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Innovation in Crop Protection: Trends in Research

Jörg Stetter* and Folker Lieb

In the absence of the remarkable levels of growth in the yields of important crops, neither the rapid increase in living standards in industrialized countries nor the adequate standard of nutrition for the greater part of the world's population would have been possible. Alongside high-yielding varieties, improved agricultural techniques, and rapid mechanization, the chemical industry has also contributed substantially to progress in agriculture since roughly the middle of the nineteenth century. From the chemists "kitchens" came two "magic weapons": artificial fertilisers and chemical agents for crop protection. Today both have become indispensable to modern yield- and quality-orientated agriculture. This review spans the development of the crop-protection industry from its earliest beginnings to the present day and attempts to portray how the research-based crop-protection industry is prepared for current and future challenges. Considerable space is thus dedicated to the discussion of trends in research.

Keywords: active substance research · chemical crop protection · pesticides · transgenic plants

1. Introduction

In "Gulliver's Travels", author Jonathan Swift says of King Brobdingnag: "…and he gave it for his opinion that whoever could make two ears of corn, or two blades of grass to grow upon a spot of ground where only one grew before, would deserve better of mankind and do more essential service to his country than the whole race of politicians put together".[1] By breeding high-yielding cereal grain varieties, dedicated scientists such as the Nobel peace prize laureate of 1970, Norman E. Borlaug, have achieved considerably more than that, yet their names are virtually unknown to the general public.[2, 3]

The first practicable crop-protection agents, such as the legendary Bordeaux mixture[4] (Figure 1), were chance discoveries, with the user reaching, in a sense blindly, into the chemicals cupboard; they represented the first modest weapons in the battle against diseases of potatoes and vines in the latter part of the nineteenth century. In the beginning the agents were generally inorganic in nature, often unselective, active only at high application rates, and frequently toxic. Arsenic-based insecticides remained the mainstay of insect control well into the twentieth century. Thus, in 1941, approximately 30000 tonnes of arsenic trioxide were still used in the USA alone for the production of arsenic-based insecticides.[5, 6]

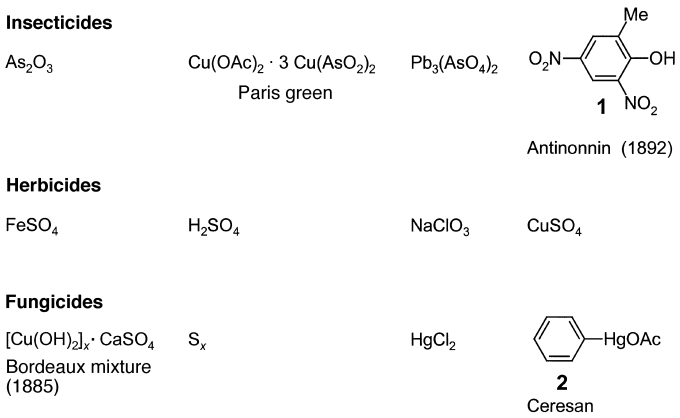

Figure 1. Historical agents of crop protection, 1st generation (1800–1930).[5]

Organised industrial research with the aim of finding less toxic, selective organochemical active ingredients for crop-protection applications first started in the 1930s. Substances which were simple to synthesize but which were nonetheless already effective, such as TMTD[7], E 605[8, 9], DDT[10–12] or 2,4-D,[13] are synonymous with the first breakthroughs achieved (Figure 2). With these substances the foundations were laid for a flourishing crop-protection industry with an estimated market volume of around 30 billion $US annually.[14]

Initially, there was an immense need to protect the increased yields, which were accruing from the rapidly developing agricultural industry as a result of improved crop

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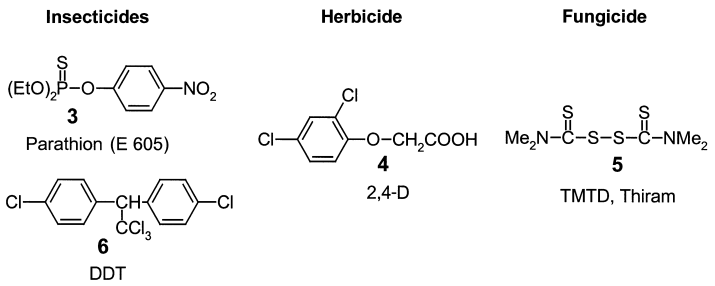

Figure 2. Historical agents of crop protection, 2nd generation (1930–1950).

varieties, artificial fertilisers, and mechanization, from the effects of pests and pathogens. User acceptance of crop-protective agents was extraordinarily high, which is attributable to the improved quality of life they brought to the farming community, as well as to the reduced economic risks. The use of crop-protective agents was an investment which yielded considerable returns. Chemistry in harmony with agriculture, an ordered world, an ideal partnership, or so it appeared for a long time. However, where there is light, there must inevitably also be shadows!

1.1. The Problem of Acceptance

With publication of Rachel Carson's book "Silent Spring",[15] if not beforehand, the relationship between agriculture and chemistry became, at the very least, tainted in the eyes of the general public. The spectrum reaches from a lack of acceptance to outright rejection, coupled with demands that the use of chemical agents in agriculture be banned. A discussion of the reasons for this is beyond the scope of this review. Two sources for the lack of acceptance should, however, be named at this juncture: first, the all pervasive "chemophobia" in contemporary culture. Irrationally, chemophobia is limited to synthetic chemicals. Ignoring their properties, the natural "environmental" chemicals, which dominate by orders of magnitude in terms of their numbers and amounts, are regarded as gentle, benign, and almost as though willed by God. This view is naive since nature makes use of an inexhaustible arsenal of chemicals, which to a large part are nothing less than chemical weapons for defence against hostile organisms.[16, 17] Even those cultivated plants which primarily serve for the nutrition of man and animals contain many secondary metabolites of proven toxicity (Table 1).[18–26]

The second reason for the lack of acceptance lies in widespread ignorance over the conditions for production in modern agriculture. Who in our modern industrialized society still faces the battle for yield and quality with which the farmer is continually confronted? With regard to the achievements of agriculture, yield and quality are either taken for

Table 1. A very healthy meal.[a]

Foodstuff	substance	action
carrots	carotatoxin	nerve poison[18]
pepper	piperine	mutagen[19]
parsley, celery	psoralen	mutagen, carcinogen[20]
raddish, onion, broccoli	thiols, disulfides[21]	inhibitors of thyroxin synthesis in the thyroid gland[22]
mushrooms	agaritine	promoter of ulcers and cancer in the stomach[23]
apples	phlorizin	cause of glycosuria[24]
mustard, horseraddish	allylisothiocyanate	cause of damage to chromosomes[25]
raspberries	coumarin	liver toxin,[26] inhibitor of blood clotting

[a] If the same safety requirements were demanded for "natural substances" or food additives as are applied to synthetic substances, the consumption of foodstuffs would have to be banned!

Jörg Stetter, born in 1945, studied chemistry in Aachen and Munich, gaining a PhD from the Institute for Organic Chemistry at the Ludwig-Maximilians University in 1973 under the supervision of Prof. Gompper. In 1974 he joined the staff of Bayer AG as the head of a crop-protection research laboratory at Wuppertal-Elberfeld. By way of various positions—section head in the Central Research Division in Leverkusen, section head of crop-protection research in Wuppertal and Monheim, and the head of process development in the Crop-Protection Division—he became head of a subject field in 1989 and then, from 1997 onwards, departmental head in the Central Research Division in Leverkusen. Since 1993 he has held an honorary professorship in technical chemistry at the Bergische Universität Wuppertal. He is a committee member of the Liebig Vereinigung für Organische Chemie and a member of the board of governors of the GDCh accreditation agency for studies in chemistry.

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granted or even seen as excessive in the present day. The consumer finds cheap, high quality foodstuffs just around the corner in the supermarket at the same time as reading about surpluses which have to be destroyed to maintain price stability. That there is little understanding for the necessity of crop-protection agents should, therefore, come as no surprise.

1.2. The Situation Today

The transformation of agrochemicals to a high-tech industry in the last few years has been almost unnoticed by the general public. In terms of scientific rigour and demand for high quality results, today's research and development in the crop-protection sector can no longer be distinguished from pharmaceutical research and development. Comparable average product-development time spans of 8–12 years speak clearly for themselves. Crop-protection chemicals today are amongst the most thoroughly investigated substances with which man and the environment come into contact. This is a fact which cannot seriously be called into question. Worldwide, there is an impressive, although dwindling, arsenal of active ingredients from which to draw, and more and more is known about them. This is because even older active ingredients are regularly tested according to the latest safety criteria in the course of re-registration procedures.

1.2.1. The Transparent Active Ingredient

The days when all that was known about a pesticide was the chemical structure and the observed beneficial effects are long gone. For example, when DDT was first introduced for crop protection, many of its properties were unknown; it was, so to speak, a "black box" (Figure 3).[27] Today the box is transparent, packed full of the latest scientific know-how in various disciplines and data generated by selected experts. "Transparency" of the active ingredient means elucidation of all relevant properties of a substance. Determination of the environmental fate of the substance and its effects in an ecosystem provides an interesting challenge for scientists from a wide variety of disciplines. How is the substance taken up and transported? How efficacious is it against the target organism and what is its mode of action? How is it broken down by plants, by animals, in water, in the soil, and in the air? Which metabolites are formed? Which residues remain? Which side-effects occur in so called "non-target organisms"? What is the toxicological profile of the substance? An army of dedicated scientists investigate these and many more questions, using the most modern methods of analytical, physicochemical, toxicological, ecological, and biological sciences, including genetically based biotechnological techniques.

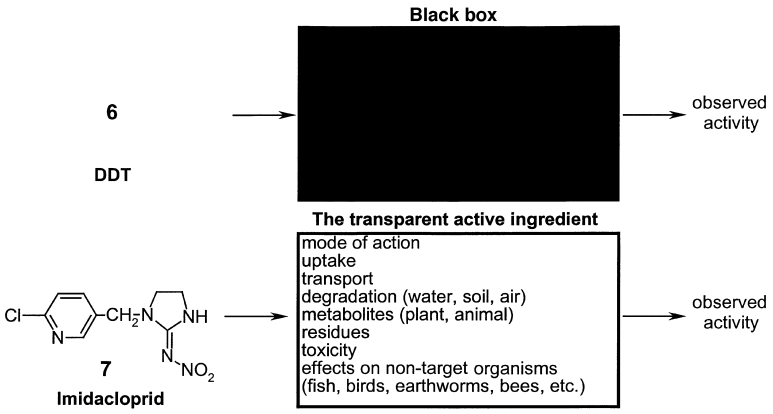


Figure 3. The transparent active ingredient.

