Part I
Methods for the Design and Optimization of New Active Ingredients

Modern Methods in Crop Protection Research, First Edition.
Edited by Peter Jeschke, Wolfgang Krämer, Ulrich Schirmer, and Matthias Witschel.

1
High-Throughput Screening in Agrochemical Research

Mark Drewes, Klaus Tietjen, and Thomas C. Sparks

1.1
Introduction

Efficient and economical agriculture is essential for sustainable food production fulfilling the demands for high-quality nutrition of the continuously growing population of the world. To ensure adequate food production, it is necessary to control weeds, fungal pathogens, and insects, each of which poses a threat of yield-losses of about 13–15% before harvest (Figure 1.1). Although a broad range of herbicides, fungicides and insecticides already exists, shifts in target organisms and populations and increasing requirements necessitate a steady innovation of crop-protection compounds.

Weeds, fungal pathogens and insects belong to evolutionary distinct organism groups (Figure 1.2), which makes it virtually impossible to have a single crop-protection compound capable of addressing all pest control problems. On closer examination, even the grouping of pests simply as insects, fungi and weeds is, in many cases, still an insufficient depiction. Although the term ''insecticide'' is often used for any chemical used to combat insects, spider mites or nematodes, the differences between these organisms are so significant that it is more precise to speak of insecticides, acaricides, and nematocides. Among plant pathogenic fungi, the evolutionary range is even much broader and oomycetes are not fungi at all, although oomyceticides commonly are also commonly referred to as *''fungicides''*. Hence, the agrochemical screening of fungicides and insecticides requires a substantial range of diverse species. The situation for herbicide screening is, in some ways, the reverse, but is no easier. Indeed, the close genetic similarity between crop and weed plants generates challenges with regards to the specificity of herbicidal compounds, in differentiating between crop and weed plants. This also results in a need to use a range of different crop and weed plants in screening programs.

In light of the above circumstances, agrochemical screening has employed, in both laboratory and glass-house trials, a wide spectrum of model and pest species. The recent developments described in this chapter, however, have allowed an even higher throughput not only in glass-house tests on whole organisms, but

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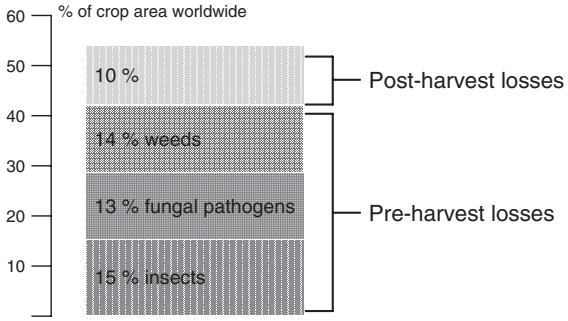

Figure 1.1 Losses of potential agricultural harvest of major crops due to different pests, diseases, and weeds [1, 2]. Non-treated, approximately 50% of the harvest would be lost.

also the exploitation of biochemical (*in vitro*) target tests. Not surprisingly, the implementation of molecular screening techniques and the ''omics'' technologies – functional genomics, transcriptomics and proteomics, etc. – into agrochemical research has been a major challenge due to the high diversity of the target organisms [5].

Molecular agrochemical research with biochemical high-throughput target screening commenced with several model species, each of which was chosen mainly because of their easy genetic accessibility or specific academic interests. These first favorite model organisms of geneticists and molecular biologists were largely distinct from the most important pest species in agriculture, however. Nonetheless, recent progress in genome sequencing has led to a steadily growing knowledge about agronomically relevant organisms (Figure 1.3 and Table 1.1).

The situation is relatively simple for weeds, as all plants are closely related (Figure 1.2). The first model plant to be sequenced, *Arabidopsis thaliana*, is genetically not very distinct from many dicotyledonous weeds, and the monocotyledonous crops are closely related to the monocotyledonous weeds which, in turn– starting several thousand years ago – formed the foundation for today's cereals species. The first sequenced insect genome of *Drosophila melanogaster*, a dipteran insect, was exploited extensively in both genetic and molecular biological research. To better reflect relevant pest organisms such as lepidopteran pests or aphids, species such as *Heliothis virescens* (tobacco budworm) and *Myzus persicae* (green peach aphid) have been investigated by the agrochemical industry, while *Bombyx mori*, *Acyrthosiphon pisum* and *Tribolium castaneum* have been sequenced in public projects (Table 1.1). Baker's yeast, *Saccharomyces cerevisiae*, has long been the most commonly used model fungus, while the ascomycete *Magnaporthe grisea* and the ustilaginomycete *Ustilago maydis* have been the first sequenced relevant plant pathogens. It is certain that, within the next few years, even the broad evolutionary range of the many different plant pathogenic fungi and oomycetes (see Figure 1.2) will be included in genome projects.

