhilinone	
B; mitosis and cell division	B1; β-tubulin assembly in mitosis	Methyl benzimidazole carbamates (MBCs)	Benzimidazoles	Benomyl Carbendazim Fuberidazole Thiabendazole	1
			Thiophanates	Thiophanate Thiophanate-methyl	
	B2; β-tubulin assembly in mitosis	*N*-Phenylcarbamates	*N*-Phenylcarbamates	Diethofencarb	10
	B3; β-tubulin assembly in mitosis	Benzamides	Toluamides	Zoxamide	22
		Thiazole carboxamide	Ethylaminothiazole carboxamide	Ethaboxam	
	B4; cell division (proposed)	Phenylureas	Phenylureas	Pencycuron	20
	B5; delocalization of spectrin-like proteins	Benzamides	Pyridinylmethyl-benzamides	Fluopicolide	43

C; respiration	C1; complex I: NADH oxidoreductase	Pyrimidinamines	Pyrimidinamines	Diflumetorim	39
		Pyrazole-MET1	Pyrazole-5-carboxamide	Tolfenpyrad	
	C2; complex II: succinate dehydrogenase	Succinate dehydrogenase inhibitors (SDHIs)	Phenylbenzamides	Benodanil	7
				Flutolanil	
				Mepronil	
			Phenyl-oxo-ethyl-thiophene amide	Isofetamid	
			Pyridinylethylbenzamides	Fluopyram	
			Furan carboxamides	Fenfuram	
			Oxathiin carboxamides	Carboxin	
				Oxycarboxin	
			Thiazole carboxamides	Thifluzamide	
			Pyrazole-4-carboxamides	Benzovindiflupyr	
				Bixafen	
				Fluxapyroxad	
				Furametpyr	
				Isopyrazam	
				Penflufen	
				Penthiopyrad	
				Sedaxane	
			Pyridine carboxamides	Boscalid	
	C3; complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (*cytb* gene)	Quinone outside inhibitors (QoIs)	Methoxyacrylates	Azoxystrobin	11
				Coumoxystrobin	
				Enoxastrobin	
				Flufenoxystrobin	
				Picoxystrobin	
				Pyraoxystrobin	
			Methoxycarbamates	Pyraclostrobin	11
				Pyrametostrobin	
				Triclopyricarb	

Continued

Table 5.1. Continued.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
			Oximino acetates	Kresoxim-methyl Trifloxystrobin	
			Oximino acetamides	Dimoxystrobin Fenaminstrobin Metominostrobin Orysastrobin	
			Oxazolidine diones	Famoxadone	
			Dihydrodioxazines	Fluoxastrobin	
			Imidazolinones	Fenamidone	
			Benzylcarbamates	Pyribencarb	
	C4; complex III: cytochrome bc1 (ubiquinone reductase) at Qi site (QiI)	Quinone inside inhibitors (QiIs)	Cyano-imidazole	Cyazofamid	21
			Sulfamoyl-triazole	Amisulbrom	
	C5; uncouplers of oxidative phosphorylation		Dinitrophenylcotonates	Binapacryl Meptyldinocap Dinocap	29
			2,6-Dinitro-anilines	Fluazinam	
	C6; inhibitors of oxidative phosphorylation, ATP synthase	Organo-tin compounds	Triphenyl-tin compounds	Fentin acetate Fentin chloride Fentin hydroxide	30
	C7; ATP production (proposed)	Thiophene carboxamides	Thiophene carboxamides	Silthiofam	38
	C8; complex III: cytochrome bc1 (ubiquinone reductase) at Qx (unknown) site	Quinone x inhibitors (QxIs)	Triazolopyrimidylamine	Ametoctradin	45

D; amino acid and protein synthesis	D1; methionine biosynthesis (proposed) (*cgs* gene)	Anilinopyrimidines (APs)	Anilinopyrimidines	Cyprodinil Mepanipyrim Pyrimethanil	9
E; signal transduction	E1; signal transduction (mechanism unknown)	Azanaphthalenes	Aryloxyquinoline Quinazolinone	Quinoxyfen Proquinazid	13
	E2; MAP/histidine kinase in osmotic signal transduction (os-2, HOG1)	Phenylpyrroles (PPs)	Phenylpyrroles	Fenpiclonil Fludioxonil	12
	E3; MAP/histidine kinase in osmotic signal transduction (os-1, Daf1)	Dicarboximides	Dicarboximides	Chlozolinate Iprodione Procymidone Vinclozolin	2
F; lipid synthesis and membrane integrity	F2; phospholipid biosynthesis, methyltransferase	Phosphorothiolates	Phosphorothiolates	Edifenphos Iprobenfos Pyrazophos	6
		Dithiolanes	Dithiolanes	Isoprothiolane	
	F3; lipid peroxidation (proposed)	Aromatic hydrocarbons (AHs), e.g. chlorophenyl, nitroanilines	Aromatic hydrocarbons	Biphenyl chloroneb Dicloran Quintozene Tecnazene Tolclofos-methyl	14
		Heteroaromatics	1,2,4-Thiadiazoles	Etridiazole	
	F4; cell membrane permeability, fatty acids (proposed)	Carbamates	Carbamates	Iodocarb Propamocarb Prothiocarb	28
	F6; microbial disrupters of pathogen cell membranes	Microbial (*Bacillus* spp.)	*Bacillus* spp. and the fungicidal lipopeptides produced	*Bacillus* spp. and the fungicidal lipopeptides produced	44

Continued

Table 5.1. Continued.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
G; sterol biosynthesis in membranes	G1; C14-demethylase in sterol biosynthesis (*erg11*/*cyp51*)	Demethylation inhibitors (DMIs) (steroid biosynthesis inhibitor (SBI) Class I)	Piperazines	Triforine	3
			Pyridines	Pyrifenox	
				Pyrisoxazole	
			Pyrimidines	Fenarimol	
				Nuarimol	
			Imidazoles	Mazalil	
				Oxpoconazole	
				Pefurazoate	
				Prochloraz	
				Triflumizole	
			Triazoles	Azaconazole	
				Bitertanol	
				Bromuconazole	
				Cyproconazole	
				Difenoconazole	
				Diniconazole	
				Epoxiconazole	
				Etaconazole	
				Fenbuconazole	
				Fluquinconazole	
				Flusilazole	
				Flutriafol	
				Hexaconazole	
				Imibenconazole	
				Ipconazole	
				Metconazole	
				Myclobutanil	
				Penconazole	
				Propiconazole	

				Simeconazole	
				Tebuconazole	
				Tetraconazole	
				Triadimefon	
				Triadimenol	
				Triticonazole	
			Triazolinthiones	Prothioconazole	
	G2; Δ^{14}-reductase and $\Delta^{8}\rightarrow\Delta^{7}$-isomerase in sterol biosynthesis (*erg24*, *erg2*)	Amines ('morpholines') (SBI Class II)	Morpholines	Aldimorph	5
				Dodemorph	
				Fenpropimorph	
				Tridemorph	
			Piperidines	Fenpropidin	
				Piperalin	
			Spiroketalamines	Spiroxamine	
	G3; 3-ketoreductase, C4-demethylation (*erg27*)	(SBI Class III)	Hydroxyanilides	Fenhexamid	17
			Aminopyrazolinone	Fenpyrazamine	
	G4; squalene epoxidase in sterol biosynthesis (*erg10*)	(SBI Class IV)	Thiocarbamates	Pyributicarb	18
H; cell wall biosynthesis	H5; cellulose synthase	Carboxylic acid amides (CAAs)	Cinnamic acid amides	Dimethomorph	40
				Flumorph	
				Pyrimorph	
I; melanin synthesis in cell wall	I1; reductase in melanin biosynthesis	Melanin biosynthesis inhibitors–reductase (MBI-R)	Isobenzo-furanone	Fthalide	16.1
			Pyrrolo-quinolinone	Pyroquilon	
			Triazolobenzo-thiazole	Tricyclazole	
	I2; dehydratase in melanin biosynthesis	Melanin biosynthesis inhibitors–dehydratase (MBI-D)	Cyclopropane-carboxamide	Carpropamid	16.2
			Carboxamide	Diclocymet	
			Propionamide	Fenoxanil	

Continued

Table 5.1. Continued.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
P; host plant defence induction	P1; salicylic acid pathway	Benzothiadiazole (BTH)	Benzothiadiazole	Acibenzolar-*S*-methyl (ASM)	P1
	P2	Benzisothiazole	Benzisothiazole	Probenazole	P2
	P3	Thiadiazole carboxamide	Thiadiazole carboxamide	Tiadinil Isotianil	P3
Unknown	Unknown	Cyanoacetamide oxime	Cyanoacetamide oxime	Cymoxanil	27
		Phosphonates	Ethyl phosphonates	Fosetyl-Al Phophorous acid and salts	33
		Benzotriazines	Benzotriazines	Triazoxide	34
		Benzene sulfonamides	Benzene sulfonamides	Flusulfamide	36
		Pyridazinones	Pyridazinones	Diclomezine	37
		Thiocarbamate	Thiocarbamate	Methasulfocarb	42
		Phenylacetamide	Phenylacetamide	Cyflufenamid	U6
	Actin disruption (proposed)	Arylphenylketone	Benzophenone Benzoylpyridine	Metrafenone Pyriofenone	U8
	Cell membrane disruption (proposed)	Guanidines	Guanidines	Dodine	U12
	Unknown	Pyrimidinone hydrazones	Pyrimidinone hydrazones	Ferimzone	U14
Multi-site	Multi-site contact activity	Inorganic	Inorganic	Copper salts	M1
				Sulfur	M2
		Dithiocarbamates and relatives	Dithiocarbamates and relatives	Ferbam Mancozeb Maneb Metiram Propineb Thiram Zineb Ziram	M3

	Phthalimides	Phthalimides	Captan Captafol Folpet	M4
	Chloronitriles (phthalonitriles)	Chloronitriles (phthalonitriles)	Chlorothalonil	M5
	Sulfamides	Sulfamides	Dichlofluanid Tolylfluanid	M6
	Guanidines	Guanidines	Guazatine Iminoctadine	M7
	Triazines	Triazines	Anilazine	M8
	Quinones (anthraquinones)	Quinones (anthraquinones)	Dithianon	M9
	Quinoxalines	Quinoxalines	Chinomethionate/quinomethionate	M10

FRAC, Fungicide Resistance Action Committee.

D. Amino acid and protein synthesis.
E. Signal transduction.
F. Lipid synthesis and membrane integrity.
G. Sterol biosynthesis in membranes.
H. Cell wall biosynthesis.
I. Melanin biosynthesis in the cell wall.
P. Activation of plant host defence.

This list includes many fundamental biochemical functions common to all organisms and shows that the key to success of fungicides is the specificity that enables fungal processes to be inhibited by compounds that do not cause undue damage to the plant hosts and other non-target organisms.

Fungicides are grouped first by target site or, if there are multiple target sites, into one of a number of multi-site clusters. Most target site groups correspond to a single formal 'group' – e.g. SDHI and QoI; other target site groups are in broad chemical groups – e.g. the B3 group is divided into benzamides and thiazole carboxamides. Abbreviations for these groups – such as AH, DMI, CAA, QoI, SBI, PA, CAA and SDHI – are widely used in the academic and promotional literature. Most groups are subdivided into a small number of chemical groups. For example, the QoI group is divided into eight chemical groups; the DMI group into five. The chemical groups are named according to the common structural element they possess.

A; Inhibition of RNA synthesis

A1; Phenylamides

These compounds include the acylalanines, butyrolactones and one member of the oxazolidinones (Fig. 5.1) and have specific activity against oomycete fungi (Table 5.2). The basis for the specificity is unknown. The acylalanine metalaxyl is the most extensively studied member of the group. Metalaxyl acts by inhibiting the synthesis of ribosomal RNA via the RNA polymerase I–template complex (Davidse, 1986), resulting in the disruption of protein synthesis. Like many fungicides, metalaxyl exists as a mixture of enantiomers. It has been established that metalaxyl-M is the more active of the two enantiomers (Nuninger *et al.*, 1996).

Phenylamides (PAs) act at specific developmental stages in the oomycete infection process. The release of zoospores from sporangia, their movement, encystment and subsequent germination, as well as the initial penetration and primary haustorium development, are relatively insensitive. However, the development of pathogens beyond the formation of the primary haustorium is well controlled. This late but specific inhibition of fungal development is explained by the biochemical MOA. In the early life cycle, sporangia and zoospores are sufficiently supplied with ribosomes to permit zoospore formation, germination, penetration and formation of primary haustoria to proceed, even in the presence of phenylamide fungicides. At later stages, continuing inhibition of the RNA polymerase I complex becomes increasingly effective and results in the thickening of hyphal cell walls and eventual cell death. These characteristic symptoms develop through an accumulation of RNA precursors, the nucleoside triphosphates, which promote the activity of fungal β(1,3)-glucan synthetase and the synthesis of cell-wall constituents (Szaniszlo *et al.*, 1985).

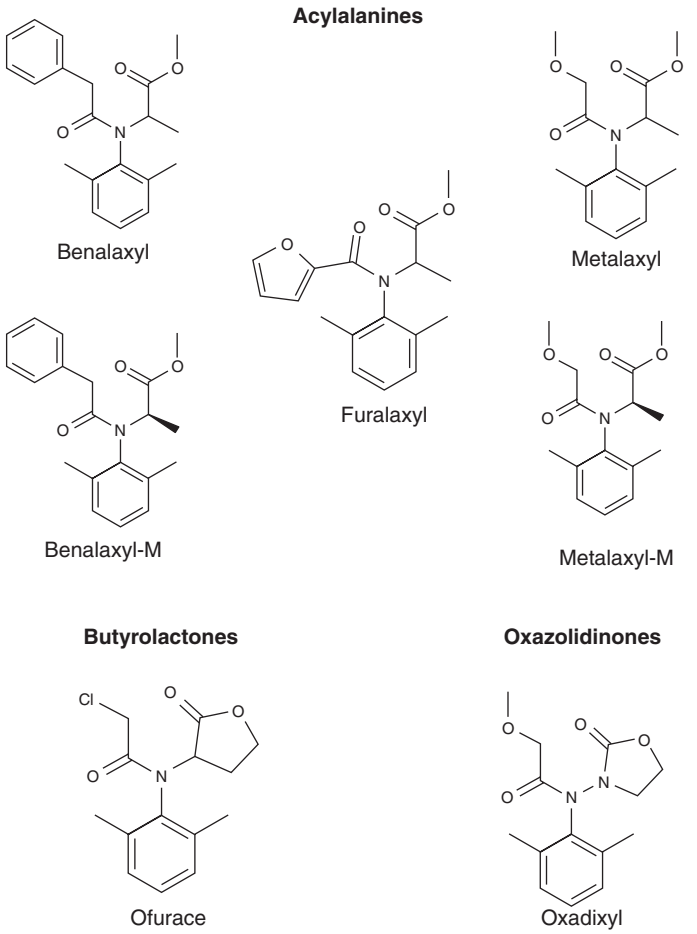


Fig. 5.1. Phenylamides.

The PA fungicides are used as protectants and curatives in seed treatments and in root and foliar applications. They are systemic, mainly via the apoplast, but metalaxyl has been reported to move to a limited extent via the symplast.

Resistance is a major problem for PA fungicides with cross-resistance between each fungicide. Even though the biochemical target has been identified, the molecular basis of the resistance is not yet known. A resistance management plan has been introduced.

A2; Hydroxy-(2-amino-)pyrimidines

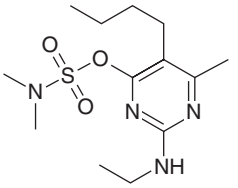
The hydroxypyrimidines (Fig. 5.2) are highly specific to the control of powdery mildews. Ethirimol is a systemic used to control powdery mildews in cereals and other field crops. It is especially active against barley powdery mildew and is used mainly as seed treatment. Dimethirimol was introduced to control powdery mildew in glasshouse cucurbits. Bupirimate is mainly used to control powdery mildews in apple and ornamentals.

Table 5.2. The spectrum[a] of different classes of fungicide.

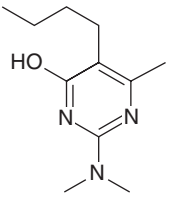
Mode of action (A1 to U)	Group name	OO	B	GFA	GSA	PM	BC	PY
A1	Phenylamides (PAs)	A	N	N	N	N	N	N
A2	Hydroxy-(2-amino-) pyrimidines	N	N	N	N	A	N	N
A3	Heteroaromatics	S	N	N	S	N	N	N
B1	Methyl benzimidazole carbamates (MBCs)	N	S	A	S	S	S	A
B2	*N*-Phenylcarbamates	N	N	N	N	N	A	N
B3	Benzamides	A	N	N	N	N	N	N
B4	Phenylureas	N	N	S	S	N	N	N
B5	Benzamides	A	N	N	N	N	N	N
C1	Pyrimidinamines	N	N	S	S	A	A	S
C2	Succinate dehydrogenase inhibitors (SDHIs)	N	S	S	S	S	S	S
C3	Quinone outside inhibitors (QoIs)	S	S	S	S	S	A	A
C4	Quinone inside inhibitors (QiIs)	A	N	N	N	N	N	N
C5	Uncouplers	A	N	N	N	S	N	N
C6	Organo-tin	S	S	S	S	S	S	S
C7	Thiophene-carboxamides	N	N	N	S	N	N	N
C8	Quinone x inhibitors (QxIs)	A	N	N	N	N	N	N
D1	Anilinopyrimidines (APs)	N	N	S	N	S	S	S
E1	Azanaphthalenes	N	N	N	N	A	N	N
E2	Phenylpyrroles (PP)	N	S	N	S	S	S	S
E3	Dicarboximides	N	S	N	S	N	S	S
F2	Phosphorothiolates	N	N	S	N	N	S	S
F3	Aromatic hydrocarbons (AHs)	N	S	N	S	N	N	N
F4	Carbamates	S	N	N	N	N	N	N
G1	Steroid biosynthesis inhibitor (SBI) Class I	N	S	S	S	S	N	S
G2	SBI Class II	N	S	S	N	A	N	N
G3	SBI Class III	N	N	N	N	N	A	N
G4	SBI Class IV	N	S	N	S	N	N	N
H5	Carboxylic acid amides (CAAs)	A	N	N	N	N	N	N
I1/2	Melanin biosynthesis inhibitors (MBIs)	N	N	N	N	N	N	A
P1/2/3	Benzothiadiazole (BTH)	S	S	S	S	S	S	S
U	Various	S	S	S	S	S	S	S
U	Arylphenylketone	N	N	N	N	A	N	N
U	Guanidines	N	N	N	S	N	N	N
Multi-site	Various	S	S	S	S	S	S	S

[a]A = all, S = some, N = none of the following pathogen subgroups: OO, *Oomycota*; B, *Basidiomycota*; GFA, general foliar *Ascomycota*; GSA, general soil or seed *Ascomycota*; PM, powdery mildew; BC, BOTCIN; PY, PYRIOR.

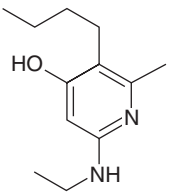
Hydroxy–(2-amino-)pyrimidines



Bupirimate



Dimethirimol



Ethirimol

Fig. 5.2. Hydroxypyrimidines.

Typically, hydroxypyrimidines inhibit germ-tube elongation and appressorium formation. Hydroxypyrimidines act through the inhibition of adenosine deaminase, an enzyme in the purine salvage pathway. Adenosine deaminase is not present in plants but is found in a wide range of fungi. However, it is only the adenosine deaminase activity from powdery mildew fungi that is sensitive to ethirimol, while the enzyme activity from other fungal species is generally not affected.

B; Mitosis and cell division

B1; β-Tubulin assembly, methyl benzimidazole carbamates

The original popularity of the benzimidazoles in the marketplace was based on their practical performance in the control of a wide range of ascomycetes and basidiomycetes. However, they lack activity against oomycetes (Table 5.2; Delp, 1995) and resistance has become a major issue in most markets. The benzimidazoles were key in the development of systemic fungicides in the 1960s and included benomyl, carbendazim, thiophanate-methyl, fuberidazole and thiabendazole (Fig. 5.3).

Benomyl has protective and eradicant activity against pathogens of cereals, vines, fruit, rice and vegetables, and is used in postharvest treatments. It is converted in plants, soils and animals to the methyl-2-yl-carbamates otherwise known as carbendazim. Thiophanate-methyl undergoes a similar conversion to carbendazim. Carbendazim, as the hydrochloride, hypophosphite and phosphate, is also used to control *Ceratocystis ulmi* (Dutch elm disease).

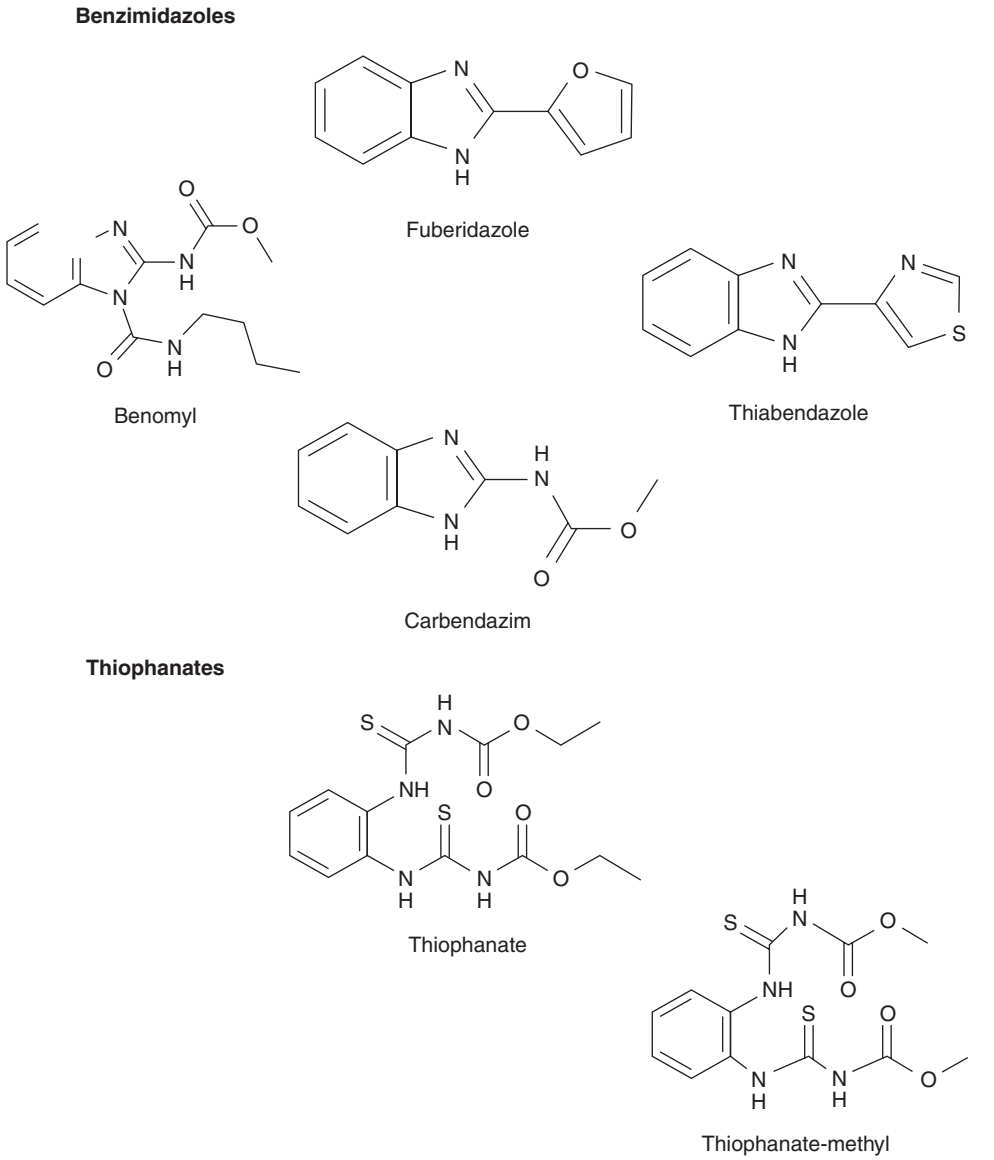


Fig. 5.3. Benzimidazoles and thiophanates.

Thiabendazole was originally introduced by Merck and Co. Ltd in 1961 as an antihelminthic. Its fungicidal and systemic properties were demonstrated in 1964, and it was widely used in the control of a range of ascomycete and basidiomycete fungi in vegetables, plantation crops, fruit, row crops, turf, protected crops and cereals. Curiously rust fungi are not controlled by this group. Fuberidazole was first prepared in 1936 but not exploited as a fungicide until 1968. It is used as a component of cereal seed treatments.

The MOA of the benzimidazoles is well researched and based on their effects on tubulin integrity. Microtubules are alternating helices of β- and α-tubulins, which

form an essential part of the cytoskeleton and are active in spindle formation and the segregation of chromosomes in cell division. Benzimidazoles disrupt mitosis during cell division at metaphase. The mitotic spindle is distorted and daughter nuclei fail to separate, resulting in cell death. These morphological changes in treated fungi correlate with biochemical studies that demonstrate the high affinity of benzimidazoles for tubulin proteins in sensitive fungi (Davidse, 1986).

Molecular biology techniques have confirmed β-tubulin as the target site (Fujimura *et al.*, 1990). Benzimidazoles are highly selective despite the highly conserved nature of β-tubulins in all eukaryotic organisms. Oomycete fungi and all plants are insensitive to the benzimidazoles. The basis of selectivity probably depends on structural differences at the binding sites of the microtubules. The modification of a single amino acid (from phenylalanine to tyrosine, F200Y; see Box 6.1 for an explanation of this nomenclature) resulting from a mutational change in β-tubulin confers resistance to carbendazim in *Neurospora* spp. In *Saccharomyces* spp., resistance is governed by a similar change, from arginine to histidine.

Resistance in the field is a serious issue for MBC fungicides. A number of fungal species developed resistance via mutation, with either the F200Y or E198A,G,K mutation being commonly found. The resistance factor (the ratio of the sensitivity of the resistant over the sensitive isolate – see Chapter 6 for details) is very high and no fitness penalty appears to ensue. For these reasons, MBC fungicides have been withdrawn from many markets. They are also under suspicion of toxic effects on animals including humans. As a result, MBCs are in decline and used only in niche markets such as legumes in Australia.

B2; Phenylcarbamates

The phenylcarbamates, as represented now just by diethofencarb (Fig. 5.4), have a similar action as the MBCs but are active against benzimidazole-resistant fungi (Ishii *et al.*, 1995). This is a rare example of negative cross-reactivity (see Chapter 6). Their gross activity in the disruption of mitosis is similar to the benzimidazoles and studies suggest the presence of a common binding region on the β-tubulin protein (Fujimura *et al.*, 1990). A mutation that results in a single amino acid change is associated with resistance to carbendazim and is the basis of negative cross-resistance between carbendazim and phenylcarbamates (Butters *et al.*, 1995).

B3; Benzamides

Microscopy studies suggest that benzamides also interfere with microtubules (Young, 1991) but in contrast to the MBCs, they only have activity against oomycetes. Zoxamide and ethaboxam (Fig. 5.5) are currently on the market (Malandrakis *et al.*, 2011).

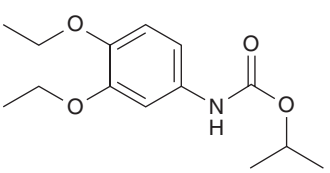


Fig. 5.4. Diethofenocarb.

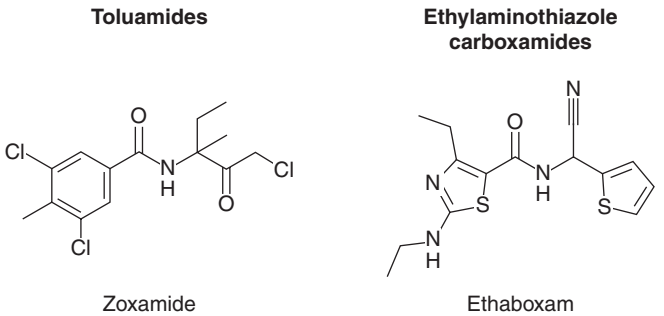


Fig. 5.5. Zoxamide and ethaboxam.

C; Respiration

The mitochondrial respiration chain has proved to be a fertile source of potent and broad-spectrum inhibitors of fungal and oomycete development. Specific inhibitors of many of the processes of mitochondrial ATP production have been modified to generate several of the currently most important fungicide classes (Fig. 5.6). The mitochondrial respiration chain is ubiquitous in both target and non-target organisms and the protein sequences in the five major complexes (I, NADH dehydrogenase; II, succinate dehydrogenase; III, cytochrome bc1 complex; IV, cytochrome c oxidase; and the proton ATPase) show high levels of conservation. Nonetheless, specific, highly active and safe inhibitors have been found.

C2; Succinate dehydrogenase inhibitors

Succinate dehydrogenase occurs in the respiratory chain as part of a complex: succinate dehydrogenase complex or complex II. The complex contains non-haem iron–sulfur proteins that act in the transfer of electrons from reduced flavin adenine dinucleotide (FAD) to coenzyme Q. Succinate dehydrogenase contributes both to electron transport and the citric acid cycle. This reaction is ubiquitous in all aerobic organisms. Inhibition of succinate dehydrogenase leads to both the starvation of ATP and the release of active oxygen. As a result the inhibitors have potent activity.

The SDHI group of fungicides has a long and interesting history and has recently undergone a major expansion resulting in a wide range of compounds with a broad spectrum and excellent activity. They now promise to rank with QoI and sterol biosynthesis inhibitors (SBIs) in importance and market size.

