*Neth. J. Pl. Path. 83 (1977 Suppl. 1) 3-13*

# EVOLUTION OF THE CHEMICAL CONTROL OF PLANT DISEASES; AN EVALUATION

G.J.M. van der Kerk

Laboratory of Organic Chemistry, State University of Utrecht, The Netherlands

During the greater part of its known history mankind has suffered from crop losses due to unpredictable, inevitable and incurable plant pests and diseases.

The idea of fighting fungal plant diseases by means of external treatment with chemicals is rather old. In particular during the past century this idea has been realized in a remarkably successful way. The great break-through occurred in the second half of the 19th century with the advent of inorganic sulphur and copper preparations. These are still of great importance but during the past decennia were superseded more and more by organic compounds.

The view that the use of chemical compounds for the protection of agricultural crops is generally to be rejected is new. It represents primarily a form of wishful thinking, somewhat related to the prayers and sacrifices of antiquity, which so far is based on expectations rather than on firm arguments. Preventive care in phytopathology cannot make use - unlike in human and veterinary medicine - of immunization techniques. It is restricted to the development of resistant varieties, crop rotation and good husbandry. Breeding for resistance is always a race against the development of pathogenic fungal variants. Although in modern agriculture crop rotation frequently is not attractive economically, good husbandry has been common practice for a long time. If these measures failed, which they usually did, mankind was in for disaster, until the modern fungicides came to the rescue. In his authoritative introductory paper at the Symposium on 'Pesticides, veterinary medicines, related compounds and their residues', held at Groningen on 8 and 9 January 1976, Dr N. van Tiel, Director of the Plant Protection Service, expressed himself as follows: 'It may not be expected

that in the foreseeable future the present levels of agricultural production can be maintained, let away be increased, without the use of chemical compounds'.

It is good to realize that anything close to the concept of 'integrated control' - which has met with some encouraging success in the combat of insect-borne plant pests - has so far never been realized in the fight against fungal plant diseases, provided of course, that we do not use this new idea just as a substitute for good old agricultural husbandry.

In fact, only one approach has been partially successful: the development and cultivation of resistant plant varieties. But here the limitations became apparent almost simultaneously, viz. the very fast selection from natural habitats of fungal strains capable of attacking the new 'resistant' varieties. And then, I still leave out of consideration the well-known high frequency of genetic changes occurring in fungi. On the basis of existing knowledge and experience and unless totally new and thus unpredictable concepts are uncovered, I feel therefore justified to state that future agricultural practice will require the continuing use of agricultural fungicides. And further, that the emphasis will continue to shift towards organic compounds with a high degree of selectivity and specificity of action.

This statement implies that on the exclusive basis of cultural and genetic measures no satisfactory suppression of fungal plant diseases may be expected. On the other hand, though I believe that organic fungicides will stay with us, I do consider it possible that organic molecules may be found which, without being fungitoxic, are able to disturb the intimate biochemical relationship between host plants and their specific fungal parasites. One may also think of compounds which increase the plant's resistance against fungal invasion and of compounds capable of inactivating the biochemical weapons of attack of parasitic fungi, viz. toxins and tissue-destructing enzymes like the pectinases. For each of these possibilities positive evidence has already been obtained, but so far these have not resulted in practical alternatives for the use of fungicides. I will not discuss these possibilities further but want to emphasize the importance of continuing research efforts in these directions.

At this Symposium important current information on internal therapy of plants will be presented. I hope and expect that there will also be some contemplation on the future of fighting fungal plant diseases. My introduction aims at offering you a modest contribution to this theme.

I have been associated with fungicide research for almost 30 years and I have given my views on agricultural fungicides at several international symposia. After such a commitment with this field I feel more or less obliged to recall some statements I have made before and to examine their value in the light of present knowledge and views. In 1958, at the Golden Jubilee Meeting of the American Phytopathological Society I pointed out that the world use of dithiocarbamates as agricultural fungicides did outrank the total use of all other organic fungicides. I also ventured to predict the improbability that more attractive general purpose protectant agricultural fungicides could be developed. Now, almost twenty years later I still hold that view. At that same occasion - which occurred well before the advent in the mid-sixties of the systemic fungicides - I made the following remark: 'It is a comforting thought that the use of the available organic fungicides is endangered less than that of any other type of biocide by the menace of the appearance of permanent resistance'.

