Session 7
Concluding Remarks

THE WAY AHEAD

K.J. Brent

St Raphael, Norton Lane, Chew Magna, Bristol, BS18 8RX, UK.

In this brief concluding contribution I was asked to assume the difficult role of pathfinder, and to discuss future priorities for research and practice. I suppose I have reasonable background for this task, having addressed fungicide resistance problems for over 25 years from the interacting but differently biassed standpoints of industrial R and D, public-sector research and independent consultancy. But it remains a daunting task, and I have been glad to be able to draw freely on all the information and opinions given over the past three days.

What have we learnt from our quarter-century of dealing with the phenomenon of resistance to fungicides? One hears totally different views on this amongst informed workers, ranging from the gloomy 'virtually nothing, we still cannot predict it reliably or stop it' to the optimistic 'we understand the problem, we know what to do, active implementation is the keynote'.

Actually I think we have achieved a great deal. We are all much more alert to the threat of resistance, so that manufacturers do now make and act upon early appraisals of risk, involving base-line monitoring, cross-resistance studies and sometimes mutation experiments. Companies are devising and implementing use strategies which must aim at a difficult compromise: using materials to the best short to medium term commercial advantage, and conserving their effectiveness for the longer term. This of course calls for inter-company co-operation regarding related compounds. Over the past twelve years FRAC has worked in this role. The speed of action of FRAC and the adequacy of some of its recommendations have been questioned from time to time, but it has to be appreciated that the tasks of securing interchange of confidential information, reconciling conflicting technical, commercial and logistic factors, and achieving consensus views, are often very hard. I believe that FRAC has done its job remarkably well, and long may it continue. Public-sector research and advisory services have also been influential, and have interacted with FRAC to a limited extent. The announcement here by its Chairman that FRAC will consult more openly with the public sector, and will support some external research initiatives, is a good omen.

The publication of data, supported by full experimental details, and statistical analysis where appropriate, continues to be of paramount importance. It was good to see the new data presented here, particularly from the industrial side. More in the future, please - especially on phenylpyrroles, methoxyacrylates, anilinopyrimidines and other new groups. In connection with publication, we still need to be very careful about defining our terms, and particularly what we mean in each particular context by 'resistance'. Some participants proposed that we adopt a narrow definition of 'resistance' to denote the loss of effectiveness in practice. In my view, any attempt to impose new semantics is unnecessary, will fail, and will cause confusion. 'Resistance' has been used over many years as an umbrella term, covering laboratory mutants, field isolates and large populations. This started in bacteriology with sulphamide and antibiotic resistance, and then in entomology with DDT resistance. But when we need to be more precise, as we often do, then we must refer specifically to

'laboratory resistance', 'field resistance' (which may be very rare or slight), or 'practical resistance' (where field resistance is frequent and severe enough to interfere with effective disease control). Fortunately, precision in reporting is gradually improving. At this meeting, 'percentage resistance' in graphs and tables tended to be replaced by 'percentage of samples unaffected by 10 ug/ml in leaf-disc test', 'percentage of sites where resistant forms were detected', or whatever was actually measured. However, more care is still needed, and must be checked by editors.

Strategies have been implemented, and most of us believe that they have helped. Most crop diseases are still under good control, but we heard of some situations where the defences are now inadequate (e.g. *Botrytis cinerea* on grapes in the Champagne region) or stretched very thinly (e.g. *Mycosphaerella fijiensis* in bananas in C. and S. America). New fungicides are urgently needed. It is heartening to know that we have reached the end of the long lull in invention, that has existed since the explosive appearance of many novel fungicide groups in the late 1960s and 1970s. It was good to hear that the UK registration authority is now welcoming increased diversity of available chemicals. This is crucially important in the battle against resistance, and is also environmentally favourable. Chemical control will be needed for many years, and it is a worrying thought that falling agrochemical profits may hinder further invention.