The first SDHIs were the oxathiin carboxamides, oxycarboxin and carboxin, and were introduced as long ago as 1966 (Fig. 5.7). They were shown to be specific inhibitors of succinate dehydrogenase (Ulrich and Mathre, 1972). The compounds had systemic activity. The genes encoding the four subunits of succinate dehydrogenase are highly conserved between organisms. It was therefore puzzling that the spectrum of the carboxins was limited to *Basidiomycota*. They were used mainly as seed treatments to control *Rhizoctonia* spp., *Ustilago* spp. and *Tilletia caries* in cereals, maize, cotton, oilseed rape and legumes (Table 5.2). Variants were tested, leading to the development of compounds like benodanil and fenfuram (1974) and

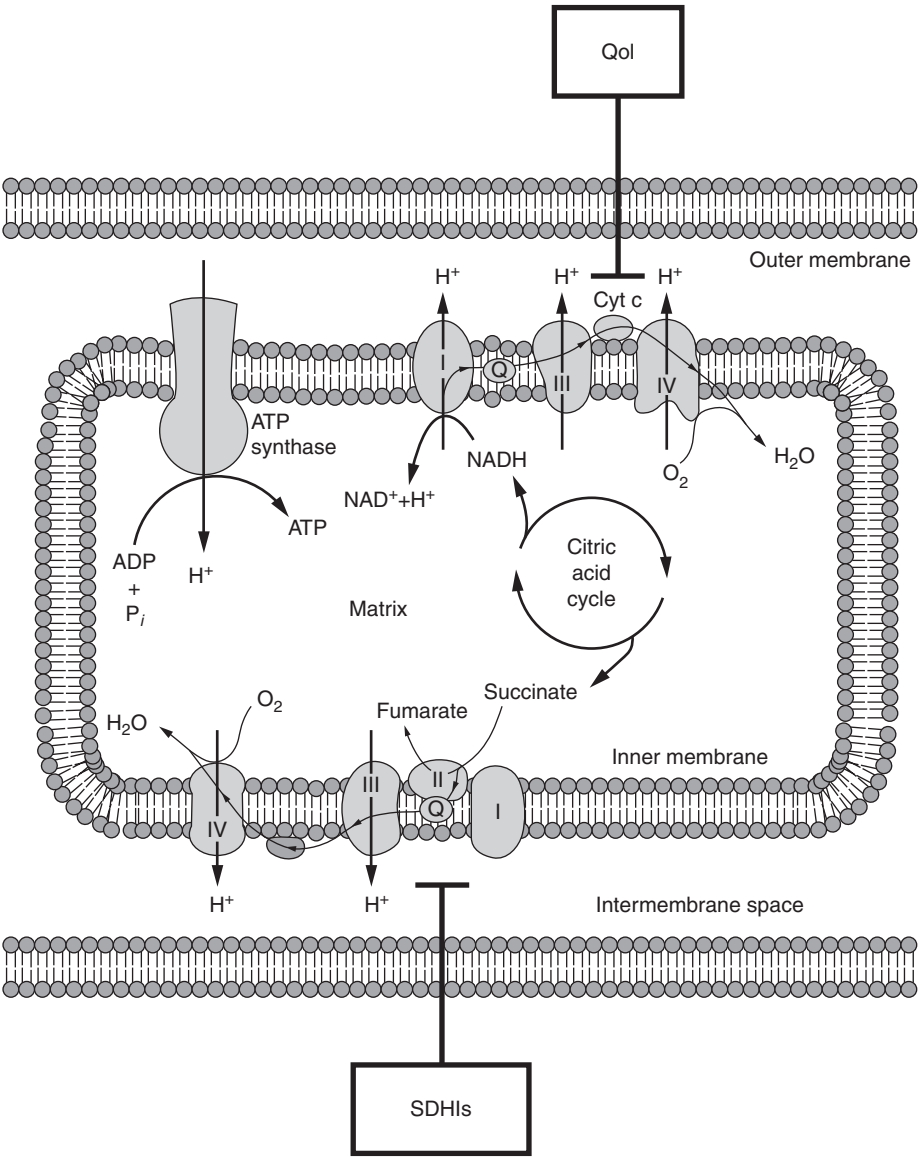


Fig. 5.6. Schematic diagram of the mitochondrion showing target sites of succinate dehydrogenase inhibitor (SDHI) and quinone outside inhibitor (QoI) fungicides. Uncouplers allow protons to re-enter the mitochondrion without passing through the ATP synthase.

methfuroxam, produced by the substitution of the 1,4-oxathiin ring by a furan moiety, and are still in use for the control of seed-borne pathogens in cereals. Similarly, mepronil (1981) is used to control *R. solani* in rice, and PUCCRT and *Typhula incarnata* in wheat. These compounds suffered from a limited spectrum and poor mobility restricting the use to seed-borne *Basidiomycota*.

A breakthrough came in 2003 with the release of boscalid, a pyridine carboxamide, by BASF. This product had broad-spectrum and foliar activity against a wide

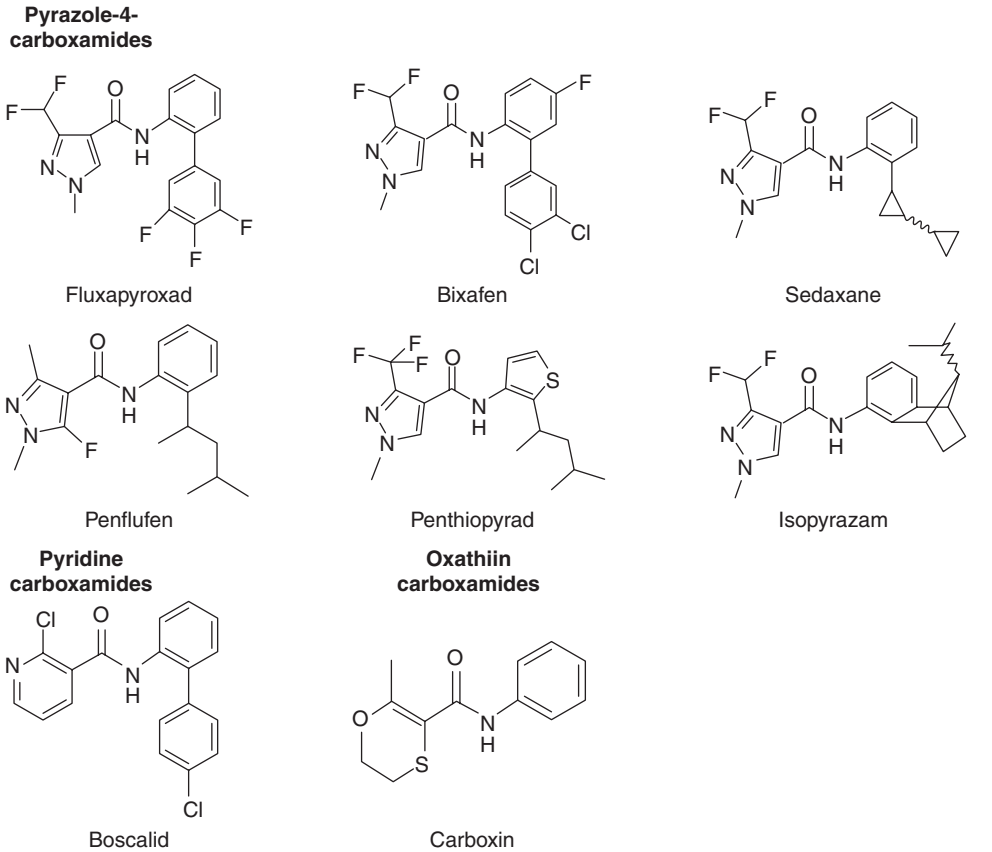


Fig. 5.7. Some succinate dehydrogenase inhibitor fungicides.

range of significant pathogens such as SEPTRI and PUCCRT. The spectrum was thus extended to *Ascomycota* pathogens, but not to oomycetes. Since then all of the major companies have released SDHIs with complementary activity and mobility characteristics. Major examples include bixafen, sedaxane, isopyrazam and penflufen. Current SDHIs mainly target foliar tissues but others such as sedexane are use in seed treatments. All of the compounds share an amide bond unit surrounded on both sides by aromatic rings of various types. Resistance is an issue for the SDHIs and so the products are normally sold in mixtures.

C3; Inhibition of complex III

This group of fungicides, formally called QoIs, but commonly called strobilurins or even 'strobis', is only a little over a decade old. The class vividly illustrates the highs and lows of the fungicide industry (Bartlett *et al.*, 2002). It includes several fungicides with annual sales approaching US$1 billion. They were inspired by a group of natural products called strobilurins. The original compounds had potent activity in the parts per billion range. Furthermore, they had an exceptional spectrum including oomycete,

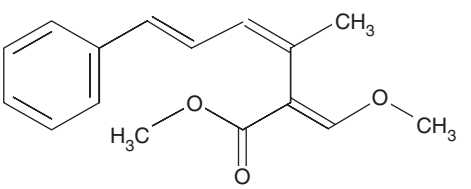
basidiomycete and all groups of ascomycete fungi. They were exceptionally non-toxic to non-target organisms including plants and animals. The acute toxicity of these compounds to animals is comparable with preservatives permitted in food. Moreover they are rapidly degraded in soil, making them environmentally benign.

The sequence of events which led to the development of the strobilurins as agricultural fungicides began in the 1960s, with the discovery by a Czech scientist, Vladimir Musilek, of a naturally occurring fungicide called strobilurin in the wood-rotting basidiomycete fungus *Strobilurus tenacellus*. This was developed for use as a medicinal agent to treat skin diseases. By the late 1970s, another antifungal secondary metabolite, oudemansin, was discovered in another basidiomycete fungus, *Oudemansiella mucida*. In 1983, BASF began to examine the potential of the strobilurins as precursors for new synthetic pesticides. By that time, the *in vitro* antifungal activity of strobilurin A was already published (Anke *et al.*, 1977; Fig. 5.8) and the MOA was shown to be the inhibition of electron transfer in complex III of mitochondrial respiration (Becker *et al.*, 1981). Although good *in vitro* activity was shown compared with synthetic standards, especially in the dark, strobilurin A possessed only weak activity *in vivo* but demonstrated an unusually broad spectrum.

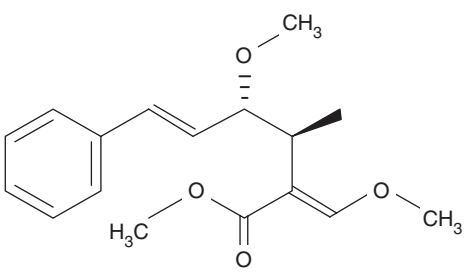
It was hypothesized that the poor transference of activity from *in vitro* to *in vivo* tests was due to the instability of the molecule, permitting rapid degradation through photolysis or metabolism. A synthesis programme was initiated to increase stability and thereby optimize *in vivo* activity.

At much the same time, studies at ICI Plant Protection (now Syngenta) investigated the activity of oudemansin A (Fig. 5.8), known to possess strong *in vivo* activity (Beautement and Clough, 1987; Beautement *et al.*, 1991). The work led to the production of a series of analogues and identified an enol ether stilbene pharmacophore as a highly active, broad-spectrum candidate (Fig 5.9).

Further work by BASF also resulted in the discovery of the enol ether stilbene pharmacophore, but showed that the molecule was photolabile. Therefore the molecule did not progress beyond tests in small plot field trials. Variations in chemical



Strobilurin A



Oudemansin A

Fig. 5.8. Strobilurin A and oudemansin A.

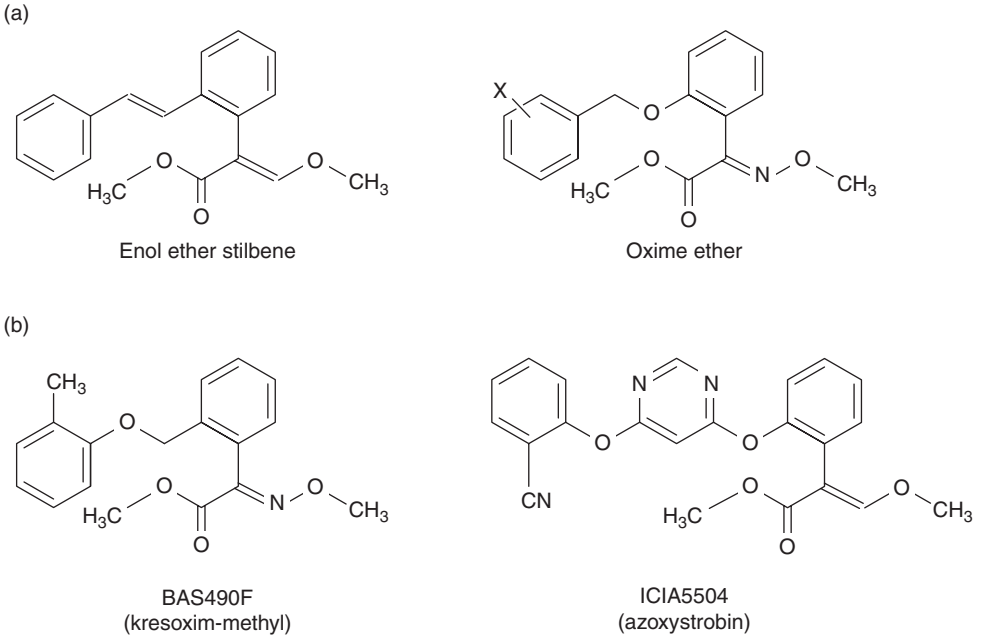


Fig. 5.9. Structures of natural (a) and released (b) quinone outside inhibitors.

structure led both companies to file patents describing the antifungal activity of the oxime ethers. Remarkably, the filings were separated by just 2 days (Sauter *et al.*, 1999).

The preferred compounds arising from the modification of the patented oxime ethers were BAS490F which was released as kresoxim-methyl (Ammermann *et al.*, 1992) and ICIA5504 which was released as azoxystrobin (Godwin *et al.*, 1992; Fig. 5.9). Both proved to be highly active compounds with broad use in a very wide range of crops and diseases. Azoxystrobin is effective against pathogens from all groups but has a particularly high potential use in the control of downy and powdery mildews of grapevine. In contrast, kresoxim-methyl is more effective than azoxystrobin against cereal powdery mildew. Although they work best as preventives, they have eradicant activity against powdery mildews.

It is remarkable that these compounds have activity against pathogens from the *Ascomycota*, *Basidiomycota* and *Oomycota* but are very safe for both plants and animals. All the other companies have developed analogues with the same MOA, though the chemical structures differ notably. The market leaders now are azoxystrobin (US$910 million sales), pyraclostrobin (US$735 million) and trifloxystrobin (US$490 million) (2009 figures; Table 5.3).

QoIs are active in the inhibition of electron transfer in complex III (bc1 complex) of the mitochondrial electron transport chain (Fig. 5.6; Becker *et al.*, 1981). Spore germination is the developmental stage of target fungi with most sensitivity to QoI, and the activity spectrum is unusually extensive. Generally, the compounds possess slow-acting systemic properties and can provide long-term disease control. Redistribution within the crop is achieved through a continuous mechanism of absorption from the waxy cuticular layer of leaves into the plant and through movement via the vapour phase and reabsorption into cuticular waxes (Sauter *et al.*, 1995).

Table 5.3. Commercialized strobilurins and other complex III inhibitors. (From Krämer *et al.*, 2012.)

Fungicide	Code number	Originator	Current owner	Launch date	Sales volume (2009, US$ million)
Kresoxim-methyl	BAS490F	BASF	BASF	1996	130
Azoxystrobin	ICIA5504	ICI	Syngenta	1997	910
Metominostrobin	SSF-126	Shionogi	Bayer	2000	<10
Trifloxystrobin	CGA279202	Ciba	Bayer	2000	490
Picoxystrobin	ZA1963	Zeneca	DuPont	2001	145
Pyraclostrobin	BAS500F	BASF	BASF	2002	735
Fluoxastrobin	HEC5725	Bayer	Bayer	2004	150
Dimoxystrobin	BAS505F	BASF	BASF	2004	50
Orysastrobin	BAS520F	BASF	BASF	2007	45
Famoxadone	DPXJE874	DuPont	DuPont	1997	60
Fenamidone	EXP10745	Rhône-Poulenc	Bayer	2001	40
Cyazofamid	IKF916	Ishihara	Ishihara	2001	50
Amisulbrom	NC224	Nissan	Nissan	2008	<10

The Achilles' heel of the strobilurins was revealed less than 2 years after release. Cereal powdery mildew isolates with very high resistance were observed and these had a consistent pattern of mutation in the cytochrome b gene. All QoIs were cross-resistant. Nonetheless, the QoI group has continued to sell exceptionally well and successful resistance management practices have been established (see Chapter 6). It was also noted that the green leaf area of the plants was extended, leading to significantly higher yields even in the absence of disease. The exact mechanism of this effect is still under discussion but it is large enough to pay for the cost of application in high-yielding situations.

C5; Uncouplers

The role of the electron transport chain is to generate the electromotive force, via displacement of protons, which will drive the synthesis of ATP. Uncouplers are compounds that interfere with ATP synthesis by collapsing the electron motive force. They do this by inserting into the inner mitochondrial membrane and providing a pathway for the transport of protons down the concentration gradient. In view of this rather non-specific MOA, it is not surprising that most uncouplers are too toxic for current use. Fluazinam, a diarylamine (Fig. 5.10), is unique among commercialized uncouplers in having low mammalian toxicity. This is due to metabolism by animal tissues into innocuous products. The compound, released in 1990, has become commercially very significant as a protectant fungicide used in the control of BOTCIN, *Sclerotinia*, *Alternaria*, *Colletotrichum*, PHYTIN and VENTIN. It also controls brassica clubroot caused by the non-fungus *Plasmodiophora brassicae*. It is not systemic but can be used both as a foliar spray and for seed treatments. The parent compounds are unstable to chemical hydrolysis and, following uptake into fungi, undergo enzymatic hydrolysis

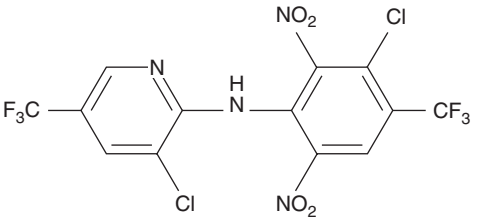


Fig. 5.10. Fluazinam.

to yield the toxic dinitrophenols, which then act as uncouplers or inhibitors of mitochondrial oxidative phosphorylation.

D; Amino acid synthesis

D1; Anilinopyrimidines

The anilinopyrimidines (APs) mepanipyrim, pyrimethanil and cyprodinil, also known as the pyrimidinamines (Fig. 5.11), are broad-spectrum fungicides and have extensive use in a wide variety of crops. Mepanipyrim and pyrimethanil are active against BOTCIN and VENTIN (Maeno and Miura, 1990; Neumann *et al.*, 1992; Daniels *et al.*, 1994). Cyprodinil has additional activity against foliar ascomycetes including powdery mildews especially for use on cereals (Heye *et al.*, 1994). The MOA has been linked to methionine biosynthesis inhibition (Masner *et al.*, 1994; Leroux and Gredt, 1995; Leroux *et al.*, 1996; Fig. 5.12). The specific target is cystathionine-γ-synthase (CGS; Fu *et al.*, 2013). Resistance is associated with alterations in the promoter of the CGS gene.

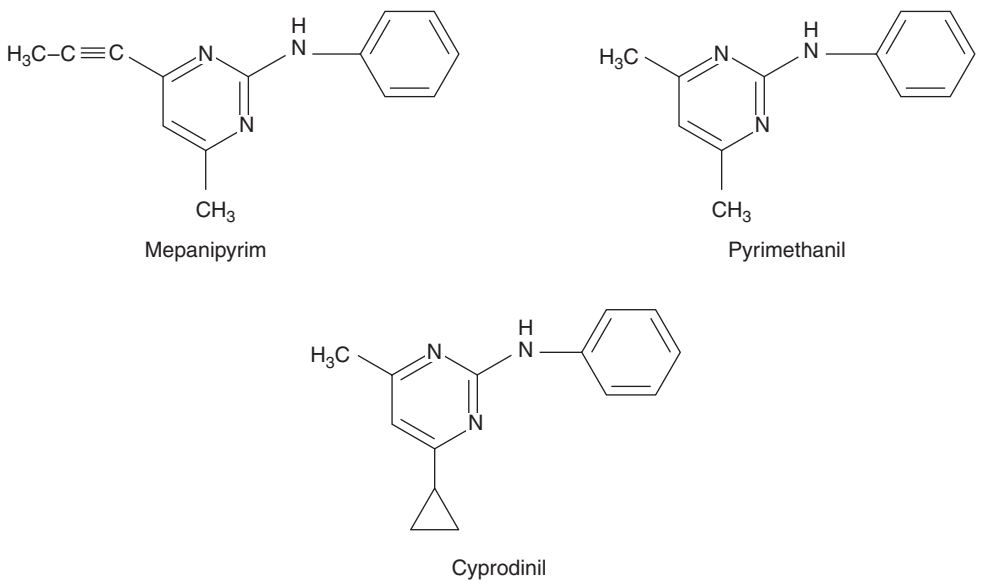


Fig. 5.11. Anilinopyrimidines.

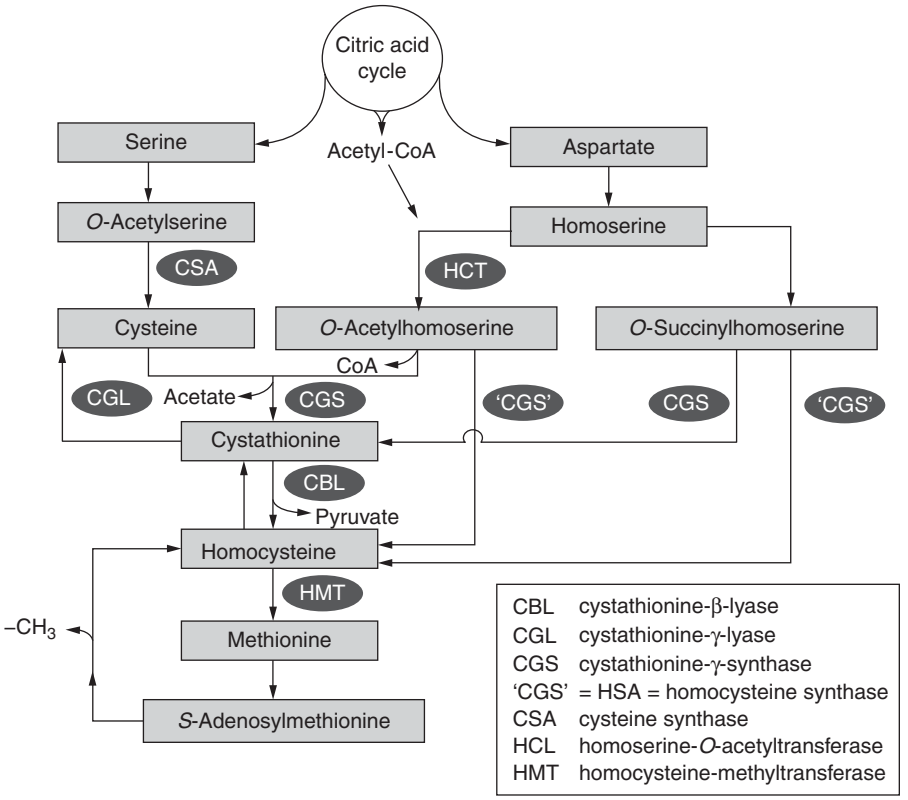


Fig. 5.12. Methionine biosynthesis pathway. (From Krämer *et al.*, 2012.)

E; Signal transduction

E1; Azanaphthalenes

Quinoxyfen and proquinazid (Fig. 5.13) are relatively recently introduced specific powdery mildewcides and they demonstrate the continuing demand for compounds to control these diseases despite the narrow spectrum. This is especially true in perennial crops like vines where genetic resistance is not available. They have little structural similarity and it remains to be seen whether they share a molecular target. Quinoxyfen was announced by DowElanco in 1996 (Longhurst *et al.*, 1996) and is unusual in its action as a systemic protectant which provides long-term control of cereal mildew. The movement of quinoxyfen through leaf sheaths to the developing basal meristem and hence to leaves not directly exposed to treatment may be involved, and other redistribution via the vapour phase may also provide a route for compound redistribution in crops. Proquinazid was introduced in 2005 by DuPont. Quinoxyfen inhibits appressorium formation by disrupting signal transduction processes (Lee *et al.*, 2008).

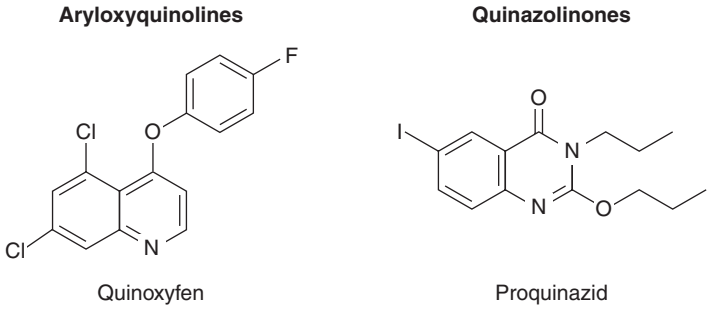


Fig. 5.13. Azanaphthalenes.

E2; Phenylpyrroles

Pyrrolnitrin is a secondary metabolite formed by *Pseudomonas pyrrocina* that has antifungal properties but is unsuitable for use in practical disease control because of its instability in light. Optimization of pyrrolnitrin led to the discovery of the commercial fungicides fenpiclonil and fludioxonil (Nevill *et al.*, 1988; Gehmann *et al.*, 1990; Fig. 5.14). The phenylpyrroles (PPs) have a broad fungal disease control spectrum but are inactive against oomycete fungi. The MOA appears to involve the MAP (mitogen-activated protein) kinase HOG1 (also known as os-2; Irmler *et al.*, 2006).

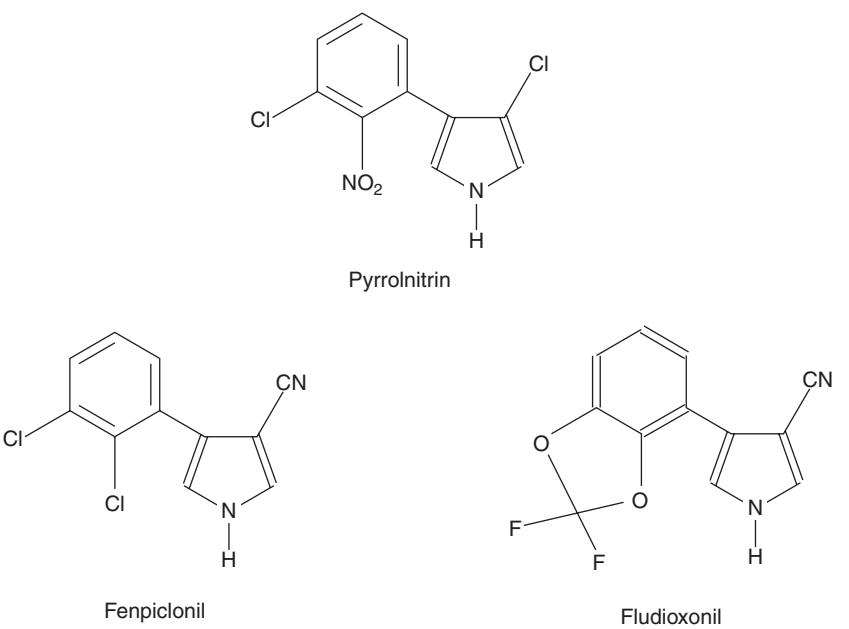


Fig. 5.14. Phenylpyrroles.

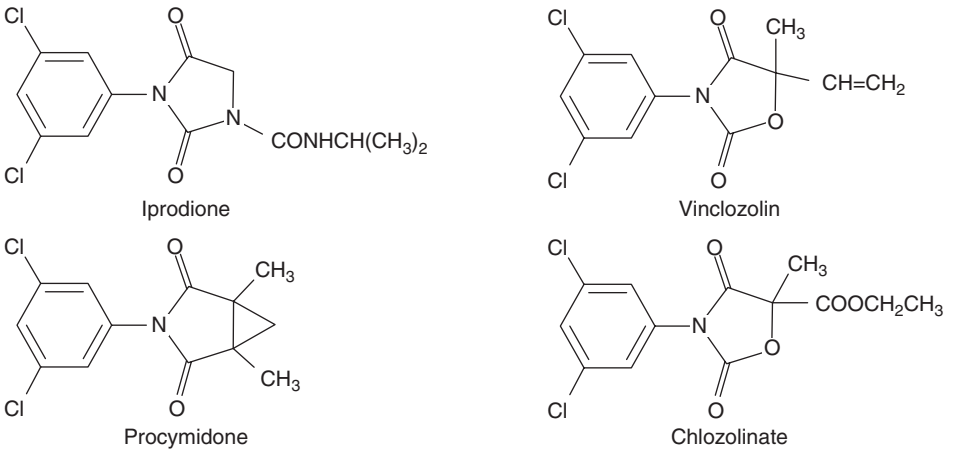


Fig. 5.15. Dicarboximides.

E3; Dicarboximides

Dichlozoline was the earliest commercial dicarboximide and was used in the control of *Sclerotinia* and BOTCIN. More recent compounds include iprodione, vinclozolin, procymidone and chlozolinate (Fig. 5.15). Their commercial strength was dependent, in part, on the occurrence of benzimidazole resistance in the target fungi *Sclerotinia* spp. and BOTCIN. The dicarboximides inhibit spore germination and cause hyphal branching, swelling and lysis. Like PPs, the MOA involves interference with kinase signalling, in this case the osmosensing histidine kinase known as Os-1 or Daf1 (Cui *et al*., 2002; Oshima *et al*., 2002).

The spectrum of the group includes BOTCIN, SEPTRI and other foliar ascomycetes in grapevine, oilseed rape, hops, ornamentals, fruit, legumes, cereals and vegetables.

G; Sterol biosynthesis in membranes

Materials that inhibit sterol biosynthesis are very effective crop disease control agents. They constitute the single largest group of fungicides in terms of both the number of individual fungicides and sales (Fig. 5.16). They are systemic and provide protectant, curative and eradicant control. They also have beneficial side-effects that seem to be unrelated to their antifungal activity. Sterols are functional components in the maintenance of membrane integrity and are present in all eukaryotes. In fungi, sterol biosynthesis is carried out *de novo* from acetyl-CoA to produce the principal sterol in most fungi, ergosterol (Fig. 5.17). Ergosterol plays a unique role in the maintenance of membrane function: a reduction in ergosterol availability results in membrane disruption and electrolyte leakage.

The biosynthetic pathway to ergosterol is a feature of all true fungi (including the *Ascomycota* and *Basidiomycota*) but is absent from the *Oomycota*, which satisfy

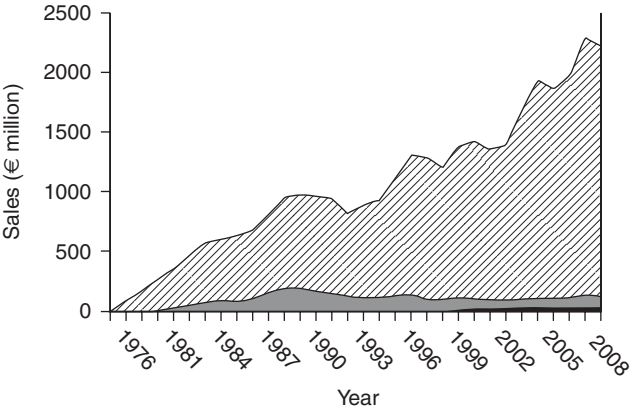


Fig. 5.16. Sales development of the sterol biosynthesis inhibitor classes in agriculture: ▨, G1; ▩, G2; ■, G3. (From Krämer *et al.*, 2012 with permission from Wiley-VCH.)

their sterol requirements directly from their hosts through mycelial uptake. This difference is the basis of the selectivity of SBIs, which cannot be used for the control of oomycete diseases. In addition, SBIs cannot be used to inhibit spore germination, which relies on stored products and can proceed in the absence of biosynthesis.