It is very discomforting that since the introduction of the systemic fungicides, and in particular following the intensive use of the benzimidazole derivatives since 1968, the occurrence of fungal resistance has become a wide-spread and dangerous reality. The first signs of this phenomenon occurred very soon after the commercial introduction of the otherwise spectacular and extremely successful systemic fungicide benomyl in 1968 by Delp and Klöpping.

Already in 1970 the reports on resistance development had become so numerous that, at the 'Conference on Biochemical and Ecological Aspects of Plant-Parasite Relations' at Budapest, I felt compelled to express the following warning: 'Until now this alarming phenomenon of the development of resistant fungal strains has never been observed at a practical level for protectant fungicides. Before proceeding with the large scale application of the new systemics the answers to the resistance problems should be known. Among the agricultural chemicals the fungicides so far have enjoyed a reasonably benevolent press. I believe that we are not allowed again, as in the case of the insecticides, to learn our lesson by bitter experience'.

At the '3rd International Congress of Pesticide Chemistry' at Helsinki in 1974, when the evidence for fungal resistance as a result of the very extensive continued application of certain systemic fungicides had become overwhelming I stated: '... we must conclude that industry has completely ignored this warning and has handled these precious new developments in a most irresponsible way. It can not be justified and, in fact, is no longer acceptable that industry flatly ignores unprejudiced scientific evidence

just to make a short-term profit under the well-known motto 'Après nous le déluge'.'

Since, things have gone on as if nothing had happened and notwithstanding the fact, that new and dramatic evidence is piling up. Just as an example I refer to the very recent and alarming paper of Fletcher and Scholefield in the Annals of applied Biology of this year on benomyl tolerance in isolates of Botrytis cinerea from tomato plants.

Well, this is where we stand now and the least one can say is that this stand requires a very careful consideration. I presume that this will happen extensively during this Symposium, but a few things I should like to say in advance.

The purposeful search for organic agricultural fungicides has been going on for almost fifty years. Tens of thousands of organic compounds, belonging to the most diversified structural types and classes have been prepared and tested on fungicidal efficiency. At present agricultural practice applies some 20 to 25 protectant fungicides and some 10 to 15 systemic fungicides, among which 3 to 5 antibiotics. Among the protectants the dithiocarbamates, in particular the ethylenebisdithiocarbamates, are outstanding. In the group of systemics the compound benomyl and a few other MBC-generating structural variants as well as some pyrimidine derivatives are preponderant. Among the modest applications of antibiotics blasticidin occupies an important position. The practically useful compounds represent very diversified classes of organic structures, with very little structural relationship. One remarkable common feature of the systemic fungicides is, however, that nearly all are heterocyclic compounds, but that otherwise they vary between moderate and extreme chemical stability and range from narrow-spectrum to very broad-spectrum agents. Taking into account the tremendous efforts one can but conclude that the number of practically important agricultural fungicides is very modest. And further, that even much greater efforts will be required to improve upon the presently available palette of useful compounds.

It is good to realize again that the purpose of fighting fungal plant diseases is to protect plants, not to kill or inhibit fungi. Originally the approach was to look for organic structures and structural variants with the highest possible direct antifungal activity. As a consequence, the spore germination test, the mycelium growth test and combinations of these, like the roll-culture test, were the tools to select the active compounds. It appeared, however, that too many compounds with high in vitro activity failed completely in the field. In many cases these failures could be ratio-

nalized and be reduced to phytotoxicity, physical and/or chemical instability and similar undesirable properties. On the other hand some compounds with moderate general in vitro activity gave excellent field performance in specific cases and a few examples are even known of compounds devoid of any significant antifungal activity which nevertheless are capable of protecting plants against fungal attack. These observations have strongly changed the approach towards finding new and effective compounds and combinations. Instead of the easy and quick tests for direct fungitoxicity one nowadays prefers the much more purposeful and reliable, but at the same time much more cumbersome tests on combinations of plants and their specific fungal parasites under field or greenhouse conditions.