1.2.2. Pressure of Costs and Market Perspective

Fascinating as all this is, the costs for the research and development of an active ingredient and suitable forms of application (formulation) have skyrocketed as a result. Today they lie in the order of 250 to 300 million DM, dimensions not far removed from the costs incurred in the development of a pharmaceutical product. However, unlike the pharmaceuticals sector, in which a single market segment such as anti-infectives has a turnover of 40 billion DM (equivalent to some 80% of the total value of the market for crop-protective agents), the segments of the agrochemicals market are substantially smaller, which severely limits the level of research and development expenditure which can be tolerated. Added to this, problems of reduced yield and quality of crops caused by pathogens, weeds, and pest animals can today in principle be more or less satisfactorily solved using existing agents of crop protection. As a rule, the introduction of a new active ingredient leads to displacement competition following the motto "the better is the enemy of the good". Better can mean many things, for example, cheaper for the user, more favorable toxicological and ecological properties, more easily broken down, less leaching behavior, a breakthrough against resistance, reduced application rates, additional systemic properties, favorable properties for beneficial organisms, or more selective. New active ingredients which open up additional markets are extremely rare.

In the pharmaceuticals field, the perspectives are quite different due to the many hitherto unsolved disease problems. New markets can be established here extremely quickly as soon as an innovative product becomes available (the most recent example is Viagra).

Products are no longer developed specifically for a particular niche pest because of the high developments costs for an active ingredient. All the research-based crop-protection companies concentrate their efforts on the large markets. The most sought after crop-protection products are those with a large potential market.

No one can sensibly argue with the fact that crops the world over will also need to be protected in order to secure yields in the future. The dynamics of world population growth allow no other choice. Even medium-term predictions demonstrate the need to act (Figure 4).[28]

2. The Future of Research and Development

The remarks so far have attempted to clarify the framework within which current and future devel-

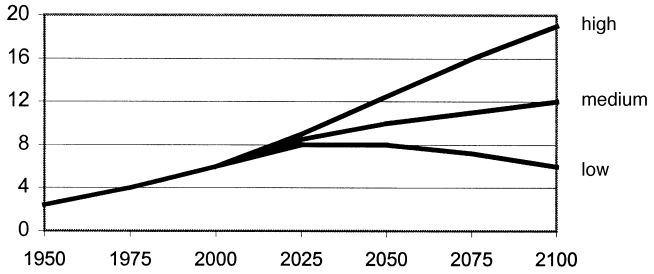


Figure 4. Previous development and predicted growth in world population.

opments in crop protection must operate. Crop-protection companies have adjusted to this with appropriate strategies for research and development. Innovation and efficiency are the magic words. Under the theme innovation and current trends in research and development, various hypotheses can be listed which will be expanded upon and illustrated with examples in the following sections.

Hypotheses for the Future

- Chemical methods will play a dominant role until well into the twenty-first century
- There will be no revolutionary change, established technologies will develop further by a process of evolution
- The process of research and development for new active ingredients will become substantially more efficient, especially through modeling, miniaturization, automation, and electronic data processing in key operations
- There will be fewer, ever safer, "bespoke" active ingredients which will be developed into saleable products
- Improved formulations and application techniques will allow a more targeted use of active ingredients, resulting not only in greater efficiency but also increased safety for the user, the consumer, and the environment
- In the context of integrated pest management, nonchemical methods will increasingly play a supporting role alongside chemical methods
- The introduction of transgenic crops, especially those resistant to herbicides and insect pests, will lead to sometimes substantial shifts in the affected markets
- Predictive and diagnostic methods will be developed much further (the concept of damage thresholds)
- Education and training of users will play an increasingly important part

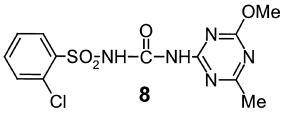
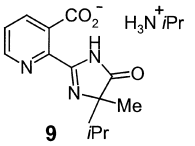
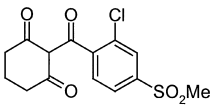
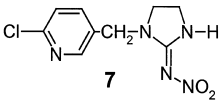
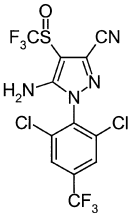
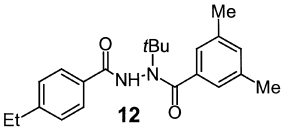
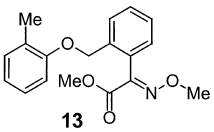
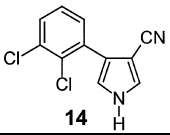
It should however, be reiterated that 'chemical methods' means those that use low molecular weight active ingredients generally, regardless of whether these are of synthetic origin or are natural products, whether they are single defined substances or mixtures, and whether they have a killing or a moderating mode of action. Stated in plain English: the use of pheromones, neem extracts, tobacco juice, and stinging nettle brews is nothing other than chemical pest control.

3. The Significance of Synthetic Chemistry for Modern Crop-Protection Research

Synthetic chemistry has not exhausted its ammunition by a long way. As the observation of new patent applications shows, the flood of new lead structures to be pursued has not yet stilled. Table 2 is a selection of interesting and important classes of structures from the 1980s and 1990s, which have yielded new and valuable solutions to problems. For every class there is an interesting story behind the invention and the inventor. It is remarkable how often luck has played a part.

It is neither to be expected or feared that the stream of valuable new lead structures and classes of active ingredients will dry up sometime in the future. The possibilities for variation offered by modern synthetic chemistry are too

Table 2. Important structural classes from the 1980s and 1990s.

Class of structure	active ingredient	indication	mode of action
sulfonyl ureas[29]	Chlorsulfuron (DuPont)  **8**	herbicide	acetolactate synthase (ALS) inhibitor
imidazolinones[30]	Imazapyr (American Cyanamid)  **9**	herbicide	acetolactate synthase (ALS) inhibitor
triketones[31]	Sulcotrione (Zeneca)  **10**	herbicide	4-hydroxyphenyl-pyruvate dioxygenase inhibitor
nicotinoides[32]	Imidacloprid (Bayer)  **7**	insecticide	agonist of the nicotinergic acetylcholine receptor (n-AChR)
arylpyrazoles[33]	Fipronil (Rhône Poulenc)  **11**	insecticide	GABA-sensitive chloride-channel (antagonist)
benzoylhydrazine[34]	Tebufenozide (Rohm & Haas)  **12**	insecticide	ecdysone agonist
MOAs[35]	Kresoxim-methyl (BASF)  **13**	fungicide	inhibitor of cytochrome reductase (in the respiratory chain)
pyrroles[36]	Fenpiclonil (Novartis)  **14**	fungicide	inhibitor of glucose phosphorylation linked to transport; inhibitor of protein kinase PK III

numerous. With the aid of techniques of automated synthesis, it should be possible to probe the expanse of chemical diversity more effectively than was possible in the past.

3.1. Successful Structural Motifs

It is noticeable that there are tried and tested structural motifs which repeatedly occur in successful active ingredients. One of many examples is the pyridine heterocycle. Table 3 shows several active ingredients containing the pyridine building block, none of which have a common mode of action and all of which have different target indications.

Frequently it is not simply the basic chemical units or the templates, in the parlance of the synthetic chemist, but often the presence of very specific substituents in certain selected parts of the molecule, which are decisive in determining the biological activity. In view of the huge, continuously increasing diversity of the chemical building set, it is obvious that many interesting classes of active ingredient await discovery. Just how the tiniest variation in substitution pattern can separate high from negligible activity or even activities in completely different indications, is illustrated by the examples shown in Figure 5. They also show, however, that in the search for a lead structure, an absolute prioritization based on chemical diversity can lead in the wrong direction. The real goal must be to find successful structure clusters in the universe of imaginable, low molecular weight, chemical compounds.

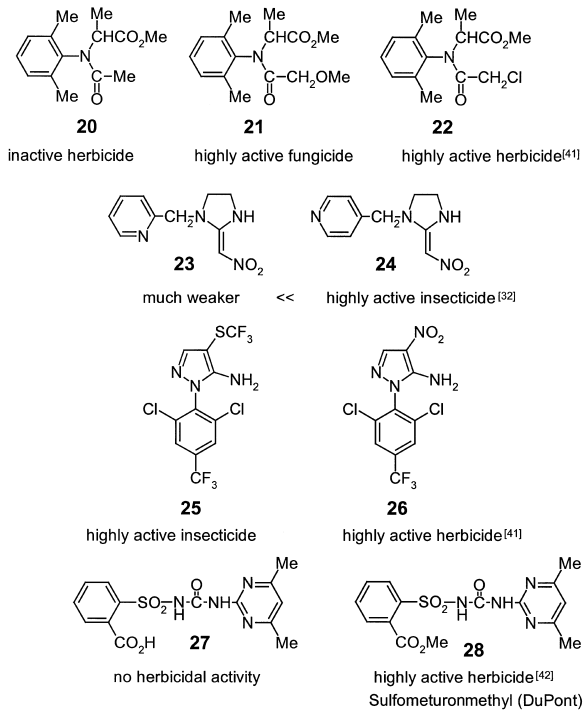
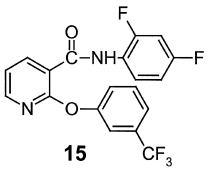
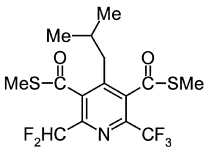
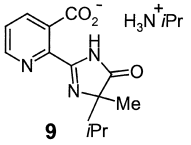
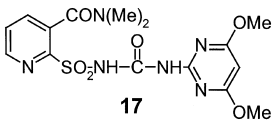
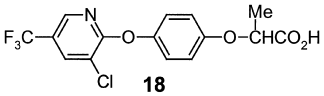
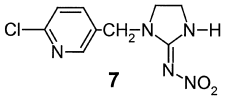
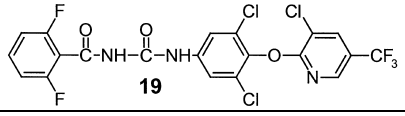


Figure 5. Chemical diversity isn't everything!

Table 3. New commercial products from the 1980s which incorporate a pyridine component.

