SYSTEMIC FUNGICIDES

Systemic Fungicides

Edited by R. W. Marsh O.B.E.
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Preface

Critics will doubtless say that this book was out of date before it was published, thus confirming the rapid progress now being made in the study of systemic fungicides. This accelerated progress started around 1968 and it will be noted that of the 1174 references listed on pp. 255–309, 591 are papers published since 1967.

Because research in this field is developing so rapidly we felt that this was not the time to produce a definitive account of systemic fungicides. Instead we have aimed at making a relatively brief survey of the position now reached, including an outline of the basic principles of systemic fungicidal action, a summing-up of the present state of knowledge in the various branches of the subject, examples of practical applications and a look at possible future developments.

Experimental work on systemic fungicides is now worldwide, but the major centres of research have been in the Netherlands, England and the United States. We have been specially fortunate in securing the authoritative contributions of Professor Dekker and Dr Kaars Sijpesteijn from the Netherlands, of Professor Wain, Professor Crowdy, Dr Woodcock, Dr Byrde, Dr Brooks, Dr Spencer, Dr Carter, Mr Maude and Dr Evans from this country and of Dr Dimond from USA. Dr Dimond's death on 4 February 1972 was a serious loss to plant pathology: his contribution to *Systemic Fungicides* was his last completed manuscript.

This book is aimed at research workers in the field of fungicides, manufacturers of crop protection products, students of agriculture and horticulture and all concerned practically with fungicides and their use.

R. W. Marsh

Long Ashton Research Station, 1972

Note added at Proof

At the time of going to press, the development of triarimol has been suspended. This action was taken by the manufacturers after undesirable toxicological effects were observed in one species of laboratory animal following long-term administration at high dietary levels.

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Contents

		Preface	ix
		List of contributors	xi
R. L. Wain and G. A. Carter	**1**	**Nomenclature and definitions**	1
		Mechanisms of chemotherapeutant activity	3
R. L. Wain and G. A. Carter	**2**	**Historical aspects**	6
		Early investigations	6
		Development from mid-1930s	8
		The assessment of therapeutic and systemic activity	10
		The search for new chemotherapeutants	23
D. Woodcock	**3**	**Structure–activity relationships**	34
		Organophosphorus compounds	35
		Antibiotics	42
		Carboxylic acid anilides	54
		Heterocyclic compounds	64
		Aromatic compounds	79
D. Woodcock	**4**	**Toxicological considerations**	86
		Organophosphorus compounds	87
		Antibiotics	88
		Carboxylic acid anilides	89
		Heterocyclic compounds	89

S. H Crowdy	**5**	**Translocation**	92
		Entry into the free space within the tissues	93
		Apoplastic movement	100
		Symplastic movement	102
		Translocation of systemic fungicides	109
		Conclusion	113
A. E. Dimond	**6**	**Effects on physiology of the host and on the host/pathogen interactions**	116
		Introduction	116
		Effects of systemic fungicides on the host	117
		Production of fungitoxicants in plants	118
		Stomatal closure as a physical barrier to host entry	119
		Systemic compounds that alter growth of the host	120
		Treatments that block pathogenic processes	126
		Future prospects	131
A. Kaars Sijpesteijn	**7**	**Effects on fungal pathogens**	
		Introduction	132
		Effect of individual compounds on the fungus	135
		Factors determining activity: mode of action; selectivity and resistance; metabolic conversion	150
J. Dekker	**8**	**Resistance**	156
		Introduction	156
		Occurrence of acquired resistance	157
		Origin of resistance	161
		Mechanism of resistance	164
		Emergence of resistance in the field	170
		Avoidance of fungicide resistance	171
E. Evans	**9**	**Methods of application**	175
		Biological activity and systemicity	175
		Seed treatment	176
		Soil treatment	178
		Leaf and stem treatments	179

		Post-harvest treatments	183
		Timing systemic fungicide treatment	183
D. H. Brooks	**10**	**Results in practice – I. Cereals**	186
		Introduction	186
		Barley and wheat	186
		Rice	204
		Maize	205
D. M. Spencer	**10**	**Results in practice – II. Glasshouse crops**	206
		Introduction	206
		Disease control in the glasshouse	208
		Use of selected systemic fungicides	209
		Methods of application in glasshouses	218
		Resistance	220
		Non-fungicidal effects of benzimidazole compounds	221
		Biological control	222
		Present trends: future needs	223
R. B. Maude	**10**	**Results in practice – III. Vegetable crops**	225
		Introduction	225
		Seed-borne diseases	225
		Soil-borne diseases	228
		Leaf diseases	232
		Post-harvest diseases	235
R. J. W. Byrde	**10**	**Results in practice – IV. Fruit crops**	237
		Introduction	237
		Temperate fruit	238
		Sub-tropical fruit	249
		Tropical fruit	252
		Conclusions	254
		References	255
		Index	311

1

Nomenclature and definitions

by R. L. Wain and G. A. Carter

Accepting the broad definition that a **fungicide** is an agent that kills or inhibits the development of fungus spores or mycelium, the fungicides used on plants may be classified as protectant, systemic and eradicant on the basis of their uptake by, and mobility within, plant tissues.