Fungicide research - but this is true as well for all other research on crop protection - has changed completely and has become an extremely costly affair. And this not only for the reasons just mentioned. It is simply true, that the total effort required to develop and introduce new, attractive and otherwise acceptable agricultural fungicides has risen almost exponentially. One factor is that notwithstanding the tremendous research efforts the discovery of new fungicides is still a matter of perseverance and of chance. It has to be admitted that so far neither studies on structure-activity relationships nor on mode of action have contributed significantly to providing a rational basis for the design of totally new classes of active compounds. In his recent penetrating analysis of possibilities for a rational approach towards new agricultural biocides, based on the presently available biochemical knowledge about essential processes in target organisms, Corbett stated that so far all efforts of this kind had met with a discouragingly low degree of success. For the planned synthesis of agricultural fungicides he did not even see any leads at all. We can but dejectedly agree with his final conclusion: 'There is no easy way to find new pesticides'.

Taking into account how much has already been done and the ever increasing demands posed upon new agricultural fungicides, we may assume that the law of diminishing return is operative here. Or, to say it otherwise, industrial research and development towards new agricultural fungicides are involved with high risks and with questionable returns.

In retrospect there have been two overwhelmingly important events in the search for organic fungicides. The first was the issue in 1934 of the Tisdale and Williams Patent, disclosing the dialkyldithiocarbamates, but in particular the classical paper of Dimond, Heuberger and Horsfall in 1943

on the ethylenebisdithiocarbamate structure. The second has been the already famous report in 1968 of Delp and Klöpping in which the systemic fungicide benomyl or Benlate was described.

The first heralded the actual beginning of the era of protectant organic fungicides, an era which is still lasting. The second marked the breakthrough towards the fulfilment of a dream: the era of the truly systemic fungicides. Now, less than ten years later, we start to realize that it has not only been the beginning of a dream but, unless we proceed very carefully, the beginning of a nightmare as well.

The need for systemic fungicides resulted from a number of clear shortcomings of protectant fungicides. The most conspicuous of these are: they give no protection after fungal penetration has occurred and are useless against vascular diseases; newly grown parts and root systems are unprotected; and they are subject to weathering and washing off. Truly systemic fungicides which are taken up by roots and foliage and which move within the plant both apoplastically and symplastically - goals that have not yet been fully attained - would in fact make up for these shortcomings. But here new conditions must be fulfilled and thus new problems may arise. Since systemics by definition act from within the plant they must be very selective in their action against fungi and plants which means that phytotoxicity becomes a very critical factor. Once within the plant the compounds are safeguarded against external decomposition and removal, but they are falling subject to the plant's biochemical processes. This new situation may give rise either to unintended and unwanted biochemical breakdown or, in case of biochemically very stable structures, to totally new residue problems. Further, the required high selectivity makes it almost inevitable that systemic fungicides should possess a high degree of specificity of action. This latter condition has already been found to have far-reaching consequences, in particular if compounds with high chemical and biochemical stability are involved.

I will elaborate this point somewhat further. Selectivity requires that a systemic fungicide clearly distinguishes between vital processes in the host plant and in the fungal parasite. Of course the ideal kind of selectivity should rest upon interference with vital processes occurring in fungi but not in plants. Examples are the interference with chitin biosynthesis by polyoxin-D and that with the biosynthesis or functioning of the specific fungal sterol ergosterol, exhibited by certain types of piperazine derivatives and polyene macrolides, respectively. The next best form of selectivity is based upon a strongly differential activity against processes occurring both in fungi and in plants. Such differential activity may depend

on:

a. differences in reaching the active sites;
b. differences in affinity for the active sites (a clear example is the difference in affinity of plant and fungal spindle tubulins for MBC);
c. differences in rate between susceptible vital processes in fungi and plants.