Some participants were rather disappointed to find us still debating some of the questions that we were discussing 25 years ago, such as mixtures versus rotations, and low rates versus high rates. These strategy problems are very difficult to research under field conditions. It generally takes several years for clear differences in resistance build-up to occur, and also to determine whether particular use strategies are of lasting advantage. Even with large plots, invasion by external populations of the target pathogen, and loss of the original population, can easily occur. When conclusive results have been obtained, these have sometimes been conflicting. We heard theoretical arguments that lower rates may be safer with regard to major-gene resistance, and higher rates safer with regard to polygenic resistance. However the doses that actually reach the target organism vary enormously in space and time, giving complex exposure sequences. More field experimentation must be encouraged, so that soundly based judgement on the best strategies for particular circumstances can be achieved.

We still lack sufficient understanding of the underlying mechanisms of resistance. In particular more research is needed on the genetic basis, and on the field behaviour of resistance genes under different selection conditions. Use of the latest molecular techniques, including DNA probes and other diagnostic agents, will greatly help us to make a much closer examination of field phenomena. For example, what is happening in potato fields where metalaxyl resistance is readily detected in bulk spore samples, but control is sustained? Are resistant and sensitive forms intimately mixed, or are there heterokaryotic populations of resistant and sensitive nuclei within the coenocyte? Will selection differ against zoospore and direct sporangial infections?

On the other hand, how much monitoring do we need to do? Year by year monitoring is expensive, and as our knowledge grows is probably giving diminishing returns. Once good base-line data are obtained, then the priority should be on continual scrutinising of field performance, if possible in a more systematic, precise and well-reported way than at present, and involving regional field trials when appropriate. Performance monitoring is

often anecdotal, and forms the weakest part of most resistance management programmes. If problems of control do arise, then sensitivity monitoring should be done as an explanatory measure. The importance of harmonising monitoring methods was stressed by some speakers. If reached by worker consensus this is fine, but the external imposition of standard methods must be avoided, first because different methods suit different circumstances, secondly because the best methods are hard to select and need to evolve by experience, and thirdly because results indicated by several different methods are actually more reliable.

Resistance management needs to be seen more clearly as a component of the broader concept of integrated crop management. When ICI first introduced ethirimol to control barley powdery mildew, I remember that we claimed that it would permit the plant breeder to stop worrying about disease resistance, and to focus better on yield and quality parameters. Even today many farmers will select a relatively disease-prone variety, relying totally on fungicide treatments to maintain crop health. Far more lasting will be the combined use of chemical and genetic defences in partnership, as exemplified in several of the papers. If only the plant breeders could find the genetic equivalent of mancozeb, at least in its stability and breadth of effect! Other husbandry components, such as disease-suppressive rotations and avoidance of excessive nitrogen fertilisation, will also form an increasingly important part of the integrated approach.

Overall, the resistance phenomenon has not been the disaster forecast by some observers, but it is an ever present threat with new cases arising and some old problems still continuing. There is much more to learn, of great practical and scientific interest, and I hope that we will all meet again in two or three years' time to discuss a lot more new and interesting data.

Syndicate Workshop Discussion Reports

Syndicate Workshop Session Discussion Reports

Five syndicates operated simultaneously, each with a chairman and assistant to capture discussion points. Each syndicate was set the following four questions under the general banner 'Resistance risk assessment and management'.

1. Resistance surveys and monitoring
   - can statistics help?

2. Resistance surveys and monitoring
   - can new technology help?

3. Predicting resistance development
   - can genetics help?

4. Anti-resistance strategies
   - mixtures or alternations

The chairman had the discretion to focus on one or more questions as appropriate.

Syndicate 1

Chairman: Dr. J.A. Lucas
Assistant: Dr. M.J. Hocart

Question 1. Resistance surveys and monitoring - can statistics help?

- What is the relevance of EC_{50} data? Values for fungicide sensitivity are calculated in different ways, sometimes based on spore populations from field samples, or genetically ill-defined mass isolates, while other estimates derive from single spore isolates, which may not be homokaryotic. In comparing data sets are we comparing like with like? What information are we trying to obtain?

- Sampling. How does one establish the baseline value, i.e. the fungicide sensitivity of the original non-selected population, against which changes in sensitivity of the population can be recognised. How many samples need to be tested?

Question 2. Resistance surveys and monitoring - can new technology help?