Inhibitors of sterol biosynthesis were discovered and developed to combat human fungal disease, but similar compounds quickly became available in crop protection and their introduction in the late 1960s heralded a radical change in the management of crop disease. The pathway for ergosterol biosynthesis has been established best in yeast. The so-called 'Erg' genes control the biosynthesis and have homologues in other species (Table 5.4). The details of the biosynthetic pathways differ slightly in other fungi. The same enzyme activity can act on lanosterol in yeast and eburicol in filamentous species. Fungicides that act through the inhibition of the sterol pathway can be divided into four major classes (G1–G4 and SBI Class I–IV) and further subdivided by which enzyme is inhibited (Table 5.5).

G1; C14-demethylation inhibitors (erg11/cyp51); SBI Class I

The most important SBIs are the C14-demethylation inhibitors (DMIs), group G1. The commercial strength of the DMIs arises from their activity spectrum and utility, which is very wide, with uses against most major ascomycete and basidiomycete pathogens (Table 5.2) but not oomycetes. There are a few problems. Their performance against powdery mildews, particularly in cereals, has been limited to mixtures, usually with morpholines, because of resistance development. Phytotoxicity can be a problem, limiting their use on legume crops.

The DMIs inhibit the removal of the C14-methyl group from 24-methylenedihydrolanosterol or eburicol (Fig. 5.18). The subsequent accumulation of precursor sterols and reduction in ergosterol is thought to be the basis of DMI activity. However, the effects of C14-demethylation inhibition are complex and still uncertain

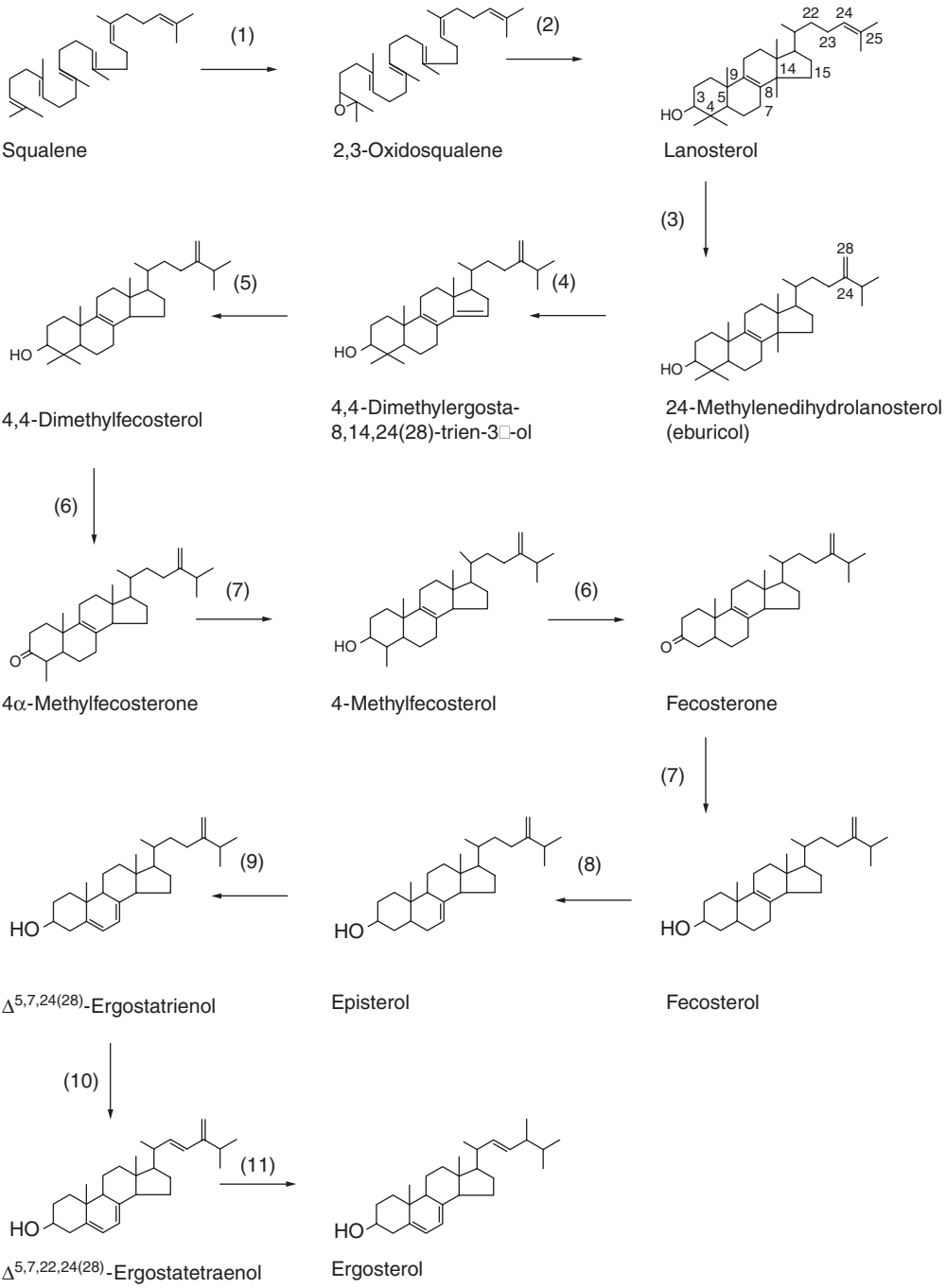


Fig. 5.17. The generic ergosterol biosynthesis pathway in fungi. Numbers indicate the enzymes listed in Table 5.4. (From Krämer *et al.*, 2012.)

Table 5.4. Enzymes and corresponding genes catalysing steps in the generic fungal ergosterol biosynthesis pathway. Fungicide MOAs are also listed. (From Krämer *et al*., 2012.)

Step no.[a]	Enzyme	Gene, other enzyme designations	Agricultural inhibitors
1	Squalene monooxygenase	*ERG1*, squalene epoxidase, oxidosqualene synthase	Target of G4 inhibitors such as allylamines; side target of some amines (G2)
2	Lanosterol synthase	*ERG7*, oxidosqualene cyclase	Side target of some amines (G2)
3	Sterol C24-methyl transferase	*ERG6*, sterol methyl transferase	–
4	Sterol C14-demethylase	*ERG11*, CYP51, lanosterol 14α-demethylase	Target of the DMI fungicides (G1)
5	Sterol C14-reductase	*ERG24*, sterol Δ^{14}-reductase	Main target of fenpropidin and spiroxamine (G2)
6	Sterol C4-methyloxidase	*ERG25*	–
	Sterol C3-dehydrogenase	*ERG26*, sterol C4-decarboxylase	
7	Sterol C3-ketoreductase	*ERG27*	Target of hydroxyanilides (G3)
8	Sterol $\Delta^{8}\rightarrow\Delta^{7}$-isomerase	*ERG2*, sterol C8-isomerase	Main target of tridemorph (G2)/secondary target of other amines (G2)
9	Sterol C5-desaturase	*ERG3*, C5-dehydrogenase	–
10	Sterol C22-desaturase	*ERG5*, ergosterol Δ^{22}-desaturase	–
11	Sterol $\Delta^{24(28)}$-reductase	*ERG4*, 24-methylene sterol (24(28))-reductase	–

MOA, mode of action; DMI, demethylation inhibitor.
[a]Step numbers are those shown in Fig. 5.17.

(Baldwin, 1983; Gadher *et al*., 1983; Baloch *et al*., 1984; Baldwin, 1990; Kelly *et al*., 1995; Senior *et al*., 1995; Lamb *et al*., 1996).

The target site of the DMIs is the Cyp51 enzyme. *Cyp51* encodes P450 monooxygenase and the fungicides appear to bind act the active site, thereby directly inhibiting access of the substrate to the enzyme (Kelly and Kelly, 2013). This both reduces ergosterol synthesis and leads to the accumulation of other, presumably toxic intermediates (Joseph-Horne *et al*., 1996). Different species of fungi have one, two or even three paralogues (copies of genes that arose from gene duplication) of the *Cyp51* gene (Fan *et al*., 2013). The presence of the different paralogues accounts for some of the variation in sensitivity in different species to different DMIs. Resistance has become a significant issue and is associated with changes in the coding sequences and overexpression of genes.

The variation in performance between DMIs may reflect differences in their binding affinities to the haem moiety of the P450 Cyp51 demethylase enzyme (see Fig 4.3 for 3D structure). Cyproconazole, for example, exists in four isomeric forms, all of

Table 5.5. Grouping of SBI fungicides in the FRAC classification. (From Krämer *et al*., 2012.)

	G: Sterol biosynthesis inhibitors			
FRAC codes	G1	G2	G3	G4
Group name	Demethylation inhibitors (DMIs)	Amines (formerly 'morpholines')	Hydroxyanilides	Squalene epoxidase inhibitors
SBI class	I	II	III	IV
Target in sterol biosynthesis	Sterol C14-demethylase	Δ^{14}-reductase and $\Delta^{8} \rightarrow \Delta^{7}$-isomerase	3-Ketoreductase	Squalene epoxidase
Chemistry	Piperazines Pyridines Pyrimidines Imidazoles Triazoles	Morpholines Piperidines Spiroketalamines	Hydroxyanilides	Thiocarbamates Allylamines

SBI, Sterol biosynthesis inhibitor; FRAC, Fungicide Resistance Action Committee.

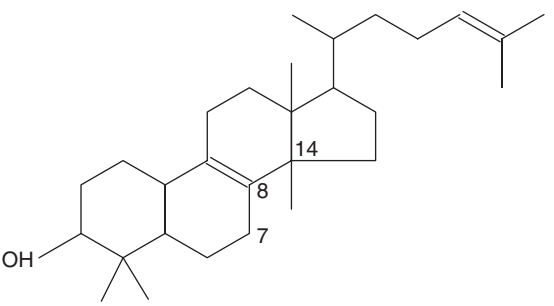


Fig. 5.18. Lanosterol, showing C7, C8 and C14 positions.

which show very high and almost equal fungicidal activity as a result of their similarity in affinity for the active site of inhibition (Gisi *et al*., 1986).

Five classes of chemical are DMIs (Table 5.5). A few pyrimidines (e.g. fenarimol) and imidazoles (e.g. prochloraz) have reached commercial importance but the group is dominated by the triazoles. This diverse range of chemistry is characterized by a nitrogen-containing heterocycle with an attendant lipophilic group. The triazole group contains more than 25 chemicals. It includes ones recommended for both seed treatment (e.g. triadimenol and fluquinconazole) and foliar treatment. Established market leaders in this group include epoxiconazole, propiconazole, tebuconazole and cyproconazole. Given that this group has been thoroughly explored since the 1970s, it was surprising that a new addition to the group, the Bayer compound prothioconazole, was launched as recently as 2004. The supplied product is a variant on the triazole theme, being a 1,2,4-triazole-3-thione. The compound is activated by exposure to the plant, losing the thio group in the process.

The imidazole prochloraz was developed initially for its potential against mildews. In field trials, the compound demonstrated useful activity against cereal eyespot and was for a while the market leader. Other major targets in cereals include SEPTRI,

BOTCIN, PYRIOR and other foliar ascomycetes. Prochloraz shows negative cross-resistance against triazoles (Fraaije *et al*., 2007).

The pyrimidines nuarimol and fenarimol are the only current examples of pyrimidin-5-ylbenzhydrols. Nuarimol is a systemic powdery mildewcide with a minor use as a seed treatment. Fenarimol, also active against mildews, has a major use in vines for the control of UNCNEC, and in apples, against *P. leucotricha*. VENTIN is also controlled by fenarimol, and its use in bananas as a resistance management tool against MYCFIJ is being developed.

*G2; $\Delta^8 \rightarrow \Delta^7$-isomerase and Δ^{14}-reductase inhibitors (*erg24, erg2*); SBI Class II*

The spectrum of disease control of the $\Delta^8 \rightarrow \Delta^7$-isomerase and Δ^{14}-reductase inhibitors (Fig. 5.19) is limited compared with the C14-demethylation inhibitors, their major use being against the powdery mildews (Table 5.2). There are only seven commercial

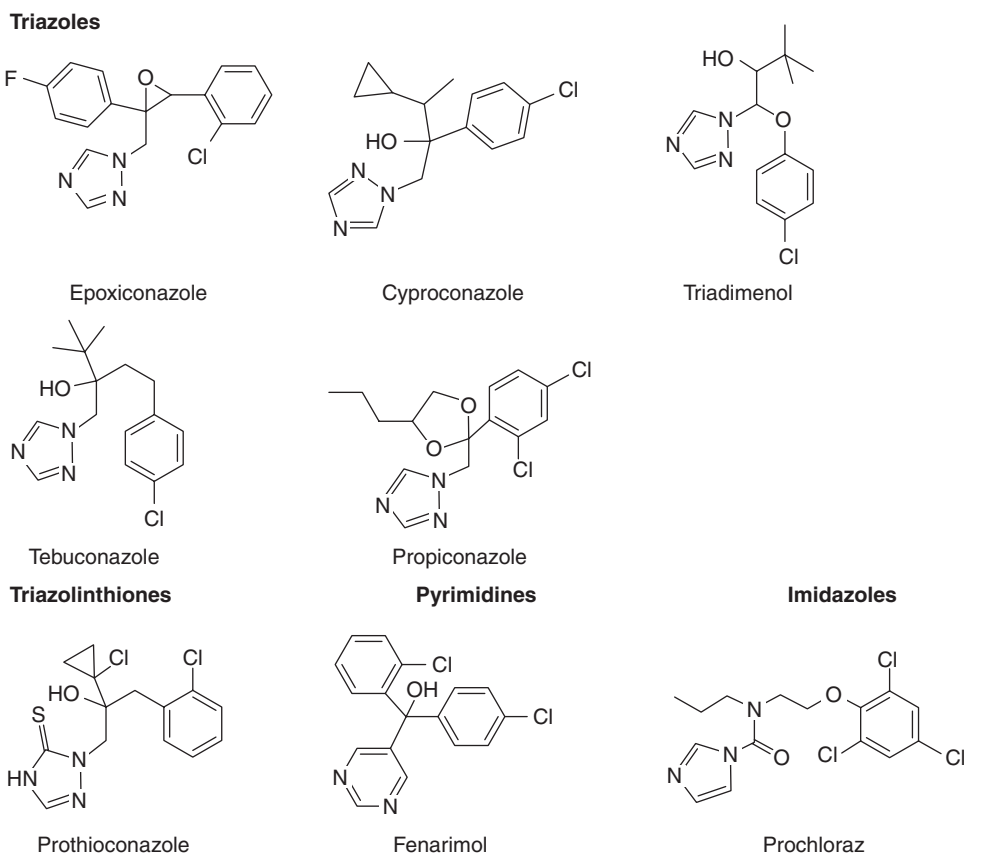


Fig. 5.19. Important triazole, triazolinthione, pyrimidine and imidazole fungicides.

fungicides in this group. They are the morpholines (fenpropimorph, tridemorph, dodemorph and aldimorph), the piperadines (fenpropidin and piperalin) and the spiroketalamine, spiroxamine (Fig. 5.20; Hollomon, 1994; Baldwin and Corran, 1995; Krämer *et al.*, 1999). Spiroxamine, the newest member of the group (1997), has preventive, curative and eradicant activity against mildew as well as significant activity against other fungi such as rusts and leaf blotches.

Extensive use of DMIs against cereal powdery mildews resulted in resistance and a reduction in control to levels below commercial acceptability. The current success of the $\Delta^8\Delta^7$-isomerase and Δ^{14}-reductase inhibitors was almost totally

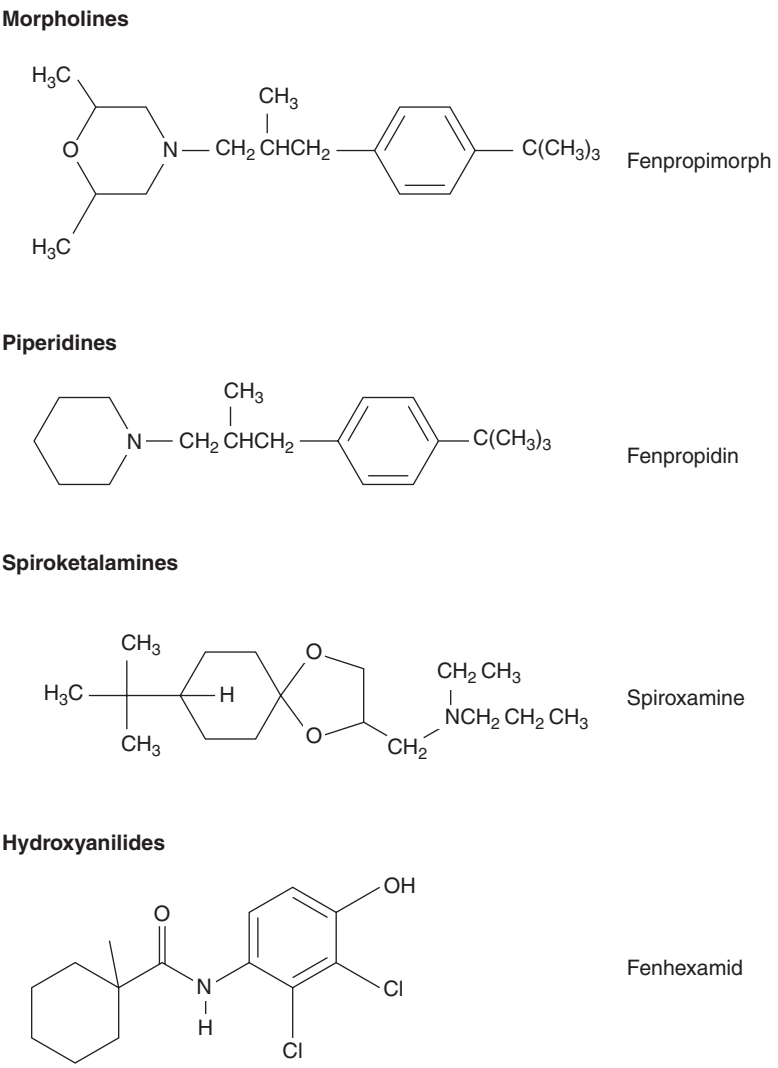


Fig. 5.20. Important morpholine, piperidine, spiroketalamine and hydroxyanilide fungicides.

dependent on the subsequent search for powdery mildew control agents with different MOAs from the azoles, and created a market for specific mildewcide products.

Although the inhibition of the isomerase and reductase has been demonstrated in laboratory studies (Baloch *et al.*, 1984; Mercer, 1991; Debieu *et al.*, 1992; Köller, 1992), the comparative importance of the two targeted steps is not well understood and the implications of inhibition are not clear (Baldwin and Corran, 1995). It is known for example, that in ERYSGH/T, the major target pathogen for this group, tridemorph is a highly active inhibitor of the isomerase reaction whereas fenpropidin has only weak activity, its major strength being the inhibition of the reductase step (Girling *et al.*, 1988). Fenpropimorph inhibits both enzymes. In addition, although some studies have been carried out that demonstrate the disruptive effects of fenpropimorph treatment on sterol levels and membrane integrity in yeast, *S. cerevisiae* (Steel *et al.*, 1989), other work showed that survival was independent of $\Delta^{8}\square\Delta^{7}$-isomerase activity (Ashman *et al.*, 1991). Morpholine inhibition of $\Delta^{24(28)}$-reductase, Δ^{24}-transmethylation and squalene-cyclization steps have also been cited as possible MOAs (Baldwin and Corran, 1995), which is reasonable considering the close structural similarities between substrates at those target sites.

The MOA is mediated by the interaction of the negatively charged enzyme site and the positively charged nitrogen atom in the fungicide molecule. Optimization of activity through structural modification extends to the choice of stereoisomer. In the spiroketal, spiroxamine, the two *cis* forms are more active than the two *trans* isomers (Krämer *et al.*, 1999).

*G3; 3-Ketoreductase (*erg27*); SBI Class III*

In a typically serendipitous manner, compounds being synthesized by Bayer as herbicides were found to have activity against BOTCIN. Optimization led to the release of the hydoxyanilide, fenhexamid, in 1998 (Fig. 5.20). It was subsequently shown that the compound inhibited a novel site in the sterol biosynthetic pathway (Debieu *et al.*, 2001). Fenhexamid had good activity against BOTCIN and the close relative *Sclerotinia* but only weak activity against other ascomycetes. It is used as a foliar product. The compound is not translocated so it is used solely as a protectant. Usage rates are high at up to 1 kg/ha.

H5; Cell wall biosynthesis; carboxylic acid amides

A diverse group of fungicides with specific activity against oomycetes were combined in a coherent group by FRAC in 2005 called the carboxylic acid amides (CAA). (Many of the important compounds in this group are cinnamic acid amides. This can give some ambiguity in the CAA abbreviation.) This rationalization occurred because research investigating fungicide resistance showed that all shared a common cross-resistance phenomenon. The group includes three cinnamic acid amides (flumorph, dimethomorph and pyrimorph), three valinamides (iprovalicarb, benthiavalicarb and valifenate) and a mandelic acid amide, mandipropamid (Fig. 5.21).

The target site was found to be the enzyme cellulose synthase. As cellulose is absent from true fungi, this explained why the spectrum only includes oomycetes.

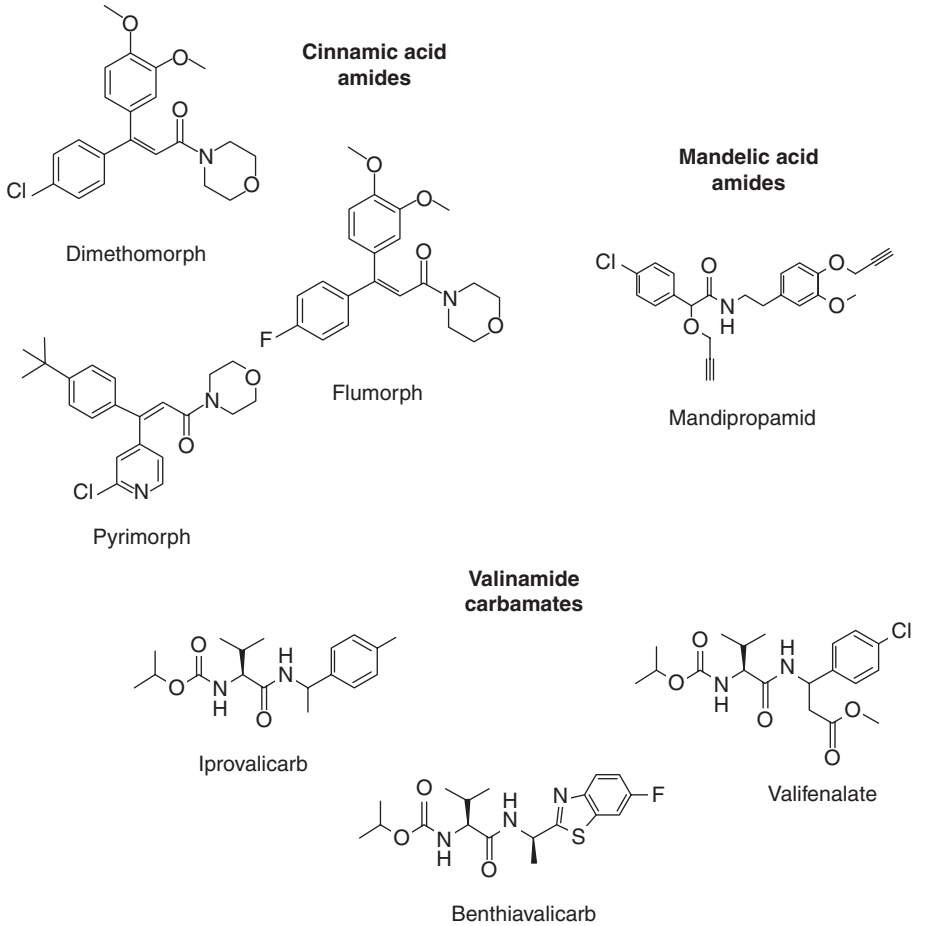


Fig. 5.21. Some carboxylic acid amide fungicides.

It is not so clear why *Pythium* species are insensitive. The application for CAAs is dominated by PHYTIN and PLASVIT.

The CAA fungicides have preventive and some eradicant activity due to some translaminar systemic movement. They operate by inhibiting germination of cystospores and sporangia, delaying elongation of hyphae and inhibiting sporulation.

The MOA was fully characterized by identifying mutations in the *CesA3* gene of PHYTIN that conferred resistance to mandipropimad (Grenville-Briggs *et al.*, 2008; Blum *et al.*, 2010). The results from other species and other CAAs are consistent with this finding.

I; Inhibition of melanin biosynthesis

The synthesis of the pigment melanin is important in fungal pathogenicity. The melanization of appressorial walls is essential for the development of infection hyphae and penetration of the host epidermis (de Jong *et al.*, 1997). Mutants of PYRIOR that

do not contain melanin are not pathogenic (Wheeler and Greenblatt, 1988). The discovery of tricyclazole initiated the development of chemicals displaying a novel MOA in pigmented ascomycetes (Fig. 5.22). Their inhibition of melanin synthesis provides excellent control of PYRIOR in rice and a significant share of the global market in fungicides.

Melanin biosynthesis in most fungi is via the DHN pathway. In this pathway, a ubiquitous polyketide synthase produces 1,3,6,8-tetrahydroxynapthalene (Fig. 5.23). Further steps convert this to scytalone, to 1,3,8-trihydroxynapthalene, to vermelone and, finally, to 1,8-dihydroxynapthalene (DHN). The melanin biosynthesis inhibitor (MBI) group of fungicides is divided into I1 (MBI-R), which inhibit the 1,3,6,8-tetrahydroxynapthalene reductase (tricyclazole, pyroquilon and fthalide), and I2 (MBI-D), which inhibit the scytalone dehydratase (carpropamid, diclycomet and fenoxanil). The compounds inhibit the enzymes by substrate mimicry.

The targets of this group are PYRIOR and *Colletotrichum*. This limited spectrum can be explained by the critical role of the appressorium in cuticular penetration by these species, which seems to be solely due to turgor pressure. This places a huge premium on extremely tough appressorial cell walls. Any inhibition by these compounds appears to be sufficient to give control. The presence of melanin synthesized from dihydroxyphenylalanine (DOPA) in fungi such as LEPTNO (Solomon *et al.*, 2004) and possibly other fungi, which is not affected by MBIs, also explains the limited spectrum.

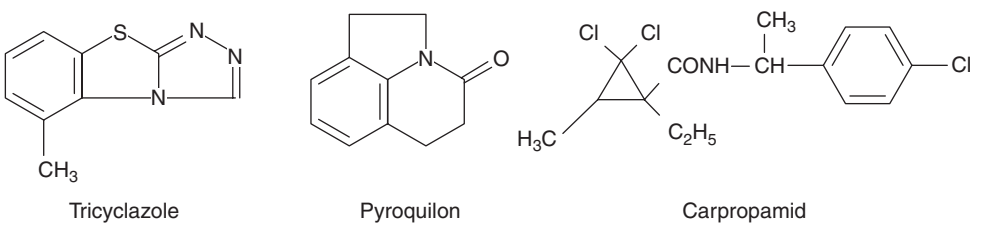


Fig. 5.22. Melanin biosynthesis inhibitors.

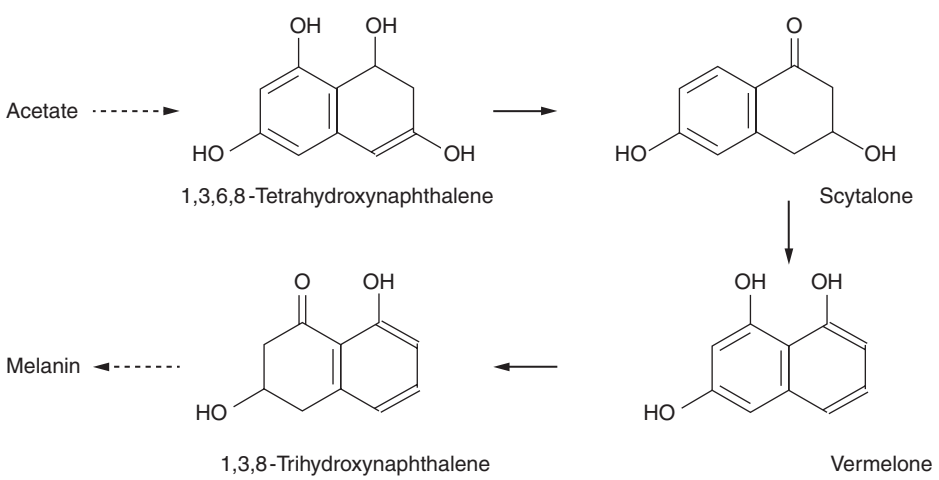


Fig. 5.23. Melanin biosynthesis (dotted arrows indicate several steps).

On tricyclazole-treated rice, the early infection stages of PYRIOR (germination of conidia and formation of appressoria) are unaffected but the melanization of appressoria and the subsequent formation of the infection peg apparatus are inhibited, effectively protecting the plant from disease. Tricyclazole is readily taken up by leaves and roots of rice plants and translocated, predominantly acropetally. Thus it is used in foliar applications and has mainly preventive activity.

P; Host plant defence induction

Most plants are resistant to most fungi. The mechanism of natural resistance has been the subject of intense study in the academic world as well as in the agrochemical industry, and a summary of the picture was given in Chapter 2. The onset of resistance following inoculation with an avirulent pathogen was found to be associated in many cases with the accumulation of salicylic acid. Furthermore, tissues remote from the inoculation site were found to also be resistance, a phenomenon known as systemic acquired resistance (SAR; Malamy *et al*., 1990; Metraux *et al*., 1990). Salicylic acid was transported around the plant and led to the induction of defence genes (often called pathogenesis-related or PR genes). Some salicylic acid was stored as the glycoside. Subsequent infection causes the release of salicylic acid from the glycoside and its accumulation at the site of infection. PR genes are induced, fungal growth is inhibited and disease expression does not occur or is slowed.

These studies suggested that agents that can induce SAR may present a new broad-spectrum means to control plant disease for extended periods. However, they have certain limitations. First, SAR is a natural phenomenon and the response to chemical inducers will be limited to the same spectrum of pathogens as in a naturally induced response. Salicylic acid induction was found to be restricted to biotrophic haustorial pathogens, whereas necrotrophic pathogens induced the accumulation of jasmonate and ethylene (Oliver and Ipcho, 2004). Secondly, by their nature, these exogenously applied chemicals have no direct fungitoxic activity, which complicates the discovery process. Nevertheless, the potential for crop disease control using chemically induced SAR responses has been explored by the agrochemicals industry has produced compounds with remarkable activity (Fig. 5.24).