Since this is not relevant for my present purpose I leave out of discussion the remarkable differences in selectivity shown by most systemic fungicides with respect to different classes of fungi, although it should be borne in mind that this type of selectivity may have important ecological consequences.

It must be realized that selectivity requirements for systemic fungicides are much higher than for protectant fungicides and it is easy to admit that exactly these requirements are met remarkably well by the presently available range of systemic fungicides. There have been times that the possibility of finding systemic fungicides was considered unlikely because of the presumed similarities between the basic metabolic processes in plants and fungi. This may be somewhat true for the catabolic processes, i.e. those involved in energy production and conversion, it certainly is much less so for the anabolic or biosynthetic processes. In this respect there are, also at the cellular level, tremendous differences between higher and lower forms of plant life. I indicated already the characteristic occurrence and functions of chitin in fungal cell walls and of ergosterol in fungal cell membranes.

A few years ago Kaars Sijpesteijn pointed to a very characteristic difference between the available protectant and systemic fungicides. Quite generally the activity of all known protectant fungicides is based on their interference with energy-producing or converting processes. As a result they are effective primary inhibitors of cellular respiration. On the other hand, almost without exception, the systemic fungicides do not primarily interfere with respiration but do so with biosynthetic processes. Moreover, all available evidence indicates that this interference with biosynthesis occurs in very specific ways.

Now, as a consequence, the protectant fungicides, being inhibitors of rather general cellular processes, are mostly working via 'multisite' mechanisms. Apart from developing effective detoxification processes or barriers against penetration, fungi have little chance of developing re-

sistance against such mechanisms. For the systemic fungicides, which are specific inhibitors of biosynthetic processes, this situation is basically different. In 1964 Dekker and Oort reported a detailed and interesting study of the experimental systemic fungicide 6-azauracil, which inhibits one particular enzyme involved in the biosynthesis of nucleic acids. They arrived at the conclusion that the very fast development of resistance to this compound is caused by mutation in just one gene and that the activity of 6-azauracil as a fungicide is based upon a single-site mechanism.

It is evident that the lower the specificity of a fungicide is, the more mutations are required to develop resistance. This implies that at equal mutation frequencies the chances of meeting a resistant mutant are much larger for the highly specific systemic fungicides than for the protectant fungicides of low or moderate specificity. All available evidence points to the fact that the presently most notorious class of systemic fungicides, viz. the benzimidazole derivatives, and in particular the compound MBC, are working via a single-site mechanism. On the basis of this knowledge the very fast development of resistant fungal strains, including cross-resistance, following the use of these compounds is after all not surprising.

Many cases of acquired resistance against the new systemic fungicides have been reported in the literature. For almost all systemic fungicides resistance can be evoked very easily under laboratory conditions but fortunately the reported cases of resistance development under field or even greenhouse conditions are much fewer. This latter observation should, however, be interpreted with care since the development of resistance under practical conditions, among other things, has a relation with the intensity of use of the fungicide in question. So far field resistance has been very wide-spread and developing very fast with the widely applied compounds benomyl, MBC and the thiophanates. Moreover, all tolerant strains exhibit cross-resistance to other types of benzimidazole derivatives. Also for the intensively used hydroxypyrimidine derivative dimethirimol field resistance has been reported under independent circumstances.

From the foregoing brief account it may be concluded that the present systemic fungicides are new and valuable tools for fighting plant diseases, which certainly have widened the possibilities available until about ten years ago. But at the same time their introduction has given rise to a few new complications and even alarming phenomena which were unknown before, viz.: the building up of resistant fungal strains, already now a most serious practical problem; ecological shifts in the soil, the consequences of

which are yet unknown but should be watched carefully; and the problem of residues in treated plants, which by the very use of systemic compounds has obtained an extra dimension.