Product	active ingredient	indication	mode of action
Diflufenican[37]	 **15**	herbicide	inhibitor of carotinoid biosynthesis
Dithiopyr[38]	 **16**	herbicide	inhibitor of mitosis
Imazapyr[30]	 **9**	herbicide	acetolactate synthase (ALS) inhibitor
Nicosulfuron[29]	 **17**	herbicide	acetolactate synthase (ALS) inhibitor
Haloxyfop[39]	 **18**	herbicide	inhibitor of acetyl-CoA carboxylase
Imidacloprid[32]	 **7**	insecticide	agonist of the nicotinergic acetylcholine receptor (n-AChR)>
Chlorfluazuron[40]	 **19**	insecticide	insect growth regulator, inhibitor of chitin biosynthesis

3.2. The Obligation to Make an Early Selection

More important in the future than ever before will be the ability for a successful, research based crop-protection company to separate the wheat from the chaff early on and to proceed with the right lead structures and candidate compounds. The key words for such a procedure are more efficient generation and identification of lead structures, and earliest possible and targeted optimization of all parameters having a bearing on development within an integrated concept. Very early knowledge about possible problems regarding toxicity, ecotoxicity and behavior in the environment are essential to-

day. The establishment and assessment of appropriate high performance, meaningful, early test systems is underway everywhere. Only when these yield the necessary certainty that the right choice has been made, can the real development work of field trials throughout the world, process development, wide-ranging experiments on animals, studies on metabolism, and the investigation of residues be justified.

3.3. Everything Revolves around the Lead Structure

The creed of the researcher seeking an active ingredient is that, at the beginning stands the lead structure. The lead structure is a defined chemical substance arising from synthesis or from a pool of natural substances, which demonstrates activity considered worth pursuing in appropriate biological tests. The more relevant a particular test within a screening cascade is to the practical application, the more valid the tested lead structure (Figure 6).

3.3.1. The Screening Cascade

The result from a tried and tested screening system based on whole organisms (insect, plant, or fungus) is of course much closer to real life than an in vitro target assay (enzyme, receptor, or ion channel). In the former, the effects of absorption, transport, and metabolism have already been taken into account. In principle, all potential targets are also tested, including those hitherto unknown. In target screening, only those substances which interact with a particular target can be found. Target screening can, however, provide valuable, additional information on shifts of activity within a class of substance or on possible toxicological risks.

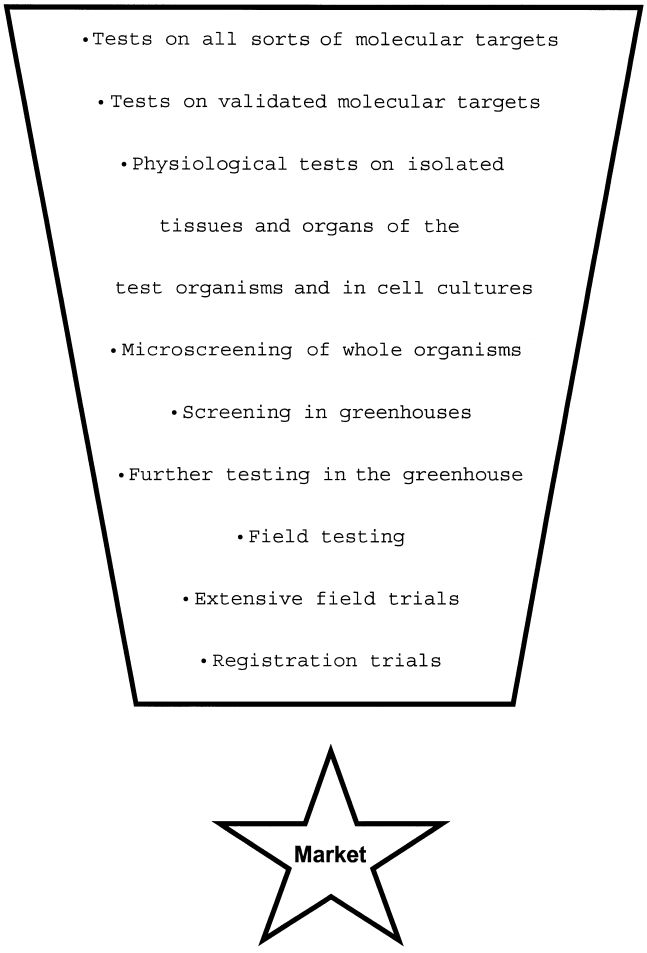


Figure 6. The screening cascade.

There is an inverse proportional relationship between the proximity of a test to the real life situation and the number of substances which can be tested with justifiable financial expenditure. One, therefore, trys to use the tests efficiently depending on the number and quality of the test substances available.

Essential as it undeniably is, the lead structure is only the very beginning of the route to a marketable active ingredient. As a rule, in the process of structure – activity optimization, hundreds and often thousands of related substances have to be synthesized in order, if successful, to identify a candidate for product development which can then be taken through to a state of readiness for the market.

A high degree of instinct and intuition is demanded of the pesticide chemist in making the very important strategic decision: Is it at all worthwhile to pursue a lead structure with weak activity? Has the structure perhaps already arrived on a plateau of activity from which no further increase is possible? It then certainly requires the healthy optimism of creative and imaginative chemists and biologists to choose this rather than another lead to follow.

3.3.2. How are Lead Structures Found?

How does one find a lead structure? In answering this central question of active ingredient research, there have been several major changes in paradigm over the last few decades (Table 4).[43] In the early phase of active ingredient research in the crop-protection industry, the costly, labour-intensive biological screening in greenhouses with a wide range of target

Table 4. At the beginning stands the lead structure.... From random chance to design and back to random chance.

The past (pre 1980)	the vision of the 1980s	the current trends
● costly greenhouse testing	● known biological targets	● all sorts of targets
● gram quantities of the test substance	● receptor – inhibitor models (lock & key hypothesis)	● high-throughput screening (HTS)
● virtually no automation	● de novo design	● automation
↓	↓	↓
● limited testing of natural products	● testing limited to designed molecules	● bottleneck: chemical synthesis of test substances
● random: chemistry from various sources		● solution: automated synthesis, combinatorial chemistry, molecular diversity
↓	↓	
● bottleneck: greenhouse testing	● deficiencies at the relevant targets	
	● lead structures may not be active in vivo	

organisms was the bottle neck in the process of searching for a lead structure. Due to the high application rates required in those days, several grams of the test compound were needed to conduct a comprehensive primary screening in all indications. All this stood in the way of a broad testing of random substances from a variety of sources (including substances from institutions of higher education and natural substances). Although working in parallel and automation of procedures were known in biological screening in rudimentary form, they were still unheard of in chemical synthesis.

Progress in biochemistry, molecular biology, and electronic data-processing led to the vision of the 1980s; the magic words were rational molecular design and molecular modeling. Based on the well known "lock and key" hypothesis for biological activity and with knowledge about the three-dimensional structure of the biological target, it should be possible after all, using molecular modeling, to design active ingredients with ideal properties of fit as potential effectors.

Many saw the role of the active-ingredient chemist reduced, as it were, to that of "cook's servant", who now merely had to make a few bespoke substances. From this collection, in depth biological testing should very quickly be able to select the candidate for development. A nice new world with relatively bad prospects for the synthetic chemist, or so it appeared.

That this could not work was actually already clear to experienced pesticide researchers at the time. First, there was too little of the necessary information about the relevant targets to allow de novo design. In addition, the in vitro activity at the level of the isolated enzyme or receptor is usually far removed from the in vivo activity of a real active ingredient against the plant, insect, or fungus target organism. In short, de novo design has so far not succeeded in generating lead structures for the chemical crop-protection industry.

Indisputable however, is the usefulness of molecular modeling as a method for lead structure optimization.[44] A quantitative structure–activity relationship (QSAR) tool already considered a classic is the Hansch analysis, which allows biological activity to be described in terms of the contribution made by physicochemical properties of individual substituents.[45] A refinement is comparative molecular field analysis (CoMFA) in which the 3-D field properties of the molecule are linked with biological activity to allow estimates of the biological activity of suggested structures.[46]

The "active analogue approach" is a widely used method which, by comparison of biologically active substances which are structurally diverse but which share a common mode of action, allows the essential common structural properties to be identified. These so called pharmacophores then serve as a template for the design of a new active ingredient.[47]

For several years now, the pendulum of lead structure generation has once again swung towards random chance. The ever-less favourable statistical quotient of the chance of finding a hit should henceforth be 'tricked' by a kind of bombardment with countless, highly diverse chemical entities. Through progress in genome research, the number of disease-relevant biological targets which are available in the form of an assay has exploded. In high-throughput screening and lately also ultra-high-throughput screening (HTS, UHTS), the bombardment mentioned above can be coped with, since several 100 000 substances can be tested in an assay in a few days.

An important question for the research-based crop-protection industry in recent years, which has been debated intensively and is also partly controversial is: Can this procedure for finding lead structures derived from pharmaceutical research be transferred to crop-protection research and should one join this shift in paradigm? The answer, at least from the large companies, was initially hesitant, then however, a clear commitment to the additional chances offered by the modern assay formats was made. This, however, did not call into question the classical screening model. One crucial difference between pharmaceutical and pesticide research should not be overlooked: while the former is compelled to rely on biochemical and molecular biological assays for the generation of lead structures, in crop protection one can, so to say, conduct tests directly on the patient. The target organism plants, fungi, and insects are relatively easy and inexpensive to rear in large numbers and are not subject to restrictions comparable with laws to protect higher animals. The test substance is applied to all targets at the same time, including those that are completely unknown. In contrast to pharmaceuticals, the target sites have to be such that an interaction with them neutralizes the target organism; the best reaction of all is when this interaction has a lethal effect. The aim in the end is, after all, to prevent further damage.