Probenazole has been sold since 1975 and is still widely used to control PYRIOR. It also controls bacterial diseases. It is used at rates of up to 3 kg/ha on paddy fields. The compound moves acropetally and is thought to act through the induction of host defence reactions. The response is specific to rice. PYRIOR can infect barley with devastating effect but is not controlled on barley treated with probenazole. In rice, the compound stimulates the accumulation of fungitoxic substances, including α-linoleic acid, following inoculation with PYRIOR. An increase in the activity of several enzymes – phenylalanine ammonia lyase, peroxidase and catechol-*O*-methyltransferase – also occurs and these enzymes, collectively, are proposed to restrict spread of the pathogen in plants. Probenazole also inhibits early fungal development stages, reducing spore germination, appressorium formation and penetration of PYRIOR in rice.

CIBA (now part of Syngenta) embarked on a directed discovery programme starting with the methyl ester of benzo-1,2,3-thiazole-7-carboxylic acid, which had activity against *Colletotrichum lagenarium* on cucumber and was accidentally synthesized

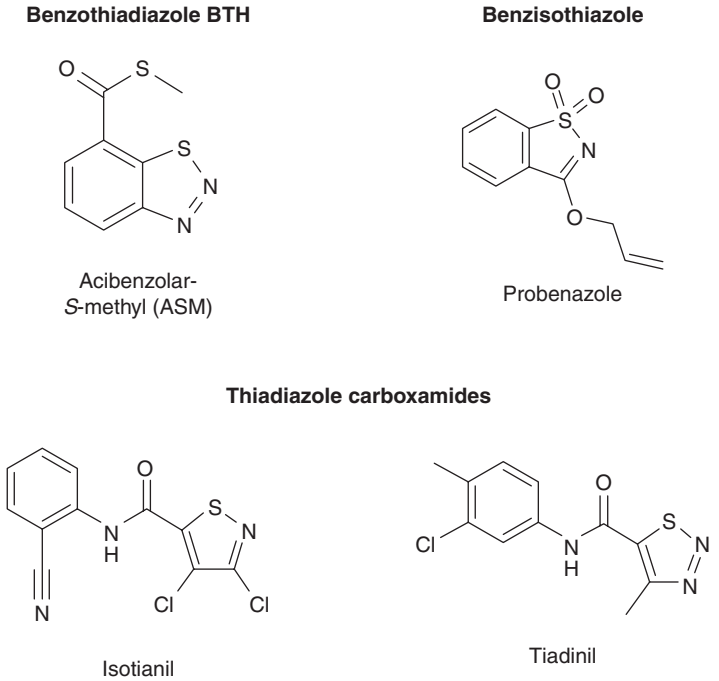


Fig. 5.24. Host plant defence induction compounds.

during a sulfonylurea herbicide discovery programme (Kunz *et al.*, 1997). The general structure of the benzothiadiazole plant activators was elucidated following extensive analogue synthesis and screening. The first compounds to have good broad-spectrum activity were dichloroisonicotinic acid and benzo-1,2,3-thiadiazole-7-carbothioic acid *S*-methyl ester, now known as ASM (acibenzolar-*S*-methyl), marketed under the trade name Bion and introduced to the market in Germany and Switzerland (Schurter *et al.*, 1990; Ruess *et al.*, 1995). It has no *in vitro* fungicidal activity, but when applied to plants it activates plant defence mechanisms against a wide spectrum of pathogens including fungi, bacteria and viruses (Kessmann *et al.*, 1996), similar to the naturally induced spectrum of disease resistance. The product is highly mobile, probably because of its weakly acidic nature, and is transported acropetally and basipetally throughout the plant, but is subject to rapid metabolism.

Further evidence that ASM is active only through the plant comes from work with mutants of the model plant *Arabidopsis thaliana* in which SAR cannot be induced (non-inducible mutants). In these and other non-inducible mutants, ASM and salicylic acid are inactive in protecting against fungal attack (Lawton *et al.*, 1996). ASM is effective against a broad spectrum of fungal, bacterial and virus diseases across a range of important crops (Table 5.6). In wheat, a single application of ASM at 30 g a.i./ha at early tillering is reported to give good protection against ERYSGT for up to 60 days. It has been suggested that the induction by ASM of the several disease resistance mechanisms that comprise SAR reduces the risk of resistance development (Kessmann *et al.*, 1996).

Table 5.6. Acibenzolar-*S*-methyl; target crops and pathogens.

Crop	Acceptable control	Suppression
Wheat	ERYSGT	PUCCRT SEPTRI
Rice	PYRIOR	
Tobacco	*Peronospora tabacina*	
Banana	MYCFIJ	
Vegetables	Oomycetes *Colletotrichum*	*Alternaria* spp.

Tiadinil and isotianil are the most recently released compounds that also seem to act via the induction of SAR. Both compounds induce PR proteins via the salicylic acid signalling pathway. A significant factor is the very much higher activity of isotianil, allowing the use of rates of about 100 g/ha compared with the 1–3 kg/ha needed for the other compounds. The spectrum is patchy, however, with good activity against ERYSGT, PYRIOR and *Colletotrichum* but none against necrotrophs such as tan spot of wheat.

The future for host plant inducers is unclear. They are widely used in rice for PYRIOR but mainly because growers fail to use resistant cultivars. They should be immune to resistance but the limited spectrum has limited use to niche applications.

Multi-site (M) and unknown (U) modes of action

U27; Cymoxanil

Cymoxanil (Fig. 5.25) is an extremely effective systemic fungicide with protectant and curative activity specifically against oomycete fungi. Cymoxanil has important uses against PHYVIT on grapevine and PHYTIN in which it is employed in a mixture with non-specific cell toxicant fungicides, for example mancozeb, as part of anti-resistance strategies to improve long-term activity and, through its curative activity, to extend the interval between sprays.

Cymoxanil is more effective against hyphal growth stages than early growth phases (the release of zoospores from sporangia and their germination). The compound inhibits nucleic acid and protein biosynthesis in *Phytophthora cinnamomi* and *B. cinerea*, but it is likely that the activity is induced via an interaction with host metabolic processes.

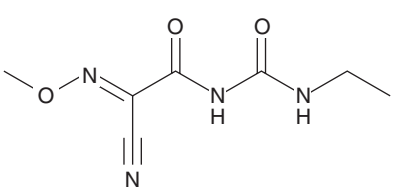


Fig. 5.25. Cymoxanil.

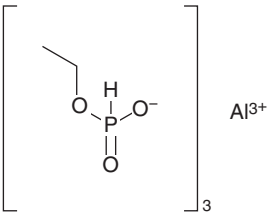


Fig. 5.26. Fosetyl aluminium.

U33; Fosetyl

Fosetyl or fosetyl aluminium (Fig. 5.26) has specific activity against the oomycete fungi, including PLASVIT, *Pseudoperonospora humuli* in hops and *Phytophthora* spp. in fruits, for example citrus. Fosetyl is a rare example of a phloem-mobile product, an action that is considered to be a function of its breakdown product, phosphonic acid (H_3PO_3), also known as phosphite, which is readily produced in aqueous solution.

The product inhibits sporangial formation and zoospore release in *Phytophthora citrophthora*, *Phytophthora parasitica*, *Phytophthora cactorum* and *Phytophthora citricola*, and oospore and chlamydospore production in *P. citricola* and *P. cinnamomi*; *Phytophthora megasperma* and *P. infestans* are comparatively insensitive (Farih *et al.*, 1981).

Recent research suggests that the MOA is complex but involves the activation of plant defensive reactions. These include both preformed and pathogen-induced defences (Machinandiarena *et al.*, 2012; Massoud *et al.*, 2012; Olivieri *et al.*, 2012; Walters *et al.*, 2013). This indirect MOA explains the low *in vitro* toxicity of fosetyl towards mycelium growth. Given that defence response does not differ significantly between the attacking pathogens, it is curious why this MOA does not protect against a wider spectrum of pathogens. There is more to be learnt about this interesting class of fungicides.

M1; Copper

Copper, as copper sulfate, was first used as a fungicide to control *Tilletia grisea* in wheat but its use was not developed until an observation by Millardet in France, in 1882, that treatments of copper sulfate and lime used in roadside situations to deter the theft of grapes were associated with the control of downy mildew PLASVIT. This led to the acceptance of Bordeaux mixture as a routine treatment for vine downy mildew.

Copper fungicides such as Bordeaux mixture and copper oxychloride (Fig. 5.27) are still employed singly, or in combination with systemics such as cymoxanil, to

$Ca(OH)_2 + CuSO_4$	$Cu(OH)_2$	$Cu_2Cl(OH)_3$
Bordeaux mixture	Copper hydroxide	Copper oxychloride

Fig. 5.27. Copper fungicides.

control several diseases in vine (PLASVIT), potato and tomato (PHYTIN), hop (*P. humuli*), banana (e.g. MYCFIJ), coffee (*Colletotrichum kahawae*) and tea (*Exobasidium vexans*). The development of copper-based products continues, and copper tallate has been described as a synergist to a range of organic fungicides (Soyez, 1992).

Copper, as Cu^{2+}, is readily accumulated by sensitive fungi. It forms complexes with enzymes that possess sulfhydryl, hydroxyl, amino or carboxyl groups, inactivating them and leading to a general disruption of metabolism and breakdown of cell integrity.

As with all immobile protectants, copper products have to be used frequently in order to achieve adequate coverage and to maintain disease control in a growing crop. Copper products have to be made relatively insoluble in water to avoid the phytotoxic effects of the copper ion. Although fungicidal efficacy may be depressed, this also reduces the loss of product by rainfall and can benefit long-term control provided the initial application is adequate and the plant is not in a phase of rapid growth.

M2; Sulfur

Sulfur was the first effective fungicide and its use may be traced back many centuries (Large, 1958). Sulfur acts as a protectant fungicide through the inhibition of conidial germination. The use of a combined sulfur and lime product to control powdery mildew in fruit was first described in the early 19th century, and sulfur products and mixtures (estimated to be over 20) are still extensively employed in apple, grapevine and other cultivated crops.

Sulfur acts against several biochemical sites. It inhibits respiration through its reduction product, hydrogen sulfide, disrupting proteins and forming chelates with heavy metals within the fungal cells. The selective activity of sulfur against powdery mildews may be attributed to their unique and exposed growth habit or to possible uptake by the lipid layers of conidia.

Sulfur also exhibits pronounced acaricidal activity, for example against spider mites, and in wet and warm (above 35°C) weather conditions may be phytotoxic.

M3; Dithiocarbamates

The discovery of the dithiocarbamate family of products in the 1930s and 1940s is usually accepted as initiating the period of organic synthesis of fungicides.

As with most immobile protectants, dithiocarbamates are broad-spectrum fungicides with uses as foliar, soil and seed treatments in fruit (VENTIN, *Taphrina deformans*), grapevine (PLASVIT), vegetables (PHYTIN, BOTCIN, *Alternaria* spp., *Septoria* spp.), sugarbeet (*Cercosporella beticola*), tobacco (*Pseudoperonospora tabacina*) and hops (*P. humuli*). The dithiocarbamates are inactive against the powdery mildews (*Erysiphales*).

Examples of the dithiocarbamates are ziram, zineb, ferbam and thiram (Fig. 5.28). Generally, dithiocarbamates are not phytotoxic but can induce damage in some crops in exceptional circumstances, for example in the use of mancozeb or zineb on zinc-sensitive plants.

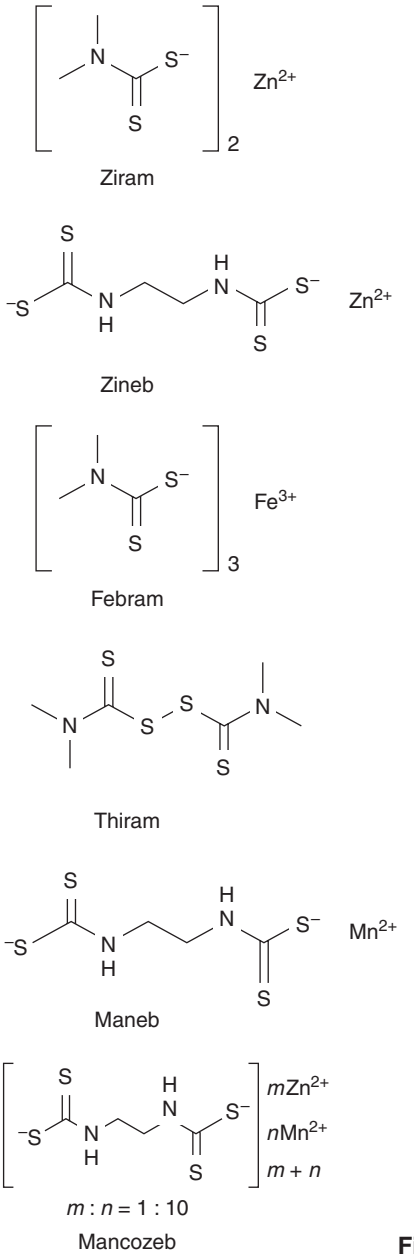


Fig. 5.28. Dithiocarbamate fungicides.

M4; Phthalimides

Phthalimides were introduced in 1952 with the announcement of captan and a close analogue, folpet (Fig. 5.29). They provide protectant control of a wide range of fungal pathogens, are used extensively as sprays, root dips and seed treatments, and are useful in the control of damping-off of seedlings. They have been used to control

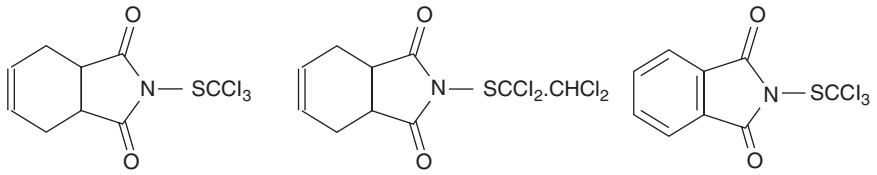


Fig. 5.29. Phthalimide fungicides.

PHYTIN, VENTIN, PLASVIT and BOTCIN and many other foliar ascomycetes. They are inactive against members of the powdery mildews.

Captan, captafol and folpet preferentially react with enzyme sulfhydryl groups but may also attack amino groups and inhibit enzymes that do not contain sulfhydryl groups.

M5; Chlorophenyls

Chlorothalonil, introduced in the mid-1960s, is a major protectant fungicide. It is recommended mainly for use alone or in mixtures to control *Septoria* spp. in cereals, *P. infestans* in potatoes and *Botrytis* spp. in vegetables and ornamentals, as well as finding uses in paints and preservatives (Fig. 5.30). Chlorothalonil binds to sulfhydryl and mercapto groups (Tillman *et al.*, 1973). It is widely used as a mixing partner with fungicides to improve the spectrum and for protection against fungicide resistance.

Fungicide Redistribution in Crops

Under conditions of continual challenge by pathogens, non-mobile compounds have to be applied several times to a growing plant in order to maintain commercially acceptable levels of disease control. In comparison, fungicides that move within the plant are generally more flexible to use because most have the inherent ability to control established pathogens, thereby providing the user with a wider window of application and higher levels of efficacy.

The same principles apply in crops. Immobile fungicides must be applied pre-infection and, in dense crops, using high spray volumes to achieve effective canopy

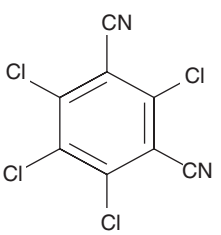


Fig. 5.30. Chlorothalonil.

penetration and foliar coverage. In rapidly growing crops such as grapevine, applications may be necessary every 10–12 days. Fungicides that redistribute within a crop allow the grower to minimize application volume and the number of treatments per season. For example in cereals, mobile fungicides may not only combat established infection through curative or eradicant activity but, through redistribution in the crop, may also provide protection from disease for 28–42 days, depending upon the product and the target pathogen.

Mobility is an important fungicide attribute and may occur in several ways.

- Interplant movement through:
  - vapour-phase activity; and
  - redistribution by rain.
- Intraplant movement through:
  - xylem transport;
  - phloem transport; and
  - diffusion.

Vapour-phase activity

Many fungicides have low vapour pressures. Some, like fenpropimorph, even possess a strong and distinctive smell which may linger for several days after treatment in the field, depending on the temperature and wind speed. For many years it has been known that some fungi are able to take up chemicals in their vapour phase (Fries, 1973) and it is likely that the redistribution and field performance of some commercial fungicides are profoundly influenced through their activity via the air (van Gestel, 1986).

The activity of sulfur is well recognized as involving a temperature-dependent volatilization process. Similarly, the activity of some immobile surface protectants may be best explained through their redistribution in the vapour phase, as coverage of leaf tissue is rarely complete in practical applications. Chlorothalonil, for example, is known to contaminate glasshouse screening tests through its redistribution in air. Some systemic compounds, however, are notoriously difficult to use in research tests carried out within the confines of a glasshouse. Many, including fenpropimorph, metalaxyl and several DMIs, are known to have vapour pressures sufficiently low to control pathogens spatially removed from their site of application (Table 5.7). Clearly, the effectiveness of fungicides through the vapour phase will be controlled by both their vapour pressure and their intrinsic activity. Compounds with relatively low vapour pressures will be effective if their intrinsic activity is high.

Fungal structures that are exposed to the air, such as powdery mildew mycelium and sporangiophores and conidiophores, are especially susceptible to fungicides active through the vapour phase. Their high lipid content may favour the uptake of lipophilic fungicides. Sporangia of PLASVIT, for example, have been shown to be particularly sensitive to metalaxyl present in the surrounding air. The antisporulant activity reported for many fungicides may be based on their vapour-phase effects on sporulating structures.

The practical value of the volatile component of fungicide redistribution in crops is difficult to quantify. Temperature and the nature of the surface impacted by the

Table 5.7. Vapour pressure of fungicides.

Compound	mPa (25°C)
Chloroneb	400
Fenpropidin	17
Tridemorph	6.4
Fenpropimorph	2.3
Tetraconazole	1.6
Propineb[a]	1
Metalaxyl	0.75
Sulfur[b]	0.527
Pencycuron[a]	0.5
Penconazole[a]	0.21
Prochloraz	0.15
Carbendazim[a]	0.09
Cymoxanil	0.08
Chlorothalonil	0.076
Fenarimol	0.065
Iprodione	0.057
Propiconazole	0.056
Cyproconazole	0.0346
Tricyclazole	0.027
Carboxin	0.025
Benomyl	0.0049
Tebuconazole[a]	0.0013
Captafol	0
Cuprous oxide	0

[a]At 20°C.
[b]At 30°C.

fungicide both affect volatilization. It may be argued that in open situations, high temperatures may lessen the performance of relatively volatile products through dilution in the vapour phase and loss of product. In contrast, the most effective use of volatile compounds may be achieved in glasshouse crops where high air concentrations of the active materials can be induced and maintained (Szkolnik, 1983; van Gestel, 1986).

Volatile fungicides are also used in the control of postharvest diseases of stored fruit or produce that has been wrapped in fungicide-impregnated and heat-sealed packages.

Phloem mobility

Few fungicides are translocated via the phloem in effective quantities, most so-called systemics being redistributed within the apoplast and hence restricted to diffusion as for example in translaminar mobility, xylem transport driven by transpiration or diffusion.

Crop physiology can modify the extent to which a fungicide can be redistributed. Materials restricted to apoplastic movement because of their physicochemical characteristics can appear to behave as phloem-mobile compounds. Such fungicides, when

applied to graminaceous crops, may in part be redistributed by a combination of droplet runoff to the base of the impacted leaves and translaminar movement to the basal apex where an accumulation of fungicide may occur. Subsequent development of the apex will then transfer the fungicide to tissues not present at the time of application. The same fungicide applied to a broad-leaf crop, for example apple, will appear to have different mobility characteristics. In this case, because little passive accumulation of the active material can occur around the apical meristems, redistribution from leaves impacted by the fungicide spray will be restricted to movement in the transpiration stream. Quinoxyfen, a new compound from DowElanco, is reported to have exceptional long-term activity against ERYSGT, displaying an ability to redistribute to leaf tissue not present at application. The compound is also active against UNCNEC, powdery mildew of vine, but in that crop appears to lack the same degree of long-term control. Reasons for this are not clear but may, in part, be attributed to differences in the passive movement and accumulation of the product in the two crops.

Access to both phloem and xylem elements of the vascular system is essential if long-distance movement and long-term control are required. Some problematic fungal pathogens may be controlled effectively only by phloem-mobile compounds. Soil-borne and root pathogens such as *Gaeumannomyces graminis* (take-all of cereals), although susceptible to many fungicides in *in vitro* tests, cannot be reliably controlled in field situations because of the spatial separation between the site of infection and the site of fungicide application. Some seed treatments, for example triadimenol, can suppress infection but acceptable control levels have not been achieved. Existing foliar fungicides are unable to redistribute to the roots via the phloem and are ineffective, even though they may possess a high level of intrinsic activity against the pathogen. The control of such pathogens will involve a significant advance in fungicide discovery and will open up new and potentially valuable markets.

When compared with the idea of using soil-applied fungicides to control root diseases, the advantages of phloem-mobile, foliar fungicides are clear. Such materials would operate at much lower application rates and would ease the potential environmental problems associated with soil absorption of fungicides and their breakdown products leaching to deeper soil layers, entry into water tables and toxicity to soil microorganisms.

Phloem transport is a common characteristic of herbicides and insecticides. In fungicides, significant phloem transport has been reported for only a few materials, notably fosetyl, and it may be that the physicochemical requirements for symplastic movement oppose those that govern uptake by, and movement in, target fungi. To date, alterations of chemical structure that favour phloem mobility have not been successful in producing fungicides that are commercially interesting. The requirements governing the binding of compounds to a hydrophobic target site are contrary to the characteristics necessary to achieve efficient movement of candidate fungicides across cuticular membranes.

Compliance with several physicochemical qualities that define levels of lipophilicity, hydrophilicity, steric parameters, ionization and distribution of charge density is necessary for a compound to enter the plant and move within the symplast (Grayson and Kleier, 1990; Kleier, 1994). The general categories that govern phloem mobility are the pK_a, or acid strength, and the log K_{ow}, or lipophilicity. Compounds that are phloem-mobile have low log K_{ow} values and are acidic (low pK_a). The two characteristics may, to some extent, balance, so that compounds that are highly lipophilic may

be phloem-mobile if they are also acidic. The problem here is that while they readily gain access to the phloem, they may be just as easily lost to the surrounding tissue.

Sugars and sugar conjugates are naturally transported in the phloem tissue. Some are actively loaded but for others, for example flavonoids, no active transport mechanism is known. Sugar conjugation with fungicidal materials may improve their mobility in target crops.

The activation of systemic natural defences in plants is mediated by an endogenous transmission mechanism which utilizes compounds such as salicylic acid that have low K_{ow} values and are acidic. Compounds that possess acidic groups or groups from which acidic groups can be derived by hydrolysis (proacids) may be phloem-mobile. Conversely, strong bases or compounds that possess quaternary nitrogen-containing heterocyclic rings may also be phloem-mobile through a reduction in lipophilicity.

Other strategies to achieve effective yet mobile products include the use of pro-fungicides and transgenic plants. Pro-fungicides are, in themselves, inactive. They rely for success on the ability of the host plant to modify their chemical structure, rendering them mobile and fungitoxic. Thus a highly lipophilic compound, for example a lipophilic ester of the fungicide, is able to penetrate the cuticular barrier but, once inside the crop host, generates a compound of reduced lipophilicity (fungicide), which is then free to be transported. However, how the structure of the fungicide is then manipulated to reverse the process in order to reach the site of action in the colonizing pathogen is not clear. Transgenic plants may be able to carry genes to generate an active agent from a phloem-mobile, low-lipophilic pro-fungicide.

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6 Fungicide Resistance

Key Points

- Fungicide resistance is a critical factor in the development and use of fungicides.
- Resistance affects the majority of current major fungicide classes.
- The study of fungicide resistance has been impacted significantly by genomics.
- Fungicide resistance can be managed by careful use of integrated disease management principles and by using minimum doses, mixtures and alternations of fungicides.

Introduction

Resistance to fungicides has grown in importance in the last 20 years and now ranks as the central preoccupation of the fungicide industry. Despite extensive fungicide use in the previous 90 years, resistance emerged as a practical problem as recently as 1970. Significantly, the incidence of resistance has been restricted largely to systemic fungicides that operate against single biochemical targets (single-site inhibitors). These were introduced from the mid-1960s onwards and include the majority of the major newer groups of fungicides (Table 6.1).

Resistance to fungicides is manifested as failures of previously efficacious products to control disease. In such circumstances, the entire economic rationale of fungicide use is removed. Fungicide resistance has united the industry because resistance to one fungicide typically affects fungicides with the same MOA regardless of whether the manufacturer is the same or different. Thus, it is in the interests of all fungicide companies, and also farmers and consumers, that the efficacy of fungicides is protected as far and for as long a period as is possible. Hence the industry has united to form the FRAC (www.frac.info) which collates information and dispenses advice.

Crop losses resulting from a breakdown in disease control can be spectacular, as occurred in northern Greece following the outbreak of benzimidazole resistance of *Cercospora beticola* in sugarbeet and in Western Europe following the loss of metalaxyl control of PHYTIN. The consequent crop husbandry and financial implications were significant, involving changes in management practice and potential yield loss (Pasquereau, 1994).

More recently, fungicide resistance was observed in barley powdery mildew populations grown in Western Australia. Very susceptible cultivars had been grown for 10–20 years. When disease problems emerged, cheap triazole fungicides, especially tebuconazole, were widely and exclusively used. As a result mutant strains of ERYSGH emerged. The resulting losses were estimated at AUS$100 million per annum or about

Table 6.1. Major instances of fungicide resistance. (From http://www.frac.info/.)

Group name (abbreviation)	Fungicide common name(s) (a selection)	Risk level; high or medium or low (current assessment)[a]	Years between introduction and emergence of field resistance[b]	Comments
A1; Phenylamides (PAs)	Benalaxyl Metalaxyl	H	2	Cross-resistance in various oomycetes
A2; Hydroxy-(2-amino-) pyrimidines	Bupirimate Ethirimol	M	2	Cross-resistance in various powdery mildews
B1; Methyl benzimidazole carbamates (MBCs)	Benomyl Carbendazim Thiabendazole Thiophanate	H	2	Resistance common; associated with target site mutations in β-tubulin gene: E198A,G,K and F200Y. No apparent fitness penalty. High resistance factors (RFs)
B2; *N*-Phenylcarbamates	Diethofencarb	H	Not known	Target site mutation in β-tubulin gene: E198K. Negative cross-resistance to MBCs
C2; Succinate dehydrogenase inhibitors (SDHIs)	Carboxin Bixafen Sedaxane Boscalid	M to H	3	Several target site mutations known; cross-resistance observed. Apparent fitness penalty. Medium RFs
C3; Quinone outside inhibitors (QoIs)	Azoxystrobin Picoxystrobin Pyraclostrobin Trifloxystrobin	H	2	Target site mutations G143A and F129L. Cross-resistance. High RFs for G143A. Intron at 143 protects against resistance
D1; Anilinopyrimidines (APs)	Cyprodinil Mepanipyrim Pyrimethanil	M	5	Target site mutations in BOTCIN

E1; Quinolines	Quinoxyfen Proquinazid	M	4	Cross-resistance known. Fitness penalty
E3; Dicarboximides	Chlozolinate Iprodione Procymidone Vinclozolin	M	5	Resistance common. Target site mutation in OS-1 I365S
G1; Demethylation inhibitors (DMIs) (sterol biosynthesis inhibitor (SBI) Class I)	Prochloraz Fluquinconazole Metconazole Propiconazole Tebuconazole Tetraconazole Prothioconazole	M to H	7	Resistance is common with many combinations of mutations in *Cyp51* gene(s), promoter mutations in *Cyp51*. Moderate RFs. Cross-resistance moderate to high within DMIs; variable and sometimes negative with other SBI classes. Also efflux pump mutation especially in BOTCIN
G2; Amines ('morpholines') (SBI Class II)	Fenpropimorph Tridemorph Spiroxamine	L to M	34	Sensitivity shifts observed
G3; (SBI Class III)	Fenhexamid	M	12	Field experiments
H5; Carboxylic acid amides (CAAs)	Dimethomorph Flumorph	H	2	Target site mutations known in *CesA8* genes
I2; Melanin biosynthesis inhibitors–dehydratase (MBI-D)	Carpropamid	M	6	Field resistance known

[a]H, high; M, medium; L, low.
[b]Data from Brent and Hollomon (2007a,b).

AUS$20/ha. Fortunately, the introduction of new fungicides from different MOAs and the replacement of the most susceptible cultivars are expected to reduce the disease to an acceptable level within a 3- to 6-year timeframe (Tucker *et al.*, 2014).

Definitions

The fungicide resistance literature has a confusing vocabulary. As in all areas of science, it is important to be clear what various terms mean.

Resistance and sensitivity

Resistance and sensitivity are different sides of the same coin. A rough test is to grow fungal isolates on a concentration of fungicide that controls wild-type strains. This dose is known as the 'discriminatory dose' (DD; Fig. 6.1). Strains that can grow on the DD are said to be resistant.

A more precise technical definition of resistance or sensitivity is the concentration of a fungicide required to inhibit growth to 50% of the level achieved in the absence of the fungicide – this is called the half maximal effective concentration or EC_{50}. EC_{10} and EC_{90} (the concentration required to inhibit growth by 10% or 90%) are also used for some purposes. EC_{50} values apply to one strain rather than a species as a whole.

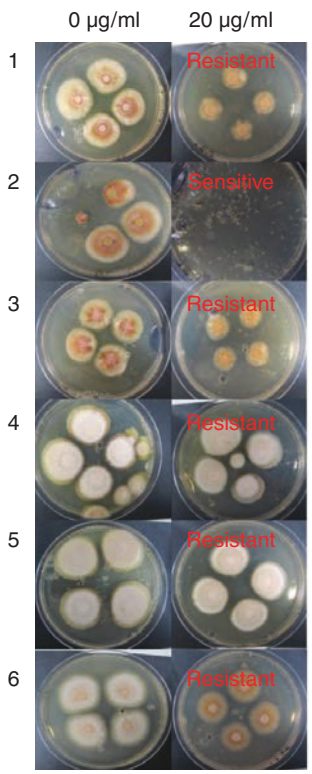


Fig. 6.1. A discriminatory dose test for six strains of *Ascochyta lentis* using thiabendazole at 20 µg/ml for 7 days. Strain 2 was classified as sensitive while the other strains were classified as resistant.

The EC_{50} values of a range of isolates of a range of pathogens are important baseline data that are required for fungicide testing and should be carried out before a fungicide is introduced into new regions.

Non-obligate fungi can be tested in *in vivo* growth measurements. These can take the form of radial growth assays in which agar plates (see Fig. 4.2) with increasing concentrations of fungicide are prepared. The fungus is inoculated into the centre of the plates, the plates are incubated for some days and the diameter measured when the control plate has reached close to the boundary. The data are plotted and the concentration at which 50% growth inhibition occurs is calculated. Radial growth assays are easy and simple and do not require the fungus to sporulate, but take a good deal of time, material and space.