The use of a **protectant** fungicide is an example of **prophylaxis**; it is a treatment intended to prevent or protect against infection. Protectant fungicides, which may be applied to seeds, soil or the plant surface, cannot penetrate into plant tissues in effective amounts. They must, therefore, act outside the plant prior to infection by the pathogen. By contrast, the use of a **systemic fungicide**, which is taken up by the plant, is one form of **therapy**, i.e. the cure of an established infection, where penetration of the host cuticle is used as the criterion of infection. A **therapeutant** is an agent that inhibits the development of a disease syndrome in a plant when applied subsequent to invasion by a pathogen. Therapy can be achieved by physical means, such as the hot-water treatment of smut-infected grain, but it is more usually brought about by chemical means; it is then termed **chemotherapy** and the active agent is a **chemotherapeutant**.

A chemical that can penetrate the plant cuticle and move through cell membranes is a potential therapeutant for any plant disease. A few fungal pathogens, notably the powdery mildews (Erysiphaceae), after penetrating their host, develop externally on the surface of the plant; non-penetrating fungicides can often be used as therapeutants for diseases caused by pathogens of this type.

Any compound capable of being freely translocated after penetrating

the plant is termed a **systemic compound** or just a **systemic**. **Translocation** can be defined as the movement of a compound within the plant body to tissues remote from the site of application. Such a compound has advantages in use over one that remains localised in tissues adjacent to the site of application, i.e. a compound working **topically.**

Topical therapeutants are more often referred to as **eradicant fungicides** or **eradicants.** Typically these are fungitoxic chemicals which, when applied at an infection site, are capable of limited penetration leading to the elimination of an established infection.

When non-systemic compounds are applied to a plant and there is no surface redistribution, the areas missed in the initial application remain unprotected, as does any subsequent growth. The surface deposits are also subject to weathering. Repeated applications are therefore necessary to ensure continuous protection. Furthermore it is difficult to protect inaccessible organs, such as roots, with non-systemic compounds.

Because they are able to move freely from applications made at readily available sites to distant, untreated tissues, systemic compounds suffer none of these disadvantages. It may be possible to delay the application of systemics until disease symptoms are apparent, thereby eliminating the need for routine and often wasteful pre-symptom applications. One application of a systemic compound cannot, however, be expected to give continuous protection to the whole plant because translocation may be limited to certain tissues and the compound will be subjected to dilution and possibly metabolic breakdown as the plant grows (see Chapters 5 and 7).

Although applications of systemic fungicides are often said to 'protect' new growth from infection, their action is usually therapeutic in that it occurs after penetration of the host even if no visual symptoms of disease arise. Systemic protectant activity could arise if a compound applied, for example, through the roots became translocated and exuded on to the plant surface. The germination of fungal spores alighting on this surface might then be inhibited in the pre-penetrative phase. The antifungal phenolics exuded by varieties of onions resistant to 'smudge' (*Colletotrichum circinans*) are thought to act in this manner (Walker and Stahmann, 1955) and fungitoxic materials have been washed from intact leaf surfaces (Topps and Wain, 1957). As yet, no externally-applied systemic compound has been shown to exert such a protectant action on the leaf surface although both griseofulvin (Stokes, 1954) and cycloheximide (Wallen and Millar, 1957) have been detected in the guttation droplets of wheat seedlings following application to the roots. Microscopic examination of wheat leaves inoculated with powdery mildew and taken from seedlings treated with three systemic anti-mildew compounds showed

that inhibition of mildew development became apparent only after penetration had started (Dekker and van der Hoek-Scheuer, 1964).

A high degree of mobility of a compound within the host plant is not always necessary for disease control; this property is more important, for example, when applying a root treatment against a leaf-attacking pathogen than when trying to control a pathogen causing a foot-rot or a vascular wilt. Chapman (1951) showed that the effectiveness of various compounds applied through the roots differed against the twig-invading Dutch elm disease pathogen *(Ceratostomella ulmi)* and the root-invading *Fusarium* wilt pathogen of tomato; the differences correlated with the mobility of these compounds in cellulose.

The direction in which the systemic chemical moves is also important. Movement is usually upwards in the xylem but for the protection of roots using foliar sprays, basipetal movement down the stem is required and this has proved very difficult to achieve.

