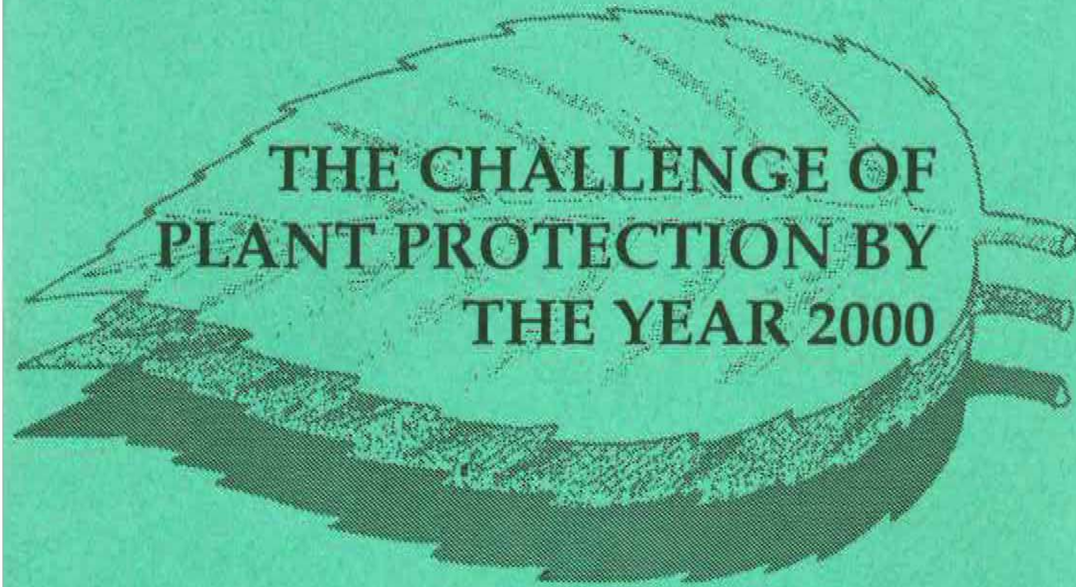

THE CHALLENGE OF PLANT PROTECTION BY THE YEAR 2000

Paolo Piccardi
Agrimont
(Montedison Group)

AGRIMONT S.p. A.
Via Medici del Vascello, 40/C
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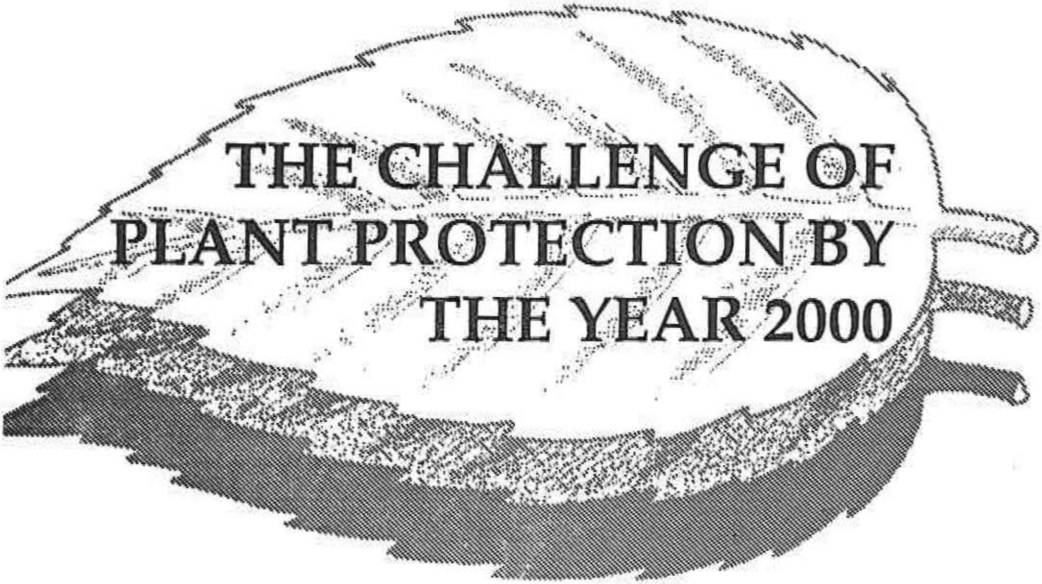

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Abstract

What is the real relevance of plant protection to man's present and future needs? The answer must take into account three important points:

- The effects of modern control agents on crop yields
- Their economics
- The feasibility and chances of alternatives.

Under present and foreseeable circumstances, the benefits from the *rational, selective use* of agrochemicals in general vastly outweigh their disadvantages. However, to continue to meet future food and fibre needs of the world population, we must overcome present constraints that impinge on our production capacity. Soil erosion and water quality, including rainfall, the genetic quality of plants, and energy limitations are all challenging incentives to develop novel management systems and practices that are economical, environmentally and ecologically sound, and socially acceptable.

The major consequences of these constraints on future plant protection research are predicted to be the following:

- *Biotechnology* will be an important component of plant protection strategy and research.
- *Biorational approaches* in the design of new active molecules will be the logical way to apply increasing knowledge of pest physiology and biochemistry, of interactions between plants and pests, and of comparative toxicology in vertebrates and invertebrates.
- *Integrated pest management* and soil conservation practices, such as reduced or zero tillage, will achieve great importance and will require new and improved products.
- *More target-oriented delivery systems* will be required for crop protection agents, and this of course will call for more specific formulations and spraying devices.

1 *Introduction*

Crystal-ball gazing is generally regarded as a difficult undertaking whose results are, at best, of uncertain value. One would say that this is even truer in the field of crop protection at a time when great expectations have been raised of quick and dramatic changes. However, two major considerations should be of help in making sensible forecasts.

Firstly, there will obviously be no change in the purpose of agriculture, which is to produce ever-increasing amounts of food, fibre and timber for a growing world population. And, since crops will always be threatened by insects, diseases and weeds, the future of plant protection can hardly be in doubt.