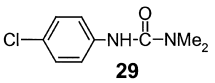
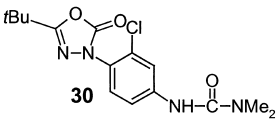
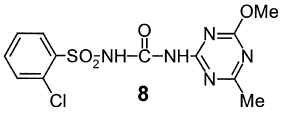
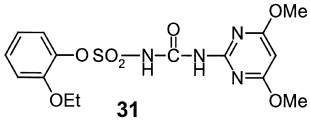
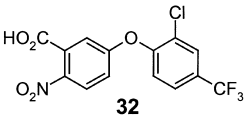
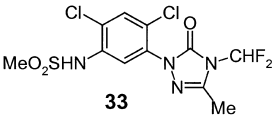
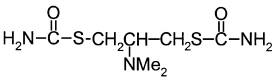
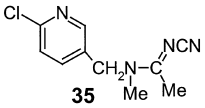
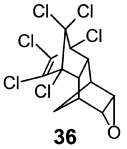
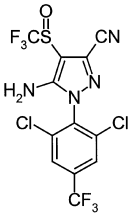
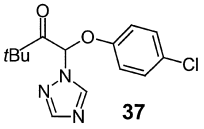
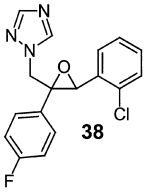
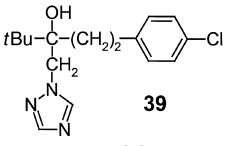
Additionally, the throughput of in vivo screenings today is incomparably higher than it was only a few years ago. Besides traditional greenhouse screening on a wide set of weeds and pest organisms, automated medium-throughput screening (MTS) with a few carefully chosen organisms, can be used in advance to select potential lead structures. The amounts of compound required lie in the range of a few milligrams.

For this reason in vitro HTS is particularly appropriate in two cases. The first is where analogue chemistry in tried and tested classes of structure brings no perceptible progress. In these cases it is of course tempting to bombard the underlying targets with large, diverse libraries of compounds once again. The hope is to discover completely new classes of substances. Here too, there is a substantial difference to the pharmaceutical industry. Targets in crop protection grow old only very slowly, if indeed at all. While a new mode of action can serve to break down resistance and can be a useful argument for the registration and sale of a new active ingredient, other parameters for the substance such as toxicity, biological selectivity, how easily it can be produced, and application rates play a much more important part. In the end after all, it is the user, the farmer, who decides about its use and for him it is the economical viability which is clearly of paramount concern. Simply stated: mechanisms of action which may already have been known for decades are not "out" just because there are various different products available on the market. Even today, a photosynthesis inhibitor which acted at low application rates, is selective for important crops, has good activity against problem weeds, is cheap to produce, and has good toxicological and environmental properties, would have a very good chance. This is despite the fact that there have been photosynthesis inhibitors for 50 years and more

than 50 different active ingredients from this class have been developed as saleable products.

The azole fungicides which act by inhibition of ergosterol biosynthesis are another of many examples. More than 15 years after the market launch of the first representatives, Epoxiconazole (Opus) from BASF and Tebuconazole (Folicur) from Bayer are two real blockbusters which have come onto the market. Examples for the longevity of mechanisms of action are shown in Table 5.

Table 5. Sites of action in crop protection.[a]

Site of action	active ingredient	
	early	late
photosynthesis (PSII)	 **29**	 **30**
	Monuron[48] (1951)	Dimefuron[49] (1978)
acetolactate synthase (ALS)	 **8**	 **31**
	Chlorsulfuron[29] (1982)	Ethoxysulfuron[50] (1996)
protoporphyrinogen oxidase (PPO)	 **32**	 **33**
	Acifluorfen[51] (1979)	Sulfentrazone[52] (1995)
nicotinergic acetylcholine receptor (n-AChR)	 **34**	 **35**
	Cartap[53] (1965) (antagonist)	Acetamiprid[54] (1995) (agonist)
GABA-sensitive chloride-channel	 **36**	 **11**
	Dieldrin[55] (1954)	Fipronil[33] (1994)
Ergosterol biosynthesis (C_{14}-Demethylisation)	 **37**	 **38**
	Triadimefon[56] (1976)	Epoxiconazole[57] (1992)
		 **39**
		Tebuconazole[58] (1988)

[a] Years of launch are given in parentheses.

The second procedure to be discussed will mean considerable rethinking and probably a change in paradigm. The approach to find potential, hitherto unknown points of attack by the analysis of genomes of pest organisms is a very interesting approach but also filled with risks. In the last few years a great deal of activity has begun here. The large research-based crop-protection companies have all got collaborative projects with new biotechnology companies in the field of genome analysis (Table 6).[59] It is hoped that by doing so an important gap will be closed. Experts predict that up to several hundred targets of relevance to crop protection will be found.

Table 6. Collaborations between genomics companies and crop-protection companies.

Genomics company	crop-protection company	year
Acacia Biosciences	Cyanamid	1998
	DuPont	1998
	Novartis	1998
CuraGen	DuPont	1998
Exelixis Pharmaceuticals	Bayer	1998
Gene Logic	AgrEvo	1998
Lion Bioscience	Bayer	1998
Lynx Therapeutics	BASF	1996
	DuPont	1998
Paradigm Genetics	Bayer	1998

There is no doubt that through the use of these targets, screening of large compound libraries will yield hits. That it is an extremely long way from such a hit to an active ingredient with a convincing overall profile in every respect is, however, crystal clear. In a favourable case, the hit identified by target screening will also have detectable activity in the later greenhouse test. Then there is clearly a motivation to undertake chemical optimization work. The decision is, by contrast, particularly difficult when there is no in vivo activity. In this case a programme of chemical work only makes sense if there are really viable concepts to overcome this dilemma. Unlike a medicine, a crop-protection agent cannot be applied to the crop orally or intravenously to increase the level of bioavailability to the target organism.

The securing of the expenditure incurred through skilled intellectual property management will be of particular strategic importance. However, the competition doesn't sleep either and, at the very latest, they "jump on the band wagon" when patents to a new class of structure have been published and try to find and develop patent-free active ingredients in the general area of the patent. Experience has shown that no company has so far managed to secure a monopoly over a class of structure and that often it is the late entrants in the game that carry away the big prizes (For example, Opus (Epoxiconazole) from BASF).[57]

4. Combinatorial Chemistry

Whether they are called MTS, HTS, or UHTS, all these "hungry" test systems require large numbers of compounds. In an analogous situation to pharmaceuticals, it is the

chemical synthesis in crop-protection research which represents the bottleneck in the search for a lead structure. Classical synthesis with an annual output of 200 – 300 new compounds per laboratory cannot meet the requirements (Figure 7).

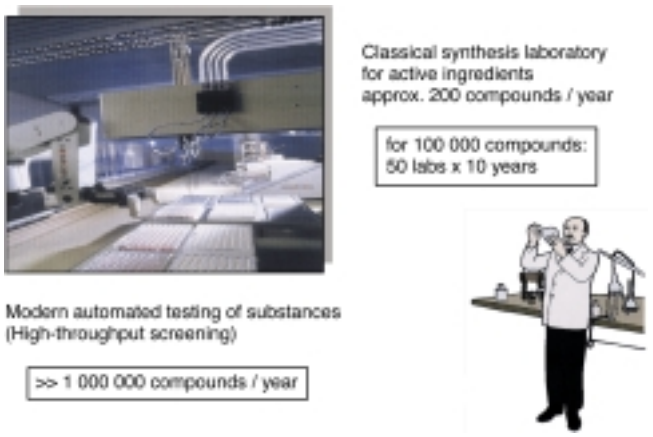


Figure 7. The challenge of high-throughput screening (HTS).

In response to the challenge of HTS, the new technology of combinatorial, automated synthesis was created several years ago. The approach has enjoyed uninterrupted popularity and is still developing at a rapid rate.[60] The basic idea is to combine small sets of synthetic building blocks to make libraries of modularly constructed molecules in large numbers (Figure 8).

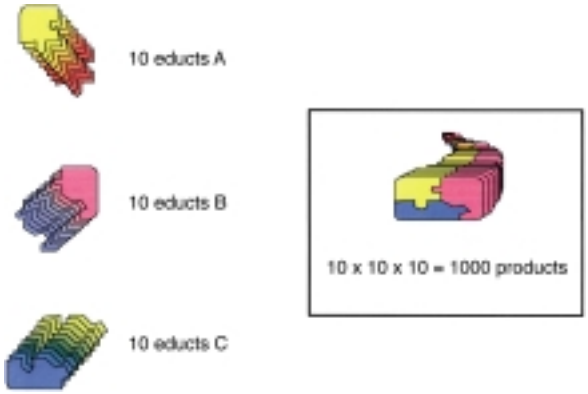


Figure 8. The combinatorial approach.

Trends in Combinatorial Chemistry

The emphasis in combinatorial chemistry has changed dramatically in the last few years. While the first libraries still impress by their immense numbers (often from 100000 to 1000000) of compounds synthesized as mixtures, the trend in the meantime is towards smaller libraries of compounds. Collections of single substances deliver more meaningful test results and because the active molecular structures can more easily be identified, the process of following up hits is speeded up. The smaller number of compounds limits the structural diversity of such a library. Computer-aided analysis of diversity is used in an attempt to optimize this process—more intelligent design of libraries ("smarter" libraries) replaces astronomical numbers. Indispensable for the design of such libraries is the knowledge of experienced pesticide researchers in making successful active-ingredient templates. At the same time, the realisation is being made that physicochemical parameters should also impact on the design of a library and that these should be investigated in HTS (Figure 9). Therefore: quality libraries instead of mass production!

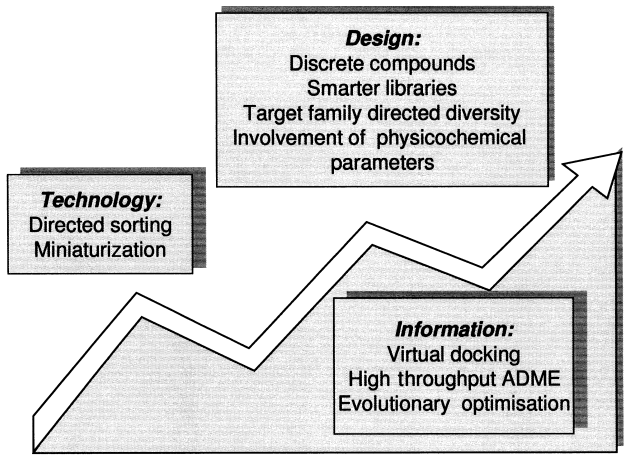


Figure 9. Trends in combinatorial chemistry.

What future has combinatorial chemistry at the threshold of the new millennium? The basic concept that producing chemical diversity can speed up the discovery of substances and reactions will undoubtedly become a reality in many areas of organic chemistry. Combinatorial active-ingredient research shows the way towards further miniaturization, chemical evolution, and the merging of synthetic and analytical chemistry, as well as screening. Analytical techniques are already being combined with methods for detection of activity. The challenge for chemistry will be overcoming the ever-narrower barriers separating parallel-working, automation, and miniaturization. In this context the original solid-phase method is today increasingly becoming augmented by combinatorial synthesis in solution. To summarize: crop-protection research has also embraced the modern or enabling technologies in the process of finding new active ingredients, albeit without doubting the worth of the established approaches.