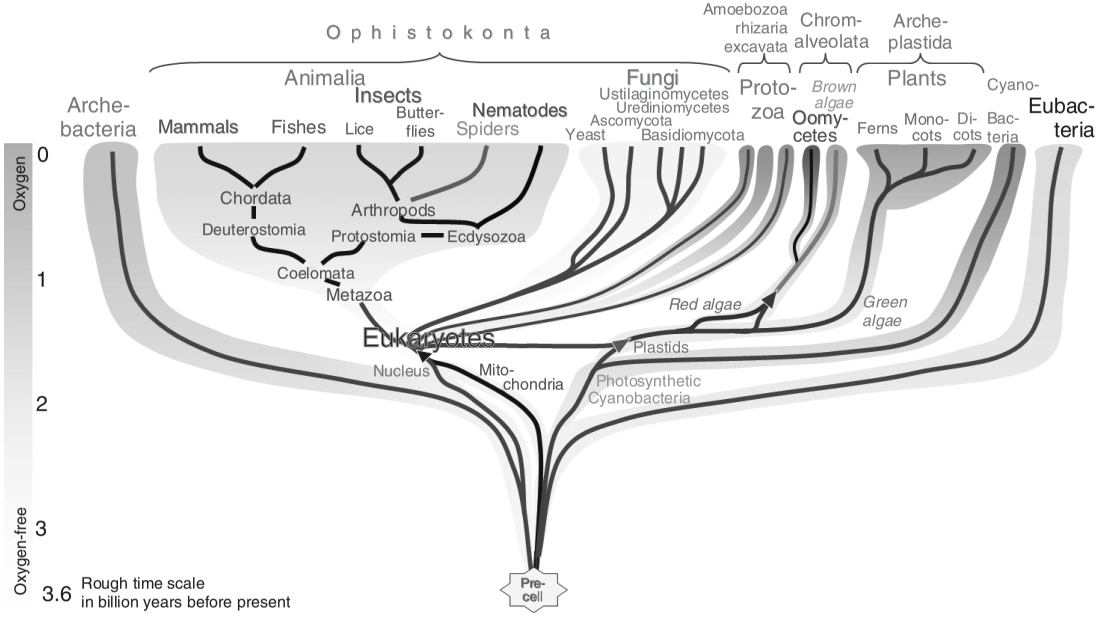

Figure 1.2 Modern evolutionary tree of life. The view is based on Refs [3, 4]; for a more detailed view of fungi, see Ref. [5].

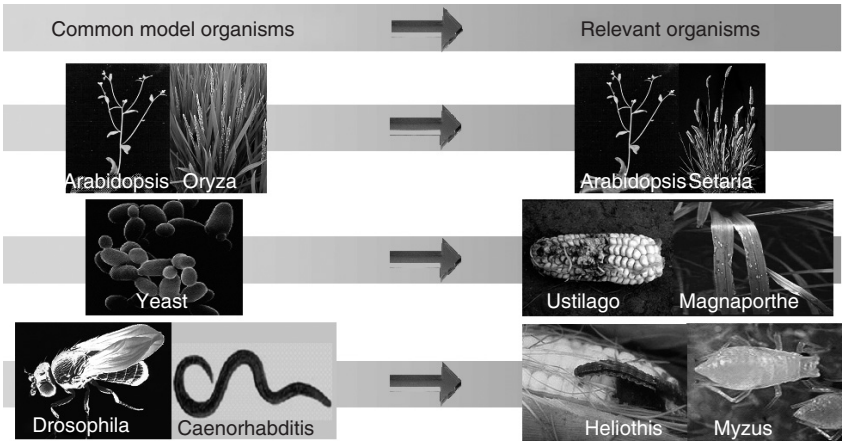

Figure 1.3 Model organisms in molecular biology and agronomically relevant target species.

1.2 Target-Based High-Throughput Screening

1.2.1 Targets

The progress of molecular biology of agronomically relevant organisms has enabled the introduction of target-based biochemical (*in vitro*) high-throughput screening (HTS), which has significantly changed the approach to the screening for agrochemicals during the past 15 years. Target-based HTS is a technology utilized in the agrochemical industry to deliver new actives with defined modes of action (MoA) [6].

Most major research-based agrochemical companies have established biochemical HTS, often conducted in cooperation with companies having special expertise in specific fields of biotechnology. The first wave of genomics – which included genome-wide knock-out programs of model organisms – indicated that about one-quarter of all genes are essential; that is, they were lethal by knock-out [6–8]. The resulting high number of potential novel targets for agrochemicals must be further investigated to clarify the genes' functions (reverse genetics) and to better understand their role in the organism's life cycle. Although the technology of genome-wide knock-out itself was highly efficient and well established, it transpired that even the knock-out of some known relevant targets were not lethal, either because of genetic or functional redundancy, counter-regulation, or because a knock-out does not perfectly mimic an agonistic drug effect on, for example, ion channels. Consequently, knock-out data are today reviewed critically with respect to as many aspects as possible of the physiological roles of potential targets and, as a result, they are taken as just one argument for a gene to be regarded as

Table 1.1 Agronomically relevant organisms with completed or ongoing genome sequencing projects.