More precise and higher-throughput assays can be achieved using microtitre plates. In these, 96 wells can be used to test one to 96 isolates at one to 96 concentrations of fungicide (Fig 6.2). Growth of the fungi is measured by turbidometric measurements using a microplate reader. Large amounts of data can be acquired directly to computer. The EC_{50} calculations can be automated and the data stored for future use. Microplates are, however, only suitable for fungi that can be induced to form spores in culture.

Obligate pathogens must be tested in *in planta* assays in which a range of fungicides is applied and the degree of fungal growth assessed in an appropriate way. Figure 6.3 illustrates such an assay for ERYSGH and tebuconazole. These assays are the most requiring of time, space and material.

Resistance can be intrinsic or acquired. Intrinsic resistance is a property of the species. Thus oomycete fungi are resistant to triazoles; intrinsic resistance is related to 'spectrum'. Acquired resistance is a property of individual strains within a species.

Resistance factor

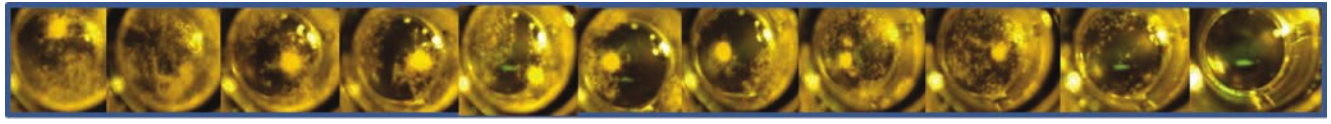
The resistance factor (RF) is the ratio of the EC_{50} of a 'resistant' isolate to that of an apparently normal or sensitive isolate. Isolates of a pathogen vary in myriad properties and so EC_{50} values will vary between isolates of a sensitive or naïve (i.e. one that has not been exposed to the fungicide) population. Such variation can be a factor of ten or 100, but would vary between an EC_{50} in the range of 10–1000 ng/ml for a useful fungicide. Hence a meaningful RF can be either between two isogenic strains of the same species or, more usually, between the EC_{50} of a suspect strain and the average EC_{50} of a set of naïve strains.

RFs can be divided arbitrarily into low (<5), moderate (5–20) and high (>20). Higher RFs occur when the mutation giving the resistance gives a very high level of resistance. In some circumstances, low or moderate RFs are termed tolerance rather than resistance. It can also be called 'lower sensitivity'.

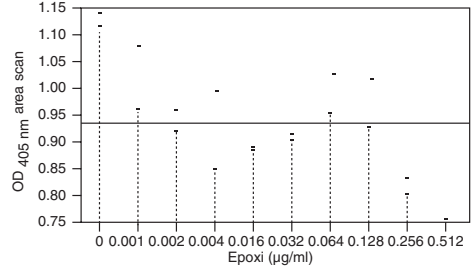
Field resistance

Field resistance is what really matters. It can be defined as the failure of a fungicide applied efficiently at the maximum permitted rate and frequency to give adequate control of the disease. Its occurrence depends on two factors:

(a)



(b)



Level		Mean
0	A	1.1290000
0.001	A B	1.0200000
0.064	A B C	0.9915000
0.128	A B C	0.9720000
0.002	A B C D	0.9410000
0.004	B C D	0.9220000
0.032	B C D	0.9085000
0.016	B C D	0.8875000
0.256	C D	0.8175000
0.512	D	0.7580000

(c)

Epoxi concn (µg/ml)	Log Epoxi concn	% inhibition	Log % inhibition
0	N/A	0	N/A
0.001	−3	0	N/A
0.002	−2.698970004	0	N/A
0.004	−2.397940009	0	N/A
0.016	−1.795880017	0	N/A
0.032	−1.494850022	31.66818044	1.500623111
0.064	−1.193820026	30.42688465	1.483257488
0.128	−0.89279003	45.9582198	1.662363198
0.256	−0.591760035	63.03360581	1.799572151
0.512	−0.290730039	87.28428701	1.940936069
1	0	95.64032698	1.980641052

(d)

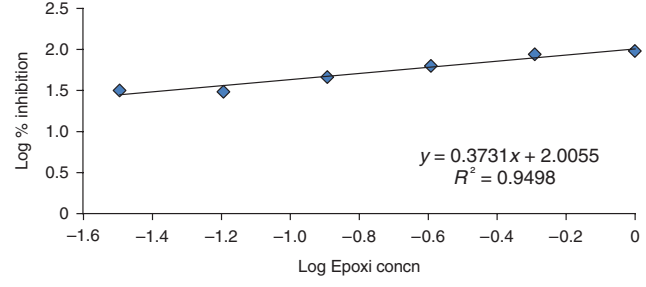


Fig. 6.2. (a) Growth of a non-obligate fungus in a microtitre plate with increasing concentrations of epoxiconazole (Epoxi) for 48 h. (b) One-way analysis of OD_{405nm} area scan versus Epoxi concentration (left) and table showing mean OD_{405nm} of replicate tests (right). (c) Table showing log transformation of Epoxi concentration and of percentage growth inhibition. (d) Plot of log percentage inhibition against log concentration and its use to calculate the EC_{50} of 0.151 µg/ml.

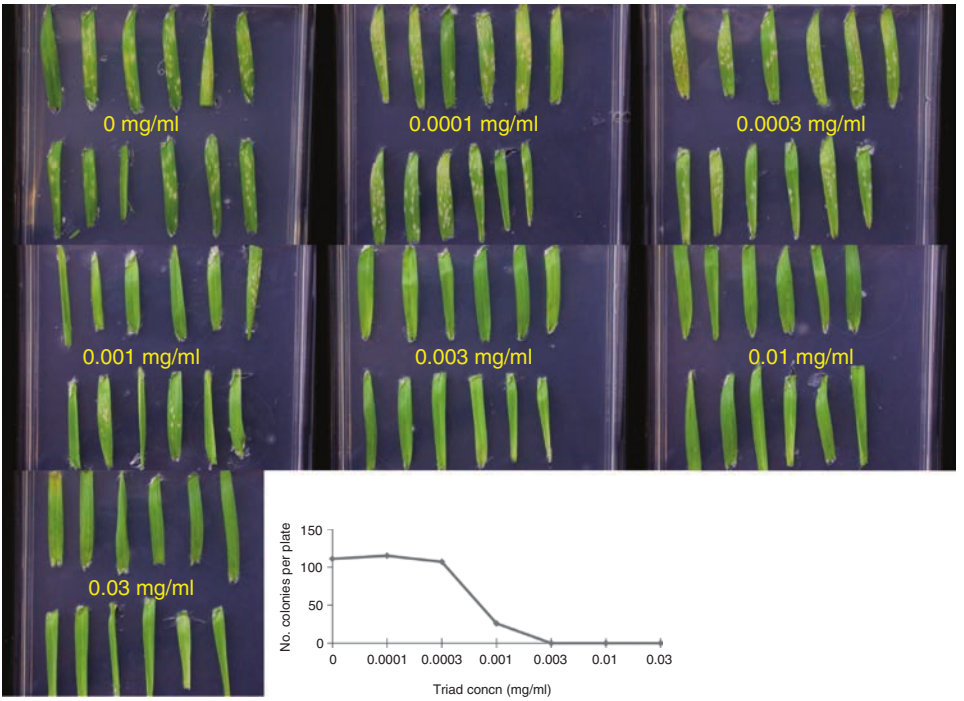


Fig. 6.3. Barley leaves infected with a single spore-derived isolate of barley powdery mildew were placed on benzimidazole agar amended with increasing concentrations of triademifon (Triad). The ED_{50} is estimated to be close to 0.001 mg/ml.

1. Whether the RF of resistant strains is high enough to protect the fungus against the field rate of the fungicide.
2. Whether the prevalence of the resistant strains is high enough to enable them to dominate the population.

Cross-resistance

Cross-resistance is the phenomenon when a strain resistant to one fungicide is found to be altered in resistance to another fungicide. The two fungicides are then said to exhibit cross-resistance. Cross-resistance is a quantitative parameter. In some cases, the RF with one fungicide is similar to another. This is typically the case with QoI and MBC fungicides. Partial cross-resistance applies when the RF with one fungicide is much lower than with another. This is the case with triazole fungicides.

Most cases of cross-resistance involve fungicides from the same MOA. Indeed, cross-resistance has often been critical evidence identifying and linking the MOAs of different fungicides as was the case with the CAA fungicides (Blum *et al.*, 2010). Cross-resistance typically involves target site mutations where mutations are found in the gene encoding the target site.

Cross-resistance is normally described as positive; that is, the resistant strain is more resistant to both fungicides than the wild-type strain. Or to put it

another way, both RFs are greater than 1. There are a few cases of negative cross-resistance. Here the strain resistant to one fungicide is more sensitive to another fungicide than the wild type; that is, one RF is >1 and the other is <1. This can occur when mutations in the target site gene alter the physical conformation of the target site. Negative cross-resistance can occur if the mutated target site binds the second fungicide more tightly than does the wild-type target site. It has been observed in fungicides that target β-tubulin and the *Cyp51* gene.

Multiple resistance

Where cross-resistance involves fungicides from different MOAs, the mode of resistance (MOR) is likely to involve non-target site mutations. These are mainly alterations in efflux pumps. Such pumps are capable of restricting the inflow of fungicides from multiple different classes and thereby decrease the intracellular concentration. Efflux pump resistance has been observed particularly in BOTCIN (Mernke *et al.*, 2011; Leroux and Walker, 2013). Unlike herbicides and insecticides, resistance due to conjugation of the pesticide to glutathione or sugars has not yet been observed in fungi.

Fitness penalty

Fungicides select for mutations in the pathogen population that confer a selective advantage on the strain in the *presence* of the fungicide. The selective advantage may be expressed as a high EC_{50}. If the mutation is significant in the field, the proportion of the pathogen population that carries the mutation will increase until it dominates the population from season to season. Such strains are said to carry a fitness advantage in the presence of the fungicide. The term fitness is used in the evolutionary sense: 'survival of the fittest', and thus applies to overall ability to reproduce and cause disease from year to year.

A very important question is whether the mutant strain is as 'fit' as the wild-type sensitive population in the *absence* of the fungicide (or in the presence of a fungicide with a different MOA). If the mutant population is less fit than the wild type in the absence of the fungicide, the resistant strain is said to carry a fitness penalty.

There are many potential reasons why a resistant population might carry a fitness penalty. It may be that the target site mutation which confers resistance has the side-effect of reducing the efficiency of the enzyme at the target site. This appears to be the case for *Cyp51* and SDHI fungicides. In the case of efflux pump resistance, it may be that the metabolic energy required to synthesize and drive the pumps represents a significant drain on the resources of the pathogen.

If the fitness penalty is substantial, removal of the fungicide should allow the re-emergence of the sensitive population of the pathogen. In this case, the previously compromised fungicide could then be usefully deployed again, for a while at least. And (it is hoped) better fungicide resistance management strategies can be applied.

Resistance Risk

The risk that resistance will develop is clearly an important parameter. It defines the sustainability of the fungicide product over several seasons. Resistance risk is affected by the properties of pathogen, the fungicide class and the way the fungicide is used in the field.

Pathogen risk factors

Fecundity; latent period; sexual reproduction

Fungicides that are mutagenic would not proceed to the marketplace. A number of stringent tests are applied to fungicides to ensure that they have no mutagenicity. Instead, fungicides merely select strains that have enhanced resistance by enforcing an evolutionary selection pressure (Paveley *et al.*, 2014). When diseases are poorly controlled the fungal population size expands rapidly to a number that is large compared with the size of its genome and the number of genes carried. Fungi typically have genome sizes of 40 to 100 million base pairs and express 10,000 to 20,000 genes. Normal processes of spontaneous mutation caused by ultraviolet or other radiation, by environmental chemicals and by failures of DNA replication repair processes would be expected to generate changes in 1×10^6 genes and 1×10^9 base pairs per nuclear generation. Thus if a billion spores are produced in a pathogen population, most base pairs in the genome would be altered in at least one strain that is present. It has been estimated that 100 m^2 of barley infected with powdery mildew would have a 95% chance of containing a strain with a given mutation (Brent and Hollomon, 2007a,b). It therefore is apparent that pathogens that produce large numbers of spores are at a higher risk of developing resistance than those that produce fewer spores.

When a mixture of the mutant strain and the wild type has been treated with a fungicide, the normal evolutionary processes come into play. A high proportion of the wild-type strain will be killed by the fungicide whereas some at least of the mutant population (and a higher frequency than the wild type) will survive and reproduce. The proportion of the population that is resistant will increase but it is unlikely to be high enough to be immediately noticeable. However, if the pathogen population reproduces frequently and the fungicide selection is reapplied, then the selection can be applied time and again and the resistant population can increase in frequency until it comes to dominate the population. The result then is field resistance. Thus pathogen species that reproduce multiple times within a season are higher risk. Or to put it another way, pathogens with short latent periods are high risk. Seed-borne pathogens that only have a single life cycle per season are low risk. In contrast, pathogens that have short life cycles and can infect for an extended period of the growing season are high risk (Fig. 6.4).

High fecundity is associated with pathogens that produce wind-borne spores primarily. Rain-splashed spores are intermediate in resistance and water-borne and soil pathogens are the lowest risk.

Some cases of fungicide resistance involve mutations in more than one gene. In other cases, the fungicide resistance mutation was in a strain that was only weakly virulent on the crop cultivar used in that field; another strain of the same pathogen had mutated to be strongly virulent on the crop cultivar but had not acquired the fungicide resistance mutation. In both these cases, combinations of genes would be much more

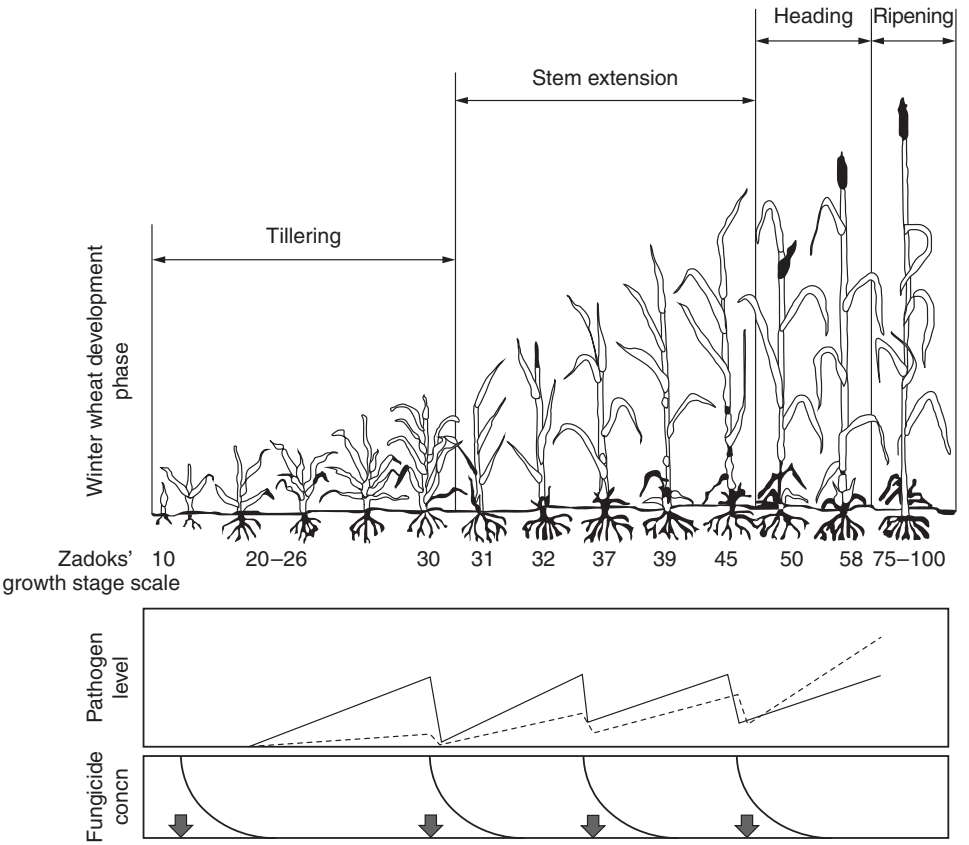


Fig. 6.4. A polycyclic pathogen with a short life cycle controlled by multiple fungicide sprays (fungicide applications arrowed) is at high risk of resistance evolution (– – –, resistant; ——, susceptible).

of a threat than the single mutations. Pathogen species that are able to undergo sexual reproduction and hence recombination therefore are more likely to evolve strains capable of combining several mutations that confer a significant selective advantage.

The asexual or epidemic growth stage of most plant pathogens is haploid. Consequently, mutational changes are expressed immediately and, provided the mutant is fit, its development in the fungal population is rapid. A notable exception is the oomycete fungi in which the asexual stage is diploid and the haploid phase is generated during the sexual stage of development. Similarly, in the *Basidiomycota*, such as the rusts, each cell is a dikaryon (binucleate) and performs as a diploid.

Based on these factors we can divide fungi into three classes: low, medium and high risk, and compare these classes with the now 30-year history of fungicide resistance. Table 6.2 summarizes relevant features of some important pathogens and their history of resistance development.

This crude analysis shows that, by and large, the theoretical prediction has been borne out by experience. BOTCIN, powdery mildews, MYCFIJ, PLASVIT and PHYTIN have consistently been the first species to display resistance to fungicides.

Table 6.2. Fungicide resistance pathogen risk factors.

Pathogen	Fecundity	Latent periods	Sexual reproduction	Resistance prediction	Resistance history
Rhizoctonia	Low	Few	No	Low	Low
Rusts	High	Many	Yes (some)	High	Low
Soil-borne pathogens; smuts and bunts	Low	Few	Some	Low	Low
SEPTRI	Medium	Medium	Yes	Medium	High
Rhizoctonia solani	Medium	Medium	No	Medium	Medium
BOTCIN	High	Many	No	High	High
Powdery mildews	High	Many	Yes	High	High
PYRIOR	High	Many	No	High	Medium
VENTIN	Medium	Medium	Yes	High	High
MYCFIJ	High	Medium	No	Medium	High
PLASVIT	High	Medium	Yes	High	High
PHYTIN	High	Many	Yes (since 1990 in Europe)	High	High

One unexpected exception is the rusts, which have many of the characteristics of high-risk pathogens – large population sizes, air-borne spores, short life cycles, sexual reproduction – but have so far failed to display significant resistance. One postulated explanation is the diploid nature of the infective organism. If the resistance mutation acts in a recessive or semi-dominant manner, mutation of both alleles would be necessary to achieve field resistance. This is of course much less likely than a single mutation. However, other diploid pathogens such as PHYTIN have a history of resistance development and the rusts are notorious for overcoming gene-for-gene based resistance, which again requires two alleles to mutate. There appears to be a discrepancy between prediction and experience for rusts that defies explanation. It would appear prudent to remain vigilant for cases of resistance in rusts.

Fungicide risk factors

History has demonstrated that the risk of resistance differs markedly between fungicide groups. Table 6.1 gives the time in years between the introduction of a fungicide and the emergence of field resistance. Some fungicides have never developed significant resistance whereas others have developed resistance in as short a period as 2 years. Understanding the reasons behind these differences has become a major goal of the fungicide industry because it might allow the design of fungicides with a lower risk of resistance.

One approach is experimental. In this scenario, a large population of a test fungus is treated with the fungicide to determine whether any spontaneous resistant mutants can be detected. To reduce the size of the population that needs to be tested, the fungus can be treated with a mutagen such as ultraviolet or gamma rays, azide or ethyl methanesulfonate. Model fungi such as *Saccharomyces* or *Neurospora* are often used for this purpose because these species are easy to handle in the laboratory and have well-developed genetic resources that can be used to determine the MOR, should resistant

mutants be detected. Other high-risk fungi such as BOTCIN and PHYTIN are also used. And despite the technical difficulties even powdery mildews have been tested.

Laboratory mutants have been found for a large number of fungicides (see http://www.frac.info/, pathogen risk list). In the majority of cases, field mutants have not so far been found. And when field mutants have been found, the genotype of mutants found in the laboratory differs from that found in the field. The successful recovery of laboratory mutants indicates the potential for that species/fungicide combination to develop resistance in the field. Failure to find field mutants resistant to the fungicide can arise from two factors. Firstly, it may be that the fungicide has not been applied to a large enough area over a long enough time for resistance mutants to develop. Secondly, it may be that the resistant mutants carry a sufficient fitness penalty that such strains die out.

Monitoring for field resistance

In the past, reports by growers of occurrences of fungicide failure were the first indications that resistance might have developed. The primary interaction was normally between the fungicide reseller and the grower. If the disease developed despite the application of the new and expensive fungicide, the grower normally wasted no time in letting the reseller know. The reseller then typically reported back to the local company representative who would then try and obtain an isolate from the affected field for analysis in the laboratory. Experience showed that the great majority of cases could not be ascribed to resistance. Much more likely were problems with the fungicide batch, adjuvants, weather conditions, spray equipment and spray coverage.

In view of these factors and because of the supreme importance of resistance to fungicide companies, monitoring for resistance for new and existing fungicides has become a much more systematic activity. Dedicated field trials are used and intensively monitored. National organizations, such as the HGCA in the UK, carry out these trials (see http://www.hgca.com/cms_publications.output/2/2/Publications/On-farm%20information/Fungicide%20activity%20and%20performance%20in%20wheat.mspx?fn=show&pubcon=9243). Each major fungicide company carries out its own trials along these lines also, although the results are not necessarily made public immediately. The trials target high-risk pathogens and use a range of concentrations to determine the efficacy graph. The trials are repeated year on year so any declines in efficacy are apparent. In addition, a large number of farmers' fields that have been treated with fungicides are inspected each year and unusual cases of disease are noted. In the UK this is called Crop Monitor (http://www.cropmonitor.co.uk/). Suspect isolates from these studies can be collected and tested under controlled conditions.

Determining the mode of resistance

Should resistant mutants be recovered from laboratory studies or the field, they can be used to determine the MOR. This field of research has been impacted significantly by recent developments in genomics (Cools and Hammond-Kosack, 2013). The goal is to identify the gene(s) that have mutated and been selected to give the resistance. Basic parameters will be collected; the frequency of mutants, the EC_{50} on the test

fungicide and whether cross-resistance is found to other fungicides. Cross-resistance of fungicides from different MOAs would indicate non-target site mutations. If the fungicide is related to known MOAs, the target site genes can be amplified by PCR and sequenced. Genetic analysis, crossing the mutant strain to a wild type, is possible in some fungi and was used to determine the MOR of CAA fungicides (Grenville-Briggs *et al.*, 2008).

If the MOR is still unknown after all these analyses have been carried out, the newer genomic methods can be applied (Cools and Hammond-Kosack, 2013). With few exceptions, the genome sequences of all major target pathogens have now been determined (for an updated list, see http://www.genomesonline.org). In principle, it would therefore be a simple matter to sequence the genome of a resistant isolate and identify changes in the genome compared with the reference genome. Unfortunately the general level of sequence variation between isolates is very high, so identifying the mutation responsible for the fungicide resistance requires further evidence. One type of further evidence is to sequence more strains, both resistant and wild type. Any sequence variations that occur between wild-type strains can be discarded. Similarly, any sequence variation in common in the resistant strains and absent in the wild type will pinpoint the likely affected site. A second type of evidence is to examine gene expression into mRNA in the wild-type and mutant strains. Gene expression data can easily be obtained using RNAseq techniques. These have largely displaced the chip-based technologies. Genes that are expressed at a higher level in mutant compared with wild type, in the absence or especially the presence of the fungicide, will gives clues both to the MOA and the MOR.

Fungicide Resistance in Different Fungicide Classes

Multi-site fungicides

Fungicides that act against several biochemical targets (multi-site inhibitors) are typically immobile, surface-acting protectants and are regarded as zero- to low-risk compounds. With few exceptions, their effectiveness has remained constant throughout many years of intensive use against a wide variety of pathogens.

Mercury fungicides were first described in the late 19th century and were used extensively as cereal seed treatments for broad-spectrum disease control. Their effectiveness against *Pyrenophora graminea*, the causal organism of barley leaf stripe, began to decline only in the 1980s, attributed to the development of resistance operating through the increased efficiency of mercury efflux from the fungus. In contrast, no resistance to copper-based fungicides has been reported even though resistance to copper toxicity has been observed in bacteria, yeasts and higher plants. This strongly suggests that the genes that govern similar resistance to copper toxicity in fungi are absent.

Fungal resistance to other multi-site inhibitors, such as the dithiocarbamates, phthalimides and sulfur, is unknown. The durability of chlorothalonil is of particular value. It is currently used as a mixing partner with high-risk fungicides such as QoI both to extend the spectrum but also to decrease the chance of resistance (Hobbelen *et al.*, 2011). Although multi-site inhibitors are severely restricted in their commercial

applications and value, their non-specific MOA has clear advantages over specific target-site fungicides in terms of resistance development.

Single-site fungicides

Fungicides that target a single vulnerable site are more prone to resistance development than multi-site fungicides. Whether field resistance emerges is dependent on the following factors:

- the RF associated with the resistant mutation(s) – this determines the ability of the mutant to grow and reproduce after treatment with field rates of the fungicide; and
- the presence and scale of a fitness penalty in the viability of mutant strains – at one extreme resistance mutations are lethal, in others the mutant is partially compromised, while in others there is no deleterious effect.

The MORs come in four forms:

1. Mutations of the target site gene rendering the gene product more insensitive to the fungicide.
2. Overexpression of the target site gene so that the total capacity of the target pathway is not severely affected.
3. Upregulation of efflux pumps such that the internal concentration of the fungicide is kept below a critical level.
4. Detoxification of the fungicide via glycosylation, or other chemical modification. In contrast to herbicides, this MOR is not important in current fungicides.

These factors are illustrated by discussing the six major fungicide classes that have been most significantly affected by resistance.

Methyl benzimidazole carbamates

The benzimidazoles were among the first systemic fungicides to be marketed. They were hailed as a magic bullet and so when resistance appeared it sent shock waves through the industry. Resistance first appeared just 2 years after their introduction.

C. beticola is a leaf spot pathogen and is prevalent in all areas where sugarbeet is grown, but causes commercially significant levels of disease only in regions with warm summers. The speed of disease establishment increases with increasing daily mean temperature. Additionally, the pathogen requires high humidity for infection and is favoured in crops where overhead irrigation is used.

Ideal conditions for the disease occur in northern Greece, where sugarbeet cannot be grown without the use of fungicides. Traditional methods of control used immobile protectant fungicides, notably fentin acetate, but under high disease pressure such products gave inadequate levels of control, especially in sprinkler-irrigated situations where fungicide wash-off from treated foliage occurred.

The benzimidazoles were among the first systemic fungicides to become available to the grower. In 1967, field testing of benomyl against *C. beticola* showed a twofold superiority in control compared with the organotins. Support grew for the replacement

of protectant fungicides with the new systemics, and by 1972 more than 3000 ha were treated exclusively with benomyl.

Previous seasons, 1970 and 1971, had been encouraging with excellent disease control being maintained by benomyl. By July of 1972, however, a catastrophic decline in control was observed. Within 20 days the proportion of infected leaves per plant increased from 5–10% to 80–100%. Increasing the application rate and frequency of application had no effect on the level of disease control. In comparison, the traditional use of organotin products, maintained in side-by-side field plots with benomyl, performed as expected (Table 6.3; Dovas, 1975).

At first the loss of disease control was attributed to the weather conditions, but soon the real cause of the phenomenon was discovered to be resistance. Prior-use patterns of benomyl in 1970 and 1971 correlated with the occurrence of resistance in 1972.

In 1973, the high selection pressure of the benzimidazoles was demonstrated in experimental plots. A low initial disease incidence of less than 5%, caused by resistant strains of *C. beticola*, increased to over 90% in less than 6 weeks, following only two applications of benomyl. Resistant strains were of equivalent fitness to the sensitive strains, in common with other benzimidazole-resistant fungi.

The genetic basis of the resistance was studied using the model fungus *Neurospora* and shown to be a single gene (Borck and Braymer, 1974). The gene was identified as that encoding β-tubulin in the yeast *S. cerevisiae* (Thomas *et al.*, 1985). The β-tubulin gene is highly conserved and with the advent of PCR and DNA sequencing techniques it was quickly shown that most resistant mutants in different species not only involved the same gene but also the same small number of DNA sequence changes. The changes most commonly seen are E198A,G,K or F200Y (see Box 6.1 for an explanation of nomenclature rules describing sequence variations). Indeed, the mutant versions of these genes were used as selectable markers in fungal transformation experiments. This absolutely verified that this mutation was the primary cause of the field resistance (Cooley *et al.*, 1991). The RFs associated with these changes are very high. Indeed, the resistant mutants are so resistant that it is hard to dissolve an inhibitory concentration of the fungicide. Furthermore there appears to be no fitness penalty. The resistant mutants are 100% of the populations in affected species.

Negative cross-resistance to the *N*-phenycarbamate diethofencarb and the new benzamide class of tubulin inhibitors zoxamide has been reported. In these cases, isolates that are resistant to benomyl are sensitive to diethofencarb and zoxamide and vice versa. It may therefore be possible to use these newer fungicides to control the MBC-resistant pathogens. An alternation strategy would seem to have great potential.

Table 6.3. The performance of benomyl and fentin acetate against *Cercospora beticola* in northern Greece, 1970–1972. (From Dovas, 1975.)

	Proportion of diseased foliage (%) in mid-August	
Treatment	1970	1972
Benomyl, 300 g/ha	5.9	85.9
Fentin acetate, 500 g/ha	19.3	39.6
Control	100	100

Box 6.1. Nomenclature for the description of sequence variations. (From den Dunnen and Antonarakis, 2001.)

A standard nomenclature has been developed that allows researchers to quickly and precisely describe nucleotide and amino acid sequence changes in genes.

Both systems refer to the number in the gene sequence. This can be confusing as homologous amino acids in different species can have different numbers because of indels in genes. Thus the SEPTRI CYP51 amino acid 524 is the homologue of the ERYGH amino acid 509.

Changes at the DNA level use the > sign. So 12T>A means the thymidine at position 12 is converted to an adenosine.

For amino acids, the one-letter amino acid code is used. Changes at the amino acid level are in the form wild-type amino acid – number – new amino acid. An example would be the CYP51 D134G. Here, the aspartate at position 143 is changed to glycine. If the amino acid is changed to several different amino acids, the form would be H272Y,R,L. If the amino acid was deleted, this is designated ΔY459; if two amino acids, this is ΔY459/G460. Insertions are designated ins. So W4_R5insK means that a lysine is inserted after a tryptophan at position 4. Frame shifts are designated with fs. So W4fsX8 means that an insertion in codon 4 causes a frame shift at codon 8. Introduction of a stop codon, X, at position 189 (e.g. G189X) would delete the entire C terminus from that point.

Amino acid	Three-letter code	One-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Deletion	Del	Δ
Stop codon		X
Frame shift		fs
Insertion		ins

Quinone outside inhibitors

Resistance to QoI fungicides appeared within 2 years of their introduction around 2000 (Bartlett *et al.*, 2002; Gisi *et al.*, 2002). The resistance was first observed in cereal powdery mildew but has since spread to affect many but not all pathogens. Significant examples are SEPTRI, UNCNEC and other powdery mildews. As before, no rusts have developed resistance. RF values are very high (>100) and while all curative activity is lost, some preventive activity remains for some fungicides in this class.