Secondly, progress in agriculture tends to be cautious and undramatic; new technology is usually introduced slowly, and with great care and effort, into existing agricultural systems. Consequently we can safely assume that the sound use of chemical agents will still be an essential part of crop protection technology by the year 2000, and that any new technology that will substantially affect agricultural productivity in ten years' time must already be known, at least within the research community.

This paper reviews the situation of innovative agrochemical products, the opportunities in crop protection research, and the likely evolution in the next decade.

2 *Characteristics of the Agrochemical Industry*

Before discussing the scientific and technical factors that are likely to limit, or to extend, innovation in the agrochemical industry, the size, shape and trends of this industry will be briefly reviewed.

It is estimated (Figure 1) (Wood Mackenzie, 1988) that the global end-user agrochemical market has grown from $850 million to $20 billion between 1960 and 1987 at an average rate of 12.4% per annum (p.a). The highest rate of growth has been achieved by herbicides (15.6% p.a. to $8.6 billion), followed by plant growth regulators and others (14.6% p.a. to $1.2 billion), insecticides (11.7% p.a. to $6.1 billion) and fungicides (9.7% p.a. to $4.1 billion).

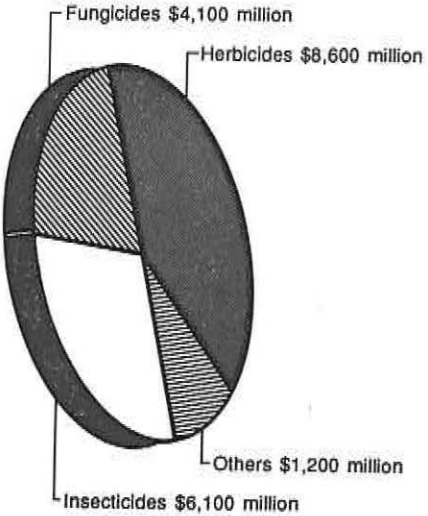

Figure 1. Distribution of the four key groups of agrochemicals in the world market in 1987 (US dollars).

No dramatic changes in the relative positions of the product groups are expected in the coming decade. The character of the market where the agrochemical industry operates, and its opportunities for new product introduction, are indicated by its geographical and crop breakdown (Figure 2). About 75% of herbicide usage occurs in Western Europe, the USA and Japan. In contrast with the herbicide sector, the major consumers of insecticides are the developing countries, due to their geographical position. In fact, tropical countries must ensure the highest possible yield and quality of the cash crops they grow. These crops include not only traditional tropical fruits, but also coffee, tea and cocoa, as well as fibre plants and sugar-cane. One should add the various spices, which have a limited, but very profitable, market. Crop protection is a must in these countries and could not be abandoned without ruining their general economy.

Virtually all agents in practical use for crop protection have been developed by industry, and it is generally agreed that industry will keep up its remarkable performance by producing new and improved products. However, agrochemical innovation is experiencing troubled times. The complexity of technical problems and the burgeoning regulatory requirements combine to make it increasingly difficult to

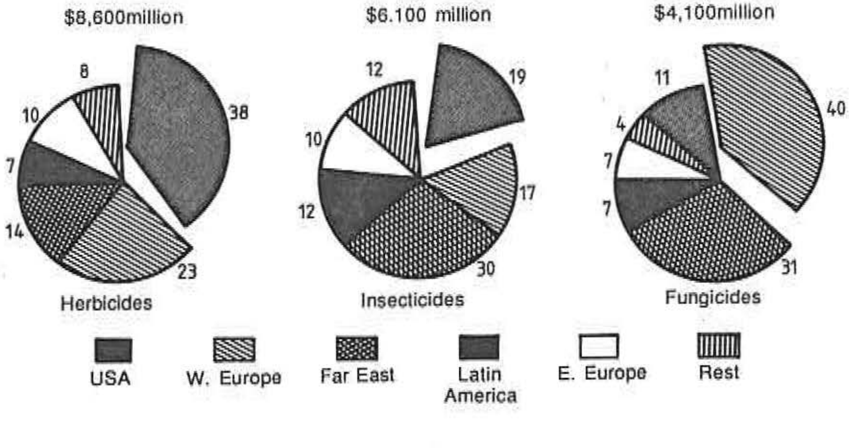

Figure 2. Percentage distribution of the three main groups of agrochemicals in 1987 (US dollars).

find profitable new products. In fact, while commonly-quoted figures are that some 8% of sales income is spent annually on R & D within the industry, an ominous change has taken place in the R & D budgets with the average expenditure for innovative research threatening to fall consistently, rather than occasionally. At the same time, all elements of so-called defensive research (studies of residues, metabolism, toxicology and environmental impact) have correspondingly increased.

The strict requirements now necessary for registration and re-registration, in most developed countries and in an ever-increasing number of the developing ones, pose severe problems for the innovation and development of agrochemicals. Now, this is not meant to criticise the attitudes and demands of registration authorities as, indeed, assurances of safety are welcomed and observed by responsible companies. But it must be realised that the development of a really new and marketable product takes at least seven years from discovery to commercialisation and may cost well above $15 million, leaving out the investment in new manufacturing facilities. One very unfortunate result of this high cost has been to make it increasingly difficult and unprofitable for a manufacturer to develop a compound that is specifically intended to be effective only in a restricted and specialised market (Herrett, 1989).

3 The Need for New Agrochemicals

In spite of the high costs and risks involved in the discovery and development of new agrochemicals, industry keeps up its efforts in this field. One may wonder what justifies the quest for new products. It should be borne in mind that, ideally, crop protection should prevent damaging effects from insect pests, diseases and weeds economically, safely and with no harm to the environment. Present methods, mainly based on agrochemicals and resistant crop varieties, have limitations. Thus the continuing development of resistance to older compounds, the lack of good solutions to some existing pest problems, the restriction or outright elimination of older, but perhaps very useful, agrochemicals for toxicological or environmental reasons, and the slowly changing pattern of pest and weed populations, are all excellent and well-acknowledged reasons for discovering new products. And although the slots where new compounds may fit will become fewer, while the criteria to judge performance and acceptability will get more stringent, this does not mean that there are not any opportunities left.