Organisms		
Plants	**Fungi and oomycetes**	**Insects and nematodes**
Dicotyledonous plants	**Ascomycetes**	**Diptera**
Arabidopsis thaliana[a]	*Saccharomyces cerevisiae*[a]	*Drosophila melanogaster*[a]
Brassica oleracea	*Alternaria brassicicola*	*Musca domestica*
Glycine max	*Aspergillus oryzae*[a]	**Aphids**
Lotus corniculatus	*Botryotinia fuckeliana*	*Acyrthosiphon pisum*
Solanum tuberosum[a]	*Gibberella zea*	**Lepidoptera**
Monocotyledonous plants	*Magnaporthe grisea*[a]	*Bombyx mori*[a]
Oryza sativa[a]	*Mycosphaerella graminicola*	**Coleoptera**
Sorghum bicolor	*Neurospora crassa*	*Tribolium castaneum*
Triticum aestivum	*Podospora anserina*[a]	**Hymenoptera**
Zea mays	*Sclerotinia sclerotiorum*	*Aphis melifera*
Brachypodium distachyon	**Ustilaginomycetae**	**Nematodes**
Setaria italica	*Ustilago maydis*[a]	*Caenorhabditis elegans*[a]
Hordeum vulgare	**Uredinomycetae**	*Meloidogyne incognita*
	Puccinia graminis	
	Phakopsora pachyrhizi	
Oomycetes	**Basidiomycetes**	
Hyaloperonospora arabidopsis	*Phanerochaete chrysosporium*	
Phytophtora infestans[a]	*Laccaria bicolor*	
Pythium ultimum	**Zygomycota**	
	Rhizopus oryzae	

[a]Completed or close to completion, otherwise: in progress.

an interesting potential target. It must also be considered that clarification of the genes' functions is a challenging and resource-consuming task, and that attention is perhaps more often focused on targets with a sound characterization of their physiological role.

The best proof for an interesting agrochemical target is the ''chemical validation'' by biologically active compounds. This is true for all the established targets. However, new chemical hits acting on such targets must have an advantage over the already known compounds. This may be a chemical novelty, a novel binding site, an increased performance, or providing a means to overcome resistance. From the standpoint of innovation and the chance to open new areas, novel targets are of particular interest, especially when active compounds are already known, such as a natural product (e.g., the ryanodine receptor for insecticides). Most interesting are novel and proprietary targets which arise from genetics programs or from MoA discovery. MoA elucidation for biological hits has, therefore, become much more important.

Modern analytical methods such as high-performance liquid chromatography/mass spectrometry (HPLC/MS), electrophysiology, imaging, and others build a gateway to today's novel target discovery. The benefit of electrophysiology for clarifying neurophysiological effects is obvious. Cellular imaging techniques complement electrophysiology and are, furthermore, a general approach for MoA studies. For metabolic targets, such as those of sterol biosynthesis, direct target identification may be possible by metabolite analysis [9, 10]. For such compounds gene expression profiling has also proved to be a valuable tool for the MoA classification [11, 12]. When used as fingerprint methods, metabolite profiling and gene expression profiling allows a rapid and reliable detection method for known MoA, and a clear identification and classification of unknown modes of action. Yet, despite the extensive progress in technology, MoA elucidation of novel targets is still – and will be for the near future – a highly demanding challenge. Only the combination of all available methodologies, with emphasis placed on traditional careful physiological and biochemical examinations, will reveal a clearly identified novel molecular target [13].

During the past decades, the identification of resistance mutations to pesticides has provided one of the most clear-cut approaches to target clarification. Although the technological progress has considerably fostered throughput in screening for mutations with a certain phenotype – so-called ''forward genetics'' [14] – it yet does not seem to be a reliable source of novel targets.

Once a target has been envisaged, further criteria for a ''good'' target are applied. Clearly, the most important criterion is the druggability of a target, which means accessibility by agro-like chemicals (see below) [15]. It is no coincidence, that the best druggable targets have preexisting binding niches, favoring ligands that comply with certain physico-chemical properties. Furthermore, the target should be relevant during the damaging life phase of a pest, and the destructive effect on a weed or pest under practical conditions should occur within a short period of time after treatment.

Having cleared all of these hurdles, an interesting target must be assayable in order to be exploited, which in turn makes assay technology capabilities a crucial asset. Overall, the number of promising targets remaining is at least two orders of magnitude lower than the number of potential targets found by gene knock-out [6]. Yet, even after having made such great efforts it still difficult to predict whether or not a new active ingredient will be identified, and whether a novel target finally will be competitive in the market.