In this respect MBC (and for that all MBC-generating compounds), notwithstanding its tremendous broad-spectrum activity, in fact combines the two most undesirable properties a systemic fungicide may have. It is a highly specific single-site inhibitor and it is extremely stable, both chemically and physically. The first property results in a very fast development of resistant fungal strains both by selection from natural habitats and as a consequence of mutations. The second property aggravates the first one since a lasting selection pressure is maintained. Moreover, the extreme stability contributes to the permanence of ecological shifts and causes additional residue problems in the harvested products. I do not believe that these undesirable properties can be kept in check sufficiently by applications spaced by time intervals and neither by combination with other fungicides having a completely different mode of action. In my opinion this class of compounds should be abandoned, or at least their large-scale use should be postponed until we have found out how to avoid or to deal with the effects which worry us at present.

Now, finally, how are we going on from here and in particular, what could be the directions of research towards improved systemic fungicides. In this contemplation I leave out of consideration efforts towards non-fungicidal means to interfere with the plant-pathogen relation indicated before, but I will start saying a few words about protectant fungicides.

1. Protectant fungicides. As already said before, notwithstanding half a century of imaginative and successful stumbling we have no real leads for the planned synthesis of yet unknown classes of agricultural fungicides. We still depend on the make-and-try (which to me sounds better and is more justified than trial-and-error) approach of yore. Some people, in particular among biologists, believe that certain phytoalexins could serve as structural examples for the organic chemist. J.M. Hirst at Brighton, 1975, put it this way: 'Might some (phytoalexins) be fungicides that the pathogen had been unable to breach with insensitivity mechanisms in millenia, whereas less than a decade seems ample for some fungicides we produce today?'. My comment is that the 'old' protectant compounds are such fungicides; they are only much better and less expensive than any phytoalexin known at present and I do not even think that these latter lend much structural inspira-

tion to the organic chemist. Let us hope for further fortuitous gold along our path, realizing, however, that the distances we will have to cover will be much larger than before.

2. Systemic fungicides. What has been said about devising new structural types of protectant fungicides is equally true for systemics; there are no real leads. The establishment that nearly all present systemic fungicides are heterocyclic compounds is not much of a hint. In the first place it is as informative as the well-known request: 'Greetings to my son in America', and secondly there is the nasty word nearly. The compounds udonkor (N-(2-cyanoethyl)monochloroacetamide) and prothiocarb (ethyl-(3-dimethylamino-propyl)-thiocarbamatehydrochloride) are just a few examples of aliphatic systemic fungicides. Thus, also here it is a matter of make-and-try.

As already indicated, it has long been a pious but almost idle wish that studies of structure-activity relationships or mode of action might offer new leads towards new classes of active compounds. Whereas such studies are highly justified, be it mainly for several other reasons, there is an imperative necessity for intensifying mode of action studies, in particular in the pursuit of systemic fungicides. In the further development of this approach towards fighting fungal plant diseases mode of action studies should be made obligatory and the results should be scrutinized as carefully as those of toxicity and residue studies.

No compound ought to be admitted unless it has been ascertained that no resistance will develop under practical circumstances. In this respect exact knowledge regarding the mode of action should constitute a most important early warning signal. According to present experiences, compounds with highly specific single-site action mechanisms are particularly suspect with respect to causing resistance problems.

It would seem that in the general zest for finding and exploiting systemic fungicides one has been overshooting the mark. For some of the apparently most successful types of systemic fungicides there is something basically wrong with their biochemical mode of action. To me it is clear that we have to take a small step back, away from the very specific single-site fungicides, towards compounds which are somewhat less specific and operate on the basis of double-site or, may be, triple-site mechanisms of action, in the hope that this change will not introduce undue phytotoxicity. In relation to what I have said before it should be realized that this suggestion does not imply even the slightest positive hint for the synthetic organic chemist, but it might prevent him and many others from entering a dead end by

eliminating certain compounds and lines of approach almost from the outset.

I am happy to look back upon my association with the 'Research Unit for Internal Therapy of Plants', in the programme of which mode of action studies have always occupied a place of prominence. But the greatest satisfaction I derive from the privilege to have experienced the stimulating cooperation with so many eminent and devoted scientists and the regular contacts with dear friends.