Besides, over the last 15 years agrochemical research itself has dramatically evolved into an intense, interdisciplinary effort binding together chemists, biochemists, biologists, toxicologists and other specialists. And when all these scientists are strongly motivated to work together, success becomes more likely.

Also, collaboration between industry and the academic world is equally important. The universities have been contributing to agrochemical science in a significant way and are expected to make further efforts to explore selected fields, particularly the physiology and biochemistry of both plants and insects. Scientists working in tropical and subtropical countries have increased in number and professional capability over the last two decades.

They should thus be able to provide precious, basic information on the peculiar features of tropical and subtropical crop ecosystems.

This information is indeed of fundamental importance in elaborating an approach to pertinent problems by more rational biochemical and chemical means (Geissbuehler et al., 1986).

4 Role of Biotechnolgy in Crop Protection

Some professional analysts are inclined to state that the so-called "era of chemical protection" is over, or has already passed its prime, anyway. While this might be true, in strictly economic terms, for certain highly developed or industrialised areas, this opinion is not acceptable from the scientific and technical point of view, as discussed above.

Nevertheless, there is a growing feeling that agriculture will benefit immensely from biotechnology. Certainly, many people believe that the potential benefits justify the diversion of research and development funds into the biotechnology field, a trend that is already quite evident in public agricultural research, in the food industry, and in the agrochemical industry itself. However, the initial excitement is now beginning to die down, as a more sober assessment of this field in general indicates that, in spite of all the glowing expectations, success is bound to be an expensive and lengthy process. In particular, it remains to be seen whether the new approaches can provide solutions to crop protection problems that will be acceptable to the farmer and his advisers for both their control effectiveness and their cost.

As things stand now, it is both likely and highly desirable that some of the basic research that is being undertaken in agricultural biotechnology may lead to the design and the development of a number of novel agrochemicals, rather than to their outright substitution. In fact, the more we learn about interactions between chemicals and about the physiological or biochemical reactions of living organisms, the better we are able to translate this information into practical and biorational solutions of pest control. In this respect, scientific knowledge is still at an early stage, but the growing number of sophisticated and quick biological tests will allow the identification of new target sites for chemical action. Three-dimensional studies of the interactions between a protein and an inhibitor should ultimately identify more effective inhibitors through the systematic application of computerised molecular modelling procedures (Piccardi, 1988). Biotechnology, in the broadest possible sense, has long been practised in agricultural science in general, and in pest control in particular. Breeding programmes have produced crops that are tolerant to diseases and insects. Biological control agents have proved their worth as components of pest control arsenals. The new biotechnologies should build upon these leads, and carry them to superior levels of efficacy. A comment on these will be made, when appropriate, in discussing the research opportunities in the control of insects, diseases and weeds.

5 *Research Opportunities in Insect Control*

5.1 THE PRESENT SITUATION

In examining the research opportunities in chemical insect control, it is appropriate to review briefly those highly effective weapons that have been added to the armoury of this sector of pesticides in the last decade (Figure 3).

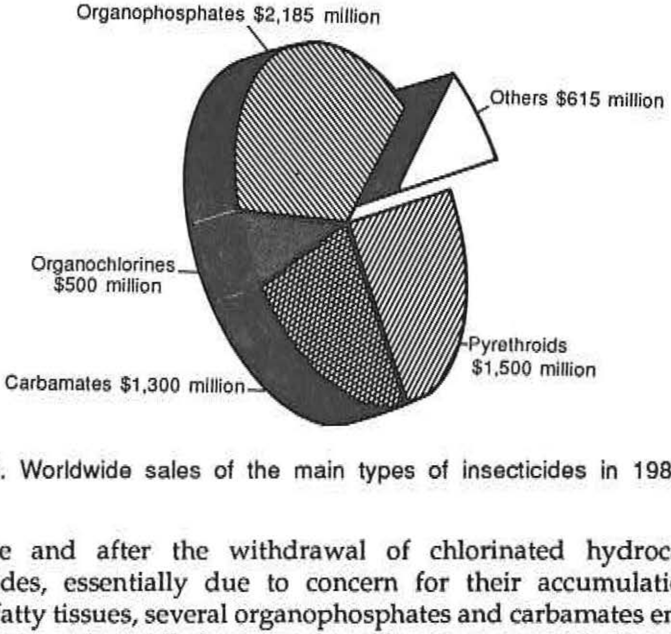

Figure 3. Worldwide sales of the main types of insecticides in 1987 (US dollars).

Before and after the withdrawal of chlorinated hydrocarbon insecticides, essentially due to concern for their accumulation in animal fatty tissues, several organophosphates and carbamates entered the world market. All these compounds act as acetylcholinesterase inhibitors and, consequently, their selective toxicities are mainly governed by differential metabolism in mammals as compared with insects. Their efficacy combines with low persistence in the environment.

However, in the seventies, the time was ripe for compounds obtained through new approaches, and by far the most important of these are the *synthetic pyrethroids*. Their introduction for agricultural use is the result of systematic chemical work, which started from the natural model of pyrethrins and, about 50 years after the first investigations, culminated in the discovery of photostable, highly efficacious compounds. The enormous interest promoted by their discovery is testified by the many reviews and symposia that have been dedicated to the subject (Crombie, 1980; Elliot, 1980).

Synthetic pyrethroids are now recognised to be — economically — the second most important group of insecticides with an estimated market size of about $1.5b in 1987. However, the profitability of this group is low because of the severe competition.

Chemistry-wise, the compounds developed in the last decade have generally kept the pyrethrin ester function. The alcohol moiety of the ester has been optimised, with minor variants, as the 3-phenoxybenzyl alcohol or, better, as the 3-phenoxy-α-cyanobenzyl alcohol, which confers higher metabolic stability to the molecules and approximately trebles activity. The very important acidic moiety has, of course, been the object of several modifications, though most of the successful products developed by the industry, particularly in Europe, are based on the dihalovinyl chrysanthemates licensed by NRDC.