- DNA-based techniques may be powerful tools for surveying and monitoring fungicide resistance, provided the nature of the variation is known in detail. For example DNA probes can identify particular mutations conferring resistance. However development of such systems requires detailed analysis and hence they may not become available until after fungicide resistance has become established in the pathogen population.

- Theoretically the technology allows rapid screening of large samples. If PCR-based techniques can be made quantitative, their potential for screening would be greatly enhanced.

Question 3. Predicting resistance development - can genetics help?

First a question was asked: "Is there any such thing as polygenic resistance?"

Fungicides with multiple sites of action represent, in general, a low risk of resistance. With site-specific fungicides the question is not "Will resistance occur?" but "When will resistance occur?" The rate of resistance development depends upon the selection pressure applied, in terms of fungicide use, but also on the biology and epidemiology of the pathogen. We know comparatively little about natural variation in sensitivity to fungicides. Hence:

- Genetic information needs to be linked to the epidemiology and biology of the pathogen. Otherwise the predictive value of any genetic information will be poor.

Question 4. Anti-resistance strategies - mixtures or alternations?

Growers perceive fungicide resistance to be "not their problem" but rather a problem for the agrochemical industry.

The use of fungicide alternations was considered difficult to implement as growers are reluctant to apply chemicals that may be less effective than the best available. Experience in vineyards in France shows, however, that this strategy can be successfully adopted provided growers are sufficiently aware of the problem of resistance; grower education is therefore important.

For agrochemical companies fungicide mixtures represent the most pragmatic approach, since the failure of the vulnerable component of the mixture, through resistance, will not result in a serious loss of disease control. But is recommendation of fungicide mixtures an anti-resistance strategy, or sometimes a ploy for continued marketing of an obsolete product?

Anti-resistance strategies must be rooted in practical disease control. For any strategy to stand a chance of success it must be implemented by growers. Consequently effective strategies will not be too complex.

Syndicate 2

Chairman: Dr. J. Gilmour
Assistant: Rosemary Collier

By popular demand Syndicate 2 addressed the questions in reverse order.

Question 4. Anti-resistance strategies - mixtures or alternations?

1. We should not be considering mixtures or alternations but mixtures **and** alternations, preferably alternations of mixtures. In Greece no problems had occurred where such an approach had been used for 10 years in contrast to the rapid appearance of resistance when new molecules were used alone.

2. There was a general feeling in favour of mixtures over alternations but programmes on fruit were cited as examples against this. It was suggested that this approach had been adopted because of the early occurrence of resistance problems, the hi-tech approach to the crop, the high value of the crop and the need for a high level of disease control.

3. There was serious doubt as to whether a farmer would really have resistance at the forefront of his mind when deciding which fungicide to use.

4. In practice most mixtures are made for reasons other than combating resistance, particularly to broaden the spectrum of control and in some cases for synergy.

5. Agrochemical companies will make mixtures of fungicides if it will be of benefit to them, i.e. if there will be a benefit they can sell to the farmer.

6. EU legislation - the group was generally content that the new Directives would appear to allow enough scope in label recommendations for the adoption of effective anti-resistance programmes. It was accepted that for new molecules this would have to be based on theory, not on evidence.

7. Finally, the danger of generalisation was stressed. It was accepted there was very little experimental evidence about the respective merits of mixtures and alternating programmes.

Question 3. Predicting resistance development - can genetics help?

1. NO. Genetic analysis could be very useful after the event but was not thought to be useful in prediction.

2. The group suggested that more effort should be directed to looking at variability within the pathogen when the new molecule is first introduced, but this raises the question of what would we be looking for and at what frequency.

3. It was stressed that mode of action studies would not be an indicator of potential mechanisms of resistance. Therefore genetic studies of mode of action are unlikely to help.

4. Artificial mutant studies were said to be able to show "everything and nothing". Such laboratory studies have not been good indicators of problems and lack of problems in the field.

Question 2. Resistance surveys and monitoring - can new technology help?

1. Molecular markers would be useful but can be used only after a resistance problem has been identified.

2. There was concern about the cost of some of the new techniques. However it was stressed that quick results from field enquiries would sometimes be very valuable and repay the higher costs incurred.