Understandably, there is no success story to report so far. Only the future will show if the game can really be won with large numbers and whether it is not merely more hits, but ultimately also innovative solutions for the challenges of crop protection, which result.

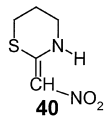
5. Successful Classes of Active Ingredient

Two classes of active ingredient from the recent past, which are particularly good examples for innovation in crop protection, were found by classical routes, either through chemically driven research centred around examples from the patents of competitors, such as the case of the neonicotinoids, or through optimization of a lead structure from natural product screening, such as in the case of the methoxyacrylates (Strobilurin). Both will be briefly presented here.

5.1. Neonicotinoids

A belief in the possibility to optimize a class of structure, which at the end of the 1970s, on the basis of unsuccessful optimization attempts up to that time, had been deemed as hopeless, was the driving force behind the discovery of a new active ingredient by Bayer, which since then has become the insecticide with the largest turnover in the world.

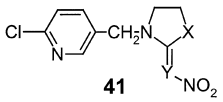
Arising from a program of chemistry with an entirely different goal, the nitro-substituted ketene aminals, discovered by Shell in the 1970s were found to act as insecticides and were worked on over a broad front. At the time nithiazin was identified as a development candidate, but it was never launched as a product in the end.[61] In the early 1980s chemists at Bayer's subsidiary company in Japan started syntheses centered around the nitromethylene lead structure once again. The introduction of a special substituent, the chloronicotinyl residue, and its coupling with the new nitroguanidine building block, brought the breakthrough to a highly potent, light-stable, low-toxicity, widely applicable, and above all systemic group of insecticides, with Imidacloprid **7** as the most interesting candidate for development (Figure 10).[32] By virtue of its marked systemic properties, **7** is eminently well suited for use in the most environmentally friendly form of application, the seed-coating treatment.



40

Nithiazine (Shell) [61]

Discovery of 2-Chloro-5-methylpyridyl-substituents:



41

a: X = S, NH
b: Y = CH, N

(CN)

Highly active, light-stable, low toxicity, systemic insecticide

Imidacloprid	NTN 33893
7	Confidor Gaucho (125-250g/ha)

Figure 10. Early in the 1980s, chemists at a Bayer's subsidiary company in Japan began research based on the nitromethylene lead structure once more.

Imidacloprid **7** is also very interesting from the aspect of its mode of action. Figure 11 shows a highly schematic diagram of the cholinergic synapse in the central nervous system. The sites of action of nearly all major insecticidal products are to be found here. While pyrethroids act on the presynaptic sodium channels and carbamates and organophosphates are inhibitors of acetylcholinesterase, **7**, a representative of the neonicotinoids, acts on the nicotinic acetylcholine receptor, like the very small group of so-called nereistoxin analogue products available on the market.[62]

With complete justification one can say that **7** is the first really widely useable, relevant product which exploits the mode of action described. As a result, insect pests which have developed resistance to insecticides from the established

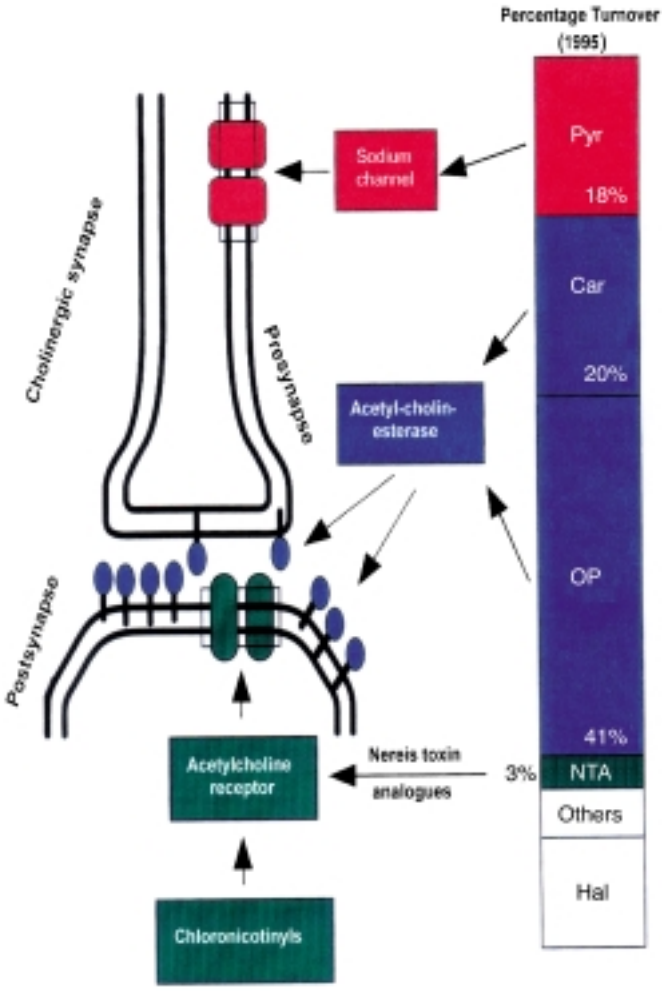


Figure 11. Mode of action of the most important classes of structure. Pyr = pyrethroids, Car = carbamates, OP = organophosphates, NTA = nereis toxin analogues, Hal = halogenated hydrocarbons.

major insecticide classes respond in a completely susceptible manner to **7**. An additional advantage lies in the selective toxicity of **7**. According to the current state of knowledge, this is attributable to the fact that the mammalian acetylcholine receptor is considerably less sensitive to imidacloprid than that of insects.[63]

The chloronicotinyl residue is a particularly important structural element for Imidacloprid and related compounds (Figure 12). At first sight it appears to be a typical chlorinated heterocycle out of the chemist's "synthetic kitchen". All the more surprising therefore, that mother nature seems to have discovered this moiety a very long time ago.[64] In 1992 a compound, which was named epibatidine, was isolated from the skin of a South American frog. Amazingly this poison from the frog contains the chloronicotinyl residue. It acts as a strong analgesic and is also an n-AchR agonist. Thus, there are particularly interesting interrelations between natural substances such as nicotine and epibatidine, which taken together with knowledge about the activity of the Shell nitromethylenes could very much earlier have led to the synthesis of Imidacloprid.

The fact that other companies have not been sleeping is demonstrated by other products which have arisen from

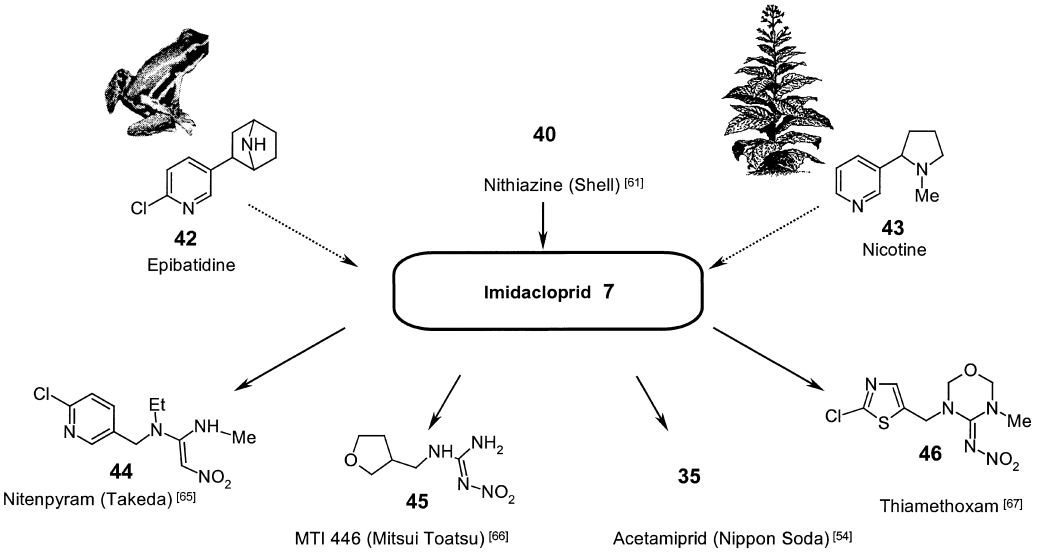


Figure 12. Development of the insecticidal nicotinoids.

analogue research centred around Imidacloprid. Besides the chloronicotinyl residue, the closely related chlorothiazoylmethyl residue and, surprisingly also, the tetrahydrofuranylmethyl residue have proven their worth. The neonicotinoids are on the way to establishing themselves as a significant new class of structure in the insecticides market. The extent of the market share which they will ultimately attain is as yet unknown. One does not, however, have to be a prophet to predict a bright future for such particularly environmentally friendly products as the neonicotinoids.

5.2. Methoxyacrylates (MOAs)

Time and again, both in pharmaceuticals research and in crop-protection research, the screening of pools of natural substances from all over the world and especially of those derived from plants, fungi, marine organisms, and microorganisms has yielded lead structures which were worth pursuing or occasionally even ones which could successfully be developed. As already mentioned, the new possibilities of MTS and HTS screening should open up additional possibilities. For various reasons (availability, costs, stability) the natural substance itself is rarely used in crop protection. However, very recent examples such as Avermectin, a product against spidermites, and Spinosad, which has excellent activity against important lepidopteran species, show that even this is possible from time to time. However, as a rule, improved synthetic analogues of the natural substance are commercialized. The synthetic pyrethroids derived from the natural insecticidal pyrethrum extract are surely the most prominent examples for the particularly successful handling of a natural substance in the past.

Similarly, a new success appears to be developing where a fungicidal lead structure from nature was followed, the synthetic analogues of the strobilurins. More than 30 of these strobilurins and the related oudemansins and myxothiazols have so far been isolated and characterized.[35]

Seen from a chemical perspective these are derived from β-methoxyacrylic acid (Figure 13). The strobilurins and oudemansins are constituents of several small fungal species, such as *Oudemansiella mucida*, which typically grow on beech trees (Figure 14).

As we know today, the fungicidal mode of action of the strobilurins is attributable to inhibition of mitochondrial electron transfer brought about by blocking a specific cytochrome b binding site.