To avoid commodity status, complex and original processes have been set up by Roussel-Uclaf for the totally resolved deltamethrin and, more recently, by Shell for the diastereoisomer alphamethrin. Other companies decided to follow a proprietary line by developing structures based on the original moiety, which can be either the alcohol, e.g. cyfluthrin (by Bayer), or the acid, e.g. the cyhalothrin compounds (by ICI), or both, e.g. tefluthrin (by ICI) and bifenthrin (by FMC, who developed permethrin as well).

An independent, original line was evolved by Sumitomo, long involved in pyrethroid chemistry, with fenvalerate, whose totally resolved form has also been developed. Similar compounds are fluvalinate (Zoecon) and flucythrinate (American Cyanamid). Two related, "non-ester" pyrethroids, MTI-500 (ethofenprox) and MTI-800, have also recently been presented.

Unlike the other sectors of the pesticide market, the great majority of important insecticides comes only from the three older groups of chemicals and the pyrethroids. Besides, all these products act on the nervous system, with the attendant grave risk of cross-resistance. This situation fully justifies the industry's keen interest in finding new compounds with radically different toxic mechanisms. One such possibility was opened up by the discovery of *benzoylphenyl ureas* (Verlop and Ferrel, 1977) a new, promising and selective class of insecticides that kill indirectly by virtue of their unique inhibitory action on chitin deposition. These compounds also show ovicidal and chemosterilant effects.

Diflubenzuron was the first product of the class and was synthesised by Philips-Duphar in the course of a programme aimed at discovering new urea herbicides. Its acute toxicity to man, fish and wildlife is very low and, due to a combination of factors, this compound is also relatively non-toxic to most beneficial insects. It is applied at field rates that can be compared with those of the most active pyrethroids. These outstanding properties soon made the product particularly welcomed in many integrated pest management programmes to protect orchards and forests. However, it did not obtain substantial market shares in the major crop sectors. It is likely that this failure was due mainly to the upsurge of the photostable pyrethroids, which overshadowed the product, and to its relatively slow killing action as compared with other insecticides. In spite of these drawbacks, the search for new benzoylureas is still under way and second-generation products are actively being developed. There are reasons to believe that, as the incidence of resistance problems in pyrethroids increases, insecticides of this type will play a more significant role in crop protection.

5.2 INSECTICIDES OF MICROBIAL ORIGIN

As part of the already-mentioned, keen general interest in finding new products that attack insect pests at novel sites and are environmentally safe, much attention is being paid to what we may broadly call microbial pest control methods. Thus, the successful use of antibiotics against human bacterial diseases has led to large-scale screening of fermentation products for crop protection properties. It is obvious that the success of this approach primarily depends on the availability of appropriate assays that are simple, sensitive and specific. Until recently, such testing systems were technically rather complex, labour-intensive, and often neither very reliable nor reproducible. However, many of these difficulties are now being rapidly overcome by continuous and impressive advances in the acquisition of basic scientific knowledge on the growth, development and reproduction of crop plants, insects, diseases and weeds. An intelligent exploitation of this knowledge will provide efficient and accurate physiological and biochemical assay procedures.

We already have pest-control agents that are produced by fermentation. A group of such products are the *avermectins* (Fisher, 1985), closely related macrocyclic lactones isolated from the mycelia of *Streptomyces avermitilis*. The increasing interest in these compounds has been stimulated by the discovery of their potent activity against a number of important human, animal and agricultural parasites. This activity is believed to arise from their interfering within invertebrate γ-aminobutyric acid (GABA) receptors and with chloride-ion channel functions. The combination of high toxicity and high specificity to invertebrates gives the compounds an enormous potential. The structure of avermectins is closely related to another group of natural macrolide products, the *milbemycines* (Mishima, 1983) which also exhibit high activity against phytophagous mites and some other pests. Almost certainly, milbemycines exert their action at the same receptor sites as avermectins. Discovery and exploitation of further such prototypes should be rewarding.

A group of microbial agents for insect control that is becoming increasingly popular are *bacterial insecticides* (Table 1). This is an area where a substantial amount of academic and government research has been conducted for many years, with some significant contribution by industry as well. The most successful bacterial insecticides have been those based on *Bacillus thuringiensis*, commonly known as *B.t.* which accounted for sales of about $50 million in 1987. The major commercial *B.t. insecticides* produced since the early seventies belong to variety "kurstaki" (serotype 3a3b of the HD-1 strain or its variants) with annual sales of about $30 million.

Bacillus thuringiensis is an aerobic, spore-forming bacillus that produces protein crystals in the course of sporulation. The large proteins of these crystals are partially broken down in the digestive tract of sensitive insects to yield other proteins that specifically bind

Table 1. Commercial bacterial insecticides

Bacterium	Target	Trade name	Company
Bacillus popilliae	Japanese beetle *(Popillia japonica)*	Doom	Fairfax
B. lentimorbus	Japanese beetle *(P. japonica)*	Milky Spore	Reuter Labs.
B. thuringiensis serotype 8A8B var."tenebrionis" and "San Diego"	Coleoptera *(Leptinotarsa decemlineata)*	Foil[1] M-One SAN 418	Ecogen Mycogen Sandoz
B. thuringiensis (HD 1) serotype 3A3B var. "kurstaki"	Lepidoptera	Bactucide Biobit Dipe[1] Thuricide Condor[1] Bactospeine InCide[3]	CRC Novo-MRL[2] Abbott Sandoz Ecogen Biochem Prods. Crop Genetics Int.
B. thuringiensis (HD1) var. "kurstaki" (SA-6 variant)	Lepidoptera *(Heliothis* spp.)	SAN 414	Sandoz
B. thuringiensis (NRD 12) serotype 3A3B var. "berliner"	Lepidoptera *(Spodoptera* spp.)	Javelin	Sandoz
B. thuringiensis serotype 7 var. "aizawa"	Lepidoptera (wax moth bee hives)	Certan	Sandoz
B. thuringiensis (HD 567) serotype 14 var. "israelensis"	Diptera (mosquitoes and blackflies)	Bactimos Skeetal[1] Teknar Vectobac Bactis	Biochem Prods. Novo-MRL Sandoz Abbott CRC
B. thuringiensis serotype 1	Diptera (mosquitoes and blackflies)	Muscabac	Farmos
B. sphaericus	Diptera (mosquitoes)	(in advanced development stage by many companies)[1]	