Often, pharmaceutical research is systematically concentrated towards particular target classes, an example being protein kinases in cancer research [16]. Thereby, know-how can be accumulated and specialized technology can be concentrated for a higher productivity [17]. A successful target triggers the attention to the next similar targets, leading to a considerable understanding of, for example, the human kinome [18, 19]. A similar approach in agrochemical research is of limited value, as there are no such privileged target classes (Figure 1.4). In fact, the common denominator of the diverse agrochemical targets often is the destructive character of the physiological consequences of interference with the

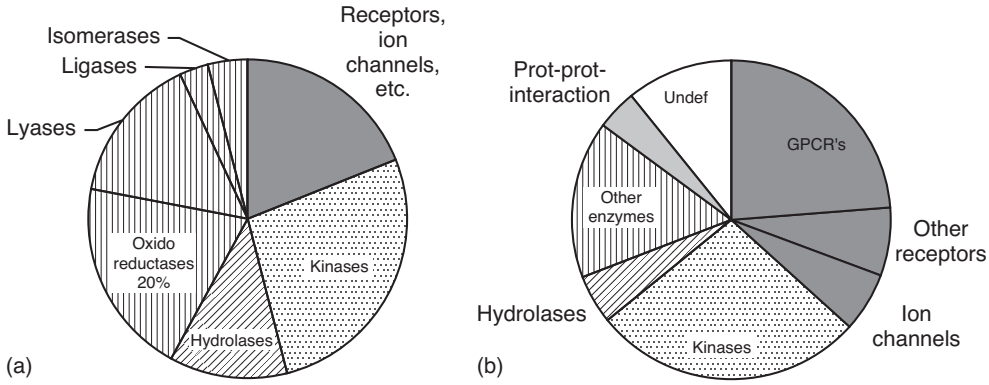

Figure 1.4 Classification by function of (a) agrochemical and (b) pharmaceutical targets (b) [23] for HTS.

target's function, sometimes even being a ''side-effect,'' such as the generation of reactive oxygen species (ROS) [6]. Nevertheless, there are exceptions – one of which is the class of protein kinases – which have been identified as a promising target class for fungicides [20–22].

1.2.2 High-Throughput Screening Techniques

In pharmaceutical research, HTS [24] has proven to be a major source of new lead structures [23], thereby motivating agrochemical research to incorporate – at least in part – this approach into the pesticide discovery process. At Bayer CropScience for example, the first HTS systems were set up during the late 1990s, after which the screening capacity expanded rapidly to more than 100 000 data points per day on a state-of-the-art technology platform. This included fully automated 384-well screening systems, a sophisticated plate replication and storage concept, a streamlined assay validation, and a quality control workflow. An expansion of the compound collection with the help of combinatorial chemistry and major investments in the development of a suitable data management and analysis system was also initiated.

The concept allows the screening of large numbers of compounds as well as large numbers of newly identified targets, thus yielding a corresponding number of hits. The simultaneously developed quality control techniques were able to separate valid hits from false-positives and/or uninteresting compounds due to various reasons (e.g., unspecific binding). Interestingly, several target assays deliver considerable numbers of *in vivo* active compounds, while for other *in vitro* HTS assays the often remarkable target inhibition was not transferred into a corresponding *in vivo* activity. In some cases, this can be attributed to an insufficient target lethality of more speculative targets or ''Agrokinetic'' factors for *in vivo* species. As discussed

earlier, the value of a thorough validation of (i) targets, (ii) assays, and (iii) chemical hits becomes evident.

The extended target validation led to increased numbers of target screens with *in vivo* active compounds. Hence, even more time could be spent on the hit validation, namely the introduction of control tests to eliminate, for example, readout interfering compounds (i.e., hits that were found only due to their optical properties or chemical interference with assay components).

The process of continuous improvement has to date shifted to an extended characterization of hits with respect to reactivity, binding modes [25] (competitive/non-competitive, reversible/irreversible, and so on [26]), speed of action and erratic inhibition due to ''promiscuous'' behavior of the compound class among others [27]. At the same time – if feasible – the hits or hit classes are submitted to orthogonal assays such as electrophysiology in case of neuronal targets, that help to further classify and validate the hits independent of the readout.

During the late 1990s, Bayer CropScience followed the trend introduced by pharmaceutical companies of conducting genomic projects in collaboration with a biotech-partner. Unfortunately, however, although this genomic approach provided more than 100 new screening assays, it did not deliver the desired output.

Hence, about five years ago the target-based screening approach was redirected, with the new direction subsequently leading to the following favorable changes:

- The screening of known MoA with validated inhibitors.
- A more stringent validation process together with indication biochemistry to ensure better starting points for chemistry.
- A cleansing of the screening library to increase the sample quality as well as the structural diversity of the collection.
- The screening of new, validated modes of action to help innovative areas such as plant stress or malaria.

Of great interest also was the observation that the relative percentage of enzyme assays compared to cell-based assays has increased (Figure 1.5) over the past 10 years. This finding reflects not only technological progress that has been made, but also the increasing back-concentration on ion channel targets for insecticides, which are especially highly validated targets.