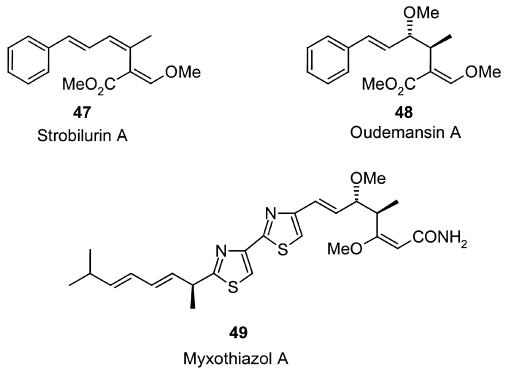


Figure 13. Natural fungicides derived from β-methoxyacrylic acid.

Figure 14. *Oudemansiella mucida*.

Apart from the strobilurins, only the newly introduced active ingredient Famoxate has the same mode of action.[35] Thus, there is only a very low risk of cross-resistance. The MOAs are remarkably safe, despite a mode of action which, in theory at least, is toxic for warm-blooded animals.[68] The natural products were never suitable as agricultural fungicides. Apart from inadequate availability, their low light stability, similar to the first Shell neonicotinoids, and too high volatility were the factors responsible.

The process of optimizing the Strobilurin A lead structure proved a slow-moving business for the companies BASF and Zeneca. Steglich and co-workers had already been able to show that making the Z-double bond more rigid through incorporation of a phenyl ring could bring about a breakthrough in terms of the light stability.[69] Industrial research, initially competitively, between Zeneca and BASF took up this idea. From several cycles of optimization, the landmarks of which are noted in Figure 15, arose the first two marketable

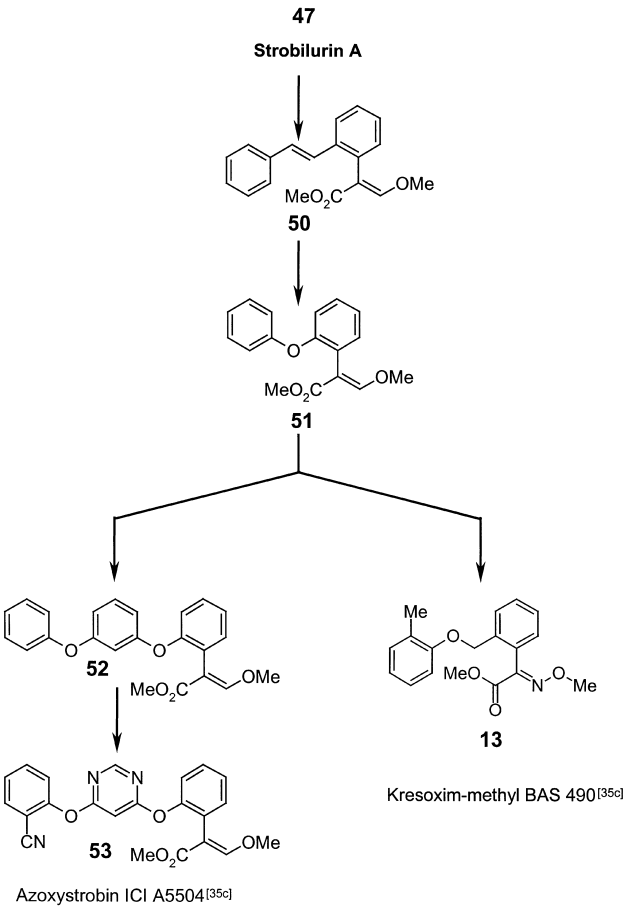


Figure 15. Evolution of the structure of Strobilurin A to Azoxystrobin und Kresoxim-methyl.

products: Azoxystrobin from Zeneca and Kresoxim-methyl from BASF. Interestingly, in the latter the methoxyacrylate toxophore is replaced by the bioisosteric methoximinoacetate residue. Both can be used in a very flexible way against a broad spectrum of fungi, in a wide range of crops such as rice, grapes for wine, fruit, and vegetables. This is a new promising class of fungicide which in the new millennium may gain equal prominence to that enjoyed by the azoles.

The neonicotinoids and methoxyacrylates have been selected here as being representative for a variety of other new developments in the area of active-ingredient research in the crop-protection industry. These examples illustrate just how important the element of chance is at the "cradle" of a new class of active ingredient following its birth, and how much a concerted scientific effort can promote the success of the optimization process to arrive at the ideal solution. The whole process of innovation and development is today conducted simultaneously across a broad front and over all the scientific disciplines involved.

6. Alternative Methods of Control

6.1. Semiochemicals

So far in this paper the emphasis has been on progress to be expected in the classical field of pest and weed control by means of chemical synthesis. Nature however, has a rich treasure trove of compounds which mediate communication between organisms of the same species and between organisms belonging to different species—the so-called semiochemicals.[70] The concept of using these substances to control pests, especially insects, is not new and, as an idea, it is fascinating. Research has been conducted in this field throughout the world and with the expenditure of considerable scientific effort for decades. Pheromones, antifeedants, and repellents have been the most frequently chosen objects for this research. In spite of all the efforts, only a very few areas of application have been found to date. In agricultural practice, aggregation and sex pheromones have predominantly been employed in traps to predict pest-populations, for the purposes of mass-trapping, or in the context of a mating-disruption approach.

Without a doubt, in future the use of semiochemicals will increase in the context of integrated crop protection. A few general advantages are worth mentioning: high species specificity with the potential to avoid harm to beneficial species, low toxicity, small amounts of the active substance, low persistence, hardly any problems with environmental residues, and the possibility for a mixed application strategy with reduced insecticide application rates acting as a synergistic and resistance-lowering method of treatment.

The development of semiochemical products requires enormous effort, especially at the point where results from the laboratory have to be transferred into the field. At least the tools to achieve this end will improve markedly through progress, particularly in the fields of molecular biology, behavioral biology, and electrophysiology. The highly volatile nature of semiochemicals will, however, require the development of technically highly sophisticated release systems. An intelligent approach from the standpoint of drawing the insect to the poison in a targeted way rather than bringing the poison to the insect, the so-called "attract and kill strategy" will briefly be discussed here (Figure 16). In the mating-disruption control strategy practised with pheromones until now, the crop which was to be protected was enveloped in a cloud of pheromone emitted from suitable release devices. As a result, male and female insects fail to meet, and copulation is, for the most part, prevented from occurring.

From results so far, the attract and kill strategy, in which the insect is led to the poison, seems more promising. The pheromone and the insecticide are both mixed into a highly viscous formulation. In the fruit orchard the formulation is then applied selectively to relatively few points on the fruit tree, for example (Figure 17).

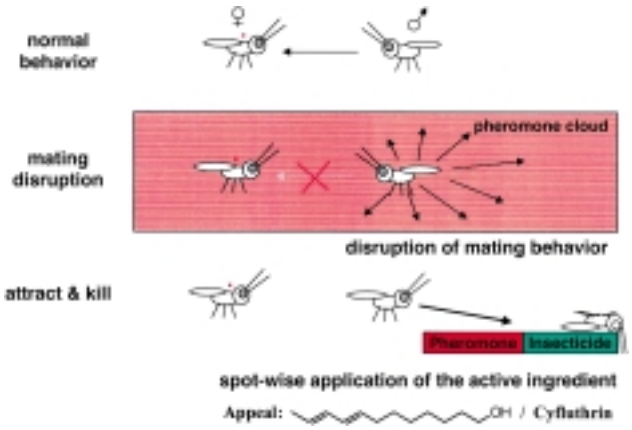


Figure 16. Behavioral manipulation of insects—the "Attract and Kill" strategy.

Attract and Kill

- Advantages:
  - intelligent combination of pheromone and insecticide
  - species specific
  - targeted application
  - no spray drift
  - protects beneficial organisms
- Disadvantages:
  - each formulation combats only one species
  - difficulties in acceptance of a new concept by growers

Aim: Bring the insect to the insecticide!

Figure 17. Attract and kill—a novel way to control insect pests.

The advantages are self evident: Only the tiniest amounts of pheromone are required and even the application rate of the insecticide used is markedly lower than in conventional spray applications. The method is highly selective, with only the major pest species which is responsive to the particular pheromone applied being controlled. It is a method therefore which should find acceptance in situations where a single pest species dominates and for high-price crops, such as fruit or grapes for wine. Two new products of this type are Sirene (Novartis)[71] and Appeal (Bayer).[72]

For the control of plant parasitizing nematodes, the concept of using so-called hatch-inducing factors is very attractive. Inducing synchronized hatching of nematodes at a time in which the host plant is not yet present or in combination with a non-persistent nematicide could provide a very good and practicable solution to the nematode problem.

6.2. Induced Immunity

Substances which enhance the immune defence of the host plant against pathogens, thereby helping to reduce the amount of fungicides applied, stand on the divide between chemical and biological methods of controlling pests and pathogens. Under the influence of so-called elicitors, which are frequently cell wall fragments of a pathogen, the plants stimulate the production of defensive substances called phytoalexins through a signal cascade, thereby preventing infection.[73] To date a variety of purely synthetic substances are known which can act as resistance inducers. One such product, known by the trade-name Bion, was commercialized by the Novartis company in 1996.[74]

The wide field of allelopathic substances produced by plants to confer a competitive advantage upon themselves in their habitat can here only be mentioned but not commented on in further depth. The border zone between chemistry and biology addressed here is scientifically highly interesting and will surely provide many surprises yet.

6.3. Biological Products

In journalism at least, much is promised of biological products, occasionally even to the extent that they will largely replace chemically based products. This view is, however, unrealistic and partly also politically motivated wishful thinking. Only in a few cases has it, so far, been possible to complete the transformation from basic knowledge into crop-protection strategies used in practice. Apart from economic obstacles such as the limited market potential, there have been specific problems with the reliability of action, which have prevented a rapid development with a major impact on the crop-protection market.

6.3.1. Bacillus thuringiensis Products

By far the majority of insecticides sold under a biological crop-protection label are *Bacillus thuringiensis* (Bt) products.[75] They have a market volume of about 200 million DM today, therefore, about 2% of the insecticides market. By way of comparison, in the USA 18% of the area of agricultural land used to cultivate maize in 1998 was already planted with a Bt maize cultivar, 14% was planted with Bt cotton and 4% was planted with Bt potatoes.[76] In future, Bt products will increase in importance, especially through expansion of their possible field of application by means of genetically modified bacteria. The number of species of insect which can be controlled using Bt products has in the meantime increased in an impressive way. The spectrum of activity of Bt products

reaches beyond the Lepidoptera market to encompass Coleoptera and Diptera as well (Table 7).