The target site of QoIs is cytochrome b. The gene encoding this protein is found in the mitochondrial genome, which led some theorists to predict that it would be protected from resistance. Instead a very consistent pattern emerged whereby the mutation G143A was found in this gene in essentially all of the affected pathogens. In a few cases, the F129L mutation has been found but this is associated with lower RFs. There was complete cross-resistance with all other QoIs but no other fungicide classes. There appears to be no significant fitness penalty associated with resistance.

Mutations in this region of the protein prevent docking of the fungicide and fully explain the resistance (Gisi *et al.*, 2002). It is interesting that the fungus that produces the lead compound, *S. tenacellus*, has a CytB with different amino acids in this region.

The identification of the MOR as a change in the sequence of the *CytB* gene led researchers to develop PCR assays to monitor populations. The *CytB* gene is very highly conserved and so degenerate primers should amplify a similar-sized region from different species. Comparison of this region in the wheat tan spot pathogen *Pyrenophora tritici-repentis* and the barley net blotch pathogen *P. teres* identified that the latter had an intron which interrupted the codon for the glycine at position 143 (Sierotzki *et al.*, 2007). Both pathogens had isolates with moderate RF with the F129L mutation; this is of little field significance. However only tan spot had the G143A mutation and these had large RFs and were uncontrolled in the field. It seems that the intron in the 143 codon of *P. teres* prevents the selection of the G143A mutation. The nucleotide change needed to alter the codon from G to A alters the splice site such that the mRNA would never be successfully processed. As *CytB* is an essential gene, such mutations would be lethal. In other words, the mutant strain has zero fitness.

This led researchers to quickly scan other target genomes for the 'blessed' intron. Introns have been found in rust mitochondrial genomes, thereby explaining their failure to develop resistance to QoIs. This was also the case in BOTCIN (Yin *et al.*, 2012). The presence and number of introns in various species vary markedly and does not follow the phylogeny of the species. Therefore it is by no means impossible that intron-free isolates of species exist somewhere in the world. We should therefore remain vigilant for resistance even for species where the examined populations contain these introns.

The early and dramatic appearance of resistance to QoIs in so many very important pathogens galvanized the industry into developing resistance management tools. The most important was to use QoIs only in combination with another fungicide, normally a triazole or chlorothalonil. Azoxystrobin is sold as a mixture with cyproconazole in the product Amistar Xtra; pyraclostrobin is sold as a mix with epoxiconazole in Opera. This both improves the spectrum and modelling studies indicate it will lengthen the effective life of the products (Hobbelen *et al.*, 2011). In addition to mixtures, alternations of fungicides are also recommended. As a result of these actions, sales of QoIs have remained very strong. With their very low mammalian toxicity, the QoIs have a secure place in the market for many years to come.

Succinate dehydrogenase inhibitors

Succinate dehydrogenase is a complex protein within the mitochondrial membrane comprising four subunits, A–D. All four proteins are encoded by nuclear genes. The original SDHIs were carboxin and oxycarboxin, which had a spectrum limited to basidiomycetes. A resistant strain of *Ustilago maydis* was found to harbour a mutation in subunit B-H272L (Broomfield and Hargreaves, 1992). Although SEPTRI was not commercially controlled by carboxin, resistant mutants could be found in the laboratory with two different mutations B-H272Y,L (Skinner *et al.*, 1998). Transformation of this tractable species with the B-272Y version showed conclusively that this mutation conferred the resistance and identified the target site.

Since 2003 a range of other SDHI fungicides has been released. Resistant mutants in species such as BOTCIN and SEPTRI have been found in the field. A number of sites are affected such as B-P225L,F,T as well as B-H272Y,R,L in BOTCIN (Veloukas *et al.*, 2013). The numbering of orthologous amino acids differs slightly between species. The mutations give general cross-resistance. RFs are moderate and early studies indicate that mutants have a significant fitness penalty (Sierotzki and Scalliet, 2013). Hence SDHI fungicides are regarded as medium to high risk. Most released products contain a second fungicide. After MBCs and QoIs, the industry is taking a cautious approach and monitoring resistance closely (Fraaije *et al.*, 2012).

Demethylation inhibitors

Resistance to G1 DMIs has crept up slowly over the last 20 years and is now a serious issue for this group of fungicides. The target site for DMIs is the *Cyp51/Erg11* gene encoding sterol C14-demethylase. DMIs have been the mainstays for disease control especially in cereals since the 1970s. Unlike the MBCs and QoIs, there were no obvious cases of catastrophic failure to catch the attention of the industry. Instead, a gradual decline in the efficacy of certain DMIs was observed and ascribed to various factors.

Research into resistance to medical DMIs and laboratory studies prepared the ground (Hippe and Koller, 1986), but it was not until the mid-2000s that resistance was linked to genetic changes in field isolates of pathogens (Cools *et al.*, 2006; Cools and Fraaije, 2013). Since then a plethora of studies have been published which detail the pattern of cross-resistance, RFs and the MORs (Cools and Fraaije, 2013).

Growers were reporting that they were having to use higher and higher doses to achieve the same level of control. When strains from these fields were examined the RFs were found to be moderate – 20–50. This explains why catastrophic failures were never found. Furthermore, whereas some older DMIs were obviously suffering from resistance, newer DMI fungicides remained as potent as upon release.

The research has highlighted three MORs.

1. Target site alteration leading to reduced sensitivity to some DMIs.
2. Target site overexpression enabling the fungus to survive higher doses of fungicide. A factor here is that some species have two or three *Cyp51* genes. Overexpression of one paralogue appears to confer resistance.
3. Non-target site mutations in efflux pump genes.

Explanations for these findings emerged as genomic technologies were applied to the problem (Cools *et al.*, 2006). Changes in sensitivity were associated with genetic changes in the *Cyp51* gene. A very large number of individual mutations were found. Some were never found singly but only in combination with other mutations.

In order to link phenotype to genotype, a yeast expression assay used previously in medical research was employed (Cools *et al.*, 2010). In this assay, the yeast gene encoding a *Cyp51* orthologue is placed under the control of an inducible promoter. A vector with the pathogen *Cyp51* is inserted into the yeast. Expression of the yeast gene is then switched off. If the pathogen *Cyp51* encodes an active enzyme, the yeast cell can grow. If so, the yeast strain is now dependent on the pathogen's *Cyp51* gene for ergosterol biosynthesis. Hence the EC_{50} values of various DMI fungicides can be tested in an isogenic context. This system can therefore be used to link the various mutations in pathogen *Cyp51* to function. It is a reductionist system that excludes the role of any mutations in other genes in the pathogen.

Using this system several mutations in the SEPTRI *Cyp51* gene have been shown to confer resistance to some of the DMIs. Examples are L50S, Y459D, Y461H, D134G, V136A, Y137F, Y461S and S524T, and the two-amino-acid deletion Δ459/460 (Fraaije *et al.*, 2007; Cools *et al.*, 2010, 2011). This work has been linked to field studies that isolated the pathogen from trial sites treated with different generations of DMI. The frequency of different mutations was compared with the fungicide used. Strains that appear in fields treated with a particular fungicide are deemed to be resistant to and selected by that fungicide. Thus it appears that early DMIs such as tebuconazole selected for the Y137F mutation whereas later DMIs such as epoxiconazole and prothioconazole selected for the S524T mutation. Some mutations appear only in combination with others. The mutation I381V also selects for tebuconazole and difenoconazole but counter selects against prochloraz (Fraaije *et al.*, 2007). RFs vary from 1 (i.e. no effect) to 50 between the different DMIs. These mutations are also found in rusts but did not result in field resistance (Stammler *et al.*, 2009). This proves that rusts are not inherently immune to fungicide resistance.

The yeast studies reveal which mutations are capable of complementing the yeast gene (i.e. they generate an active enzyme) and how well the yeast strain grows. Overall it appears that the Cyp51 enzyme cannot change by single steps into forms that both retain full levels of activity and exhibit high levels of resistance. Combinations of mutations have been selected that represent a compromise between these two parameters. Further combinations of these mutations encode genes with even higher RFs and adequate enzyme activity. These combinations of combinations would be highly unlikely to arise from scratch but can accumulate in a stepwise fashion when DMI use is continued despite a noticeable drop-off in efficacy. The solution to this 'escalator of resistance' is presumably to use other MOAs instead of DMIs. In practice that may mean using mixtures and alternations of fungicide MOAs.

Overexpression of the *Cyp51* gene has also been linked to resistance (Cools *et al.*, 2012). This phenotype is linked to insertions in the promoter of the gene. The RFs are in the range of 7–15 and the same regardless of which DMI is tested. The interpretation is that the Cyp51 enzyme is working at near full capacity during fungal growth. Inhibition by a DMI therefore has a noticeable effect on flux through the pathway and this can be detected as both a reduction in growth rate and the

accumulation of toxic sterols (Bean *et al.*, 2009). Overexpression of the gene produces more enzyme and therefore compensates for the reduction in specific activity. The insertions in the promoter have been found in several species.

The *Cyp51* gene is present in one, two or three copies (paralogues) in different species (Hawkins *et al.*, 2014). All species have at least one *Cyp51* and this appears to be an evolutionarily very old enzyme (Kelly and Kelly, 2013). *Rhynchosporium* has three genes and one, *Cyp51A*, is upregulated in DMI isolates (Hawkins *et al.*, 2014). Similarly there are three genes in *F. graminearum* and this explains why *Fusarium* is not well controlled by DMIs because it is necessary to inhibit all three. Each one has a different profile of sensitivity to DMIs, giving it in-built insensitivity to field rates of these fungicides.

Carboxylic acid amides

It was only when studies of resistance to CAA fungicides were concluded that the MOR and the MOA were identified. CAA fungicides are specific to oomycete pathogens and had been suspected of interfering with cell wall biosynthesis. Resistance was detected in PLASVIT within 2 years of use but had not been detected in PHYTIN even after prolonged use. The resistant PLASVIT mutants were cross-resistant to all CAA fungicides, mandipropimad, dimethomorph and iprovalicarb, indicating a target site mutation. Laboratory PLASVIT resistant mutants were crossed with the wild type (Gisi *et al.*, 2007). Genetic mapping focused attention on the cellulose synthase gene *CesA3*. This identified the MOA. Single nucleotide polymorphisms in the gene segregating with resistance identified the MOR (Blum *et al.*, 2010). The mutation G1105S required two nucleotide substitutions. Resistance to CAA fungicides is regarded as moderate risk mainly because of the features of the target organisms. A resistance management plan is in place.

Acylalanines

The acylalanines are specifically active against oomycete fungi. Acylalanines inhibit RNA biosynthesis through their interference of the activity of a nuclear, α-amanitin-insensitive RNA polymerase–template complex.

The repeated use of (and dependence on) metalaxyl, applied in the field to provide growers with flexible control of downy mildews, established a continuous and high selection pressure that favoured the development of resistance. Resistant strains spread very rapidly. Some cases of resistance in PHYTIN on potatoes, PLASVIT on grapevine, *Pseudoperonospora cubensis* on cucumbers and *Peronospora hyoscyami* f. sp. *tabacina* on tobacco developed within a single season.

In 1984, it was shown that nucleic RNA polymerase isolated from a metalaxyl-sensitive strain of *P. megasperma* f. sp. *medicaginis* could be partially inhibited by metalaxyl, whereas the RNA polymerase from a similar isolation using a metalaxyl-resistant strain was unaffected (Davidse *et al.*, 1984). The mechanism of resistance, therefore, is associated with a mutational change in one of the RNA polymerases. However the mutation responsible for the resistance has not been identified.

The Management of Resistance

Fungicide resistance is now recognized as a fact of life for the fungicide industry. Therefore a series of practices has been recommended by fungicide manufacturers and national agricultural advisory services. A typical example is the advice collated by the UK-based Fungicide Resistance Action Group (FRAG; see http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/Resistance-Action-Groups/frag).

Its advice is based on the premise that 'Good resistance management is based on limiting the level of exposure of the target pathogen to the fungicide'. Hence FRAG advises the following nine concepts.

1. Fungicide input is only one aspect of crop management and other control measures should always be used, such as good hygiene through disposal of crop debris and control of volunteer crops which may harbour disease.
2. Always aim to select varieties exhibiting a high degree of resistance to diseases known to be prevalent in your area, in addition to the main agronomic factors you desire.
3. Avoid growing large areas of any one variety, particularly in areas of high disease risk where the variety is known to be susceptible.
4. Only use fungicides in situations where the risk or presence of disease warrants treatment.
5. Use a dose that will give effective disease control and which is appropriate for the cultivar and disease pressure.
6. Make full use of effective fungicides with different MOAs in mixtures or as alternative sprays.
7. Ensure that mixing partners are used at doses that give similar efficacy and persistence.
8. Monitor crops regularly for disease and treat before the infection becomes well established.
9. Avoid repeated applications of the same product or MOA and never exceed the maximum recommended number of applications.

Some of these pieces of advice have been validated by experiment or by modelling whereas others are considered to be self-evident. The premise 'Good resistance management is based on limiting the level of exposure of the target pathogen to the fungicide' recognizes the truism that selection for fungicide resistance can only ever occur when the pathogen is exposed to the fungicide, although it is clear that this normally applies to all fungicides with the same MOA. Herein lies the conundrum. A farmer will only use a fungicide if it gives useful control and this inevitably exposes the pathogen to the fungicide. The goal is to achieve satisfactory disease control while delaying or preventing the development of resistance.

Good hygiene

Several of the pieces of advice aim to reduce the total amount of the pathogen in the environment of the crop. Thus Advice #1 recommends destroying volunteer crops and infected crop debris and using clean seeds. The retention of crop debris is clearly

associated with several important diseases (Jørgensen and Olsen, 2007). However, limited tillage techniques are critical for the success of farming in most of the drier arable zones around the world.

Integrated disease management

Advice #2 and #3 acknowledge that genetic disease resistance is a critical part of disease management even when a pathogen is well controlled by the fungicide. Plant breeders have to combine a multitude of traits in order to generate successful cultivars. Disease resistance is only one of these traits and by no means the highest priority in most cases. It is rare therefore for a crop variety to be adequately resistant to *all* the pathogens likely to infect it. A farmer may feel obliged to use a fungicide if even only one disease threatens the crop. And as most fungicides are broad-spectrum, it may be considered that the genetic disease is superfluous.

A further conflict can arise if a crop variety that is resistant to the pathogens of importance has a lower yield than one that is susceptible in the absence of disease. This is known as a 'yield trade-off' (Brown, 2002, 2003). A farmer may calculate that a $20 fungicide spray on a susceptible cultivar may be more profitable than using a cultivar that is resistant but gives a 20 kg lower yield.

The advice on growing a single resistant variety is based on the risk that the pathogen may evolve virulence and thus create an epidemic. This advice underpins the concept of integrated disease (or pest) management. IDM (or IPM) embodies the advice that all control methods should be applied simultaneously. In this way, the fungicide protects the genetic disease resistance because any strain that evolves virulence would be controlled by the fungicide; vice versa, any strain that evolved fungicide resistance would be controlled by the genetic disease resistance.

Dose rate

Advice #3 and #4 can be summarized as using the minimum quantity of fungicide that gives adequate disease control. In the absence of disease, there is clearly no need to use any fungicide. To some extent, this conflicts with Advice #8 to spray before the disease gets established. In practice, most growers will know from experience which diseases are likely to occur and which weather patterns promote their spread. In these cases, spraying early is prudent and conforms with the overall premise of 'limiting the level of exposure of the target pathogen to the fungicide'. Spraying early reduces the total number of pathogen spores (and hence nuclei) that get exposed to the fungicide and hence the chance that a resistant mutant will be subjected to the selection pressure.

The effect of dose on the emergence of resistance has been the subject of intense debate (Shaw and Pijls, 1994; Zziwa and Burnett, 1994). It is now established for the great majority of cases that the lower the dose the lower the risk of resistance. This result is supported by both modelling and experience (Van den Bosch *et al.*, 2011). Rationalization of this finding stems from the simple idea that the resistant isolates of the pathogen survive with higher frequency at all doses of the fungicide (Fig. 6.5). In Fig. 6.5 the selection pressure is represented by the vertical arrows and is higher at higher doses.

Figure 6.5a models a fungicide resistance with a moderate RF. Figure 6.5b represents a high RF; the selection pressure still increases with increasing dose. Figure 6.5c represents a fungicide resistance with a significant fitness penalty. Here the selection pressure is negative at low doses and increases with dose. Figure 6.5d represents a situation not yet seen in fungi but seen in weeds where the survival frequency converges at very high doses. In this case the selection pressure varies both up and down with dose.

The concept that low dose equates to low risk was counterintuitive and contrary to the advice for herbicide resistance. With weeds, a high dose can eradicate a weed population and therefore a grower can be sure that no resistant mutant has survived. If a weed survives a herbicide spray, it can be detected and killed by another herbicide, by mowing, grazing or even burning. Pathogen populations are huge and invisible and so no prior warning of resistance occurs.

More important, however, is the effect of ploidy. Weeds are normally diploid and most herbicide resistance traits are semi-dominant. So if one allele of a herbicide tolerance gene

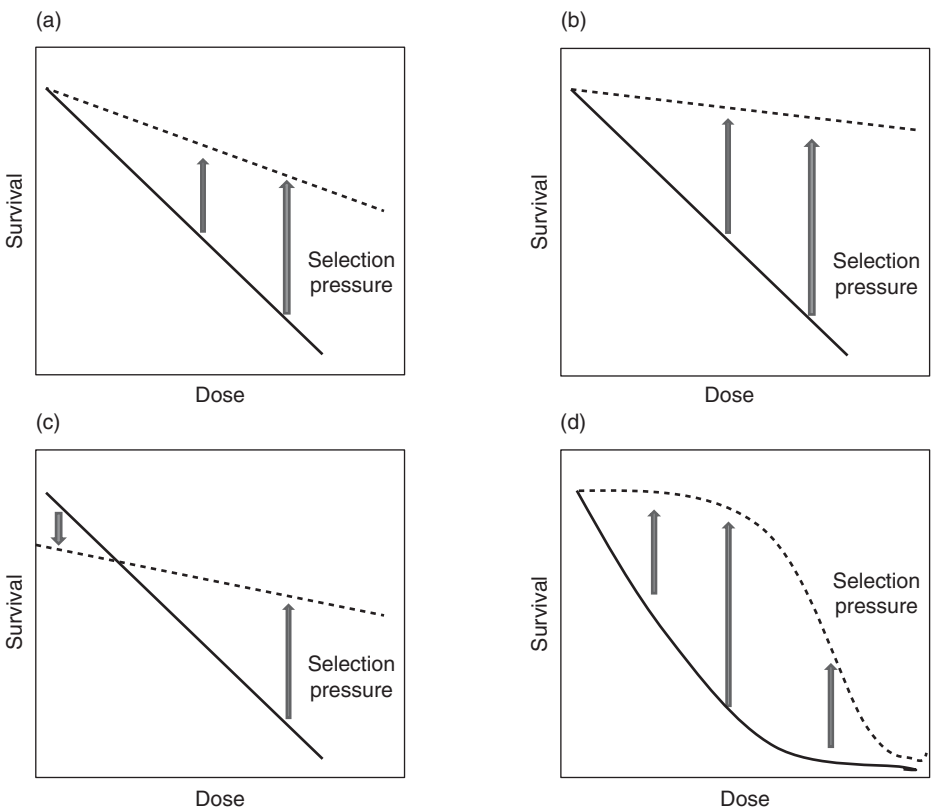


Fig. 6.5. Schematic dose response curves for wild type (——) and resistant mutant (– – –). Panel (a) represents a mutant with a moderate resistance factor (RF) and shows that the selection pressure (vertical arrow) is higher at higher doses. Panel (b) shows a mutant with a high RF; the selection pressure still increases with increasing dose. Panel (c) represents a mutant with a fitness penalty at low dose; the selection pressure at low dose is therefore negative. Panel (d) represents a scenario in which the survival of the mutant and wild type converge at high dose; in these conditions (so far not observed in fungi although seen in weeds) the selection pressure may decrease at high dose.

mutates, this heterozygous plant would survive a moderate dose, higher than the homozygote sensitive but lower than homozygote resistant. The chances of both alleles mutating are tiny. Hence growers are advised to use a dose of the herbicide that would kill the heterozygous resistance plant. If such plants were allowed to grow, some would cross-pollinate and this would create homozygous mutants that can tolerate much higher doses. Most (but not all) pathogens are haploid and so the concept of heterozygous resistance does not apply.

Mixtures and alternation

Advice #9 argues against the repeated use of the same MOA. Accordingly Advice #6 advises using either mixtures or alternation with different MOAs. At a simplistic level, it is easy to rationalize that using different fungicides in either alternations or mixtures would delay the emergence of resistance. Repeated use of the same fungicide MOA applies the selection pressure repeatedly to the already selected population. Regulatory authorities therefore legislate for the maximum number of times an MOA can be used in a given period.

Mixtures or alternations should be a good way to prevent resistance (Hollomon and Kendall, 1997). If a strain resistant to one fungicide survived treatment with that fungicide, it would be killed by the other fungicide. For this to be true the MORs need to be different. Hence fungicide companies are increasingly selling fungicides as mixtures; for example of QoI and DMIs, or QoIs and chlorothalonil. On the other hand, use of a mixture might be thought to promote the selection of mutants resistant to both the fungicides. This has so far not been observed (Hobbelen *et al.*, 2013; Spolti *et al.*, 2013). Mixtures of DMIs may provide protection as different DMIs seem to select different mutations (Fraaije *et al.*, 2007).

Modelling studies have supported the notion that mixtures provide several years of protection against the emergence of resistance (Hobbelen *et al.*, 2011). In that study, mixtures of high risk (e.g. QoI) and low risk (e.g. chlorothalonil) were found to be effective in delaying resistance. The dose of the two fungicides was optimal when the low-risk fungicide was used at the maximum rate and the high-risk one was used at the minimum dose compatible with adequate disease control. This finding equates with Advice #7 requiring 'that mixing partners are used at doses that give similar efficacy and persistence'. It is self-evident that a fungicide can only contribute to resistance management if it is being used at a dose that would have a significant effect on disease if used on its own. Hence it is necessary for researchers to monitor populations of pathogens for loss of sensitivity to solo fungicides even if that fungicide is only used in a mixture in commercial products. Detection of resistance to one mixing partner would remove the rationale for the mixture.

It might be argued that there is a higher risk of developing resistance to both fungicides, either by selection of pump-based resistance or of both single-site mutations. However no cases of this scenario have so far been detected.

Mixtures are relatively easy for the farmer as the product is normally sold as such. Farmers can also 'tank mix' fungicides and add in other pesticides if appropriate. Alternations of fungicides require extra work on the farm. Theoretical studies suggest that both strategies decrease the risk of resistance for rather similar time periods.

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7 Strategy and Tactics in the Use of Fungicides

Key Points

- Fungicides are effective tools to control disease and thus improve the yield and quality of a crop.
- Growers are faced with numerous decisions on how best to use fungicides.
- Strategies are plans fixed at the beginning of the growing season; tactics are plans altered in response to factors emerging during the season. The tactics and strategies are needed to decide:
  - where to apply fungicide – to seed, soil or foliar tissue;
  - which type of fungicide to use;
  - what fungicide dose to use; and
  - when to apply fungicide.
- The optimum answers to these questions will be dependent on myriad factors, only some of which are under the control of the grower. Factors not under farmers' control include:
  - the weather – and how it affects both crop growth and pathogen growth;
  - rainfall during and after fungicide application; and
  - the incidence of disease pressure coming from outside the farmer's property.
- Factors more or less under farmers' control include:
  - disease resistance status (for all relevant diseases) of the crop cultivars used;
  - the timing of sowing;
  - details of seeding – row spacing, seeding rate, row orientation; and
  - fertilizer use.

Where to Apply Fungicide

Seed-applied fungicide

Seed treatment by fungicides has many inherent advantages that favour its widespread use. The first uses of chemicals to protect crops against disease were seed treatments (see Chapter 1). As the seeds can be mixed with fungicides in a closed container, all the seeds should receive a uniform coverage and none of the fungicide can escape into the environment. The fungicide is not brought into contact with soil and so undergoes slower degradation than in the case of in-furrow applications. Furthermore, as the fungicide is automatically physically close to the seed-borne pathogen it has only to penetrate a few cell layers to reach its target. In addition, systemic fungicides will travel up the shoots as the plant germinates and provide some degree of protection for the vulnerable early leaves. Some of the fungicide will leach into the soil where it can suppress soil-borne pathogens.

Seed-borne diseases are very effectively controlled by seed treatments and the treatment is highly cost-effective (Murray and Brennan, 2009, 2010). Theory and experience confirm that seed treatment by fungicide is unlikely to lead to fungicide resistance. Many diseases, such as the bunts and smuts and some powdery mildews, have been effectively neutralized by the current range of seed fungicides.

Soil or in-furrow application

Soil or in-furrow application is more problematic. In this case, the fungicide is mixed with the seeds as they are planted. The targets are pathogens such as take-all and *Rhizoctonia*. These pathogens exist as free-living species in soil and so the goal of the in-furrow application is to suppress the hyphae as they attempt to invade the germinating seed. The fungicide is inevitably bound on to soil particles and suffers microbial degradation due to the soil microflora. As a result, in-furrow applications are restricted to some specialized situations where the disease pressure is high and no options for rotations are available.

Foliar application

The great majority of fungicide applications are to above-ground tissues, including stems, flowers as well as leaves, and referred to as foliar use. Foliar diseases are very obvious to a grower and so there is a large incentive to respond to the presence of the pathogen. In contrast, root diseases are to a large extent 'out of sight and out of mind'.

Which Type of Fungicide to Use

The primary driver to consider when using a fungicide is to reduce or eliminate disease. In principle this would mean spraying the maximum amount of the most effective fungicides at the most frequent permitted intervals. Such a strategy is very unlikely to be the most profitable strategy. It is also likely to be a strategy that promotes the development of fungicide resistance. Hence there has been a great deal of effort in the development of strategies and tactics that aim to use fungicides in the most cost- and time-effective manner. This means that there is no one tactic or strategy that is optimum. The tactics and strategy will vary according the crop species, the variety used (and its resistance to disease), the presence of inoculum and the weather conditions that accompany the growing season. Hence this is a complex subject, requiring the advice of specialist advisors who are knowledgeable about local conditions.

Diagnosis

There is a clear need to identify the fungus causing the disease as different fungicides have different spectra. The use of fungicides that are ineffective against the pathogen

that is present would clearly be a waste of time and money. Prochloraz, noted for its activity and utility against *Pseudocercosporella herpotrichoides* and SEPTRI, is often used in attempts to control *Puccinia* spp., a genus that is not sensitive. Similarly, the same product is used to control ERYSGT – a pathogen that is sensitive to prochloraz – but because of the relatively immobile nature of the chemical, only disease suppression may be achieved, inferior to the control given by a morpholine fungicide.

Products may be wrongly used because of poor commercial advice, but a significant factor may be the inability of many growers to recognize the cause of crop disease. The most common pathogens tend to be recognized successfully, but even in those cases may be confused with totally unrelated organisms. In cereals in 1986, powdery mildew was not positively identified by 11% of farmers; only 80% correctly diagnosed true eyespot, and 28% confused *P. hordei* with *R. secalis* (Smith and Webster, 1986; Table 7.1). More recently, misdiagnosis of herbicide damage as a fungal disease has become common.

Identification aids, usually comprising a series of photographs of symptoms, are now available to growers. The agrochemicals industry also provides information on the identification of pathogens as a promotional tool. Some computer-based packages incorporate diagnostic modules. Others are specific aids to diagnosis, for example the Muskmelon Disorder Management System (MOMS), which caters for the diagnosis of 17 crop disorders including fungal infections, nutrient deficiencies and environmental damage. This system includes the additional sophistication of a capacity to use uncertain data and will provide a probability of successful diagnosis if the situation prevents the complete expression of symptoms. However, it is the need to identify pathogens during their early biotrophic and non-symptomatic growth phase that has stimulated the development of sensitive and highly specific diagnostic aids.

The accurate and timely identification of the causal organisms of crop disease is vital to effective crop protection. The array of fungicides available to the grower and the range of their possible uses against specific pathogens, in combination with other products or in an integrated farming programme, increase the need to make correct diagnoses.

Table 7.1. Ability of farmers to recognize cereal diseases. (From Smith and Webster, 1986.)

Disease	% of farmers correct	% of farmers incorrect	% of farmers who 'don't know'	Disease most often confused with
ERYSGT	89	3	8	SEPTRI
Eyespot	80	4	16	*Fusarium*
PUCCRT	72	5	23	Scald
Yellow rust (early)	13	28	59	Insect
Yellow rust (late)	57	18	35	PUCCRT
SEPTRI	28	27	45	PUCCRT
LEPTNO (early)	24	23	53	*Fusarium*
LEPTNO (late)	23	38	39	Bunt/smut
Foot rot (*Fusarium*)	12	39	49	SEPTRI
Stress disorders	37	35	28	SEPTRI
Nutrient deficiencies	8	38	54	PUCCRT

Methods of identification are worthless if they are not reliable. At worst, they must provide the grower with a good probability of success and list possible alternatives. In addition, diagnostic techniques must operate under a variety of environmental conditions.

The performance and, to some extent, the availability of suitable fungicides are linked to the stage of growth of the pathogen. Regardless of their reliability, diagnostic tests that are slow to carry out may be of little value to the grower if the appropriate control measures cannot be employed in a timely manner. The urgency attached to diagnosis is made more acute in regions that are subject to sudden weather change. In Europe, for example, there are very few days in the growing season that are suitable for the efficient application of foliar fungicides. Reliance by farmers on a tardy diagnostic technique may force them to apply products in suboptimal conditions or at a stage of fungal development that is not ideal, resulting in poor disease control.

Diagnostics must be easy to use and the results obtained by a farmer sitting on the back of a tractor, or by a company representative in a car, must be equivalent to those achieved under laboratory conditions. Traditionally, growers diagnose disease by eye. The method requires experience but is very rapid and allows immediate and appropriate action to be taken. Diagnostic techniques that are inferior in practice to currently accepted methods are unlikely to be adopted. After all, in many cases the farmer may choose to apply a broad-spectrum protectant product rather than commit to the use of a complex diagnostic programme and risk missing a good spray window or increase his overall input costs by having to spray different fields at different times.

In other instances, especially in cereals, the appearance of one pathogen may signal to the grower the need to apply a broad-spectrum product. For example, mildew infection may be controlled using a specific product such as fenpropidin. However, it is more usual for the farmer to combine a fenpropidin/fenpropimorph treatment with a triazole. The second component provides the farmer with an insurance against attack by other pathogens, hence reducing the number of excursions into the crop and lowering total cost.