At the same time, the chemical libraries at Bayer CropScience became more diversity-oriented, with major efforts being made to further increase the quality of the compound collections (see below), with especially careful quality checks of the hit compounds.

All of these measures together have greatly increased the proportion of true hits, so that finally the chemistry capacities are concentrated on fewer, albeit well-characterized, hit classes with a clearly increased likelihood of a successful hit-to-lead optimization.

The huge amount of data and information generated during the various phases of HTS and subsequent validation processes has triggered the development of sophisticated data analysis tools [28] that help biologists and chemists to select and

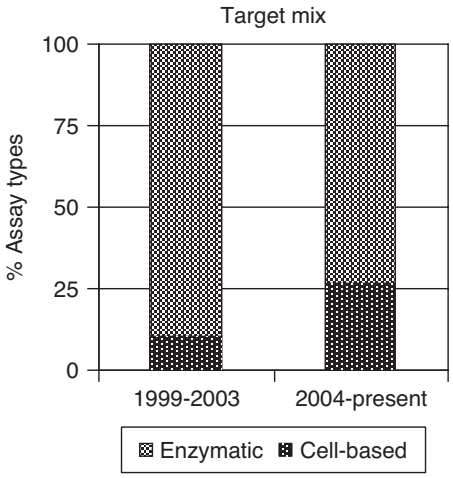

Figure 1.5 Proportions of assay types screened at Bayer Crop Science HTS facility. Between 1999 and 2003 (age of genomic targets) these included 10% cell-based assays (including cell-growth assays) and 90% enzyme assays. From 2004 to the present day (age of chemically validated targets), they included 26% cell-based assays (exclusively ion channels) and 74% enzyme assays.

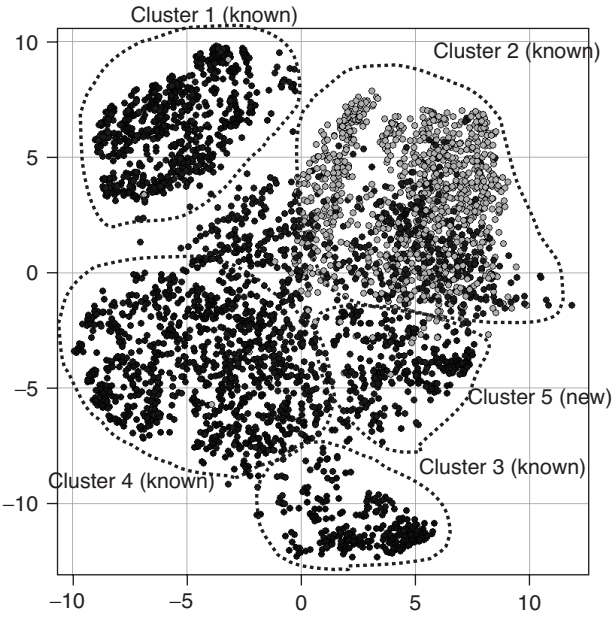

Figure 1.6 Example of the visualization of the chemical space of hits and similar inactive structures from a target assay. Light gray = inactive; gray to black = decreasing IC_{50}.

prioritize the most promising hits or hit classes (cluster of similar compounds) (Figure 1.6).

Biochemical *in vitro* screening may deliver compounds that, despite a clear target activity, are unable to exhibit *in vivo* activity due to, for example, unfavorable physico-chemical properties (lacking bioavailability), rapid metabolism, insufficient stability, or a poor distribution in the target organism. Nevertheless, these chemical classes are still of interest to chemists because such properties reflect the characteristics of the compounds that may be overcome by chemical optimization. As a consequence, ''agrokinetics'' has led to the identification of pure *in vitro* hits as such, and also helped to elucidate the reasons for failure in the *in vivo* test, thus guiding the *in vitro* to *in vivo* transfer of hit classes. Such observations underline the fact that the *in vitro* and *in vivo* screening processes can be complementary, and together can be used to broadly characterize the activity of test compounds within the early discovery process.

Currently, two trends can be observed among the high-throughput community: (i) miniaturization into the nanoliter dispensing regime; and (ii) new high-content screening (HCS) techniques. The small-volume screening (either on 1536-well plates or the recently introduced low-volume 384-well plates) clearly is also of interest for agrochemical research, since the enzymes and substrates of new target proteins are often difficult and costly to produce in larger quantities. Due to the above-mentioned screening strategy this process is not so much driven by the need to further increase the capacity, but rather by cost efficiency, the standard reaction volume having decreased from more than 50 µl to 5–10 µl (Figure 1.7). Moreover, further reductions are possible with new pipetting equipment having now reached a robust quality with inaccuracies of below 5% in the 1 µl range.