3. It was suggested that new diagnostics for the presence of pathogens may be more beneficial. In support of this the example was cited from Chile where diagnostics for Botrytis are used in combination with a forecasting technique that has allowed vine growers to reduce their fungicide

programme from typically nine sprays to only two sprays per season.

Question 1. Resistance surveys and monitoring - can statistics help?

1. Some concern was expressed about the wide range of different techniques being used. It was thus not always clear whether the results obtained by different workers were the same or different.

2. It was pointed out that there are some recognised protocols (especially EC_{50}), though the need for different approaches was appreciated.

3. It was apparent that there was some need for more education and training in the use of the techniques that already exist.

Syndicate 3

Chairman: Dr. M.W. Shaw
Assistant: Dr. R. Beresford

Question 4. Anti-resistance strategies: mixtures or alternations?

There was broad agreement that both strategies were usable, but that mixtures were far more attractive, because they offered many advantages as well as a possible reduction in the rate at which resistance evolves. These included the fact that the strategy could be sure of implementation, because it depended on manufacturers rather than growers; that it provided coverage against a wider range of pathogens; that it could give "insurance" against failure of one of the components; and that it would often allow reduction in the rates of the individual components. For the most part, participants favoured mixtures of an "at risk" fungicide with an historically safe chemical such as chlorothalonil or dithiocarbamate. Appropriate application rates became a central theme of the discussion: what rates should be used in a mixture? If full rates were used, the main attraction of alternation was that it involved substantially less active ingredient overall. A majority opinion emerged, although there were dissenting voices. This opinion was that if the resistance to which a fungicide was at risk was monogenic and large, as with metalaxyl or MBC, the rate used should be as low as possible; if the resistance were continuously distributed and polygenically controlled, the rate should be as high as possible. (Our attention was drawn to the paper of Stevas in these proceedings, which supported the latter idea). However, it was also pointed out that pathogen biology needs to be considered separately for each individual case, and that "rate" by itself has little meaning: what matters is the percentage survival of the pathogen, and the spray coverage.

Question 3. Predicting resistance development - can genetics help?

Laboratory studies of the genetics of resistance were felt to be useful, but not

very useful. Their main use was as a negative guide: if it was easy to get resistance in the laboratory, there must be deemed to be a considerable danger in the field. However, this could be misleading - the example of MBC resistance in *Rhynchosporium* was cited - and the failure to get laboratory resistance was no guarantee of safety in the field.

Statisticians know a lot about sample surveys, and one would be foolish to undertake one without taking statistical advice, mainly about sampling strategy. The sample size required depends on the question, and quite small samples can be useful for some questions. You are unlikely to get early warning of monogenic resistance arising in the field, but you might for polygenic, because of the different dynamics in the two cases.

There was very cautious support for the promise of new technology. Some of the new techniques are so specific that they might miss a novel resistance mechanism. More importantly, development of such techniques takes a long time, and the circumstances in which they will then be useful are restricted, as it will often be too late. DNA techniques seemed to offer little for polygenic resistance, simply because too many genes are supposed to be involved.

Syndicate 4

Chairman: Dr. J.K.M. Brown
Assistant: Dr. B.J. Nielsen

This workshop concentrated on questions about the population biology of fungicide resistance which are so far unresolved. Participants discussed current areas of controversy and the research that would be needed to resolve them. In discussing the question, "Predicting resistance development - can genetics help?" several participants pointed out that the prediction that strong control will necessarily select resistance is not always valid. For example, resistance to tridemorph has not developed in barley powdery mildew or black leaf streak of banana, despite the extensive use of this fungicide. Four points which might mitigate the development of resistance were raised. One is the pathogen's reproductive biology. It was argued, on one hand, that sexual reproduction could recombine resistance genes and so promote resistance, and on the other hand, that sex could break up combinations of resistance genes, thus slowing the evolution of resistance. This question clearly requires further study. Secondly, migration might overwhelm selection. The relative importance of these two factors is poorly understood in plant pathology in general. Thirdly, the mode of action of the fungicide may be such that resistant mutants occur at a very low frequency. This might be a factor in the continuing effectiveness of tridemorph. A final issue is the fitness of resistant isolates. There is little good data on whether or not resistance adversely affects fitness, and little understanding of the significance of fitness in the population genetics of many pathogens.