Regardless of the undoubted success enjoyed by Bt products, their use is limited almost exclusively to high-value crops and niche markets. The reasons for this lie in the biology of

Table 7. Commercial Bt products.

Pest	market or target organism	strain or source	product	company
Lepid-optera	vegetables	*Bacillus thuringiensis* (*B.t.*) *kurstaki*	Bactospeine	Novo
	fruit		Biobit	Novo
	cotton		Condor	Ecogen
	maize		Cutlass	Ecogen
	forestry		Dipel	Abbott
			Forey	Novo
			Javelin	Sandoz
		Pseudomonas fluorescens	MVP	Mycogen
	Plutella xylostella	*B.t. aizawai*	Cen Tari	Abbott
			Florbac	Novo
		transconjugate (*B.t. kurstaki*/*B.t. aizawai*)	Agree	Ciba
Cole-optera	potatoes	*B.t. tenebrionis*	M-One	Mycogen
	vegetables		Trident	Sandoz
	forestry		Novodor	Novo
		transconjugate (*B.t. kurstaki*/*B.t. aizawai*)	Foil	Ecogen
		Pseudomonas fluorescens	M-Trak	Mycogen
Dip-tera	mosquito	*B.t. israelensis*	Acrobe	ACC
	blackfly		Bactimos	Novo
			Skeetal	Novo
			Teknar	Sandoz
			Vectobac	Abbott

Bacillus thuringiensis and in the properties of its insecticidal crystalline toxins, such as:

- Very selective action which is limited to a few species
- Inadequate longevity of action on leaves
- Inadequate longevity of action in the soil, due to microbial degradation

These serious disadvantages of Bt products demand more frequent applications, more complicated forms of application, and intensive monitoring and controlling of the crop to determine the optimum timing for applications. In other words, the costs and the expenditure of time are generally higher for a Bt application than for conventional insecticides. For this reason Bt products are used above all in organic farming and in the production of fresh vegetables. Still only sporadically, if at all, are Bt products used by growers of the most important crops such as maize, cereals, cotton, and oil seeds.

When looked at it detail however, the application of the Bt toxins, "packed-up" in the dead bacterium so to speak, is nothing other than chemical pest control, albeit using a highly specific active ingredient. Whether or not the very low level of toxicity to warm-blooded animals can also be guaranteed when genetically modified bacteria are used for toxin production must certainly still be investigated carefully. In the meantime, there have also already been reports of resistance to Bt products.[77]

6.3.2. Other Biological Products

The true and original arsenal of biological control agents for combating pests and pathogens consists of living bacteria, viruses, fungi, nematodes, and predatory insects which can parasitize and kill the pest organisms.[78] The intelligent use of such principles is a stimulating challenge time and again. These methods of control are particularly appealing when used together with chemical agents or semiochemicals in the context of an integrated concept for crop protection. In using biological products, one should however acknowledge their limits:

- Narrow spectrum of activity due to high host specificity
- Reliable effect only under controlled conditions
- Efficacious only up to certain levels of infestation

These constraining factors have the following consequences:

- The use of biological products alone is normally insufficient
- Regions with extreme weather conditions are unsuitable for the use of biological products
- Reliable control cannot be guaranteed under intense infestation

In cases where these constraints are acceptable, such as under controlled conditions in a greenhouse or in the relatively stable microclimate of the soil, biological products are an interesting alternative.

In the meantime bio-insecticides based on the entomopathogenic fungi of the geni *Beauveria*, *Metharhizium*, and *Verticillium* have been developed by various companies (Table 8).

A very important point in the development of biological products is the question of registration. As the markets in question are generally small, development is only worthwhile if the expenditure incurred is substantially lower than that for a synthetic pesticide. Here there is still no coherent legislation and a hesitant stance on the part of the regulatory authorities.

7. Transgenic Plants

Through a process of breeding and selection based on the available gene pool, man has created the crops of today over the course of many thousands of years. Without the intervention of humans these would therefore not exist, as they would not have been capable of surviving had nature been left to its own devices. It was only in the middle of the nineteenth century that Gregor Mendel introduced scientific rigour into the process of plant breeding which until then had been based on intuition and visual, phenotypic selection.[79] The rapid progress made in green biotechnology in the last two decades, today makes it possible to introduce desired properties (traits) into the plant in a targeted way by the transfer of particular genes. Illustrated in Table 9 are some of the landmarks on the way from Mendel's laws of inheritance to the first commercially available transgenic crop plants.[80]

While the use of gene and biotechnology is now widely accepted in the sphere of human health, green biotechnology has still had to battle against a strong headwind of opposition, particularly recently.[81] As in the case of synthetic pesticides,

Table 8. Examples of entomopathogenic fungi (commercial products and products in development).

Fungus	product and/or company	indication	formulation
Aschersonia aleurodis	Koppert (Netherlands)	*Trialeurodes vaporariorum* *Bemisia tabaci* (whitefly)	wettable powder
Beauveria bassiana	Naturalis/Troy Bioscience (USA)	whitefly, thrips, white grubs	liquid formulation
Beauveria bassiana	Conidia/AgrEvo (Germany, Columbia)	*Hypothenemus hampei* (coffee berry beetle)	suspendible granules
Beauveria bassiana	Mycontrol-WP/Mycotech Corp. (USA)	*Trialeurodes vaporariorum* *Bemisia tabaci* (whitefly)	wettable powder
Beauveria bassiana	Ostrinil/Natural Plant Protection (France)	*Ostrinia nubilalis* (European corn borer)	microgranules of mycelium
Beauveria brongniartii	Betel/Natural Plant Protection (France)	*Hopochelus marginalis*	microgranules of mycelium
Metarhizium anisopliae	Bio-Path/EcoScience (USA)	*Blattella germanica* (German cockroach)	conidia on a medium placed in trap/chamber
Metarhizium anisopliae	Biogreen/Biocare Technology Pty. Ltd (Australia)	*Adoryphorus couloni* (redheaded cockchafer)	conidia produced on grain
Metarhizium anisopliae	Bilogic;Bio1020/Bayer AG (Germany)	*Otiorhynchus sulcatus* (black vine weevil)	granules of mycelium
Verticillium lecanii	Mycotal/Koppert (Netherlands)	*Trialeurodes vaporariorum* *Bemisia tabaci* (whitefly) *Frankliniella occidentalis* (thrips)	wettable powder
Verticillium lecanii	Vertalec/Koppert (Netherlands)	*Aphidus* sp. (aphids)	wettable powder

Table 9. Significant landmarks in the story of transgenic crop plants.

1865	Mendelian laws	Mendel
1869	first discovery of DNA in cells, called "nuclein"	Miescher
1944	DNA identified as the substance involved in genetic inheritance	Avery, MacLeod, and McCarty
1953	discovery of the double-helix structure of DNA	Watson and Crick
1965	complete description of the genetic code	(1961 – 1965)
1969	restriction enzymes	Arber, Cohen, and Boyer
1977	sequencing of DNA (in vitro)	Maxam, Gilbert, Sanger
1982	first transgenic plants	Horsch, Rogers, and Fraley; Chilton; Schell
1987	first field trials with transgenic plants	
1994	first commercially available transgenic crop plant	
From 2000	many crop plants with many new characteristics (stacked traits)	

one of the principle arguments is the threat of polluting the "natural" foodstuffs and the environment with unknown risks for health and species diversity. The following two statements illustrate the incompatibility of views for and against gene technology. "Genetic engineering has the potential to become the most highly refined but grossest form of bestiality yet discovered"[82] and "Applied to gene technology in medicine and agriculture, the comparative risk analysis—risk of using transgenic organisms vs risk of deliberately not introducing gene technology—is so much in favour of employing transgenic systems that it would be inhuman to refrain from using gene technology".[83]

Regrettably one must conclude that, in their enthusiasm for what can be achieved and over the expected economic gains to be had, industrial supporters of green biotechnology have somewhat lost touch with the interests of the food industry and the consumer. Alarmed by the massive resistance on the part of consumers, well-known companies in the foodstuffs sector, who initially had a positive view of the matter, have now stated that they must have the approval of consumers before they can process ingredients derived from transgenic plants.[84]

A more in depth discussion of arguments for and against would be beyond the scope of this review. However, the analogy is unmistakable: on the one hand, gentle natural products and "natural" crops, on the other hand, hard synthetic chemicals and genetically modified plants. A widely misunderstood nature is often elevated to an unchanging God-given principle and is placed in opposition to technological progress.

Let us, however, return to the main theme of this paper. The potential of green biotechnology extends far beyond the application to crop protection. It is important to differentiate between the so-called "input traits" such as herbicide resistance, resistance to insects, fungi, and viruses, stress-tolerance, improved photosynthetic performance, and nitrogen fixation and the "output traits" such as the accumulation of constituents of value for human and animal nutrition, human health, or industrial use (proteins, amino acids, oils, carbohydrates, vitamins, vaccines, and valuable pharmaceutical substances). Linked to the key word "quality", the reduction of toxic or allergenic food components should also be mentioned (Table 10).[80] The currently fashionable "buzzwords" here are nutraceuticals, novel food, functional food, and pharming.

Green biotechnology can therefore build new bridges linking the chemical industry with the foodstuffs industry through seed companies. On the march from agrochemicals to "novel foods" by way of the seed, the Monsanto company has been a pioneer for a number of years now.[85] The company has concentrated on biotechnology in a very radical way. Other leading crop-protection companies have been far more cautious in this respect and have conducted such activities as an addition, without calling their core business in chemical crop protection into question.

Table 10. Predicted development of transgenic properties.