Other very important aspects of ion channel screening are the recently developed automated and medium-throughput patch clamping systems that perfectly meet the increased demand for in-depth hit characterization. Yet, the future role of HCS – fully automated confocal life cell microscopy imaging systems – is less clear than in pharmaceutical research, where it has become *the* validation and screening method development of the past few years [29]. Nonetheless, the applicability of HCS to agrochemical research will need to be evaluated in the future.

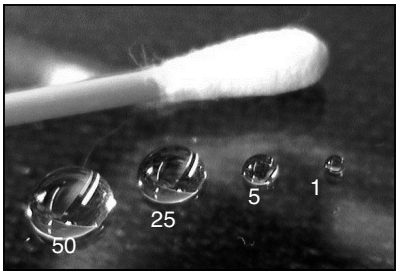

Figure 1.7 Size comparison of water drops between 50 and 1 µl as compared to a cosmetic tip.

1.3
Other Screening Approaches

1.3.1
High-Throughput Virtual Screening

During the past two decades, computational chemistry has become a key partner in drug discovery. Indeed, one of its main contributions to high-throughput methods is that of virtual screening [24, 30], a computational method that can be applied to large sets of compounds with the goal of evaluating or filtering those compounds against certain criteria, prior to or *in lieu* of *in vivo* or *in vitro testing*. In this regard, some methods consider target structure information while others are based solely on ligand similarity to complex model systems. Additionally, when three-dimensional information is incorporated into an analysis, the calculation becomes more demanding, especially if a flexible target protein is considered. Although massive screening with fully flexible models is not yet feasible, the so-called flexible *docking* of huge (both real and virtual) compound collections into a rigid binding pocket has today become routine [31]. The most obvious advantage of the latter method over the relatively fast similarity searches is that any compound which has binding site complementarity will be identified, and that no similarity to a known ligand is needed. This stands in contrast to similarity-based screening, where completely new scaffolds are rarely found.

In order to have a reasonable hit enrichment when using docking methods, computational chemistry must start with high-resolution protein structures; if possible, more than one ligand co-crystal would be used to construct the binding domain. In addition, some programs are capable of handling a certain degree of target flexibility through *ensemble formations* of binding domains from various experimental structures [32]. Whilst the quality of the results will obviously improve, a greater computational effort will be required as a consequence.

Virtual-target-based screening can be applied in many ways, the most obvious being the screening of huge libraries in order to prioritize the synthesis, acquisition and/or biochemical screening, or to select reactants for combinatorial libraries that show highest hit likeliness. These applications do yield target-focused libraries, and can be extended to families of targets, such as kinases or G-protein-coupled receptors (GPCRs).

1.4
In Vivo High-Throughput Screening

Since the very beginning of the search for new agrochemicals, *in vivo* screening has been the primary basis for agrochemical research, leading to the identification and characterization of new active chemistries and their subsequent optimization. In 1956, 1800 compounds needed to be evaluated for every one that became a product, a number that had risen to 10 000 by 1972 [33]. By 1995, the number has risen

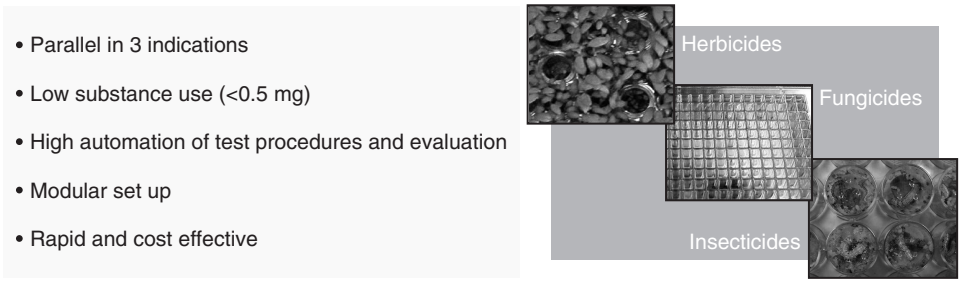

Figure 1.8 The advantages of high-throughput screening.

to more than 50 000, and today is about 140 000 compounds tested per product discovered [34]. In part, this rise is due to the increasing demands with regard to the need for increased biological activity, improved mammalian and environmental safety, as well as a variety of economic considerations. Beginning in the mid-1990s, most of the major agrochemical companies established *in vivo* HTS systems [15, 35–38], an interest which coincided with the development and expanding use of combinatorial libraries. In the HTS systems, the numbers of compounds screened each year are reported as ranging between 100 000 and 500 000, with most programs utilizing less than 0.5 mg of substance to produce relevant answers for a targeted set of plants, insects and fungi, using either 96-well or 384-well microtiter plates (MTPs) (Figure 1.8). Such HTS systems can produce a large number of hits, all of which are dependent on the screening dose, pass criteria, and the number and type of test species used. The quality of the hits from the HTS can be improved through the addition of extra dose rates and replicates [15], which can in turn improve the quality of the hits delivered to relevant follow-up screens.