MECHANISMS OF CHEMOTHERAPEUTIC ACTIVITY

A chemotherapeutant can modify, and be modified by, the tissues of the plant to which it is applied. It does not necessarily, therefore, reduce disease by direct fungitoxic action against the pathogen and so need not itself be fungicidal. The postulated mechanisms of therapeutic activity are very varied but three broad categories have been distinguished; direct toxic action against the pathogen, inactivation of toxins produced by the pathogen and enhancement of resistance of the host.

Direct activity This requires that an effective level of a fungitoxicant accumulates at the infection site. The compound applied need not itself be fungitoxic *in vitro* as it might be converted to an active compound by metabolism within the plant.

To inhibit a fungal pathogen inside the plant a fungitoxic chemical must be effective within the tissues – and these present a very different environment from that of the standard laboratory tests for *in vitro* fungitoxicity. Certain **systemic fungitoxicants**, e.g. rimocidin and pimaricin (Oort and Dekker, 1960), can be detected by bioassay in extracts made from treated plants yet show no therapeutic activity in those plants. Antagonism by cell constituents (Gottlieb, 1957) and irreversible binding to adsorption sites can render a powerful *in vitro* fungitoxicant inactive *in vivo*.

A direct-action therapeutant must show selective activity – it must be toxic to the pathogen but innocuous to the host plant (Dimond, 1963b). In practice this has proved difficult to achieve because the basic bio-

chemistry of fungi and higher plants has much in common. Indeed, the mechanism by which a fungicide exerts its toxic action may well determine whether or not it is a potential chemotherapeutant (Kaars Sijpesteijn, 1970).

Some workers in the field of chemotherapy of both plant and animal diseases have measured selectivity in terms of a therapeutic index, this being calculated by dividing the minimum curative dose by the maximum tolerated dose. It is not possible, however, to assign a single index to each therapeutant for, as pointed out by Dimond (1962), some plant tissues, e.g. leaves, are more susceptible to toxic damage than are others, e.g. seeds.

The presence of a reactive toxophore in the molecule of a fungitoxicant may render it both poorly translocated and phytotoxic. A derivative of such a molecule, however, may possess improved translocation and therapeutic activity (Dimond and Davis, 1953; van Raalte *et al.*, 1955; Hamilton *et al.*, 1956; Pluijgers, 1959) although *in vitro* fungitoxicity is often lost. Such compounds have been referred to as **masked fungicides** (Kaars Sijpesteijn, 1961b) the intention being that the original fungitoxic molecule be released within the plant, preferably only at infection sites.

Inevitably most chemotherapeutants will be metabolised within plant tissues and where the metabolites are inactive this will result in a steady loss of effectiveness. This subject is dealt with in Chapter 7 and has also been reviewed by Kaars Sijpesteijn and van der Kerk (1965), Wain and Carter (1967) and Kaars Sijpesteijn (1969).

While the term **systemic fungicide** is often employed to describe therapeutants with a direct toxic action, some pathologists use the term synonymously with **systemic therapeutant**, thereby employing it in a wider sense. This usage has been criticised (see e.g. Horsfall, 1956) on the grounds that an indirect-action therapeutant is not itself a fungicide: if this view is accepted, the term 'systemic fungicide' should be restricted to direct-action systemic therapeutants.

Toxin inactivation This topic is reviewed by the late Dr Dimond in Chapter 6. There is evidence that toxins secreted by the pathogen are at least partially responsible for inducing the disease symptoms in the host (Braun and Pringle, 1959; Ludwig, 1960; Wheeler and Luke, 1963; Deverall, 1964; Pringle and Scheffer, 1964). Certain chemotherapeutants are thought to antagonise these toxins, thereby reducing host symptoms without appreciably affecting the growth of the pathogen. The therapeutic action of 8-quinolinol salts against Dutch elm disease, for example, is thought to be due to toxin inactivation (Horsfall and Zentmyer, 1942).

In some ways related in their action to these fungal toxins are the extracellular hydrolytic enzymes, secreted by many fungi and known to induce

disease symptoms, notably in the vascular wilts (Wood, 1960). Grossmann (1962d) showed that several *in vitro* inhibitors of fungal pectinases can alleviate symptoms in tomato cuttings infected with the *Fusarium* wilt organism.

Enhancement of resistance of the host plant It has frequently been suggested that a chemotherapeutant operates indirectly by altering the metabolism of the host plant in such a way as to render it more resistant to disease. Widely differing mechanisms by which this increased resistance can be brought about have been suggested (see Chapter 6 and Dimond, 1963a, b, 1965; Grossmann, 1968b). These include alterations to (*a*) the host surface; (*b*) the nature of pectic substances (Edgington *et al.*, 1961); (*c*) wood morphology (Beckman, 1958); (*d*) carbohydrate levels (Horsfall and Dimond, 1957); and (*e*) phenolic constituents (Kaars Sijpesteijn and Pluijgers, 1962; Holowczak *et al.*, 1962). Several 'masked fungicides' appear to exert their therapeutic activity indirectly rather than by liberation of the free fungicidal molecule (Kaars Sijpesteijn, 1961b; van Andel, 1962b; Dekhuijzen, 1964).