[1] Genetically altered strain produced by bacterial conjugation.
[2] MRL = Microbial Resources Ltd.
[3] The product consists of maize endophyte CG 102, incorporating the *B.t.* toxin gene.

themselves to a receptor site of the epithelial cell membrane, whose ionic balance is consequently disrupted. These events lead to feeding inhibition within a few minutes and to eventual disintegration of the gut wall (Luethy and Ebersold, 1981). Since the active toxin is only formed in the midgut of sensitive insects, the protein crystals as such do not harm non-target organisms. Formulations of *B.t.*-toxin have been on the market for years to control the larvae of a number of lepidopteran, dipteran and coleopteran species of economic importance (Aronson et al., 1986). Recently a variety of "tenebrionis" — serotype 8a8b — has been isolated that controls Colorado potato beetle (Krieg et al., 1984; Herrnstadt et al., 1986). Bacterial insecticides offer advantages over chemical ones, since they show little or no toxicity to mammals, do not usually cause a build-up of resistance in insects, and are considered to be environmentally safe.

In spite of these favourable characteristics and of the importance of biological control systems within the frame of integrated pest management (IPM), it is perhaps surprising that the *B.t.* share of the total market for insecticides is less than 1%. This commercial weakness is due to the major shortcomings of these products, such as a lack of persistence in field situations, a highly specific activity (which often makes their usage uneconomic in the simultaneous control of two or more species), and a critical timing of application, because they must be ingested by insects in order to kill them, combined with their slowness of action, which allows pest damage to continue.

Recent advances in genetic engineering techniques may be applied to overcome some of these constraints. *In vitro* recombination of different genes, or their mutagenesis, might improve not only the spectrum of application, but also the potency per unit of toxin produced, which might result in a more rapid toxic action.

Many companies are developing formulation technologies of *B.t.* products to avoid the easy inactivation of the protein toxin on storage, or after its release into the environment. A unique microencapsulation technique is based on a delivery system consisting of dead, genetically-engineered, *Pseudomonas* cells that contain *B.t.* thus creating a kind of biological package that protects the fragile toxin (Kim, 1987).

A second group of microbial insect control agents are *viral insecticides*, based on viruses that infect and kill some species of insects (Table 2). Unlike bacteria, which can exist free in the environment, viruses exhibit very low persistence and are incapable of replication outside their hosts. This provides an added margin of safety to non-target organisms. Most work has been done on baculoviruses, because they are only lethal to invertebrates and their viral particles are coated by proteinaceous crystals that protect them from rapid inactivation on leaf surfaces.

Table 2. Viral insecticides

Virus	Target	Trade name	Company
NPV[1] of *Neodiprion sertifer*	Pine sawfly	Virox Preserve	Novo-MRL[3] MicroGeneSys
NPV of *Mamestra brassicae*	Vegetable caterpillars	Mamestrin	Calliope
NPV of *Lymantria dispar*	Gypsy moth	Gypcheck	USDA Forest Service
NPV of *Orgya pseudotsugata*	Douglas fir tussock moth	Biocontrol-1	USDA Forest Service
NPV of *Heliothis zea*	Cotton caterpillars	Elcar	Sandoz
GV[2] of *Cydia pomonella*	Codling moth	Decyde	MycroGeneSys

[1] Nuclear polyhedrosis virus.
[2] Granulosis virus.
[3] Microbial Resources Ltd.

Although several pathogens have been approved for use in the USA, the nuclear polyhedrosis viruses of the European pine sawfly (*Neodiprion sertifer*) and of cotton bollworm (*Heliothis* spp.) are the only ones currently being sold, though in very small quantities (Klausner, 1985). The high degree of specificity of viruses and their high production costs have greatly limited their commercial success. Besides, these organisms generally act slowly and may thus be unable to prevent significant crop damage in cases of high pest infestation.

In the future these problems will probably be mitigated by biotechnology. Thus the introduction of an insect-specific toxin into the viral genome would quicken the rate of action and broaden the host range. However there is still a long way to go before any such engineered viral strains can be regarded as commercial products. Also, significant concern has been expressed over the release of genetically engineered viruses into the environment, and this may result in additional restrictions on research and product commercialisation.

It is generally agreed that in the coming decade the sales of microbial pesticides should increase at a healthy rate. However, it has been pointed out (Finney, 1988) that starting from their present very narrow base, the new biological products are probably not going to account for more than 5% of the total crop protection market by the year 2000.

5.3 BIORATIONAL APPROACH

The possible role of biotechnology in the discovery of new pest control agents has been dealt with briefly. The new century might see the development of entirely new classes of insecticides — more generally, of pesticides — through the knowledge gained from metabolic and other related biochemical studies in living organisms and from isolated enzyme systems. This novel approach is usually referred to as the *biochemical design* of new pest control agents (Piccardi, 1987).

Indeed, whenever a vital biochemical process of a pest organism can be identified, it should, in principle, be possible to rationally design, and hence synthesise, compounds that interfere with this process. For example, once a key enzyme has been singled out, the shape and reactivity of its active site might be defined. To this end, it would be of great help to know the structure of natural inhibitors and substrates of the enzyme, which may provide a sort of rough cast of the site. On these bases, and with the help of molecular modelling, it should be possible to design and then synthesise a range of potential inhibitors of the target enzyme. In the insecticide sector, examples of attractive targets for such studies might be the regulation of the GABA complex (Casida, 1986), or a full understanding of insect neuropeptide structures and their physiological degradation (Schooley et al., 1986), and of the neural, calcium-sensitive, phosphorylation systems.