HTS programs are based on automation, miniaturization, and often also the use of model organisms or systems which are easy to handle and adaptable to the MTP format. In pesticide discovery programs, model systems using *Aedes aegypti*, *D. melanogaster*, *A. thaliana*, *Caenorhabditis elegans* [39] or cell-growth-based fungicide assays can be successful in identifying a large number of hits. These model systems, using species that can be highly sensitive, are primarily intended to identify biological activity. However, in follow-up tests with agriculturally relevant species the number of interesting compounds often decreases dramatically due to a weak translation between the model organisms and the real pest species. As such, HTS systems with model organisms can potentially miss relevant hits (Figure 1.9).

As a consequence of this less-than-ideal translation, there has been an evolution among *in vivo* HTS systems to incorporate more relevant target organisms [40], particularly for insecticides and fungicides. For example, 96-well MTP assays involving pest lepidopteran larvae are widely used [15, 41, 42], while leaf-disc assays have been developed [6, 33, 43] that have been adapted by many companies for sap-feeding insects such as aphids.

HTS systems for fungicides utilize cell growth tests, but also cover only a part of the relevant target organisms; all obligate pathogens such as mildews or rusts cannot be tested. Additionally, such cell tests do not test the relevant phases of the

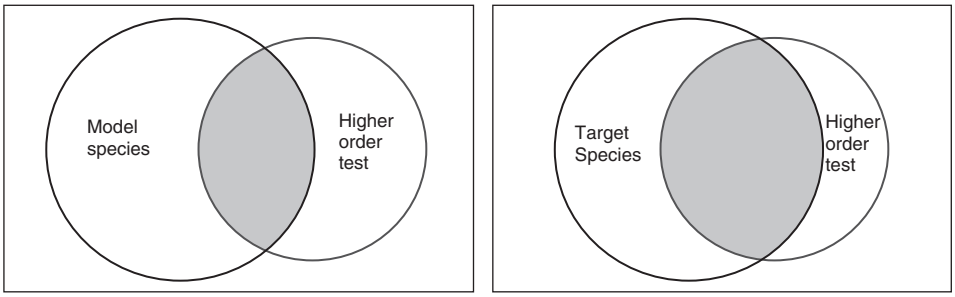

Figure 1.9 The overlap of mutual active chemical hits found in model species tests versus target species tests.

development of fungal pathogens on living plant tissues. However, this gap can be closed by using leaf discs [44, 45] or whole plants with relevant fungal species.

The development and further improvement of the more relevant HTS assays using target pest species for insecticides and fungicides is an on-going challenge. In many cases, these assays can be significantly more complex, and the time and effort required to run target organism assays can be greater than was required for previous model systems. As such, the number of species screened in an *in vivo* HTS has often been reduced to just a few, with one or two model species as general indicators of biological activity, plus perhaps a couple of specific pests that represent major product areas. For example, in the case of insecticides many discovery programs focus on one or two lepidopteran species that serve as indicators for a broad range of chewing pests, and an aphid species that is an indicator for a broad range of sap-feeding insect pests. While these two product areas do not denote the total insecticide market, they do capture the largest segments. Thus, the use of these more complex HTS systems requires a balance relative to throughput and dedicated resources for an *in vivo* HTS program. The net result is that better-characterized compounds with a more relevant biological profile are derived from HTS programs that focus on representative pest insects.

1.4.1
Compound Sourcing and *In-Silico* Screening

In order to achieve the ambitious goals of HTS, a large number of compounds are needed to satisfy the capacities of the tests. Consequently, many of the major chemical companies – both pharmaceutical and agrochemical – began to buy large numbers of ''off-the-shelf'' compounds [46] from so-called ''bulkers'' on a world-wide basis. Further, the boom triggered by combinatorial chemistry also helped to satisfy the need for large numbers of new substances, and this in turn led to the founding of several new companies that synthesized such materials (e.g., ArQule, BioFocus, or ChemBridge) to meet the demand. The compounds initially purchased were predominantly driven by availability and convenience. However, in spite of the increased throughput of compound screening, the number of new

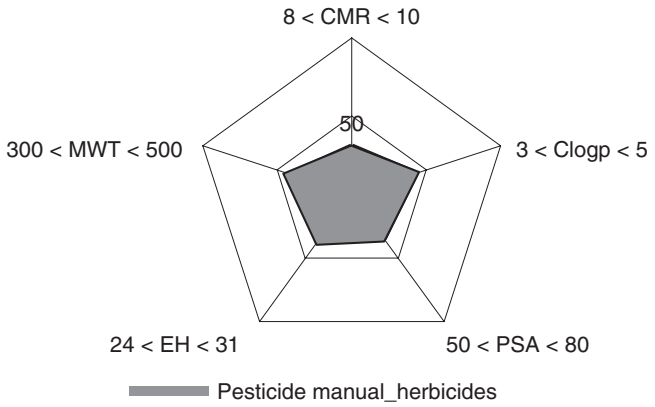

Figure 1.10 Percentages of herbicides in the *Pesticide Manual* [50] within constraint range. CMR, molar refractivity; EH, equivalent hydrocarbons; PSA, polar surface area.