The use of diagnostics is valuable in improving the reliability of disease identification but has to provide the farmer with cost benefits that are at least equal to those that are currently achieved. However, in those situations where fungal infection is difficult to detect or to diagnose correctly, or when a decision is made to use fungicides only as required, modern diagnostic methods present the grower with highly accurate methods of identification.

Immunology

Immunological detection techniques are of increasing importance in the management of crop diseases (Fox, 1993; Schots *et al.*, 1994). Adapted from similar methods used in medicine, they are rapid and rely upon the detection of an antigen from the fungus under test, visualized as a colour change in the assay. An antigen is any material that can induce an immune response, resulting in antibody production, and immunological methods use the characteristic of antigens to bind specifically with corresponding antibodies. Fungi produce characteristic molecules, often on their surfaces or readily accessible by straightforward extraction methods. The role of immunodiagnostics in plant disease diagnostics is to infer the presence of a potential pathogen

when such fungus-specific antigens are detected through their interaction with an appropriate antibody.

Immunoassays can be used to identify pathogens, for example SEPTRI and LEPTNO, which elicit latent or indistinct symptoms within the host. The tests may be poly- or monoclonal based. Polyclonal-based assays contain a cocktail of antibodies manufactured by test animals challenged by the antigen, usually presented as an injection of a crude extract of the test fungus. Polyclonal assays are non-specific, being able to recognize all fungi containing the components of the antigen. However, many fungi, including non-pathogenic species, produce non-specific antigens, thereby reducing the diagnostic value of polyclonal-based tests. Monoclonal-based assays are highly specific, permitting the detection of particular fungal species or strains. Combinations of the two, of known specificity, can be constructed to support the identification of a predetermined spectrum of pathogens.

Several test kits have been produced, enabling results to be obtained within a timescale that permits a flexibility of response by the grower (Miller *et al.*, 1992; Fox, 1993). In a matter of hours, the presence or absence of a disease threat can be determined, with the extent of any colonization of the tissue under test being proportional to the intensity of the colour change in the assay.

In particular, ELISA techniques have allowed immunodiagnostic methodology to be used successfully in practical situations. Several methods are available but two, the multi-well assay and the dip-stick assay, are probably the most widely adopted. The first employs 96-well microtitre plates, each well coated with the antibody specific to the pathogen to be assayed. Test samples from plants suspected to be infected by the fungus are dispensed into the wells. If the fungus is present, antigens in the extract conjugate with the antibodies on the walls of the microtitre wells. The result is developed after the addition of a second aliquot of antibody, complexed with an enzyme capable of producing a colour change in the test medium. The second method, the dip-stick assay, effectively transfers the ELISA technique to a convenient form. The assay involves the use of nitrocellulose sticks coated with the appropriate antibody. When dipped into a crude extract from plants suspected to be infected, a colour change on the surface of the stick quickly reveals the presence of the pathogen. The technical challenges behind these techniques can be said to have been solved, but except in specialist situations the products remain too expensive for routine use in crop pathology.

Nucleic acid-based diagnostics

Nucleic acid-based methods have inherent advantages over antibody-based tests. The identity of any organism is essentially in the organization of its relatively stable nucleic acid, or genetic code. Methods based on PCR can reveal a one-base-pair difference in one molecule of DNA. Thus DNA-based methods have extreme sensitivity and specificity (Fox, 1993).

The basis of such diagnostic techniques lies in the slight but constant genetic variations that occur between unrelated organisms. Despite this, practical applications of molecular diagnostics are few and far between (Ophel-Keller *et al.*, 2008). Methods remain too slow and certainly too expensive for general use. The costs of a test would seem to be insignificant when considering a decision to buy a farm. Infestation of the land with *Rhizoctonia* would seem to be a very credible reason to use the available

test. However, such kits have not been a commercial success and their use is very largely restricted to researchers.

What Fungicide Dose to Use

All applications of fungicide follow a dose–response or 'efficacy' curve. Figure 7.1 illustrates a typical efficacy curve. The amount of disease declines with increasing fungicide dose. The curve is steep at first but is subject to diminishing returns at higher dose. Reducing disease typically (but not always) has a noticeable effect on yield, which rises to a plateau with dose. The extra yield will translate into greater farm-gate value. The cost of fungicide includes both the application costs (including the use of the spray equipment and staff time) plus the direct costs of the fungicide product. Subtracting the cost of fungicide from the farm-gate value generates a curve that peaks at a middle dose and declines at both low dose, when disease reduces yield, and at high dose, when uneconomical fungicide use reduces overall profit. In most cases, growers will use the fungicide dose that maximizes profit. The maximum dose that can be used might be limited by legislation or by the need to limit the risk of resistance.

The exact value of the parameters illustrated in Fig 7.1 will vary depending on the value of the crop, the cost of fungicide and the progress of the season. All parameters will be subject to year-by-year variability. Some crops, such as many horticultural products including grapes, are sufficiently valuable that the costs of fungicide applications are comparatively minor. In these cases, growers are likely to take a 'safety first' approach and apply fungicide regardless of the prospects for disease. In others, such as broad-acre cereal crops and especially where yields are less than 2–3 t/ha, fungicide costs are significant and therefore controlled tightly. Growers will think carefully before deciding whether to apply or not.

When to Apply Fungicide

Having decided whether to apply a fungicide, the next question is when to apply the product. Three strategies are in use: (i) to apply when the crop reaches a certain

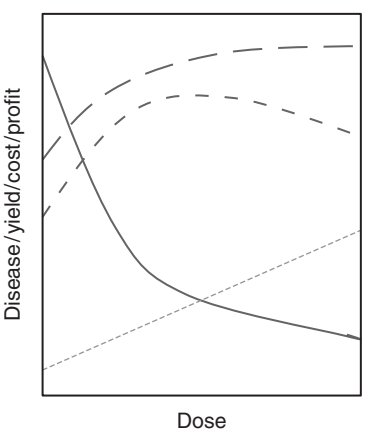

Fig. 7.1. An idealized efficacy curve in which the *x*-axis plots dose and the *y*-axis plots disease (——), yield (— — —), fungicide cost (· · · · ·) and profit (– – –). The amount of disease will decline with increasing fungicide dose. The resultant yield will increase with dose and plateau. The cost of fungicide increases with both dose and number of applications. The resultant profit curve will typically peak at a level at which some disease is apparent.

growth stage; (ii) to await a certain threshold level of disease; and (iii) to use a disease-threat model.

Growth stage strategies

Growth stage strategies are best suited when both the crop value and the probability of disease are high. Figure 7.2 illustrates the options for a cereal crop. The principle underlying this strategy is to place a dose of fungicide on the plant so that the most productive leaves are protected from disease by the pathogens. In higher-yielding areas with dense canopies, the flag leaf contributes the dominant fraction of the yield and so strategies to protect this stage are optimal. On the other hand, an earlier spray may prevent the multiplication of the inoculum and so lead to less disease overall.

Disease threshold strategies

Disease threshold strategies rely on monitoring of the crop to determine when the threat level is high enough to warrant the application of the fungicide. Clearly a threshold strategy is dependent on the vigilance of the grower detecting the disease and the rate of development of the epidemic from this point forward. It also depends on the speed with which the grower can mobilize a spray programme. Spraying requires appropriate weather conditions; too much or too little wind can prevent spraying as can imminent rain.

Detecting the disease can be as simple as walking through the grower's field or talking to neighbours. Vineyards have traditionally grown roses at the ends of rows. Rose mildew is caused by a different pathogen but the conditions that cause it are

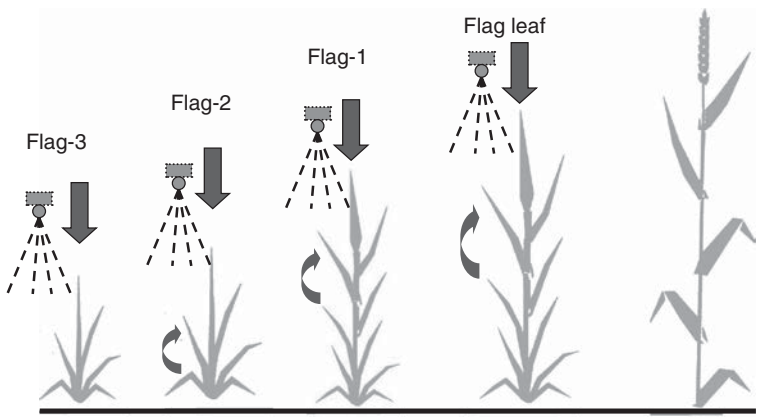

Fig. 7.2. Disease control across a cropping season; time and growth stage. The growth of cereals is accompanied by the emergence of successive layers of leaves up until the flag leaf. Growth stage fungicide strategies apportion fungicides to protect the most productive leaves.

similar to those that cause grape mildew. It therefore is simple (and pleasant) to walk through a vineyard and examine the roses for mildew. Detecting can also take the form of spore traps operated from research stations, vehicles or even flying drones. Detection and monitoring of pathogen populations is a very active area of research.

This strategy depends on the principle that prevention is better than cure. Fungicides differ in their preventive and curative activities, as described in Chapter 5. In general, preventive activity is much better than curative. However, too early application of a fungicide means that it will decay in concentration due to solar radiation and being washed away by rain, before the pathogen arrives. Too late and the disease level will have built up so that it overwhelms the fungicide. The weather prediction is also important. Most diseases are promoted by wet weather, so if rain is not forecast it may be safe to ignore a small level of disease. Conversely, a forecast of rain may justify a fungicide spray.

Disease-threat models

Disease-threat models use weather data to predict when particular diseases are likely to reach a level when spraying is warranted. Such models input parameters of temperature and humidity in real time. The parameters are best worked out for the downy and powdery mildew diseases of grapes. The parameters predict hours of leaf wetness, and when a threshold is reached, spraying is initiated. Experience has shown that such models need to be reduced to simple rules of thumb before they are well adopted. In the future we can expect mobile phone alerts initiated by state or university researchers, advisory services or even fungicide resellers to become the norm.

Frequency of application

Most fungicides have a relatively short window during which they are effective. Sun, rain and transport through the plant will reduce the effective concentration of the fungicide over a period of time most likely to be less than 2 to 3 weeks. Therefore if the disease threat spans a longer time period it is likely to be economic to spray more than once; indeed in high-value crops and high disease situations, sprays may be a frequent as weekly. If sprays are close together, the effective concentration never declines to zero (Fig. 7.3). Wider spacing may allow a window during which infection can initiate. The timing of the individual sprays can, like for a one-spray strategy, be driven by growth stage, disease levels or prediction models.

Multiple sprays are more expensive than a single spray and therefore must be justified by greater yields. The cost of multiple sprays can be mitigated by applying a reduced dose – so-called split-dose strategies. In most countries, labels specify the total concentration in the growing season, rather than at any one spray. Furthermore, residues in the final crop may be an issue that prevents sprays occurring after a certain period.

Having decided to spray more than once, it is generally a good strategy to alter the fungicide that is used, ideally using a different MOA. Using different fungicides improves the spectrum of disease that is controlled and mitigates against resistance (Hobbelen *et al.*, 2013).

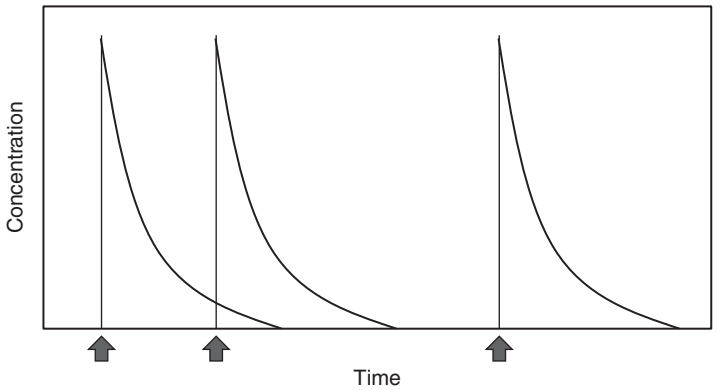

Fig. 7.3. Fungicide concentration as a function of application frequency (fungicide applications indicated by arrows).

The different fungicides can be incorporated into the spray programme as mixtures or as alternations (Fig. 7.4). Most likely the dose will be split between several applications. It is clear that there are essentially infinite parameters than can be altered. The success of a particular strategy can only be predicted with moderate confidence, even after extensive field trials. Thus the strategy used will be a combination of prediction, experience and convenience.

Interaction with Fertilizers

Crop nutrition has always been recognized to interact with crop protection. Indeed, right through the latter part of the 19th century the concept that disease was due to poor crop nutrition rather than microbial pathogens was maintained by Lawes (Money, 2006). It is not surprising that he was a fertilizer manufacturer, but we can be grateful that his fortune was used to establish the research centre at Rothamsted (http://www.rothamsted.ac.uk/), a key site of disease and fungicide research for over 170 years.

The basis of the controversy lingers because of the evidence that crops that have suboptimal fertilizer regimes are more susceptible to disease. This is a complex area and firm conclusions are hard to make. However, there is some support for the view that over-fertilized crops (and particularly for nitrogen) are hypersusceptible to pathogens and especially biotrophs. Effects on necrotrophic pathogens are less clear (Solomon *et al.*, 2003). A particular effect of potassium on disease has been noted (Brennan and Jayasena, 2007). The clear message is to make sure the fertilizer regime is balanced and at an appropriate level.

Apart from direct or indirect interactions between fertilizer and pathogens, there is a deeper level of interaction expressed as optimizing the levels of both fertilizer and fungicide. Fungicides generally increase yields by reducing the losses caused by pathogens. However they can also improve yield by increasing the length of time that green leaf area is maintained by crops. Green leaf area duration (GLAD) improvements due to fungicides are very significant in some areas and some crops (Dimmock and

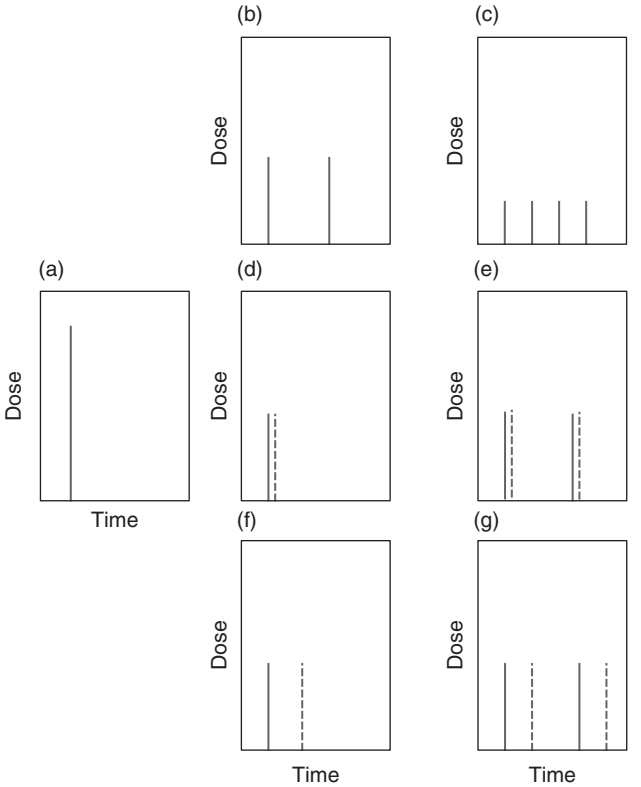

Fig. 7.4. Different strategies of mixtures and alternations: (a) single dose; (b) 2× split dose; (c) 4× split dose; (d) mixture; (e) 2× split mixture; (f) alternation; (g) 2× split alternation.

Gooding, 2002; Ruske *et al.*, 2003; Pepler *et al.*, 2005; Berdugo *et al.*, 2012). They are poorly understood but they are associated with QoI and SDHI fungicides in particular and less so with DMIs.

If the fungicide increases the yield, the dose of fertilizer that is optimum will also increase (Berry *et al.*, 2010). Nitrogen response curves will show an economic optimum; however if the yield effects of the fungicide are high, the optimum nitrogen level can be shifted towards higher levels. This is turn may justify a further increase in the intensity of the fungicide regime (Fig. 7.5). Similar curves will no doubt also apply to phosphate and potassium levels.

Summary

In summary therefore, fungicides are best seen as part of a package of inputs used by the grower to produce the most profitable crops. Numerous interactions apply between the inputs. It will never be possible to delineate the optimum strategy even for one farm due to changing weather conditions and pathogen loads. Risk-averse farmers are likely to err on the side of reduced inputs as a small decrease in fungicide or fertilizer level is very unlikely to have a serious negative impact on

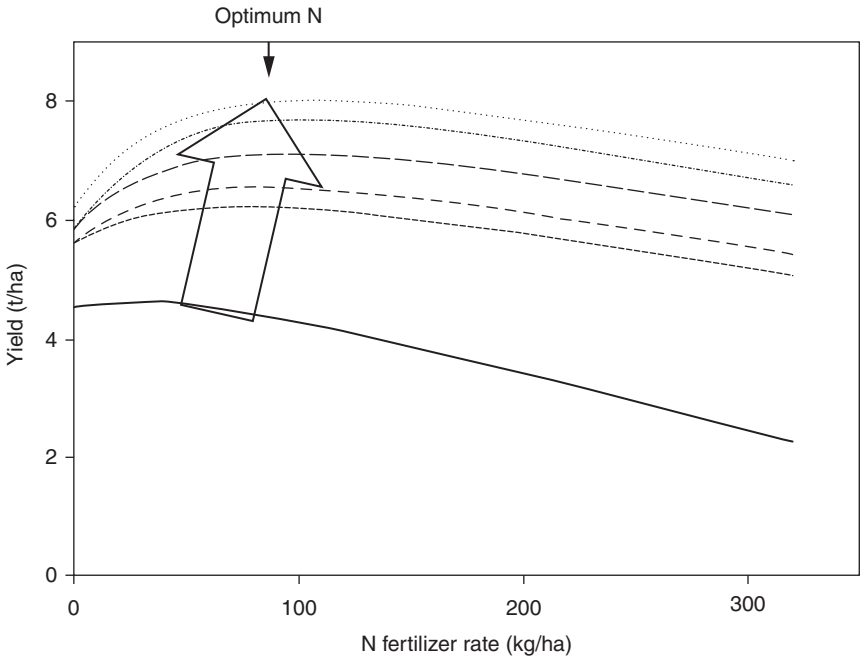

Fig. 7.5. Disease and fertilizer interactions. The optimum nitrogen rate (arrow) increases from 25 to 100 kg/ha as the dose of fungicide increases (relative fungicide dose rate: ——, nil; - - -, 0.125; – – –, 0.25; — — —, 0.5; – · – · –, 1; · · · · ·, 2) allowing greater canopy development. (From Berry *et al*., 2010 with permission.)

profitability. In contrast, maximizing profitability may require higher inputs. Careful, locally based research can provide clear guidelines for growers and limit the range of parameters that are most likely to be close to optimal. It will then be up to the grower to choose a strategy and apply tactics that fit the risk level s/he is willing to endure.

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8 Legislation and Regulation

Key Points

- Fungicide companies operate within a strict and detailed legislative and regulatory framework covering the safety and efficacy of products and manufacturing processes. The laws and regulations differ around the world.
- Regulatory regimes are subject to political as well as scientific factors.
- Fungicide users also operate within a strict regulatory framework designed to protect the environment, the farmer and the general public and to produce food that is free from damaging residues of pesticides.
- Consumers generally do not appreciate the safety of current fungicides or their importance in maintaining food security.
- Conventional farming must coexist with the so-called 'Organic' movement, which bans the use of most modern systemic fungicides.

Introduction

The legislative requirements of fungicide registration are primary concerns for fungicide companies. The combined cost of registration, environmental testing and toxicology can add up to US$170 million per launched product or more than two-thirds of the total cost. A great deal of thought and experimentation goes into predicting and testing the properties of lead compounds so as to minimize the time and effort spent on compounds that are destined to fail to secure registration. It would be bad enough to have to abandon a compound late in development after perhaps US$200 million has been spent on its development. But far worse would be if a compound was released and subsequently found to have some deleterious effect. The loss of reputation and the payment of compensation to damaged parties could threaten the very viability of the company.

The purpose of legislation is to allow benefits to be obtained while incurring the least possible risk to the manufacturer, user, consumer and the environment. For pesticides, this includes a spectrum of activities from the patenting of a candidate product derived from synthetic or natural sources to the examination of its potential short- and long-term effects on humans, animals, plants and the environment.

Traditionally, legislative procedures and regulations have differed between countries. The current goal of standardizing pesticide registration regulations across nations ('harmonization') is intended to improve the effectiveness of industry and government resources and lower the costs associated with risk assessment that are eventually financed by the consumer.

Registration Requirements

The legal requirements that define the process of fungicide development and use also apply generally to pesticides.

Effective pesticides, and particularly fungicides, are difficult to discover and predictably are subject to many more rigorous toxicological and environmental tests than pharmaceuticals before they can be sold. By comparison with pharmaceuticals, the action of using a fungicide to control a crop disease is equivalent to the selective and safe treatment of headaches using aspirin dissolved in water and sprayed in low volume from an aircraft over a town in which some of the sufferers are either inside buildings, and therefore protected from the application, or have not yet arrived on the scene. Fungicides are not usually applied to single, captive plants in the same manner as a pharmaceutical is used on a single patient. Consequently, factors other than safety to an individual become important in determining their safety. An outline of the testing processes and the timescale is given in Fig. 8.1.

Prior to their sale in any country, new and effective products must be shown to be safe to:

- the operator who handles and applies the product;
- the consumer of the treated crop;
- the environment; and
- the crop.

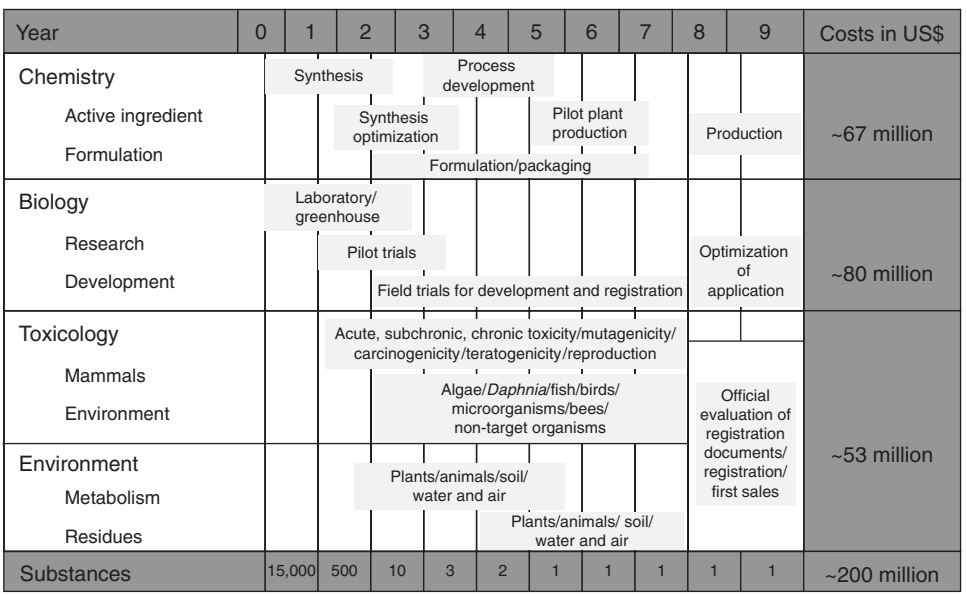

Fig. 8.1. Development of a crop protection product. (Courtesy of Andy Leadbeater, Syngenta, based on data from an ECPA study carried out by Phillips McDougall. © Phillips McDougall.)

In some countries, the product must be shown to be efficacious; that is, promote a significant yield increase. This requires the use of field trials for each crop and each pathogen in a representative range of agroecological zones.

More recently, regulations have been introduced that promote practices designed to prevent fungicide resistance and thus prolong the effective life of the compound. Initially, the acute toxicology of new compounds is determined so that advice may be given to researchers conducting chemical, biological and formulation studies and, if appropriate, to make decisions with respect to further development. As the candidate proceeds through the various stages of biological evaluation, the programme of studies widens to support the development of the compound and ultimately to satisfy the regulatory authorities.

The emphasis on global markets means that studies to define the safety of candidates must comply with the requirements of all the major regulatory authorities. Detailed guidelines are produced by individual countries and by international organizations such as the World Health Organization, the FAO and the Council of Europe.

Toxicology

Toxicology studies are exercises in prediction. They are also extremely expensive and form the major component of the total development budget for a new fungicide. Consequently, tests are carried out only as they become necessary to progress a candidate towards registration.

A broad range of tests is employed, which examine the safety of new compounds in rats, mice, dogs and primates in a stepwise procedure, depending on the stage of development of the fungicide candidate. As this process is the most expensive of all development costs, the agrochemicals industry has good reason to welcome the development and acceptance of animal-free toxicology tests. However, the debate that questions the use of animals in toxicological tests has failed, so far, to produce an alternative that is acceptable to regulatory authorities.

Acute toxicology testing involves the derivation of the lowest dose resulting in 50% mortality (LD_{50}). LD_{50} values are ranked according to toxicity. Values of less than 5 mg/kg body weight (bw) are very toxic; values between 5 and 50 mg/kg bw are toxic; those between 50 and 500 mg/kg bw are harmful. The LD_{50} values for fungicides are generally high, demonstrating very low oral toxicities (Table 8.1).

LD_{50} values are used to design subacute studies for longer-term evaluations of toxicology. These include 90-day feeding studies and others of up to 2 years' duration which explore possible chronic, oncogenetic (tumour-inducing), mutagenic and reproductive effects. The metabolic fate of the new fungicide in animals is also examined. Tests are planned strategically to coincide with nodal decision points corresponding to the maturity of other tests in the development programme (Fig. 8.1). It is current policy to review the toxicology of pesticides every 10 years.

Environment

Fungicide use is intimately involved in ecosystem dynamics and new compounds are assessed for their potential impact in a variety of environments.

Table 8.1. Acute toxicology of a range of fungicides.

Compound	LD_{50} (rats) (mg/kg bw)
Benomyl	10,000
Captan	9,000
Chlorothalonil	10,000
Cyproconazole	1,020
Cyprodanil	2,000
Fenpiclonil	5,000
Fenpropimorph	3,000
Fentin	140–298
Iprodione	3,500
Kresoxim-methyl	5,000
Mancozeb	5,000
Metalaxyl	669
Polyoxin	21,000
Propiconazole	1,517

LD_{50}, lowest dose resulting in 50% mortality; bw, body weight.

Most fungicides are applied as foliar sprays. Some are used as seed treatments. Logically, a significant proportion of the fungicide used to control disease finds its way into the soil where it may be degraded by microbial action or through direct chemical reaction, or move in the soil water and in direct runoff to water courses or to the underlying water table. Fungicides entering water courses may adversely affect aquatic life or the wildlife associated with a water environment. Likewise, fungicides may affect soil microorganisms or may be consumed by animals and introduced into food webs. It is necessary, therefore, that all new compounds at an appropriate stage of development are investigated with respect to their environmental fate and safety.

The first tests are straightforward, determining water solubility, lipophilicity, adsorption/desorption characteristics and hydrolytic capacity. With prior knowledge of the parameters that govern mobility of compounds in soil, reasonable predictions can be made of the potential environmental impact of the new compound. Subsequent tests probe the breakdown and metabolism of the candidate fungicide and its metabolites in soil and water.

The potential of a compound to leach is extremely important, and there is legitimate public concern about the presence of pesticides in drinking water. Leaching studies carried out in the laboratory may overestimate the potential of a fungicide to move in soil water but are useful in comparative tests with compounds of proven mobility. The use of lysimeters is now standard practice and can provide realistic measurements of fungicide movement over extended periods in a variety of soil types. In 1980, a European directive set the acceptable limit for individual pesticides in water at 0.1 ppb, although there is no toxicological basis for that level. Proof that fungicides are present at levels below 0.1 ppb often stretches the limits of the available analytical methods.

Lysimeter methodology, combined with the use of radio-labelled compounds, can also be used to investigate the fate of the parent and its degradation products in soils,

in the presence and absence of crops. The effects of light, temperature, rainfall, moisture content, pesticide concentration and soil type in aerobic and anaerobic conditions may be determined over time and used to establish the half-life, and hence the time to 90% disappearance, of the fungicide.

Because of the possibility of runoff into water courses and, in the case of rice fungicides, the use of products in paddy environments, the toxicology of new compounds to aquatic fauna and flora is determined using fish (trout and carp), *Daphnia* and algae.

Tests on birds are routine and include both acute and chronic studies designed to mimic the effects of scavenging activity in seedling crops and at harvest. Other studies include those on beneficial insects, for example bees, earthworms and soil microorganisms. The effects of candidate fungicides are also assessed on non-target plant species (Table 8.2; Pilling *et al.*, 1996).

Predictions of the field performance of candidate compounds in the environment are based on the accumulated data, either directly or by the use of one of the many available mathematical models, for example the leaching estimation and chemistry model (Hutson, 1992). However, ultimately it may be necessary to confirm the results of laboratory and lysimetry experiments in field trials.

Table 8.2. Higher plants tested for azoxystrobin safety. (From Pilling *et al.*, 1996.)

Family	Species
Dicotyledons	
Amaranthaceae	*Amaranthus retroflexus* (pigweed)
Chenpodiaceae	*Beta vulgaris* (sugar beet)
	Chenopodium album (fathen)
Compositae	*Bidens pilosa*
	Xanthium strumarium (cocklebur)
Convolvulaceae	*Ipomoea lacumosa* (morning glory)
Cruciferae	*Brassica napus* (oilseed rape)
Euphorbiaceae	*Euphorbia heterophylla* (spurge)
Leguminaceae	*Glycine max* (soybean)
Malvaceae	*Abutilon theophrasti* (velvetleaf)
	Gossypium hirsutum (cotton)
Polygonaceae	*Polygonum aviculare* (knotgrass)
Rubiaceae	*Galium aparine* (cleavers)
Monocotyledons	
Cyperaceae	*Cyperus esculentus* (yellow nutsedge)
	Cyperus rotundus (purple nutsedge)
Gramineae	*Alopecurus myosuroides* (blackgrass)
	Avena fatua (wild oat)
	Digitaria sanguinalis (crabgrass)
	Echinochloa crus-galli (barnyardgrass)
	Oryza sativa (rice)
	Setaria viridis (green foxtail)
	Sorghum halepense (johnson grass)
	Triticum aestivum (wheat)
	Zea mays (maize)

An example of the process is seen in studies using quinoxyfen which showed the parent compound to be resistant to leaching and to be stable. Metabolic products were identified in a variety of different soil types and other environmental situations. The principal compounds were 5,7-dichloro-4-(4-fluorophenoxy)-3-hydroxyquinoline (3-OH-DE-795) in soil and water/sediment tests and 2-chloro-10-fluoro(1) benzopyrano(2,3,4-de)quinoline (CFBPQ) in water and air. A minor metabolite, 5,7-dichloro-4-hydroxyquinoline (DCHQ), which formed only under acid conditions (pH 4.2) in soil and water/sediment, was judged as irrelevant to the study (Fig. 8.2; Reeves *et al.*, 1996).