Another approach, which has not yet been fully traced out but has nevertheless been named, is the *biorational design* of pest control agents (Geissbuehler et al., 1986; Piccardi, 1988). Broadly speaking, it calls for the isolation and characterisation of the many still-unknown compounds that are synthesised in minute amounts by living organisms to affect, very selectively, their growth, development and reproduction (as well as behaviour and defence against pathogens or parasites). The same process could be applied to any natural substance that may depress, or block altogether, the production of the above-mentioned powerful compounds. Any lead thus obtained might then be suitably modified by chemists working in close partnership with biochemists.

The outstanding example of work already carried out along these lines is the synthesis of molecules that interfere with the endocrine processes that control metamorphosis and reproduction in insects. Disappointingly, however, the very considerable effort in juvenile hormone analogue research did not lead to any commercial success. Methoprene is the only such compound on the market and is used to control mosquito larvae in floodwater and stagnant water.

Rather than applying mimics of juvenile hormone, it would theoretically be preferable to inhibit hormone production in insects. From a common bedding plant, *Ageratum houstonianum*, Bowers (Bowers, 1986) isolated compounds that caused precocious metamorphosis in a few hemipteran species, thus providing a model for intensive follow-up synthesis in several industrial and university laboratories. Little success has been achieved so far. The general impression is that if new control agents that work by disrupting the endocrine systems of insects are to be found, then more basic research into insect physiology and biochemistry will be required. This will allow the identification of possible sites of action that are susceptible to disruption in a way that would confer a broader and more useful spectrum of activity on the compounds.

6 *Research Opportunities in Fungal Disease Control*

6.1 THE PRESENT SITUATION

Progress in the protection of plants from fungal diseases was relatively slow up to the late sixties, but has undergone a dramatic acceleration since then. The chemicals developed up to that time were protectant fungicides, many of which are still widely used today (Figure 4). Most are unspecific inhibitors of respiration, acting at many sites on germinating spores, and are harmless to plants, which they cannot penetrate. The new fungicides, instead, can penetrate the plant cuticle and translocate within the plant to sites of infection. These *systemic fungicides* must discriminate between the plant and its

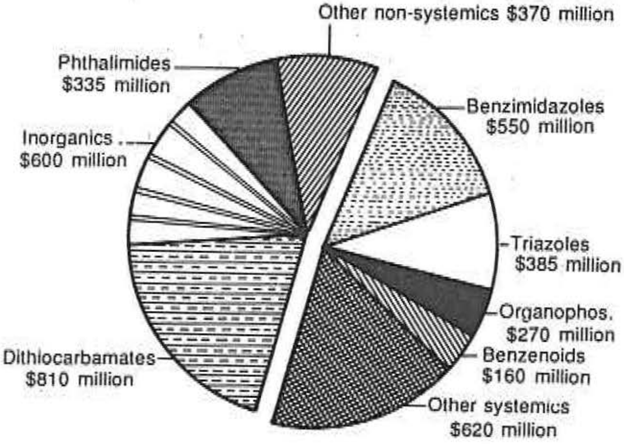

Figure 4. Worldwide sales of the main types of fungicides in 1987 (US dollars).

pathogen, in order to circulate within the plant itself without damaging it. Generally, this is possible by virtue of their affinity or binding with one site of an enzyme protein that is peculiar to the fungal pathogen and absent in its host. This specificity entails a certain vulnerability to structural variations of the binding site, leading to selection of resistant strains. Since it occurred extensively with the use of early systemic fungicides, resistance had been the object of intense research and careful control.

On the other hand, the considerable advantages of systemic fungicides (such as their favourable impact on the environment and very low rates of application) and the consequent market requirements have prompted the discovery and investigation of new chemical classes, thus making this sector of crop protection very dynamic and open to new prospects.

Among truly systemic fungicides, two classes have polarised academic and industrial interest in the last decade: the so-called *N-phenylamides*, and *azoles* or *ergosterol biosynthesis inhibitors* (EBIs). Compounds belonging to the first class are used in the control of diseases caused by *Peronosporales* (downy mildews) in important crops. They are all structurally connected, within the general formula of their class, but can further be grouped into three chemical families, namely alaninates, butyrolactones and oxazolidinones (Gozzo et al., 1985). Their common primary mode of action appears to consist in the inhibition of an endogenous RNA-polymerase in sensitive fungi. Secondary modes of action, probably due to effects on fungal cell membranes, have been clearly observed with benalaxyl and cyprofuram (Davidse et al., 1988). To avoid spreading resistant fungal strains, all compounds of this class are used as mixtures with protective fungicides, mainly dithiocarbamates.

However, it is the second class, the azole derivatives, which control a broad spectrum of Ascomycetina, Basidiomycetina and Deuteromycetina, that has attracted the greatest attention by far. This is witnessed by the impressive number of patents, reviews and papers published on EBIs and by the continuing interest in developing new products (Leroux and Benveniste, 1985; Koeller, 1987).

Following the pioneering work by both Bayer and Janssen in the antimycotic sector this area was given special impetus by the successful introduction of two groups of triazole derivatives (triadimefon/triadimenol and propiconazole) that were able to control major cereal diseases, such as powdery mildew and rusts, at very low dosages. As a result of so many companies actively pursuing success in this area, several new compounds have been, and are being, developed that are either more active, or have a broader spectrum, or both.

In common with other heterocyclic compounds, the azole fungicides have been shown to act as sterol inhibitors by preventing oxidative 14-α-demethylation of an early precursor in the biosynthetic pathway from lanosterol to ergosterol. This inhibition brings about cessation of mycelial growth. The 14-α-demethylase involved is a specific form of cytochrome P-450, the protohaem iron atom of which binds to the *meta* nitrogen of the triazole, thus excluding oxygen which is the natural ligand.