Today ➡	2005 ➡	2010
herbicide tolerance	non-Bt insecticides	wide ranging protection of crop plants
Bt genes	fungicidal proteins	reductions in fertilizer application rates
viral coat proteins	resistance gene	increased stress tolerance
more valuable oils	carbohydrates	improvements in photosynthetic yield
lysine	proteins/amino acids	increased knowledge about relevant genes (genome analyses)
ripening	fatty acids/oils	industrial enzymes
	polymers	new polymers
	modified secondary metabolites	new secondary metabolites
	gene stacking	pharmaceuticals
		nutritive supplements

7.1. Herbicide-Resistant Crops

The transgenic plants introduced onto the market so far almost all possess properties which are exclusively relevant to crop protection. The development in the USA has been impressive. As shown in Figure 18, the area cultivated with herbicide- and insect-resistant crops increased more than tenfold between 1996 and 1998 and already accounts for a significant proportion of the total area cultivated with maize, soya, and cotton. The herbicide resistance sector predominates and has already caused considerable turbulence in the American herbicides market.[80]

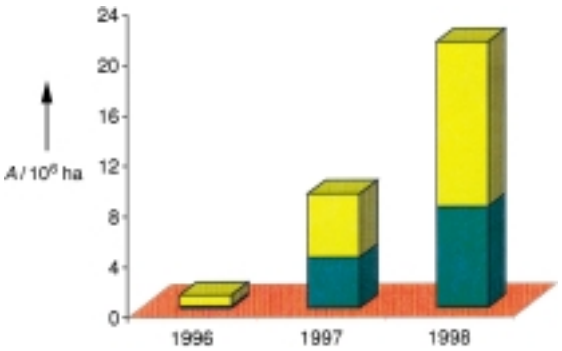
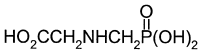


Figure 18. Estimate of the area under cultivation with transgenic crop plants in the USA. Yellow = herbicide resistance, green = insect resistance.

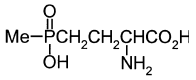
Glyphosate-resistant, "Roundup Ready" varieties of soya, maize, and cotton account by far for the largest slice of the market, followed some way behind by glufonsinate-resistant Liberty Link cultivars of maize, soya, and rape. The resistant varieties which, it is thought, will be introduced to the market in the next few years are summarized in Table 11.

7.2. Insect-Resistant Crops

Insect-resistance in crop plants is still synonymous today with the expression of *Bacillus thuriengiensis* protein crystals in maize and cotton. The notion that the Bt toxin does not have to be applied to the surface of the plant by means of a spray application, but instead could be produced as a secondary metabolite by a suitably modified crop plant is, of course, captivating. Several of the drawbacks pertaining to classical Bt products mentioned above are thereby obviated (see Section 6.3.1.).



54
Glyphosate



55
Glufosinate

In 1996, Bt cotton (Bollgard) with resistance to *Heliothis* and Bt potatoes with resistance against the Colorado potato beetle were first introduced in the USA. Since then Bt maize resistant to the European corn borer has come onto the market (Table 12).[80] Unless the inherent problem of resistance to the toxins successively devalues the crops, it is anticipated that there will soon be virtually no species of crop which is not at least partially protected by inclusion of the appropriate genes.

Table 12. Crop plants with resistance to insects.

Resistance	Company	Crop plant
Lepidoptera	AgrEvo	cereals
Lepidoptera	Novartis	cereals
Lepidoptera	Monsanto	cotton
Coleoptera	Monsanto	potatoes
Colorado beetle	Monsanto	potatoes
Lepidoptera	Monsanto	tomatoes

To broaden the spectrum and to avoid resistance, research is increasingly directed towards the identification of further insecticidal proteins and to express these in crop plants.[86] Virus-resistant transgenic varieties of tobacco, tomatoes, and pumpkins have in the meantime been commercializ-

Table 11. Crop plants with resistance to herbicides: Commercial products and development products from 1996 – 2002.

Resistance	company	crop	method
Roundup Ready (Glyphosate)	Monsanto	soya, cotton	gene technology
Liberty Link, BastaR	AgrEvo (PGS)	rape, maize, soya	gene technology
imidazolines	ACC	maize	selection in cell cultures
sulfonyl ureas	DuPont	soya	selection in cell cultures
bromoxynil	Calgene (Monsanto)	cotton	gene technology
sethoxydim	BASF	maize	selection in cell cultures
Roundup Ready (Glyphosate)	Monsanto	rape, maize, rice	gene technology
Roundup Ready (Glyphosate)	Monsanto	sugar beet, rice	gene technology
imidazolines	ACC	maize, soya, rape	gene technology
sulfonyl ureas	DuPont	maize, soya, rape	gene technology

ed. Fungus-resistant genetically modified crops still lag far behind.

Naturally, the approaches listed here cannot provide solutions covering all the problems of crop protection. Impressive as the perspectives for plant gene technology may be, it is certain that the technology will not be able to produce a plant with only positive features, including the genetic potential for high yield, and, at the same time, one which is resistant to every conceivable pest and pathogen. The war against pest organisms, which are able time and again to adapt to changing conditions, will never end and in principle cannot be won. The very valuable genetically modified plants will need particular protection with the result that novel active ingredients will then certainly be required.

8. Formulation and Application

So far this article has only dealt with the huge effort invested in the search for new active ingredients and new modes of action with improved biological and ecological properties. Besides the actual active ingredient with its intrinsic properties, the form which the application takes is also of utmost importance. The formulation plays an important part in determining the efficacy of an active ingredient against the target organism and also affects its environmental impact. By means of oral and intravenous application a comparatively high level of bioavailability can be achieved for medicines. The scope in this respect is much more limited for agents of crop protection. An intelligent formulation is, therefore, all the more important.

With respect to improved bioavailability, lower risk for the user, and reduced environmental impact, the industry today expends considerable effort in formulation research. The declared aim is to establish a rational basis for the optimization of compositions, a process which in the past was, by and large, conducted empirically. Listed below are a few trends:

- Regulated droplet size with the aim of achieving a more targeted application (avoidance of the spray drifting)
- Formulations which are either low in, or even free from, solvents (suspensions instead of emulsifiable concentrates)
- Low dust formulations (water dispersed granulates instead of powders)
- Improved seed coating treatments (such as gels)
- Use of additives (optimization of activity)
- New, innovative types of formulation and application techniques (attract and kill, trunk application, pills, nanoparticles)
- Slow-release formulations (microencapsulation, etc.)
- Recyclable containers
- Water-soluble packaging
- Direct injection-based dilution and application of the spray mixture, all within a synchronized procedure (no remains of the spray mixture)

Following the theme of reduced risks for the user, the trend in formulations today is away from emulsifiable concentrates and water-dispersible powders towards suspended concentrates and water-dispersible granules. The advantages for the user are obvious—no risks from dust during preparation of the spray mixture and less solvent introduced into the environment.

Another type of formulation which in future will increase in importance is the seed-coating treatment. Here too, the advantages are self evident: the active ingredient is only found where activity is desired, the seed-treatment process itself is carried out professionally by specialists, and the user is hardly exposed to the active ingredient at all. Figure 19 shows the dramatic differences in area of coverage achieved during the treatment of a hectare plot of arable land, when the application was by means of broadcast spray treatment, furrow treatment, or by application of the coated-seed treatment.

Application	Type of product	Area treated [m^2]
Seed-coating treatment	seed coating	approx. 58
Furrow treatment	granulate	approx. 500
Broadcast spray	sprayable product	10 000

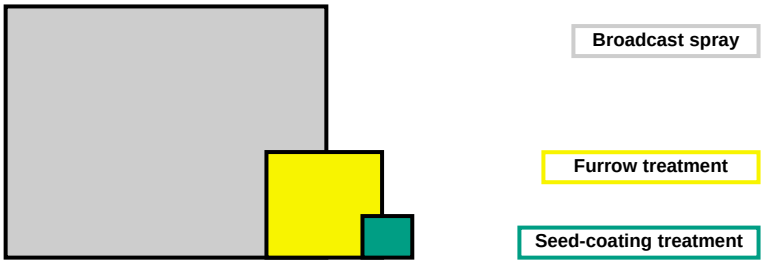


Figure 19. The treated area seen in relation to the method of application used, illustrated by the example of NTN 33893 (Imidacloprid, **7**).

Within the remit of the article it is only possible to expand upon a few of the mentioned trends in formulation technology. Significant progress is also constantly being made in the fields of machine technical development and packaging technology.

Methods for the diagnosis and prediction of pest and predator infestation have improved considerably in the last few years. Thanks to powerful computers, a mass of various measured data can now rapidly be channeled into guiding the appropriate actions. The timing, intensity, and type of such actions can be optimized in relation to the location and variety of the crop involved. Viewed from the perspective of economic damage thresholds, the following maxim can be applied: as much as necessary but as little as possible! Through schooling and training of users, this concept is increasingly becoming the principle focus of companies acting in a responsible manner.

9. Outlook

So, there is then a great deal involved in the subject: "Innovation in Crop Protection: Trends in Research". While

the aim of this review article is to give an overview which is as up-to-date as possible, the subject can not nearly be dealt with exhaustively. As is to be expected from the background of the authors, the examples chosen come largely from fields of research which are centered around chemistry. Colleagues from other disciplines would almost certainly have chosen somewhat different emphases.

Regarding agricultural practice, the available technologies will develop in an evolutionary way; revolutionary changes are not to be expected. In the final analysis, the strength of this prediction is unlikely to be diminished even by increases in the cultivation of transgenic crops.

One concept supported by all crop-protection companies and regulatory authorities is that of integrated crop production and crop protection; that is, the involvement and use of all possible relevant measures which help to maintain a productive, environmentally and resource-friendly agricultural industry. In this regard, chemical crop protection is one of the possible measures which has proven to be particularly efficient. Increasingly it will be joined by agricultural, breeding (gene technological), biological, and physical approaches. The disciplines involved are well prepared for this task.

However, one aspect above all must be considered: A responsible, targeted use of the available methods assumes an ever better level of technical expertise and training on the part of the user. It is becoming clear that there is an enormous need for action on the part of state institutions and industry in this regard. At the same time however, the widening chasm between industrialized and developing nations is growing ever more obvious. Without huge amounts of support from industrialized nations, the latter will hardly be able manage the step into the high-tech world that is modern crop protection.

All the research efforts described here can, in the final analysis, only be of use if society is prepared to accept the results. The illogical and unfortunate contrast which sadly seems deeply rooted in the consciousness of the general public, between biological and chemical, between nature as a benign entity and the evil of chemistry must, with the utmost care, be resolved responsibly by those of us in the scientific community. The demands for "zero risk" must be exposed for what they are: a nonsense endangering the progress of humanity.

We are indebted to our colleagues Dr. N. Griebenow, Dr. R. Hain, Dr. G. Hessler, Dr. J. Konze, Dr. W. Krämer, Dr. B.-W. Krüger, Dr. E. Kranz, Dr. K. Naumann, Dr. H.-G. Rast, Dr. D. Wollweber and Dr. H.-J. Wroblowsky for their valuable suggestions.

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