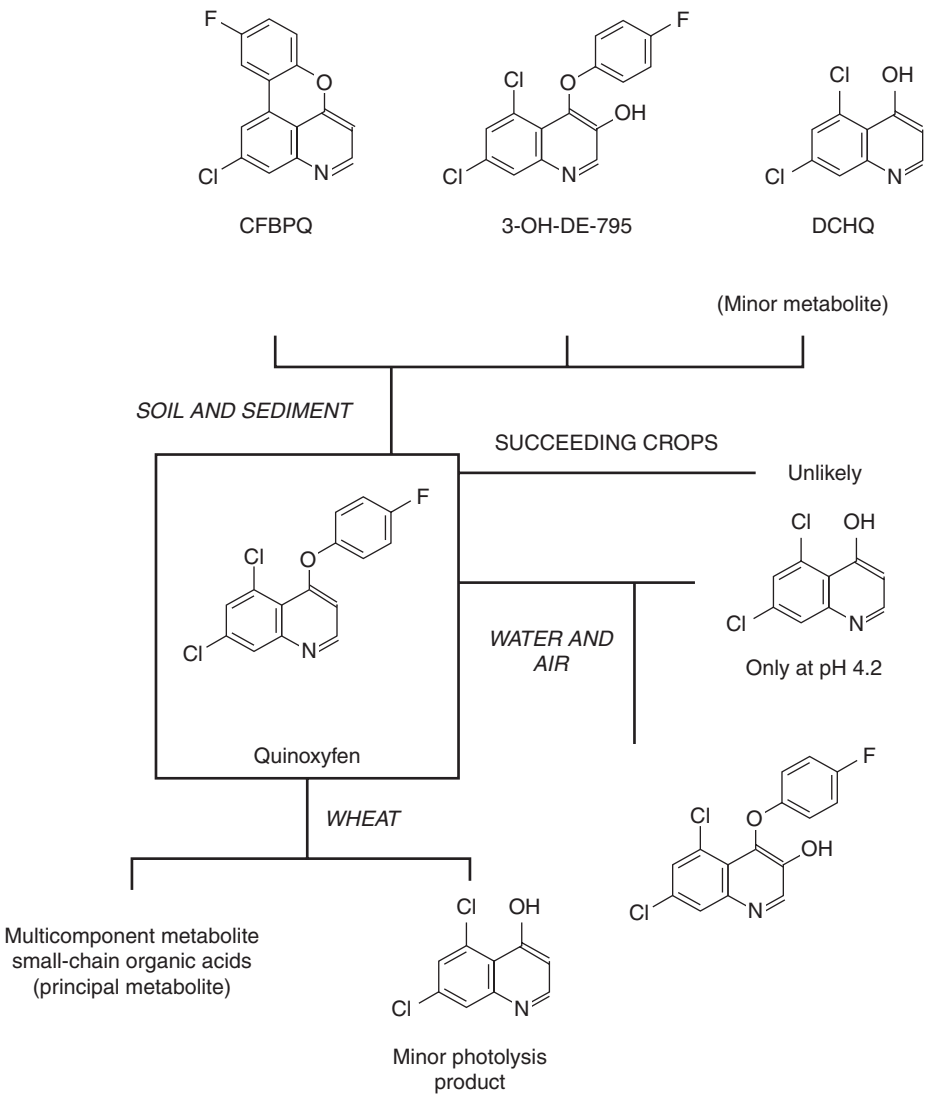

Fig. 8.2. Metabolites of quinoxyfen (CFBPQ, 2-chloro-10-fluoro(1)benzopyrano(2,3,4-de) quinoline; 3-OH-DE-795, 5,7-dichloro-4-(4-fluorophenoxy)-3-hydroxyquinoline; DCHQ, 5,7-dichloro-4-hydroxyquinoline). (From Reeves *et al.*, 1996.)

Residues

The main point of exposure of the general public to any crop pesticide is at the time of consumption of the treated crop. For that reason, the quantity and quality of pesticide residues in the crop at harvest are determined. Additional studies on the fate of residues in cooking, baking, refining and processing, including taint testing, may be carried out.

Residue trials are conducted in field crops in a variety of environments over at least two seasons. As with crop phytotoxicity studies, residue trials employ twice the maximum optimum rate of application of the test compound. Furthermore, the potential for accumulation in meat and milk is determined. Any major metabolites of the parent compound that are discovered undergo an independent series of toxicology and environmental tests.

For example, the principal residues in wheat treated with quinoxyfen are predominantly the parent compound and a mixture of small-chain organic acids. Photodegradation of the parent on leaf surfaces produces a third and minor metabolite, DCHQ, which is present at much less than 0.4 mg/kg plant material. Studies on subsequent crops showed that quinoxyfen is unlikely to be taken up via the roots. It was also demonstrated that quinoxyfen was the only significant residue in edible plant tissue (Reeves *et al.*, 1996).

Several immunodiagnostic assays are available for the detection of certain fungicides in food, food products and the environment. The permitted levels for most fungicides are of the order of 1–20 ppm. Diagnostic assays, based on ELISA technology, have detection capabilities to 1 ppb. Benomyl, because of its widespread use and public safety issues, has attracted the most immunodiagnostic work (Charlton *et al.*, 1991). However, systems are developed for metalaxyl (Newsome, 1985), triazoles (Newsome, 1986; Forlani *et al.*, 1992), procymidone (Ferguson *et al.*, 1993), iprodione (Newsome, 1987) and fenpropimorph (Jung *et al.*, 1989). More recently, mass spectrometry methods have come to the fore.

Residue levels are dependent on the particular agricultural systems that apply in each country. Sunlight, rainfall and temperature conditions, soil types and crop storage methods differ between each country. Hence many countries require residue testing to be carried out under local conditions.

In 1992, a UK survey carried out by the Ministry of Agriculture, Fisheries and Food established that out of 3500 samples of various foodstuffs, less than 2% contained more than the maximum residue levels of pesticide, none of which was a fungicide.

Operator safety

Operator safety is assessed in a series of experimental exposure studies carried out under practical conditions of fungicide application. In the UK, the Control of Pesticides Regulations (1986) require that persons handling pesticides, engaged in their distribution or applying them to crops are suitably qualified by validated examination.

Under the EU harmonization legislation guidelines for the setting and application, an operator exposure level (AOEL) has been established (http://ec.europa.eu/food/plant/protection/resources/7531_rev_10.pdf).

Long-term risks

The highest concentration of the candidate fungicide that over the normal lifespan of test animals causes no observable effects (NOEL) is used to derive a value for an acceptable daily intake (ADI) for a person. Using residue data and a knowledge of the daily intake of various food crops, the ADI and the toxicological characteristics of the fungicide can be compared. Only if the ADI differs from the NOEL by at least a factor of 100 is the candidate considered to present no long-term risk to consumers of treated crops (Table 8.3).

In most cases, the consumption of synthetic pesticides in food is less than 10% of the ADI, even assuming an excessive intake of treated crops.

Resistance risk

It is a requirement for registration of new fungicides under European Community legislation that an assessment of resistance risk, including details of a monitoring programme and baseline response data, and, if appropriate, a resistance management strategy should be supplied (Anon., 1991; Anon., 1993; Furk and Slawson, 1994).

European Union regulation

The European Union (EU) has taken a vigorous stance on pesticide risks. It has promoted implementation of Council Directive 91/414/EEC (see http://ec.europa.eu/food/plant/protection/index_en.htm) and its successors.

Moves to unify national registration requirements are designed to allow the entry of pesticides to all EU countries operating under the legislation (Lynch and Feeley, 1992). The directive enforces a review of all existing products and, recognizing

Table 8.3. Acceptable daily intake (ADI) and no observable effect level (NOELs) for a range of fungicides.

Compound	ADI (mg/kg bw)	NOEL (rats) (mg/kg diet)	NOEL (dogs) (mg/kg diet)
Benomyl	0.0200	2500	500
Captan	0.1000	2000	–
Chlorothalonil	0.0030	60	120
Fentin	0.0005	2	5
Iprodione	0.3000	1000	2400
Mancozeb	0.0500	–	–
Metalaxyl	0.0300	–	250
Triadimenol	0.0500	125	–
Flusilazole	0.0010	10	5
Vinclozolin	0.0700	27.1	–

bw, body weight.

the need for a balance between the essential role of pesticides in food production and the social and political constraints, will work towards:

- removal of confidentiality of testing;
- minimal use of vertebrates in testing;
- ensuring that no unnecessary pain or suffering is caused;
- maintenance of the precedence of safety and the environment over the need to produce crop protection agents;
- ensuring that candidate pesticides can provide real benefit; and
- promotion of the principles of integrated management.

Implementation of the European directive and of comparable schemes in the USA (Jellinek and Gray, 1992) has been subject to considerable delay and debate, which has affected the progress of new materials through to registration and has impeded the re-registration of older products.

In 1992, the Organization for Economic Co-operation and Development initiated a pesticide programme with the aims of harmonizing pesticide assessment and control procedures, speeding the process of re-registration of established products and reducing risk. Comparative studies are in progress to evaluate differences between member countries. For example, iprodione is one of seven pesticides chosen as benchmarks in the assessment of data review procedures and involves the cooperation of authorities in the USA, UK, Canada, Australia and Finland, and the FAO (Grandy and Richards, 1994).

The European Parliament now espouses the need to eliminate compounds that pose a particular *hazard* to the public or the environment. Previously, the evaluation process attempted to quantify the *risk* of a deleterious effect. A compound is defined as hazardous if it generates a deleterious effect at any concentration. One of the most contentious hazards is so-called 'endocrine disruption'. Endocrine disruption is manifested as, for example, alterations in sex organ development in molluscs (Bielza *et al.*, 2008; Gisi and Leadbeater, 2010). The fungicide industry argues that the concentration of compound that causes disruption should be compared with the concentration of the compound that is likely to be found in contaminated land, water courses or food products, but this proviso is not recognized by the authorities. Furthermore the agrochemical industry argues that elimination of the pesticide might lead to increased disease losses, lower food yields and higher food prices, which might be much more damaging to the health of the population than the fungicide. In response to this argument, the EU has introduced the notion of 'substitution'. This device states that if a 'hazardous' compound could be substituted by a compound with the same or similar crop protection properties, then the hazardous compound can be withdrawn. The result of these regulations has been the wholesale withdrawal of compounds from the market. About 50% of the 400 relevant products have been withdrawn (Bielza *et al.*, 2008). Many of these compounds were old and out of patent. The decision to withdraw was taken in some cases not because of toxicity but because the cost of maintaining registration could not be covered by future predicted sales. Hence some useful products for small markets may have been inadvertently lost.

The Danish government has added an extra layer of regulations designed to reduce the use of pesticide in its country. Around 2000 it introduced a simple regulation limiting the total *mass* of pesticide that can be applied to the fields. This straightforward but blunt measure had the effect of promoting the use of

compounds with high specific activity regardless of whether the compound was hazardous at the standard rate.

A more complex system has recently been introduced (L.N. Jorgensen, 2013, personal communication). Under this system, the fungicide is scored by the authorities for a range of toxic properties. The score is then used to set a tax for the pesticide. Hence a farmer needs to weigh up the extra cost of a fungicide versus the control that a particular compound affords. This system has placed a particular burden on the DMI group of fungicides that are coincidentally the mainstay of cereal disease protection. It is estimated that if DMI fungicides were withdrawn, food prices would rise by 20% and the EU would cease to be a wheat exporter.

Resistance in medical fungi

A new and urgent threat to agricultural fungicide use has emerged from studies of fungicide resistance in medically important fungi (Arendrup *et al.*, 2010; Camps *et al.*, 2012; Chowdhary *et al.*, 2012). The main culprit is the fungus *Aspergillus fumigatus,* which is the cause of invasive aspergillosis (IA) in humans. This disease has increased in importance in recent years due to the prevalence of immune-compromised patients emerging from transplant surgery and HIV/AIDS treatment. DMIs have been used, and indeed were their original use, to control IA. The disease is hard to control and patients receive DMI treatment for weeks or months. Recently isolates of the pathogen recovered from affected patients were shown to display resistance to the triazoles. Genomic studies highlighted mutations in the *cyp51* gene.

IA is believed to result from the inhalation of *A. fumigatus*, which is a ubiquitous environmental fungus. The threat to the DMIs comes from the finding that environmental isolates of the fungus also have the mutations in the *cyp51* gene. The epidemiology of IA suggests that the fungus never sporulates in humans and so cannot transfer from human to human. Hence selection for DMI resistance in humans cannot be blamed. It is therefore suggested that the mutations in environmental samples have been selected by the use (or misuse) of agricultural fungicides. However, sources within the fungicide industry point out that DMI fungicides are also widely used in domestic situations more likely to be relevant for immune-compromised patients. These uses include paints, carpets and other textiles used both in the home and hospital. The resolution of this debate may decide the fate of DMI fungicides.

Organic Farming

Organic farming is a broad term that includes a number of official and private schemes that are united by their rejection of synthetic fertilizers and modern pesticides, in particular fungicides. The movement grew rapidly in the 1980s and 1990s but seems to have peaked following the 2007 global financial crisis. In Europe, the number of farms adopting an organic farming regime increased from 7800 to over 55,000 between 1986 and 1996, with an area expansion from 0.12 to over 1.3 million ha (Lampkin, 1996). Similarly, in Germany and Austria organic farming developed from a proportional land use of about 0.5 to 4.6% overall (l.6 and 7.6%, respectively). Financial incentives are available to farmers in many European

countries to convert to organic food production. In support of this policy, further provision has been made to carry out research and development programmes, together with educational and training initiatives.

However, contrary to the amount of publicity that accompanies organic farming, the sales of organically produced food have not been significant. In 1991, sales were estimated to be between 1 and 5% of the total production, with a forecasted growth to 10% by 1997 (Lunt, 1991). The reasons for the discrepancy are uncertain, given the strength of public opinion against the use of pesticides, but Lunt includes:

- conflicting interests in the retail trade between the maintenance of their conventional sales compared with the promotion of a niche market;
- high cost of food production, created by high labour and distribution costs and uncertain yields, that are subsequently supported by the consumer;
- the imposition of premium prices for organically grown produce may deter buyers;
- variability of supply and quality will impact upon shelf life for retailers and purchasers;
- the poor appearance of produce, particularly fruit, is of concern especially to those who buy from conventional sources, affecting the readiness of uncommitted buyers to purchase organic produce; and
- the authentication of food sources may be uncertain and the buyer has no clear understanding of what constitutes organically grown food.

Organic agriculture relies on the use of natural inputs and a self-styled sympathetic view of nature to support the claim that the food produced is more wholesome, of greater quality and generated in an environmentally friendly manner. Part of that philosophy relies on the conviction that 'natural' means healthy and, by implication, 'man-made' is unhealthy or corrupt. Several reports address this issue in detail (see Lunt, 1991) but, in terms of crop disease control and the value of fungicide use, the salient points are outlined here.

Several fungi produce mycotoxins which in their various forms can be carcinogenic, teratogenic (induce malformation of the fetus) and directly affect the nervous system. They are not uncommon and infect a range of crops including rice, legumes, onions, celery, marrow, peanuts and tomato (Moreau and Moss, 1979; Riemann and Bryan, 1979; Canning and Lansdown, 1983; Lunt, 1991). Aflatoxin, with a lethal dose of 0.25 mg, is 2000 times more toxic than the insecticide parathion and is produced in large quantities in diseased legumes, especially peanuts. Patulin, produced by *Penicillium expansum*, a common fruit-rotting fungus, induces acute and chronic disorders in animals and has been reported as a contaminant of fruit juice and drinks. The fungus *C. purpurea* causes ergot of graminaceous hosts. Ingestion of infected grain, particularly rye, which is especially susceptible to infection, results in internal haemorrhaging, abortion and death. The symptoms gave rise to the name St Anthony's fire and the disease was commonplace throughout Europe during the Middle Ages. Fortunately, poisoning due to mycotoxins is rare because of quality control measures. The benefits of fungicide use in combating the infections that led to mycotoxin production cannot be ignored, however. Although fungicides are not used for the specific control of *C. purpurea*, it is interesting that the number of cases of St Anthony's fire in Europe is increasing and it is tempting to speculate that the removal of fungicides from niche market cereal production, such as organic rye cultivation, may be encouraging this revival.

However, the argument that promotes the use of fungicides as a safeguard against the contamination of food by mycotoxins is not robust, as shown in studies on *Fusarium* diseases in cereals. Several mycotoxins are associated with *Fusarium* in cereals and they are known to be harmful to animals, including humans. Although the use of fungicides to remove the risk from mycotoxin poisoning is valid when infection is completely prevented, fungicide applications to established infections may affect the level of mycotoxin produced, depending upon the active ingredient and the toxin (D'Mello *et al.*, 1996).

Laboratory studies using *in vitro* cultures of *Fusarium* demonstrate the stimulation by fungicides of some mycotoxins concurrent with a decline in others (Table 8.4). Under field conditions the results of similar studies are conflicting, but the conclusion must be that the long-held view that fungicides are always beneficial by virtue of their preventive action against mycotoxin contamination in food is not well founded, and should be investigated further.

In addition to the toxins produced by the pathogenic fungi, plants also respond to invasion by releasing a powerful array of chemical defence mechanisms. In evolutionary terms, this is to be expected: because of their physical inability to escape attack, plants, unlike animals, have developed chemical means to evade damage. The majority of these compounds have not been studied but some, like the glycoalkaloids present in potatoes at levels up to 500 ppm, would prevent the sale of potatoes if the rules that govern fungicide and pesticide registration were applied.

Some natural toxins, for example nicotine, have been known for many years, and some have been incorporated into folklore. There is a certain contradiction in the argument that promotes 'natural' farming systems on the grounds of safety because it places no reliance upon synthetic toxicants to control pests, but then advises the use of highly toxic chemicals, albeit of natural origin. However, there are few fungicides of natural origin. Lime, copper and sulfur mixtures, including Bordeaux mixture, are commonly recommended as protectant materials. Copper-based products are effective fungicides and have a widespread use in crop protection in high-input farming systems as well as in organic agriculture, reflected in their global sales value. They are not without problems; copper poisoning can lead to neurological and kidney dysfunction. If used incorrectly, crops can be severely damaged, high levels of copper may accumulate in soils and their overuse in fruit crops may result in taint problems. In contrast, sulfur is less toxic but can be phytotoxic and can cause skin and eye irritation.

Table 8.4. *Fusarium* spp. mycotoxin production in response to fungicides.

Fungicide	Pathogen	Effect on toxin production
Dicloran	*Fusarium graminearum*	DAS reduced or inhibited
Iprodione	(PDA broth culture)	ZEN reduced or inhibited
Vinclozolin	(PDA broth culture)	ZEN reduced or inhibited
Tridemorph	*Fusarium sporotrichioides*	T2 inhibited at 6 μg/ml
		DAS inhibited at 6 μg/ml
		T2 stimulated at 36 μg/ml
Carbendazim	*F. sporotrichioides* (PDA agar)	T2 increased at 5 μg/ml
Difenconazole	*Fusarium culmorum* (PDA agar)	3-ADON increased at 0.1 μg/ml

DAS, diacetoxyscirpenol; ZEN, zearalenone; T2, T2 toxin; 3-ADON, 3-acetyl deoxynivalenol.

Much of the publicity that surrounds the use of organic farming suggests that it is an advanced and enlightened method of crop production. In fact, most of the world uses methods of food production that are more like organic farming than high-input systems and, historically, organic farming was the only method of food production.

Organic farming is characterized by variable yields, occasional crop failure and, in extreme cases, famine. Only 150 years ago, European agriculture was suffering under the threat and, for some, the reality of crop failure. Crop disease epidemics, notably potato blight, went unchecked and the course of history was changed. Organic agriculture is not new and the potential problems associated with it are still present.

It is difficult not to draw a comparison between the historic and current geographic distribution of food-insecure people and the use of low-technology farming. In a recent report, it was stated that 'Organic methods simply cannot produce the quantities and qualities of food required by 20th century society' (Lunt, 1991). The same conclusion was reached in a recent symposium on the use of crop protection products and food quality (Anon., 1997). The growth of organic farming represents, and should remain, no more than a minor part of total crop production if we are to avoid the disasters that, in Europe at least, we appear to have forgotten.

Consumer Values and the Agrochemicals Industry

It is difficult to quantify the effect of consumer pressure on the activities of the agrochemicals and fungicide manufacturing industry. However, a poll of boardroom managers in the chemicals industry identified a growing concern for environmental issues. Over 60% reported that, despite the current restrictions on resources, environmental matters have become a strong factor in investment strategy, with nearly half committed to significant spending to comply with environmental obligations. In the agrochemicals industry, the demands of registration authorities already account for much of the expense involved in pesticide discovery. The controls associated with the production of agrochemical products are much stronger and more established than in other sectors of the chemicals industry, arguably even more robust than in the pharmaceuticals industry. However, the increasing demands of legislative authorities and public pressure groups ensure that no one can afford to be complacent.

The changes in legislation are the main impetus behind the increasing boardroom awareness of the importance of environmental issues. While nearly 40% of managers acknowledge the influence of current and future legislative requirements, social responsibility was cited by only 25% of managers as a determining factor. Clearly, in the upper levels of management the consumer movement has yet to make a major contribution to discovery strategy.

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9 The Future Prospects for Fungicides and Fungal Disease Control

Key Points

- The incessant rise in food demand means that all reliable methods of crop protection must be deployed at full efficiency.
- Global warming and biosecurity failures are likely to further impact crop protection.
- Many existing fungicides are likely to be phased out due to regulatory challenges.
- Fungicide resistance demands that resistance management strategies are used – this increases the need for new actives with new MOAs.
- The pipeline for new actives is working but at ever-increasing cost. Genomics and molecular modelling are likely to have an increasing impact.
- IDM will become standard practice. Better methods to select genetically resistant crops will bear fruit.
- Transgenic (GM) methods to deliver disease resistance have not developed due to public reluctance to accept transgenic crops.
- Developing genetically modified (GM) traits to replace or more likely supplement fungicides will require a major research effort.

Food Demand and Disease Threats

The world's population is growing at a faster pace than ever before and looks set to increase until at least 2050. The population needs to be fed and needs somewhere to live. Hence more food needs to be grown on less land with less water. To reduce the levels of food insecurity that already exist in parts of the world and to prevent food deficits occurring in more productive regions, efficient and effective methods of crop production must be introduced and maintained.

There are many reasons to believe that the disease pressure on the crops will increase. Global warming will have varied and rather unpredictable effects on crop diseases (Carlton *et al.*, 2012; West *et al.*, 2012) but generally will decrease food security. Global warming and ever-increasing international travel and trade will reduce or even eliminate the power of national quarantine agencies to keep exotic pathogens out of their countries. History teaches that plant pathogenic fungi will always challenge our ability to produce food in quantity and of an acceptable quality. In adopting the highly efficient practice of crop monoculture, the risk of crop failure from plant diseases has increased from something of occasional and marginal importance that could be sustained in an unsophisticated society, to a serious and continual problem often resulting in devastating yield losses and widespread social disruption.

Although the introduction of monocultures provided crop pathogens with an ideal environment in which to multiply, the situation in some crops was exacerbated by techniques that were subsequently adopted to manage other problems. In cereals, the drive to increase yield through the use of improved varieties and higher fertilizer inputs highlighted the value of good weed control. The ensuing spiral towards higher yields through the increasing use of fertilizers and herbicides eventually hit the yield-limiting factor of plant disease. Fungicides allowed yet more fertilizer to be used, to achieve even greater yields.

The effects of crop disease cannot be trivialized because they are never far away. Current estimates suggest that without fungicides we would lose up to one-third of yield, depending on the crop. In some circumstances, total loss is possible. Even in Europe, famine and food shortage were only a few harvests ago and the threat of their return has not disappeared. This reality necessitates the use of crop protection management systems that contain fungicides as an integral component.

The development and use of fungicides in crop protection is a success story. It is a story that has developed from their earliest and crude application in agriculture and horticulture, through a series of technological evolutionary steps, to a point where products are able to exert safe, broad-spectrum control for extended periods, or to work precisely to protect against attack by specific pathogens, or even to influence the host itself to combat infection. However, the process of improvement in crop disease management continues and the next 20 years are likely to witness even greater changes in fungicide technology and use.

Loss of Existing Fungicides

We have already seen (Chapter 8) that regulations initiated in Europe have led to the withdrawal of many active compounds. The ever-tightening regulatory demands, at least in Europe, will increase the pressure on the remaining compounds. The DMI group is already under serious threat and its loss could have a massive impact on the quantity and quality of food production worldwide.

Fungicide resistance preceded the withdrawal of the MBC class of fungicides by some years. Other fungicides afflicted significantly by resistance (see Chapter 6), including the DMI, QoI, PA, CAA and SDHI groups, remain in use. Indeed, predictions that QoIs would become useless through resistance have proved very wide of the mark. Instead fungicide resistance management strategies have ensured their continued use. The strategies involved mixtures and alternations of fungicides. Hence there is a strong demand for new fungicides to fulfil roles in resistance management.

The Discovery Process

The pace of fungicide discovery shows no sign of slowing up (Chapter 4). Instead, new actives and new MOAs are being as released as fast as ever. This may be because the consolidation of fungicide discoveries into ever fewer but larger companies (Syngenta, BASF and Bayer) has increased the efficiency of the discovery processes.

Nonetheless, the low-hanging fruit have been picked. The unique biomolecules in fungi, particularly the ergosterol biosynthesis pathway, have been thoroughly

examined for fungicide targets. It seems inevitable that newer fungicides will require a more expensive discovery pathway than existing ones.

Genomics has not yet had a profound impact on the processes of fungicide discovery. However we now have the situation in which the genome sequences of all relevant organisms, both the target fungi and the non-targets, are obtained or could be with only trivial effort. It is therefore possible to imagine a genomics-led discovery process in which molecules will be designed only to bind and inhibit key enzymes in the fungi and have no effect on non-target organisms. This is theoretically more straightforward than designing a pharmaceutical for a non-infectious human disease. Such a development would require a major effort in genomics and automated protein structure prediction.

Genetic Disease Control

Crop diseases are exceptional events, as all plants have natural defence systems to repel most fungal challenges. Molecular plant breeding allows breeders to combine in one cultivar all the best alleles of disease resistance genes as well as other desirable traits, as long as markers for the genes of interest have been discovered. Despite the fact that genome sequences for many major crops are now available, this process has not progressed as fast as was predicted and, to date, only major resistance gene markers are in general use. The quantitative and minor genes typical of so many resistance phenotypes have been harder to pin down. Developing the understanding of pathogenicity mechanisms in more fungi and better genomic resources for more crops will accelerate this process.

Transgenic (GM) Disease Control

Mechanisms that permit the transfer of alien genes into plants have been available for over 25 years (Binns, 2002). Several characteristics have been researched in breeding programmes, such as nitrogen fixation, drought tolerance and the modification of protein components and their storage. The GM technologies were new and deemed to be commercially risky, so the chemical companies pursued only the biggest markets with the greatest profit potential. Hence the great majority of GM crops released to date involve genes for herbicide resistance and for insect tolerance. Resistance to viruses has also been successfully deployed.

As long ago as 1991, it was shown that the expression of alien genes controlling hydrolytic enzyme activity in transgenic tobacco and oilseed rape resulted in increased resistance to infection by *R. solani* (Broglie *et al.*, 1991). Many other traits have been examined and tested in laboratory-scale experiments but, to date, no commercial crops with transgenic disease resistance have been released (Logemann *et al.*, 1992; Toubart *et al.*, 1992; Gurr and Rushton, 2005).

The reasons for this glaring failure are partly scientific but also partly political. Developing a GM disease resistance trait is beset with many of the same difficulties as developing a new fungicide; the GM trait should generate good levels of disease resistance against a wide spectrum of pathogens and should be safe. Research was carried out on a wide scale in both university and chemical company laboratories.

Indeed, many chemical companies bought seed companies so as to have a route to market the new disease resistance traits.

The first major disease resistance genes (R-genes) were cloned and analysed around 1994 (Jones *et al.*, 1994; Hammond-Kosack and Jones, 1997). The first thought was to express these genes in other plants to see whether they conferred resistance. However, it soon became apparent that R-genes were very specific and only worked in the species or at best the family from which they were derived (Gurr and Rushton, 2005). Hence this route has limited spectrum and has not attracted sufficient commercial interest.

Activation of resistance genes during infections leads to the production of a defence response which somehow kills the fungus (Anderson *et al.*, 2005). So-called PR (pathogenesis related) genes producing chitinases and glucanases were among the induced genes. The release of active oxygen was also involved. Hence many people pursued the idea that enhanced expression of these genes would lead to resistance. This strategy has been undermined to some extent by the growth reductions seen in plants expressing PR proteins that outweigh the potential benefit of disease resistance.

Another line of thought was to deploy antifungal proteins in transgenic plants (Jach *et al.*, 1995). These are diverse proteins with potent activity against several fungi. However such traits have failed the very stringent animal toxicology tests.

The latest research involves the use of RNA interference to inhibit the expression of fungal genes essential for infection (Nowara *et al.*, 2010; Duan *et al.*, 2012; Panwar et al., 2013). The mRNA is targeted by a short RNA molecule that is complementary in sequence. This creates a short stretch of double-stranded RNA (dsRNA). dsRNA is efficiently detected in plants by a set of enzymes that cleave the RNA and inactivate it before it is translated into proteins. This is a very promising technology that can be delivered either by direct delivery of RNA molecules instead of a chemical fungicide or via expression of the RNA in the infected plant tissue. The proponents of this technology predict its widespread use in the next 5–15 years. It seems likely that a combination of chemical, conventional genetic and GM traits using antifungal genes, signalling molecules and RNA interference will become the norm in the next decades.

Developments in GM disease resistance have so far failed to progress to market. The scientific questions are tough but surely would have been solved had the level of investment present through the 1980s and 1990s been maintained. However the backlash against GM products that emerged in Europe in 1996 following the 'mad-cow disease' outbreaks caused both public- and private-sector organizations to cut back investments in this area. GM herbicide- and insect-resistant crops have been grown on a huge area and no deleterious effects have been reported. Nonetheless, no relaxation of the regulations has been forthcoming albeit there are signs of reduced anxiety at the moment. We will see whether investment now increases to exploit the potential of the GM disease resistance market.

Market Development

The last decades have seen a major consolidation in the fungicide market. Currently only three major companies are engaged in the full range from discovery to marketing.

A further merger within these companies seems unlikely. It is also hard to imagine how a new company could enter the market for conventional fungicides. Generic manufacturers are increasing in number and global importance, especially as China, India and many other tropical and semi-tropical countries become both fungicide users and manufacturers. There is the potential for new companies to enter the arena through the provision of GM traits, but the extremely demanding regulatory burden makes this unlikely. Clearly, the future for fungicide discovery is firmly fixed within a few very large companies.

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