Selectivity of this interaction is important for two main reasons. Firstly the ergosterol inhibitors may also affect plant membranes, with consequent phytotoxicity. Secondly they may interfere with the biosynthesis of gibberellins by inhibiting oxidative demethylation of kaurene, which is also mediated by cytochrome P-450, with attendant growth retardation and other physiological effects. These may often be segregated from antifungal effects, for a given compound, by separation of its diastereoisomers or enantiomers and made unpredictably more or less pronounced by trivial modifications of the basic structure. In fact some triazoles have been developed as plant growth regulators.

6.2 BIOCHEMICAL APPROACH

Research on the mode of action of fungicides and on the mechanisms whereby fungi become resistant will continue as part of a rational effort to identify new target sites within fungal organisms. This will also provide needed and reliable guidelines in the correct and sustained use of established products. There are useful research approaches that can be pursued such as the practical use of some systemic products in combination, or in formulation, with synergistic chemicals.

The search for new fungicides may be viewed from other perspectives. Novel approaches could be directed towards the activation of either phytoalexin synthesis or other natural defence mechanisms of the plant by the use of the so-called elicitors of host resistance (Bailey, 1986). After all, many higher plants are impervious to fungal attack. However, prospects for the discovery of such alternative chemicals seem to be limited. At present, the origin and the chemical composition of the signal, or signals, involved in these systemic defence mechanisms are largely unknown. Their further investigation should be worthwhile. If chemically accessible, such signal substances may provide a new method of plant disease control in the future. Investigations leading to compounds that affect fungal development and differentiation may also be rewarding.

In general, fungi organise their living structures differently from their hosts. For example, fungi reproduce through spores and some fungal cell walls are made of chitin. A few assays have been developed to exploit these differences, such as those that enable the selection of antisporulant and anticonidiophore compounds. But more work will have to be done to achieve practical results.

7 *Research Opportunities in Weed Control*

7.1 THE PRESENT SITUATION

We have already seen that, generally, a knowledge of biochemical modes of action is obviously of the utmost importance in understanding crop protection. In the case of *herbicides*, their modes of action have often been very difficult to elucidate, because several physiological functions are usually altered, thus making it difficult to distinguish primary effects from induced, or secondary, effects. Most older families of herbicides (Figure 5), covering about 50% of the marketed products, work by inhibiting that most peculiar of plant functions, photosynthesis. Surprisingly, perhaps since their chemical structures differ considerably, the majority of them act at the same site, which is located in the membrane of chloroplasts. Completely different modes of action can be found instead in many newer, very effective products.

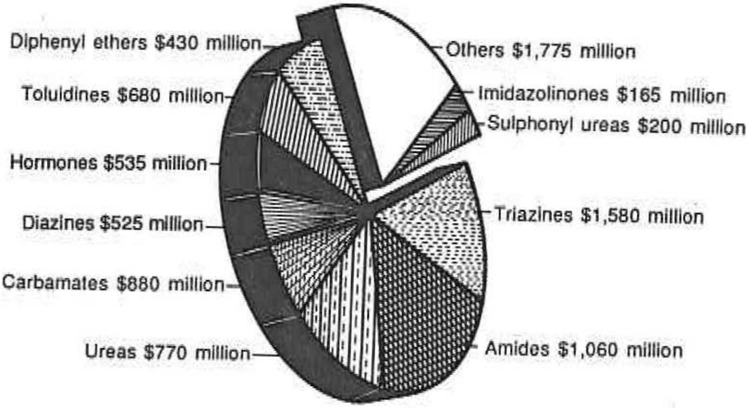

Figure 5. Worldwide sales of the main herbicides in 1987 (US dollars).

It is generally of primary importance that a good herbicide be selective, that is capable of discriminating between crops and weeds. Nevertheless *glyphosate*, an outstanding herbicide that has been developed by Monsanto for use on unwanted vegetation, is essentially non-selective. Glyphosate is environmentally safe since it is a non-toxic compound that is quickly inactivated by the soil. Its action is slow and takes place in the meristematic tissue of both shoots and roots, which are readily reached by this product on translocation through the phloem. Cell death occurs following inhibition by glyphosate of 5-enolpyruvylshikimate-3-phosphate synthase with consequent starvation of essential aromatic aminoacids. The chemistry of glyphosate and related compounds has been thoroughly investigated by Monsanto but no new product has emerged, apart from *glyphosine* a compound that has been developed as a plant-growth regulator.

Although glyphosate appeared to be unique, as a herbicide, among the phosphoromethylated-aminoacids, a new phosphorus-bearing aminoacid, *phosphinothricin*, was independently isolated by two research groups from culture broths, as a tripeptide. Both phosphinothricin and its tripeptide showed non-selective herbicidal properties. The former has been developed, starting from new organophosphorous intermediates, as *glufosinate* (Hoechst) and seems to act by inhibition of glutamine synthetase. The tripeptide is being made by fermentation and developed as *bialaphos* by Meiji Seika Kaisha.

Two other families of herbicides, developed in the eighties, appear to inhibit the biosynthesis of some aminoacids and share some similarity of symptoms with glyphosate, as they primarily affect the meristematic tissues. The first of these is represented by a number of *sulphonylureas* (Levitt, 1983) which can kill broad-leaved weeds at incredibly low rates (5–35 g/ha) in both pre- and post-emergence treatments. The only major crops that normally resist their action are cereals, which can quickly metabolise sulphonylureas.

The extensive investigation of this class by Du Pont has resulted in the development of several compounds with complementary efficacy, the first concern being, apparently, the allowance for rotational crops. Recently new compounds of the same family have been introduced by other companies.

The mode of action of sulphonylureas has been thoroughly investigated and it was soon recognised that they were potent inhibitors of cell division. On further investigation, this was shown to occur through inhibition of acetohydroxyacid synthase, a key enzyme which controls the biosynthesis of the branched-chain aminoacids, leucine, isoleucine and valine in plants and micro-organisms.