A treatment that reduces disease without actually enhancing the disease resistance of the host has been demonstrated by Király *et al.* (1962). This operates by modifying the plant's growth so as to shorten the susceptible phase of development.

Although a classification of fungicides in terms of where, when and how they act in controlling plant disease is both feasible and useful, uptake, translocation and mode of action can be influenced by many factors as will be apparent in later chapters. Therefore, such a system of classification must inevitably be less rigid and precise than one based on the chemical structure of the fungicidal molecule. It must also be remembered that a compound which is a successful systemic therapeutant for one disease frequently fails to be so when a different host or pathogen is involved.

2

Historical aspects

by R. L. Wain and G. A. Carter

EARLY INVESTIGATIONS

Since this topic has been discussed in the reviews of Müller (1926), Roach (1939), Stoddard and Dimond (1949) and Horsfall (1945, 1956), only a brief outline will be given here.

The idea of introducing substances into plants goes back at least to the twelfth century when solid materials such as spices, medicines and colouring matters were inserted under the bark or into a borehole in a tree with the aim of imparting new odours, tastes or colours to the developing fruit. Such experiments were carried out spasmodically for several hundred years and in the fifteenth century Leonardo da Vinci describes the use of injections of arsenic into fruit trees to render the fruit poisonous. In 1602 an anonymous writer describes methods for improving the flavour and colour of fruits by inserting spices and dyestuffs into the pith; he also mentions the idea of killing 'wormes', i.e. larvae of wood-boring beetles, by inserting a mixture of pepper, laurel, incense and wine into boreholes. Over 150 years later Wilson records the use of liquid mercury inserted into boreholes in branches of trees to combat insect pests. This early work was, inevitably, empirical – there was at that time little knowledge of the uptake and movement of compounds in plants, although pioneer work on movement of sap was performed by Hales in 1726.

During the eighteenth century, when the study of plant physiology developed rapidly, studies were made on the movement of injected dyestuffs and mineral salts. These experiments revealed that certain com-

pounds could be transported readily within the plant but great differences in the extent of uptake and translocation were observed. A further impetus to these studies was provided by the work of Liebig and other agricultural chemists in their pioneer investigations on the mineral nutrition of plants which led to the recognition of nutrient deficiency diseases. In attempts to cure such diseases, and especially chlorosis resulting from iron deficiency, injections of mineral salts were made. This work was pursued by workers in France, Germany, Russia and the USA, culminating in the studies in this country by Roach (1934, 1938, 1939).

Mokrzecki (1903) observed that certain nutrient solutions when injected appeared to alleviate attacks by pests and diseases. Slightly earlier Ray (1901) had injected various liquids, some of biological origin, into leaves via capillary tubes with the specific intention of rendering the leaves immune from disease. In 1906 Bolley reported that injections of copper sulphate, iron sulphate and formaldehyde reduced attacks of *Taphrina* on fruit trees and ten years later Norton reduced the incidence of *Septoria lycopersici* on tomato plants by injecting copper sulphate solution.

At the turn of the century in Russia and Italy, poisonous substances, particularly potassium cyanide, were injected into plants in the hope of combating insect pests. Work with cyanide was continued in the USA where its movement and persistence in the plant were studied in some detail.

Early this century Brooks and his co-workers began investigations on the silver leaf disease of plum incited by *Stereum purpureum* and Brooks and Bailey (1919) found that several dyes and disinfectants, when injected into diseased trees, allowed some recovery. Later Brooks and Storey (1923) reported the very high *in vitro* fungitoxicity of 8-quinolinol sulphate towards *S. purpureum* and this compound also proved effective against silver leaf when injected into the host plant (Roach, 1939).

In the USA Rumbold (1920a, b) made extensive studies on the injection of sweet chestnut trees as a method of curing blight, caused by *Endothia parasitica*. She found that injections of lithium salts could check disease development but the effect was only transient. Later Scherer (1927) and Jacobs (1928) reported the efficacy of injecting a solution of thymol for the control of both fungal and bacterial pathogens.

Throughout most of the early studies on therapy of disease, injection directly into the host plant was the preferred method of application. However, attempts were made to eradicate established infections with surface-applied compounds; Viala (1893) reported that copper sulphate had an eradicant action against anthracnose *(Elsinöe ampelina)* of the vine and Bolley (1891) controlled scab *(Actinomyces scabies)* on potato tubers with mercuric chloride. Later it was shown that zinc chloride could