biologically active classes of herbicides, fungicides and insecticides did not increase correspondingly. It was quickly recognized that for both pharmaceutical and agricultural compounds, certain constraints were needed on the types of compound acquired to obtain an effective level of relevant biological activity (Figure 1.10). Subsequently, in pharmaceutical research two general approaches emerged to resolve these problems, namely fragment-based screening and diversity-oriented synthesis [47, 48]. Agrochemistry commonly favors diversity to be early, in accord with the constraints posed on compounds. These constraints, along with (substructural) fingerprints as descriptors [49] for molecular similarity, have been applied to select chemical collections for agrochemical discovery.

A further refinement of the agro-like constraints [51, 52], assisted by *in-silico* screening, has further improved the diversity [53] of the collections. Importantly, with these and other *in-silico* approaches to refining and targeting the types and numbers of desired molecules [54], the requirement for screening vast numbers of compounds has been potentially reduced. Thus, improvements in the quality and relevance of the inputs to an HTS program should increase the number of potentially interesting compounds that emerge from that program.

In the area of combinatorial chemistry a significant realignment has occurred, with the starting points used for the libraries having changed from ''blue sky'' chemistries to more relevant scaffolds with a biological background [6, 55, 56]. Such considerations entail more intricate synthetic routes, which in turn can lead to a reduction in the size of the libraries. However, various studies have indicated that with a correct design, very large libraries are unnecessary for the adequate sampling of a desired chemical space, and that smaller libraries can be just as effective [55, 57]. With these considerations, the probability of obtaining better-quality hits is improved, thereby providing a better path forward in the early phases of lead finding. In the future, it is likely that a combination of agro-likeness tools and

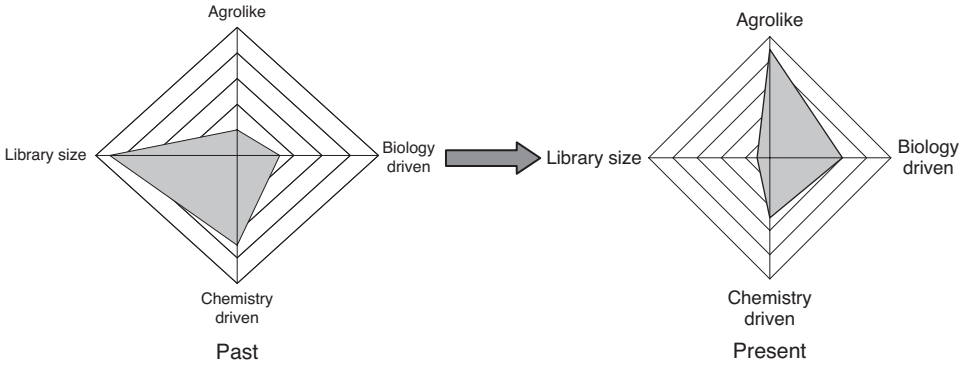

Figure 1.11 Higher input of agro-likeness and biological input in combinatorial chemistry scaffolds.

carefully chosen biological scaffolds will be among the approaches giving rise to new leads and, ultimately, to products for the agrochemical industry (Figure 1.11).

1.5 Conclusions

During the past 15 years, HTS has been adopted by the agrochemical industry as an essential component of the early discovery phase, in part to address the increasingly challenging requirements in the development of new pesticides and the declining success rates in the identification and development of new products. In contrast to the pharmaceutical industry, which extensively employs *in vitro* target-based HTS in its discovery programs, the agrochemical industry has the added advantage of being able to capitalize on *in vivo* HTS using, in part, the pest species of interest. The *in vivo* HTS programs have been developed using the experience of classical and well-established biological screening. In agrochemical research, the broad diversity of the target organisms presents a specific and complex challenge which must be carefully considered and addressed for each screening program. Fed by high-throughput chemistry, functional genomic projects and significant progress in robotic screening systems, procedures have been successfully established that allow agrochemical companies to test large numbers of compounds very efficiently and with a broad set of test organisms, including newly identified and well-established targets.

As an effective pesticide discovery program is continuously evolving, it is essential to continuously evaluate and incorporate the experiences concerning the advantages and limitations of new and established technologies and approaches. With modern agrochemical research platforms undergoing continuous and dynamic changes, adjustments to such platforms must be aimed at integrating the most promising parts of the many approaches that are currently available.

With the continued implementation of new technologies into the standard screening and testing workflows for both early and late research phases, a broad knowledge has been gained which by far exceeds the specific HTS approach alone. Moreover, such knowledge is being translated to overall improvements in agrochemical research. Finally, it is to be expected that, as a result of these new technologies, innovative products will emerge to meet the needs of modern agriculture.

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