At about the same time, another class of new herbicides, the so-called *imidazolinones* (Los, 1986), were also found to act by inhibiting acetohydroxyacid synthase. Chemically, they are altogether different. The free carboxylic acid is considered to be the phytotoxic principle and when esterified (as in *imazethabenz*) selectivity appears to depend on its liberation in weeds, while in cereals other metabolic pathways are dominant. Imazaquin and imazethabenz showed selective control of grassy and, especially, broad-leaved weeds incorporated in pre-plant, pre-emergence, and post-emergence treatments of leguminous crops, where the metabolism was stated to be more rapid. The nicotinic acid derivative *imazapyr*, which has no substituent available for easy metabolic attack, is a non-selective herbicide introduced for total vegetation control.

7.2 BIOTECHNOLOGY IN WEED CONTROL

All these classes of herbicides that kill plants by inhibiting highly specific enzymes cannot do any harm to fish, insects and mammals. Also, they either break down readily in the soil or do not leach appreciably into ground water, and are thus highly desirable from an environmental perspective. However, their very broad spectrum of activity, often bordering on lack of selectivity, is an obstacle to their widespread use. They are not likely to be used to their full potential unless resistant crops can be made available.

Recently, gene transfer and cell selection techniques have been successfully used to create crop plants tolerant to these products (Shah et al., 1986; Fillatti et al., 1987). The rapid progress that has been made in the engineering of selective herbicide tolerance has been really surprising. This research has been greatly helped by knowledge of the mode of action of the herbicides involved, and by the identification and molecular cloning of genes that encode herbicide-sensitive and herbicide-insensitive target proteins or by encoding the enzymes that detoxify the herbicides. As the methods for gene transfer and for trait incorporation into commercial germplasm become routine, the engineering of selective herbicide tolerance will become an accepted and essential strategy for the development of weed control systems.

7.3 FUNGI AS WEED KILLERS

Natural infections by fungi are known to play an important role in the control of many pests, but their use in crop protection is very limited (Table 3).

Table 3. Commercial mycopesticides

Fungus	Target	Trade name	Company
Verticillium lecanii	Aphids	Vertalec	Novo-MRL[3]
V. lecanii	Whiteflies *(Trialeurodes vaporariorum)*	Mycotal	Novo-MRL
Beauveria bassiana	Colorado beetle *(Leptinotarsa decemlineata)*	Boverin	(USSR)
Beauveria brongniartii	Cockchafer larvae in woodland	([1])	Abbott
Hirsutella thompsonii	Citrus rust mite *(Phyllocoptruta oleivora)*	Mycar[1]	Abbott
Metarhizium anisopliae	Spittle bug, sugarcane frog hopper	Metaquino	Embrapa (Brazil)
Paecilomyces lilacinus	Nematodes	Biocon	Asiatic Technologies (Manilla)
Pseudomonas spp.	Seed cotton fungicide	Dagger	Ecogen
Trichoderma barzianum	Seed treatment fungicide	([2])	Eastman Kodak Bio-Technology General Makhtesheim
Phytophthora palmivora	Milk weed vine *(Morrenia odorata)*	Devine	Abbott
Colletotrichum gloeosporoides	Northern joint vetch	Collego	Nor-Am (Schering)
Alternaria cassiae	Sicklepod *(Cassia obtusifolia)*	Casst[2]	Mycogen
Fusarium lateritium	Velvetleaf plants	Velgo[2]	Mycogen

[1] Commercial trials in Europe.
[2] Under application for registration.
[3] Microbial Resources Ltd.

Current research has been focused primarily on the use of *fungi as weed pathogens*, since fungal diseases of weeds are readily observed and provide an easy source of material. Recently, a mycoherbicide has captured the attention of the media (Klassen, 1987). This product, which is under development, is based on a fungal pathogen, *Alternaria cassiae*, and it has been shown capable of effective, post-emergence control of sicklepod in glasshouse and field trials, when applied alone or in combination with chemical week killers. Sicklepod is a resistant weed that infests about 4 million hectares of soybeans and peanuts in the southeast USA. The problem of poor control under dry conditions, common to all mycopesticides, is being tackled by using different formulations.

As in the case of biological insecticides, several of the benefits of mycoherbicides can also be seen as disadvantages, when compared with chemical products. For example, low persistence of any toxic residue in the environment is a bonus, but this may entail a far greater number of applications of a biological herbicide than are necessary with a conventional product to achieve residual control of weeds. Even if product costs per hectare of application were comparable (an unlikely situation, since the technology for their large-scale production is difficult), total product costs per season would be higher. The second drawback results from the very high degree of selective action, which is almost contrary to the goal of a herbicide. Although only one weed may be dominant in a field, its elimination would simply help other weeds to flourish.

Suppliers of mycoherbicides are attempting to overcome these limitations by developing formulated products that are compatible with commonly used chemical herbicides, so that they may be used in tank-mix combinations.

8 *Conclusions*

It can be confidently predicted that agrochemicals will still play a very important role in crop protection by the year 2000.

The industry is in good shape and will certainly do its best to introduce novel agrochemicals, in the face of scientific and regulatory difficulties, because highly effective, environmentally-safe, new products are, in fact, badly needed.

Biotechnology will not wipe out pesticides, but will play an increasingly important role mainly by stimulating basic research that may lead to the discovery of new and better agrochemicals.

In the field of insect control, highly efficacious compounds are not available, but insecticides with new modes of action are needed and may indeed become available through biochemical and biorational design. Microbial insect control agents and integrated pest management will become increasingly important.

Research has produced an impressive array of systemic fungicides and resistance is being carefully studied and controlled. Work is in progress everywhere to identify new target sites in pathogenic fungi and to find ways of stimulating plant resistance.

And, finally, weed control has recently achieved remarkable progress with the introduction of products that can be applied at extraordinarily low rates and have new modes of action. Knowledge of the latter will be useful in the rational design of new herbicides.

In order to make full use of the potential of the very active modern compounds now available, rapid progress is being made in the engineering of crop plants tolerant to these products. For some of the mycoherbicides now being produced, there is the prospect that they might be improved by biotechnology.

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