Much of the discussion of a second question, "Resistance surveys and monitoring - can new technology help?" was motivated by an outline of a test for benzimidazole resistance in scald of barley, using an oligonucleotide probe, presented by Dr Derek Hollomon (Wheeler et al., 1994). This method has greatly increased the throughput of tests for resistance, but several questions about the general applicability of molecular tests were raised. One problem is that, in order to develop such a test, the mechanism of resistance must be known. These methods may not, therefore, be capable of predicting whether or not resistance will occur in future, but may assist in monitoring the development of resistance once it has been detected, and in evaluating strategies for its control. A further limitation is that, in some cases, the phenotype - resistance or susceptibility - of an isolate would not be fully predicted by genetic variation in a single DNA sequence. Finally, some participants doubted that a molecular test would always save time or money. Despite this, the test for benzimidazole resistance in scald indicates the potential of these techniques.

By contrast, a discussion of the question, "Resistance surveys and monitoring - can statistics help?", quickly reached a consensus. Participants agreed that it should be possible to detect resistance which is serious enough to be a practical problem without elaborate analysis. To quote one participant, "if you need a mathematician to tell you if you have resistance, then you don't really have resistance". However, statistical analysis should be used in planning experiments, for instance in choosing dose rates and sampling schemes, and in summarising results, by presenting a few figures which describe the major conclusions, by testing the significance of the conclusions and by allowing comparisons with control isolates used in different experiments.

REFERENCE

Wheeler, I.; Kendall, S.; Butters, J.; Hollomon, D. (1994) Rapid detection of benzimidazole resistance in *Rhynchosporum secalis* using allele-specific oligonucleotide probes. *These proceedings.*

Syndicate 5

Chairman: Dr. C.E. Caten
Assistant: Dr. S. Heaney

Although Syndicate 5 was a relatively small group of around 12, we had a lively and varied discussion with the result that only two of the four questions posed by the Symposium organisers were addressed.

We started by considering whether statistics can help interpret data from resistance surveys. Such surveys usually aim to answer two questions:

1. Is there a shift in sensitivity between the test population and some baseline, control population?

2. If there is a shift, is it of practical significance?

There was broad agreement that while suitable statistical procedures to tackle these questions exist, they are not sufficiently employed. One reason for this may be the lack of familiarity of many biologists with all but the most basic statistical procedures. Resistance survey data frequently pose special statistical problems, e.g. the parameters (LC_{50}, MIC, etc.) are themselves complex and may not be normally distributed, sampling methods and sample sizes may be critical, bioassays contain many potential sources of error. For all these reasons it is important to first define the objectives of the study and then adopt methods appropriate to these objectives. Statistics can not compensate for a poorly designed survey and therefore, where results are likely to be complex, statisticians should be consulted from the outset of the study. Our conclusions were summed up by one member of the group as "statistics can help but only if used prophylactically".

The group then turned its attention to whether genetics can help predict resistance development. There was unanimous agreement that *in vitro* mutagenesis experiments can reveal the potential for resistance. Furthermore, it was agreed that the nature of the genetic control of resistance, whether major gene or polygenic, is a useful indicator of the likely speed of development and magnitude of resistance problems, and could suggest the use of different anti-resistance strategies. However, it was noted that the type of genetic control is a property of the particular strains being crossed; not the character or organism in question. Thus resistance to fungicide X in species Y may be determined by a major gene in one cross, but under polygenic control in another. While user-friendly model systems will continue to be important in studies of the genetics of fungicide resistance, there is a need to check the results from such studies on actual target pathogens. Up to this point the group had been of one mind in its view of the value of genetics. (Perhaps this was to be expected from a group dominated by fungal geneticists!) However, this cosy equanimity was destroyed when the question of the effect of regular sexual recombination on the development of polygenic resistance was raised. Will it facilitate the acquisition of quantitative resistance by combining individual resistance polygenes, as some members thought, or slow it down by breaking up those synergistic combinations that do arise, as others felt? Given the lateness of the hour, we were happy to agree on the need for more studies on this point, before retiring gracefully to the bar.